



XXX CONGRESSO BRASILEIRO DE VIROLOGIA

XIV ENCONTRO DE VIROLOGIA DO MERCOSUL

16 A 19 DE OUTUBRO DE 2019 - CUIABÁ, MT

ANAIS 2019





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Scientific Programming

WEDNESDAY | OCTOBER 16th

3:00 p.m. / 6:00 p.m. - FLOWERS AUDITORIUM

PRE-CONGRESS ACTIVITY # 1

Chairperson: Luciana Barros de Arruda, UFRJ
ASM WORKSHOP ON SCIENCE COMMUNICATION AND OUTREACH
American Society for Microbiology

3:00 p.m. / 6:00 p.m. - TREES AUDITORIUM

PRE-CONGRESS ACTIVITY # 2

Chairperson: Fernando Spilki, Feevale
MINISTÉRIO DA SAÚDE (DECIT)

3:00 p.m. / 6:00 p.m. – 11th ROOM

PRE-CONGRESS ACTIVITY # 3

Chairperson: Fernando Spilki, Feevale
MINISTÉRIO DA SAÚDE (DECIT)

6:00 p.m. / 6:30 p.m. – WELCOME COFFEE

6:30 p.m. / 8:00 p.m. - FLOWERS AUDITORIUM

OPENING SESSION

CONFERENCE 1

Chairperson: Bergmann Morais Ribeiro (UNB)
PLANT VIRUSES TRANSMITTED BY BREVIPALPUS SAGA
Elliot Watanabe Kitajima (ESALQ/USP)

8:00 p.m. / 10:00 p.m. - CONVENTION CENTER HALL

CONFRATERNIZATION COCKTAIL



THURSDAY | OCTOBER 17th

9:00 a.m. / 10:15 a.m. - FLOWERS AUDITORIUM

CONFERENCE 2

Chairperson: Dr. Eurico de Arruda Neto, FMRP
**INTERPLAY BETWEEN DENGUE AND ZIKA:
WHAT AN ENDEMIC AREA CAN TEACH US**

Maurício Lacerda Nogueira (FAMERP)

9:00 a.m. / 10:15 a.m. - TREES AUDITORIUM

ORAL PRESENTATIONS SESSION 1

Chairperson: Dr. Abelardo Silva Junior, UFV;
Dra. Maria Isabel M. Coelho Guedes, UFMG; Dr. Mathias Martins, UNOESC
VETERINARY VIROLOGY 1

10:15 a.m. / 10:45 a.m. – COFFEE BREAK AND VISIT TO EHBITS

10:45 a.m. / 12:00 a.m. - FLOWERS AUDITORIUM

CONFERENCE 3

Chairperson: Dra. Maria Isabel M. Coelho Guedes
VETERINARY IMPORTANT ENTEROPATHOGENIC VIRUSES

Amauri Alcindo Alfieri (UEL)

10:45 a.m. / 12:00 a.m. - TREES AUDITORIUM

ORAL PRESENTATIONS SESSION 2

Chairperson: Prof. Iranaia Assunção Miranda, UFRJ;
Prof. José Luiz Proença Módena, UNICAMP; Prof. Renato S Aguiar UFMG
BASIC VIROLOGY 1

12:00 a.m. / 1:30 p.m. – LUNCH BREAK

1:30 p.m. / 3:15 p.m. - FLOWERS AUDITORIUM

ROUND TABLE 1

Chairperson: Dra. Gislaine Fongaro, UFSC
**ENVIRONMENTAL VIROLOGY
HEPATITIS E VIRUS: AN EMERGING FOODBORNE PATHOGEN?**

David Rodriguez-Lazaro (Un Burgos)

ROTAVIRUS AND VACCINATION IMPORTANCE

Marcelle Figueira Marques da Silva (UFG)

VIROLOGICAL RISK MANAGEMANT

Maria Tereza Pepe Razzolini (USP)



1:30 p.m. / 3:15 p.m. - TREES AUDITORIUM

ROUND TABLE 2

Chairperson: José Luiz Proença Módena, UNICAMP

OMICS VIROLOGY

OMICS TO REINFORCE LABORATORY-BASED SURVEILLANCE

Thiago Moreno Lopes e Souza (Fiocruz)

VIRAL METAGENOMIC DIVERSITY IN COASTAL AND MARINE ENVIRONMENTS

Gustavo Bueno Gregoracci (UNIFESP)

VIRUS HUNTING IN THE GENOMIC ERA

William Marciel de Souza (Unicamp)

3:15 p.m. / 3:45 p.m. – COFFEE BREAK AND VISIT TO EHBITS

3:45 p.m. / 5:15 p.m. - FLOWERS AUDITORIUM

ROUND TABLE 3

Chairperson: Daniel Mendes Pereira Ardisson de Araújo (UFSM)

INVERTEBRATE VIROLOGY

MOLECULAR BIOLOGY OF CHRYSODEIXIS INCLUDES VIRUSES

Bergmann Morais Ribeiro (UNB)

FUNDAMENTAL STUDIES ON BACULOVIRUS ACMNPV: IMPACT ON THE DEVELOPMENT OF BIOTECHNOLOGICAL TOOLS

Victoria Alfonso (INTA Argentina)

A NANOVIRUS TURNS ITS APHID VECTORS INTO WALKING-DEAD

Stephane Blanc (INRA França)

3:45 p.m. / 5:15 p.m. - TREES AUDITORIUM

ORAL PRESENTATIONS SESSION 3

Chairperson: Dra. Paula Rahal, UNESP; Dra. Isabel M.V.G. Carvalho Mello, BUTANTAN

HUMAN VIROLOGY 1

5:15 p.m. / 6:30 p.m. - FLOWERS AUDITORIUM

CONFERENCE 4

Chairperson: Dr. Flavio Guimarães da Fonseca, UFMG

VIRAL INTERACTION, IRNA / ARBOVIRUS

João Trindade Marques (UFMG)

6:30 p.m. / 8:00 p.m. - CONVENTION CENTER HALL

POSTERS SESSION 1



FRIDAY | OCTOBER 18th

9:00 a.m. / 10:45 a.m. - FLOWERS AUDITORIUM

ROUND TABLE 4

Chairperson: Dr. Mathias Martins, UNOESC

SMALL ANIMAL VIRUSES

"PANORAMA DE MORBILLIVIRUS FELINO NO BRASIL"

Alice Fernandes Alfieri (UEL)

EPIDEMIOLOGICAL, CLINICAL-PATHOLOGICAL AND GENETIC FEATURES OF CANINE PARVOVIRUS (CPV-2) IN DOGS IN SOUTHERN BRAZIL

Pablo Sebastian Britto de Oliveira (UFSM)

DRUG AND GENE THERAPY FOR CANINE MORBILLIVIRUS

Abelardo Silva Júnior (UFV)

9:00 a.m. / 10:45 a.m. - TREES AUDITORIUM

ROUND TABLE 5

Chairperson: Dra. Isabel M.V.G. Carvalho Mello, BUTANTAN

VIRAL GLOBAL SPREAD

CHIKUNGUNYA VIRUS AND NEUROLOGICAL SPECTRUM DISORDERS

Soniza Vieira Alves Leon (UniRio)

HANTAVÍRUS AND ARENAVIRUS IN BRAZIL: AN UNPREDICTABLE AND LATENT HUMAN HEALTH THREAT

Elba Regina Sampaio de Lemos (Fiocruz)

VIRUSES THAT WE CARRY AROUND: PUZZLES OF HUMAN LYMPHOID TISSUE VIROME

Eurico de Arruda Neto (USP)

10:45 a.m. / 11:15 a.m. – COFFEE BREAK AND VISIT TO EXHIBITS

11:15 a.m. / 12:30 p.m. - FLOWERS AUDITORIUM

CONFERENCE 5

(Chairperson: Renato S Aguiar UFMG)

ARBOVIRUSES REAL TIME SURVEILLANCE

Luiz Carlos Junior Alcântara (Fiocruz)



11:15 a.m. / 12:30 p.m. - TREES AUDITORIUM

ROUND TABLE 6

Chairperson: Rafael Elias - LNBio

VIRAL STRUCTURE AND PATHOGENESIS

STRUCTURE-GUIDED REVERSE VACCINOLOGY APPROACHES TO PROTECT AGAINST ENVELOPED VIRUSES

Felix A. Ray (Instituto Pasteur)

ARBOVIRUS ENVELOPE LIPID COMPOSITION AND VIRUS CELL-INTERACTIONS

André Marco de Oliveira Gomes (UFRJ)

TARGETING FLAVIVIRUS NON-STRUCTURAL PROTEINS FOR DRUG DISCOVERY

Andre S. Godoy (IF-USP)

12:30 p.m. / 2:00 p.m. – LUNCH BREAK

2:00 p.m. / 3:30 p.m. - FLOWERS AUDITORIUM

ORAL PRESENTATIONS SESSION 4

Chairperson: Prof. Iranaia Assunção Miranda, UFRJ;

Prof. José Luiz Proença Módena, UNICAMP; Prof. Renato S Aguiar UFMG

BASIC VIROLOGY 2

2:00 p.m. / 3:30 p.m. - TREES AUDITORIUM

ORAL PRESENTATIONS SESSION 5

Chairperson: Dra. Caroline Rigotto, FEEVALE; Dra. Gislaine Fongaro, UFSC

ENVIRONMENTAL VIROLOGY

3:30 p.m. / 4:00 p.m. – COFFEE BREAK AND VISIT TO EHBITS

4:00 p.m. / 5:15 p.m. - FLOWERS AUDITORIUM

CONFERENCE 6

Chairperson: Poliane Alfenas Zerbini, UFV

ORIGIN OF VIRUSES: PRIMORDIAL REPLICATORS RECRUITING CAPSIDS FROM HOSTS

Valerian Dolja (Oregon St. University)



4:00 p.m. / 5:15 p.m. – TREES AUDITORIUM

ROUND TABLE 7

Chairperson: Dr. Abelardo Silva Junior, UFV

EMERGING VIRUSES

PATHOGENESIS AND TRANSMISSION OF VIRULENT NEWCASTLE DISEASE VIRUSES

Helena Lage Ferreira (USP)

DETECTION OF WEST NILE VIRUS IN EQUIDS IN BRAZIL:

CHALLENGES AND PERSPECTIVES

Érica Azevedo Costa (UFMG)

SENECAVIRUS

Amauri Alcindo Alfieri (UEL)

5:15 p.m. / 7:00 p.m. - FLOWERS AUDITORIUM

ROUND TABLE 8

Chairperson: Dra. Paula Rahal, UNESP

ONCOLITIC IMMUNOTHERAPY

HTLV-1 INFECTION: A NEGLECTED HEALTH PROBLEM IN BRAZIL

Marzia Puccioni Sohler (UFRJ)

**ACTIVE IMMUNOTHERAPY: A NOVEL THERAPEUTIC CONCEPT TO CONTROL HPV
INDUCED TUMORS**

Luís Carlos de Souza Ferreira (USP)

TRANSPOSON-BASED CANCER IMMUNO-GENE THERAPY

Martin Hernan Bonamino (INCA)

5:15 p.m. / 7:00 p.m. – TREES AUDITORIUM

ROUND TABLE 9

Chairperson: Poliane Alfenas Zerbini, UFV

PLANT AND INVERTEBRATE VIROLOGY

TOSPOVIRUS-HOST INTERACTION

Renato de Oliveira Resende (UnB)

POLEROVIRUS HOST INTERACTION

Maite Vaslin de Freitas Silva (UFRJ)

IFLAVIRUS HOST INTERACTION

Daniel Mendes Pereira Ardisson de Araújo (UFES)

7:00 p.m. / 8:30 p.m. - CONVENTION CENTER HALL

POSTERS SESSION 2



SATURDAY | OCTOBER 19th

9:00 a.m. / 10:30 a.m. - FLOWERS AUDITORIUM

PRESENTATION OF WORK

Chairperson: Joao Pessoa Araujo Jr., UNESP

HELIO GELLI PEREIRA AWARD

9:00 a.m. / 10:30 a.m. - TREES AUDITORIUM

ORAL PRESENTATIONS SESSION 6

Chairperson: Dr. Abelardo Silva Junior, UFV;

Dra. Maria Isabel M. Coelho Guedes, UFMG; Dr. Mathias Martins, UNOESC

VETERINARY VIROLOGY 2

10:30 a.m. / 11:00 a.m. – COFFEE BREAK AND VISIT TO EHBITS

11:00 a.m. / 12:30 a.m. - FLOWERS AUDITORIUM

ROUND TABLE 10

VIRUS-CELL INTERACTIONS

Chairperson: Iranaia Assunção-Miranda, UFRJ

FLAVIVIRUS MODULATION OF CELLULAR LIPIDS METABOLISM

Glenn C Randall (University of Chicago)

EPIGENETIC REGULATION OF CD8 T CELLS BY POLYCOMB PROTEINS

DURING LCMV INFECTION IN MICE

Renata de Meirelles Santos Pereira (UFRJ)

COMMON CELLULAR PATHWAYS AND GENE REGULATION EXPRESSION MECHANISMS

MODULATED BY ENCEPHALITIS CAUSING ARBOVIRUSES (Zika, Chikungunya,

Oropouche, and Mayaro)

Renato Santana de Aguiar (UFMG)

11:00 a.m. / 12:30 a.m. - TREES AUDITORIUM

ORAL PRESENTATIONS SESSION 7

(Chairperson: Dra. Isabel M.V.G. Carvalho Mello, BUTANTAN; Dra. Paula Rahal, UNESP)

HUMAN VIROLOGY 2

12:30 p.m. / 2:00 p.m. – LUNCH BREAK



2:00 p.m. / 3:15 p.m. - FLOWERS AUDITORIUM

ROUND TABLE 11

ANTIVIRALS

(Chairperson: Prof. Renato S Aguiar UFMG)

NEW DESIGNS, NEW APPROACHES, NEW TARGETS, NEW VIRUSES

Luciana Jesus da Costa (UFRJ)

A NOVEL APPROACH TO HCV THERAPY

Bruno Moreira Carneiro (UFMT)

**ANTIVIRAL ACTIVITY OF NATURAL PRODUCTS FROM
MATO GROSSO STATE**

Carla Regina Andrighetti (UFMT)

TAKEDA DENGUE VACCINE

Eduardo J. M. Nascimento (TAKEDA Vaccine Business Unit)

2:00 p.m. / 3:15 p.m. - TREES AUDITORIUM

ORAL PRESENTATIONS SESSION 8

**Chairperson: Dr. Daniel M. P. Ardisson-Araujo, UFSM, Poliane Alfenas Zerbini, UFV
VIROLOGY OF PLANTS AND INVERTEBRATES**

3:15 p.m. / 3:45 p.m. – COFFEE BREAK AND VISIT TO EHBITS

3:45 p.m. / 5:30 p.m. - FLOWERS AUDITORIUM

CONFERENCE 7

Chairperson: José Luiz Proença Módena, UNICAMP

HEPATITIS C VIRUS HOST INTERACTIONS

Glenn C Randal (University of Chicago)

3:45 p.m. / 5:30 p.m. - TREES AUDITORIUM

ROUND TABLE 12

Chairperson: Dr. Daniel M. P. Ardisson-Araujo, UFSM

PLANT VIRUS DIVERSITY AND EVOLUTION

**NEW CONCEPTUAL FRAMEWORKS ARE REQUIRED TO UNDERSTAND THE BIOLOGY OF
MULTIPARTITE VIRUSES**

Stephane Blanc (INRA França)

ORIGINS AND EVOLUTION OF THE GLOBAL RNA VIROME

Valerian Dolja (Oregon St. University)

EVOLUTIONARY DYNAMICS OF BIPARTITE BEGOMOVIRUSES:

ONE GENOME, TWO HISTORIES

Francisco Murilo Zerbini Junior (UFV)



5:30 p.m. / 6:00 p.m. - FLOWERS AUDITORIUM

**AWARDS
CLOSURE**

6:00 p.m. / 7:00 p.m. - FLOWERS AUDITORIUM

SBV GENERAL ASSEMBLY

8:00 p.m. – CLOSURE PARTY BY ACCESSION



List of Speakers:

Conferences:

- 1-) Dr. Elliot Watanabe Kitajima, UnB
- 2-) Dr. Mauricio Lacerda Nogueira, FAMERP
- 3-) Dr. Amauri Alcindo Alfieri, UEL
- 4-) Dr. João Trindade Marques, UFMG
- 5-) Dr. Luiz Carlos Junior Alcântara, Fiocruz
- 6-) Dr. Valerian Dolja, Oregon State University, EUA
- 7-) Dr. Glenn C. Randal, University of Chicago, EUA

Round Tables:

Round table 1

- Dr. Gislaine Fongaro (Universidade Federal de Santa Catarina, UFSC) - Chair
Dr. David Rodriguez-Lazaro (University of Burgos, Spain)
Dr. Marcelle Figueira Marques da Silva (Universidade Federal de Goiás, UFG)
Dr. Maria Tereza Pepe Razzolini (USP)

Round table 2

- Dr. José Luiz Proença Módena (Universidade de Campinas, UNICAMP) - Chair
Dr. Thiago Moreno Lopes e Souza (Fiocruz, Rio de Janeiro),
Dr. Gustavo Bueno Gregoracci (Universidade Federal de São Paulo, UNIFESP)
Dr. William Marciel de Souza (UNICAMP)

Round table 3,

- Dr. Daniel Mendes Pereira Ardisson de Araújo (Universidade Federal de Santa Maria, UFSM) - Chair
Dr. Bergmann Morais Ribeiro (Universidade de Brasília, UNB)
Dr. Stephane Blanc's (Institut national de la recherche agronomique, INRA, France)

Round table 4,

- Dr. Mathias Martins (Universidade do Oeste Catarinense, UNOESC) - Chair
Dr. Alice Fernandes Alfieri (UEL)
Dr. Pablo Sebastian Britto de Oliveira (UFSM)
Dr. Abelardo Silva Junior (Universidade Federal de Viçosa, UFV)



Round table 5

Dr. Isabel M.V.G. Carvalho Mello (Instituto BUTANTAN) - Chair
Dr. Soniza Vieira Alves Leon (Universidade do Rio de Janeiro, UniRio)
Dr. Elba Regina Sampaio de Lemos (Fiocruz, Rio de Janeiro)
Dr. Eurico de Arruda Neto (USP)

Round table 6

Dr. Rafael Elias (Laboratorio Nacional de Biosciências, LNBio) - Chair
Dr. Felix A. Rey's (Instituto Pasteur, France)
Dr. André Marco de Oliveira Gomes (Universidade Federal do Rio de Janeiro, UFRJ)
Dr. Andre S. Godoy (USP)

Round table 7

Dr. Abelardo Silva Junior (UFV) - Chair
Dr. Helena Lage Ferreira (USP)
Dr. Érica Azevedo Costa (UFMG)
Dr. Amauri Alcindo Alfieri (UEL)

Round table 8

Dr. Paula Rahal (Universidade do Estado de São Paulo, UNESP) - Chair
Dr. Marzia Puccioni Sohler (UFRJ)
Dr. Luís Carlos de Souza Ferreira (USP)
Dr. Martin Hernan Bonamino (Instituto Nacional do Câncer, INCA)

Round table 9

Dr. Poliane Alfenas Zerbini (UFV) - Chair
Dr. Renato de Oliveira Resende (UnB)
Dr. Maite Vaslin de Freitas Silva (UFRJ)
Dr. Victoria Alfonso (Instituto Nacional de Tecnología Agropecuaria, Argentina)

Round table 10

Iranaiá Assunção-Miranda, (UFRJ) - Chair
Dr. Glenn C Randall (University of Chicago, USA)
Dr. Luciana Jesus da Costa (UFRJ)
Dr. Renato Santana de Aguiar's (UFMG)

Round table 11

Dr. Renato Santana de Aguiar (UFMG) - Chair
Dr. Luciana Jesus da Costa (UFRJ)



Dr. Bruno Moreira Carneiro (Universidade Federal de Rondonópolis, UFR)
Dr. Carla Regina Andrighetti (Universidade Federal de Mato Grosso, UFMT)
Dr. Eduardo J. M. Nascimento (TAKEDA Vaccine Unit, England)

Round table 12

Dr. Daniel M.P. Ardisson-Araujo (UFSM) - Chair
Dr. Stephane Blanc (INRA, France)
Dr. Valerian Dolja (Oregon State University, USA)
Dr. Francisco Murilo Zerbini Junior (UFV)



Presentation

In 2019, the 30th Brazilian Congress of Virology was held in Cuiabá, capital of the state of Mato Grosso, a city located on the edge of one of the richest and most threatened biomes in the world, the Pantanal. The first time that Brazilian virologists met in this city was full of symbolism, the need to better understand the relationship between the stability and conservation of natural ecosystems to prevent the emergence of new viruses, a new pact between the human species and the nature that could represent a slowdown in the accelerated process of arrival of new pathogens to humans and domestic species and the transshipment of viruses from our ecosphere to wild species.

It was a Congress of struggle and resistance, at a time of historic decline in financial resources and government stimulus for science and technology. We actually had one of the least attended events in our history, given the lack of financial support for students and the general economic crisis itself. Nevertheless, we had a hot Congress (typical of the climate of Cuiabá), with fraternization among peers and of the a clear demonstration of the strength of Brazilian Virology, in its all areas, from human to plant virology, and the excellence of our research is well represented in this book of abstracts.

Support





Index

Ficha Catalográfica	02
Diretoria.....	03
Scientific Programming	04
List of Speakers	13
Presentation	16
ENVIRONMENTAL VIROLOGY	33
HOUSEHOLD-BASED BIODIGESTERS PROMOTE THE REDUCTION OF ENTERIC VIRUSES AND BACTERIAS IN VULNERABLE AND POVERTY RURAL AREA	34
MICROBIOLOGICAL EVALUATION OF MAMPITUBA RIVER - TORRES / RS	35
MICROBIOLOGICAL EVALUATION OF WATER AND SAND OF NORTH RIO GRANDE DO SUL BEACHES.....	36
A NEW DSRNA MYCOVIRUS INFECTING THE PHYTOPATHOGENIC FUNGI MYCOSPHAERELLA FRAGARIAE	37
WIDESPREAD DISTRIBUTION OF PROPHAGES SIGNALING THE POTENTIAL FOR ADAPTABILITY AND PATHOGENICITY EVOLUTION OF RALSTONIA SOLANACEARUM COMPLEX GENOMES	38
EXPLORING THE VIRAL DIVERSITY OF SINGLE-STRANDED (SS) DNA VIRUSES IN DAIRY CATTLE RUMEN.....	39
THE INVOLVEMENT OF VIRAL SRNAS IN THE CONVERSION OF THE PHYTOPATHOGENIC RALSTONIA PSEUDOSOLANACEARUM INTO A COMMENSAL BACTERIUM BY AN INOVIRUS.....	40
RELATIONSHIP BETWEEN ZIKA VIRUS INFECTION AND PLUVIOMETRIC PRECIPITATION IN MATO GROSSO, 2016.	41
INFECTION RISK ASSESSMENT OF HUMAN MASTADENOVIRUS SPECIES C AND F IN CONTAMINATED WATERS FROM SOUTHERN BRAZIL	42
MICROBIOLOGICAL EVALUATION IN THE COMPOSTING PROCESS FROM A DRY TOILET UNIT	43
HUMAN MASTADENOVIRUS IN ENVIRONMENTAL WATER SAMPLES DETECTED BY REAL TIME PCR AND IMMUNOCHROMATOGRAPHIC.....	44
WATER AND SEWAGE COLIFAGES AS MICROBIOLOGICAL INDICATORS OF FECAL CONTAMINATION	45
MOLECULAR DETECTION OF OROPOUCHE VIRUS IN MOSQUITOES FROM MATO GROSSO, BRAZIL, 2017	46
RISK OF ZIKA VIRUS INFECTION IN MATO GROSSO MUNICIPALITIES, BRAZIL, 2016.....	47



CONTAMINATION OF THE SOIL FROM A PUBLIC PARK BY HUMAN AND CANINE MASTADENOVIRUSES	48
COMPARISON OF IMMUNOMAGNETIC SEPARATION AND ULTRACENTRIFUGATION AS CONCENTRATION METHODS FOR HUMAN MASTADENOVIRUS IN WATER SAMPLES	49
PROSPECTION OF NEW ENTEROBACTER AEROGENES BACTERIOPHAGES FOR BACTERIA CONTROL PURPOSE.....	50
BACTERIOPHAGES ISOLATION AGAINST KLEBSIELLA PNEUMONIAE AND PROTEUS MIRABILIS FOR ENVIRONMENTAL APPLICATION	52
BRAZILIAN CATTLE BACTERIOPHAGES ISOLATION.....	53
VIRUCIDAL POTENCIAL OF MICROALGAE EXTRACTS CULTIVATED IN SWINE MANURE.....	54
DOE OPTIMIZATION PROCESS OF A T4-LIKE BACTERIOPHAGE USING THE ROTATIONAL CENTRAL COMPOSITE DESIGN (RCCD) METHODOLOGY TO DETERMINE OPTIMAL CARBON SOURCES AND CULTIVE CONDITIONS.....	55
EXPANDING THE REPERTOIRE OF AMOEBA GIANT VIRUSES: ISOLATION AND CHARACTERIZATION OF ORPHEOVIRUS BRASILIENSIS IN VERMOAMEBA VERMIFORMIS...	56
INFLUENCE OF FAECAL CONTAMINATION FROM THE CAMBORIÚ RIVER ON THE MICROBIOLOGICAL QUALITY OF WATER IN A BIVALVE SHELLFISH PRODUCTION AREA.....	57
EVALUATION OF LYTIC PHAGE POTENTIAL IN DECREASE BIOFILM FORMATION OF ENTEROBACTERIA.....	58
DETECTION OF HUMAN BOCAVIRUS RECOMBINANT STRAINS IN SEWAGE FROM URUGUAYAN CITIES	59
EDETECTION, QUANTIFICATION AND MICROBIAL RISK ASSESSMENT OF GROUP A ROTAVIRUS IN RIVERS FROM URUGUAY.....	60
ENTERIC VIRUSES DETECTION IN ENVIRONMENTAL WATER FROM MIDWEST-BRAZIL AFTER ONE DECADE OF ATTENUATED VACCINE AGAINST ROTAVIRUS INTRODUCTION	61
INFLUENCE OF PHAGE VB_ECOM-UFV13 ON BIOFILM FORMED BY CONSORTIUM P48SEP ..	62
ROTAVIRUS A IN WILD AND DOMESTIC ANIMALS FROM AREAS WITH ENVIRONMENTAL DEGRADATION IN THE BRAZILIAN AMAZON.....	63
PREVALENCE AND INCIDENCE DE DENGUE AND ZIKA IN THE PARTICIPANTS OF THE PROSPECTIVE COHORT STUDY IN SAO JOSE DO RIO PRETO, SP.	65
HIV-1 TAT MODULATES M1-M2 ACTIVATION PHENOTYPE OF BV-2 MICROGLIAL CELLS.	66
INFECTION OF ENDOTHELIAL CELLS BY DENGUE VIRUS INDUCED ROS PRODUCTION BY DIFFERENT SIGNALING PATHWAYS, AFFECTING VIRUS REPLICATION, CELLULAR ACTIVATION, DEATH AND VASCULAR PERMEABILITY	67
PHENOTYPE OF VIRUS-LIKE PARTICLES (VLP) AND HPV-POSITIVE CARCINOMA CELL LINES BY ELECTRON MICROSCOPY	68
IN VITRO EVOLUTION OF ZIKA VIRUS IN INSECT CELLS.....	69
HUMAN INTERFERON-INDUCED PROTEIN WITH TETRATRICOPEPTIDE REPEATS 5 (IFIT5) INHIBIT RABIES VIRUSES	70



RABIES VIRUS ISOLATION IN HUMAN EMBRYONIC KIDNEY (HEK-293T) CELL LINE: AN ALTERNATIVE FOR RABIES DIAGNOSIS AND RESEARCH	71
ANALYSIS OF THE EFFECT OF ANTIOXIDANT ACTIVITY OF A CARBON-BASED NANOMATERIAL ON ZIKA VIRUS INFECTIONS.....	72
HUMAN BETAHERPESVIRUSES 6 AND 7 SALIVARY SHEDDING IN RENAL TRANSPLANTATION RECIPIENTS: LONGITUDINAL STUDY REVEALS ACTIVE REPLICATION.....	73
THE ROLE OF P53 PROTEIN IN HCMV REPLICATION IN U138 GBM CELL LINE.....	74
THE HOST PROTEIN AP1 IS RELEVANT TO HIV-1 NEF ANTAGONISM AGAINST SERINC5	75
IMMUNOMODULATION OF MONOCYTES AND LYMPHOCYTES BY HYDROXYPROPYL-BETA-CYCLODEXTRIN (HP- BCD) AS A POTENTIAL STRATEGY TO CONTAIN HIV-ASSOCIATED CRHONIC IMMUNE ACTIVATION	76
DIFFERENTIAL MODULATION OF TYPE I IFN RESPONSE BY DISTINCT ZIKA VIRUS ISOLATES AND ITS ROLE FOR VIRUS REPLICATION AND DISSEMINATION TO THE CENTRAL NERVOUS SYSTEM	77
INVESTIGATION OF THE ROLE OF VARIATIONS IN E AND NS1 PROTEINS IN ZIKA VIRUS PATHOGENESIS.....	78
THE PROTEIN COMPLEX MTORC MAY INFLUENCE CHIKV INFECTION IN MURINE DENDRITIC CELLS.....	79
THE ROLE OF INNATE RECOGNITION PATHWAYS IN PLACENTAL CELLS AFTER OROV INFECTION.....	80
CELLULAR ALIX PROTEIN: A HIV INFECTIVITY PROMOTER	81
MOLECULAR, BIOLOGY AND CLINICAL CHARACTERIZATION OF CHIKUNGUNYA VIRUS STRAIN RJ-IB1 FROM RIO DE JANEIRO, BRAZIL.....	82
NOVEL QUINOLONE DERIVATIVE COMPOUNDS: BIOTECHNOLOGICAL APPLICATION AS ANTI-MAYARO AGENTS.....	83
HIV-1 NEF PROTEIN REGULATES VIRAL PROTEASE ACTIVITY TO INCREASE VIRAL INFECTIVITY VIA ALIX.....	84
A POSSIBLE ROLE OF RAB27A/B IN OROPOUCHE VIRUS REPLICATION CYCLE.....	85
ZIKA VIRUS INHIBITION BY COPAIBA (COPAIFERA OFFICINALIS) OIL NANOEMULSION	86
A NEW (?) PHLEBOVIRUS ISOLATED FROM AMAZONIAN SANDFLIES	87
HUMAN HERPESVIRUS 6 (HHV-6) AND HUMAN HERPESVIRUS 7 (HHV-7) EXCRETION IN ORAL FLUIDS OF PATIENTS WITH CHRONIC HEPATITIS C	88
POXVIRUS-HOST INTERACTIONS: THE ACTIVATION OF COMPONENTS OF THE HOST'S UNFOLDED PROTEIN RESPONSES (UPR) DURING INFECTIONS BY THE VACCINIA VIRUS STRAINS GUARANI P1 AND PASSATEMPO	89
IDENTIFICATION OF DIFFERENTIALLY EXPRESSED MIRNAS IN HUMAN PROSTATIC CELLS INFECTED WITH ZIKV	90
FULL GENOME CHARACTERIZATION OF GROUP II CONFIRMS THE DICHOTOMY BETWEEN BRAZILIAN VACCINIA VIRUS.....	91



ILHEUS VIRUS IDENTIFIED IN THE CEREBROSPINAL FLUID OF A PATIENT WITH CEREBRAL HEMORRHAGE IN AN ARBOVIRUS ENDEMIC AREA.....	92
IN-DEPTH ANALYSES OF THE REPLICATION CYCLE OF ORPHEOVIRUS EVIDENCED MORPHOLOGICAL CHANGES IN VERMAMOEBIA VERMIFORMES	93
MOLECULAR CHARACTERIZATION AND PHYLOGEOGRAPHIC ANALYSIS OF THE FIRST COMPLETE GENOMES OF SUBTYPE 2B HEPATITIS C VIRUS IN LATIN AMERICA.....	94
GENETIC DIVERSITY AND MOLECULAR EPIDEMIOLOGY OF HIV-1 AMONG THERAPEUTIC FAILURE PATIENTS FROM SANTA CATARINA STATE, SOUTHERN BRAZIL.	95
VERTICAL NATURAL INFECTION IN CULICIDAE FROM MATO GROSSO, BRAZIL.....	96
GENE EXPRESSION MODULATION INDUCED BY OROPOUCHE VIRUS INFECTION IN ENDOTHELIAL CELLS	97
THE ANTIBODY PRODUCTION AND INNATE IMMUNE RESPONSE BY B CELLS ARE ESSENTIAL FOR RESTRICTION OF OROPOUCHE VIRUS PRIME-INFECTION.....	98
QUANTITATIVE COMPARISON BETWEEN VERO-76, C6/36 AND BHK-21 CELL LINES USED AS FLUORESCENT FOCUS ASSAY SUBSTRATE FOR FLAVIVIRUS TITRATION.	99
STANDARDIZATION OF FLUORESCENT FOCUS ASSAY AND COMPARISON WITH THE “GOLD STANDARD” PLAQUE ASSAY FOR DENGUE, YELLOW FEVER AND ZIKA VIRUS TITRATION USING VERO-76 AND BHK-21 CELL LINES.....	100
COMPARISON OF IMMUNE RESPONSE IN MICE INTRACRANIALY INFECTED WITH DIFFERENT ZIKA VIRUS ISOLATES.....	101
PRODUCTION OF HUMAN CYTOMEGALOVIRUS UL111A TRANSCRIPTS IN FIBROBLASTS AND GLIOBLASTOMA CELL LINES: IDENTIFICATION OF A NEW TRANSCRIPT	102
QUANTIFICATION OF THE UL111A HUMAN CYTOMEGALOVIRUS TRANSCRIPTS IN PRODUCTIVE AND LATENT INFECTED CELLS	103
OROPOUCHE VIRUS INFECTION OF HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS IN VITRO.....	104
THE CRISPR/CAS9 COMPLEX AS A NEW ANTIVIRAL THERAPY AGAINST HERPES SIMPLEX TYPE 1.....	105
PHYLOGENETIC AND STRUCTURAL ANALYSIS OF THE 5' AND 3' UNTRANSLATED REGIONS OF THE MAYARO VIRUS GENOME	107
MOLECULAR ANALYSIS OF DENGUE VIRUS TYPE 4 INTRODUCED IN 2012 IN MATO GROSSO, BRAZIL.....	108
HUMAN VIROLOGY.....	109
YELLOW FEVER VIRUS DETECTION BY RT-QPCR IN Aedes SCAPULARIS MOSQUITO, SÃO PAULO, BRAZIL	110
MOLECULAR EPIDEMIOLOGY OF NOROVIRUS AND CIRCULATION OF THE EMERGENT RECOMBINANT STRAINS GII.P16- GII.4 AND GII.P16-GII.2 IN BRAZIL, 2017-2018.	111
PREVALENCE OF NOROVIRUS AND OTHER ENTEROPATHOGENS AMONG CHILDREN HOSPITALIZED FOR ACUTE GASTROENTERITIS IN BELÉM, PARÁ, BRAZIL	112



ROLE OF TAM RECEPTOR LIGAND GAS6 IN THE PATHOGENESIS OF ZIKA VIRUS INFECTION	113
EPIDEMIOLOGICAL PROFILE OF WOMEN AND RESEARCH OF ARBOVIRUS IN THEIR MILK DONATED TO THE HUMAN MILK BANKS OF CUIABÁ-MT.....	114
FREQUENCY OF DRUG RESISTANCE MUTATIONS USED IN THE TREATMENT OF HIV INFECTION OBTAINED BY GENOTYPING TEST, CARRIED OUT IN THE PERIOD 2013 TO 2015 IN THE STATE OF PARÁ.....	116
DETECTION OF COINFECTION WITH CHIKUNGUNYA VIRUS AND DENGUE VIRUS SEROTYPE 2 IN SERUM SAMPLES OF PATIENTS IN STATE OF TOCANTINS, BRAZIL.....	117
DEVELOPMENT OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAYS FOR CONFIRMATORY DIAGNOSIS OF HTLV-1/2 INFECTIONS.....	118
MUTATIONS IN HTLV-1 TAX-RESPONSIVE ELEMENTS IN HAM/TSP PATIENTS ARE ASSOCIATED WITH LOWER PROVIRAL LOAD BUT NOT TO DISEASE PROGRESSION	119
SUSCEPTIBILITY TO MEASLES AND RUBELLA IN ADOLESCENTS, YOUTH AND ADULTS IN THE MUNICIPALITIES OF BELÉM AND ANANINDEUA.	120
EPIDEMIOLOGICAL ANALYSIS OF ZIKA AND DENGUE IN CITY OF LARANJAL DO JARI, STATE OF AMAPÁ, BRAZIL USING REAL TIME (RT-QPCR) AND SANGER-BASED SEQUENCING.....	121
EPIDEMIOLOGY OF VITAMIN D RECEPTOR POLYMORPHISMS IN THE CITY OF MACEIÓ/ALAGOAS AND THEIR RELATIONSHIP WITH ZIKA AND CHIKUNGUNYA VIRUS INFECTIONS.....	122
VIRUCIDAL INFLUENCE OF IMIDAZOLIUM IONIC LIQUIDS AGAINST TO ZIKA VIRUS.....	123
ANTIHERPES EVALUATION OF EXTRACTS AND FRACTIONS OF ILEX GUAYUSA LOES. LEAVES	124
STUDY OF 17 PATIENTS WITH GUILLAIN-BARRÉ SYNDROME OCCURRED IN CUIABÁ-MT.....	125
MOSQUITO POOL SAMPLES DO NOT INHIBIT ZIKV dPCR DETECTION	127
ANALYSIS OF SPATIAL DIFFUSION OF YELLOW FEVER EPIDEMIC IN STATE OF SÃO PAULO, 2016 TO 2019.	128
MAYARO VIRUS (MAYV) DETECTION IN PARÁ WESTERN.....	129
INFLUENCE OF THE REPLACEMENT RADICAL OF IMIDAZOLIC IONIC LIQUIDS ON CYTOTOXICITY AND ANTIVIRAL ACTIVITY AGAINST MAYARO VIRUS.....	130
EVALUATION OF ANTIVIRAL ACTIVITY OF TWO IMIDAZOLIC ION LIQUIDS AGAINST CHIKUNGUNYA VIRUS	131
MOLECULAR EPIDEMIOLOGY AND SURVEILLANCE OF CIRCULATING ADENOVIRUS IN BRAZILIAN PATIENTS WITH GASTROENTERITIS, 2008-2011.....	132
DETECTION OF FLAVIVIRUS RNA IN CEREBROSPINAL FLUID OF CHILDREN WITH NEUROLOGICAL SYMPTOMS IN MINAS GERAIS.....	133
DYNAMIC PROTEOMICS OF MAYV-INFECTED AEDES AEGYPTI CELLS.....	134
MOLECULAR CHARACTERIZATION OF HEPATITIS C VIRUS QUASISPECIES IN LIVER TISSUE OF PATIENTS WITH HEPATOCELLULAR CARCINOMA	135
ANTI-CANCER DRUG DELIVERY BY A LYTIC EUKARYOTIC VIRUS EXPRESSED IN PLANTS	136



ANTIHERPETIC EVALUATION OF PLANTS BELONGING TO THE GENUS BACCHARIS	137
DEVELOPMENT OF AN IMMUNOCOMPETENT ANIMAL MODEL FOR THE STUDY OF HETEROTYPIC DENGUE VIRUS INFECTION.....	138
MORPHOGENETIC ANALYSIS OF THE SLEV INFECTION MECHANISMS AND ITS EFFECTS IN A MURINE MODEL OF PLACENTAL DEVELOPMENT.....	139
ZIKA VIRUS TRANSMISSION THROUGH MICE BITE, A CASE REPORT	140
RESPIRATORY VIRUSES IN SECONDARY LYMPHOID TISSUES.....	141
EVALUATION OF ANTIVIRAL EFFECT OF HYBRID COMPOUNDS CHLOROQUINE-SULFADOXINE AGAINST ZIKV	142
EVALUATION OF THE SENSITIVITY OF REALSTAR® HEV RT-PCR KIT 2.0 WITH THREE COMMONLY USED EXTRACTION METHODS.....	143
HUMAN TROPHOBLASTS 3D CULTURES FOR ZIKV AND HHV-2 INFECTIVITY AND ANTIVIRAL STUDIES.....	144
PARVOVIRUS B19 INFECTION DURING PREGNANCY: A CASE REPORT	145
EVALUATION OF THE ANTI-RABIES VIRUS RIBONUCLEOPROTEIN POLYCLONAL IgG ANTIBODY IN DIRECT RAPID IMMUNOHISTOCHEMISTRY TEST FOR THE RABIES DIAGNOSIS	146
FERRITIN, ERYTHROCYTE SEDIMENTATION RATE AND C REACTIVE PROTEIN IN PATIENTS WITH CHIKUNGUNYA VIRUS INDUCED CHRONIC POLYARTHRITIS	147
CLINICAL-EPIDEMIOLOGICAL PROFILE OF PEOPLE LIVING WITH HIV-1 SUBMITTED TO VIRAL GENOTYPING TEST IN THE PERIOD FROM 2013 TO 2015, IN THE STATE OF PARÁ	148
CYTOKINES PRODUCTION AND GENETIC VARIABILITY OF HEPATITIS B VIRUS (HBV): INFLUENCE ON THE COURSE OF INFECTION IN PATIENTS WITH ACUTE, CHRONIC AND OCCULT HEPATITIS B	149
PRODUCTION OF YFV AND HIV VIRUS LIKE PARTICLES (VLPS) USING BACULOVIRUS EXPRESSION SYSTEM AND INSECT CELLS	150
SURVEILLANCE AND VIRAL DETECTION PROGRAM OF YELLOW FEVER IN NONHUMAN PRIMATES IN METROPOLITAN REGION OF RECIFE - PE.....	151
HEV PREVALENCE AND SEROCONVERSION AMONG KIDNEY TRANSPLANTED PATIENTS IMMUNOSUPPRESSED BY TACROLIMUS.....	152
CARDIOVASCULAR FINDINGS IN CHIKUNGUNYA CHRONIC ARHTROPATHY PATIENTS DURING AN EPIDEMIC IN CENTRAL- WESTERN BRAZIL.....	153
PREVIOUS CHIKV EXPOSURE INDUCES PARTIAL CROSS-PROTECTION AGAINST SECONDARY MAYV INFECTION IN MICE	154
RECONSTRUCTING THE DISSEMINATION DYNAMICS OF THE MAJOR HIV-1 SUBTYPE B NON-PANDEMIC LINEAGE CIRCULATING IN BRAZIL	155
GENOMIC SURVEILLANCE REVEALS HIDDEN DIVERSITY OF CHIKUNGUNYA VIRUS CIRCULATING IN THE METROPOLITAN REGION OF RIO DE JANEIRO	156
INFLAMMATORY CHEMOKINES IN THE SERUM AND CEREBROSPINAL FLUID OF HTLV-1- INFECTED INDIVIDUALS: INSIGHTS INTO THE DEVELOPMENT OF HTLV-1-ASSOCIATED MYELOPATHY	157



MOLECULAR CHARACTERIZATION OF OCCULT HEPATITIS B VIRUS IN PATIENTS WITH HEPATITIS C BEFORE TREATMENT WITH ORAL DIRECT-ACTING ANTIVIRALS (DAAS)	158
DEVELOPMENT OF A MULTIPLEX REAL-TIME PCR WITH HIGH RESOLUTION MELTING (HRM) ANALYSIS FOR THE SIMULTANEOUS DETECTION OF SIX HUMAN HERPESVIRUSES.	159
THE RISK FACTORS ASSOCIATED IN THE HIGH FREQUENCY OF MUTATIONS L91M IN CORE PROTEINS HEPATITIS C VIRUS (HCV) OF RIO DE JANEIRO.....	160
EVALUATION OF ZIKA VIRUS MARKERS DETECTION IN DIFFERENT SAMPLES FROM PREGNANT MONKEYS UNDER SOFOSBUVIR TREATMENT.....	161
INTERLEUKIN 27 PROMOTES DIVERGENT EFFECTS ON HIV-1 REPLICATION IN PERIPHERAL BLOOD MONONUCLEAR CELLS THROUGH BST-2/TETHERIN.....	162
INJURY IN MULTIPLE ORGANS OF FATAL CASES DENGUE IN CHILDREN: VIRAL DETECTION AND PROFILE OF CYTOKINES.....	163
DEVELOPMENT OF AN ARBOVIRAL LUMINEX SEROLOGICAL ASSAY BASED ON NON-STRUCTURAL PROTEINS.....	164
DEMYELINATING DISEASES ASSOCIATED TO CHIKUNGUNYA VIRUS INFECTION IN RIO DE JANEIRO/BRAZIL: ACUTE DISSEMINATED ENCEPHALOMYELITIS AND TRANSVERSE MYELITIS	165
PREVALENCE AND MOLECULAR CHARACTERIZATION OF HUMAN BOCAVIRUS IN ACUTE GASTROENTERITIS CASES IN BRAZIL, 2016 - 2017.....	167
MOLECULAR INVESTIGATION OF ENTERIC VIRUSES IN THE ETIOLOGY OF THE CENTRAL NERVOUS SYSTEM VIRAL INFECTIONS IN CEREBROSPINAL FLUID SAMPLES FROM THE HOSPITAL DE BASE OF SÃO JOSÉ DO RIO PRETO - SP, 2016- 2017.....	168
PREVALENCE AND GENOTYPING OF HUMAN PEGIVIRUS-1 (HPGV-1) IN BLOOD DONORS AND HCV AND HIV INFECTED INDIVIDUALS FROM RIO DE JANEIRO, BRAZIL.....	169
IDENTIFICATION AND GENOTYPICAL CHARACTERIZATION OF ROTAVIRUS IN ISOLATES OF COPROSCOPIC ANALYSIS OF HIV-INFECTED ADULT INDIVIDUALS IN PORTO VELHO - RONDÔNIA.....	170
DIFFERENT TYPES OF RAW MEAT ACQUIRED IN COMMERCIAL ESTABLISHMENTS CONTAMINATED WITH ROTAVIRUS A AND MASTADENOVIRUS	171
STUDY OF HUMAN ASTROVIRUSES IN THE IMPLEMENTATION OF EPIDEMIOLOGICAL SURVEILLANCE NETWORK OF CASES OF CHILDHOOD GASTROENTERITIS: PREVALENCE, CO-INFECTION AND MOLECULAR CHARACTERIZATION.....	172
EPIDEMIOLOGICAL TRANSITION OF HEPATITIS A VIRUS IN BRAZIL: NEW CHALLENGES.....	173
GENOMIC INVESTIGATION OF HAV OUTBREAKS INTO EPIDEMIOLOGICAL AND ENVIRONMENTAL SURVEILLANCE FRAMEWORK: A VALUABLE MODEL FOR MONITORING WATER-BORNE VIRAL DISEASES.	174
IN VITRO ANTIVIRAL ACTIVITY AGAINST CHIKUNKUNYA VIRUS FROM A NATURAL PRODUCT OF THE BRAZILIAN BROWN SEAWEED DICTYOTA MENSTRUALIS.....	175
IN VITRO ANTIVIRAL ACTIVITY AGAINST CHIKUNKUNYA VIRUS FROM A NATURAL PRODUCT OF THE BRAZILIAN BROWN.....	176



SEAWEED <i>Dictyota menstrualis</i>	176
DEVELOPMENT OF A SELF-ASSEMBLING NANOVACCINE FOR ZIKA VIRUS.....	177
DRUG RESISTANCE VARIANTS DETECTED IN HIV-MULTIEXPERIENCED PATIENTS USING NGS	178
IDENTIFICATION AND GENOTYPIC CHARACTERIZATION OF NOROVIRUS ISOLATED FROM COPROSCOPIC ANALYSIS OF INFECTED ADULTS BY HIV IN PORTO VELHO, RONDÔNIA.	179
PREVALENCE AND CHARACTERIZATION OF NOROVIRUS INFECTIONS IN PERNAMBUCO, NORTHEAST BRAZIL.....	180
ANTIVIRAL SCREENING OF XANTHENODIONES AGAINST MAYARO VIRUS.....	181
DEVELOPING A QUICK R\$1 TEST TO DIAGNOSE ZIKA IN HUMANS AND MOSQUITO SAMPLES	182
PREVALENCE AND MOLECULAR CHARACTERIZATION OF HTLV-1/2 INFECTION AMONG PATIENTS WITH HEMATOLOGICAL DISEASES IN MANAUS, AMAZONAS.	183
EVALUATION OF RESPIRATORY SYNCYTIAL VIRUS (RSV) MOLECULAR DETECTION WITH VIASURE FLU-A, FLU-B & RSV REAL-TIME PCR DETECTION KIT (CERTEST BIOTEC), IN HOSPITALIZED INFANT PATIENTS WITH RSV RELATED BRONCHIOLITIS.....	184
RAPID SELECTION OF ZIKA VIRUS VARIANTS UPON SERIAL PASSAGES IN MOUSE BRAIN	185
PROCEDURE STANDARDIZATION OF SEMEN SAMPLES FOR DETECTION OF ZIKV USING REAL-TIME PCR.....	186
MAYARO FEVER: REPORT OF CHRONIC ARTHRITIS CASES IN MATO GROSSO, MIDWESTERN BRAZIL, 2018	187
INVESTIGATION OF 2'C-METHYLCYTIDINE ANTIVIRAL PROPERTIES AGAINST ILHÉUS VIRUS INFECTION IN VITRO AND IN VIVO.....	188
RETROSPECTIVE STUDY OF HUMAN RABIES CASES CONFIRMED BY LABORATORY DIAGNOSIS AT THE INSTITUTO PASTEUR, SÃO PAULO, BRAZIL, IN THE PERIOD 2003-2016.....	189
RETROSPECTIVE STUDY OF HUMAN RABIES CASES CONFIRMED BY LABORATORY DIAGNOSIS AT THE INSTITUTO PASTEUR, SÃO PAULO, BRAZIL, IN THE PERIOD 2003-2016 ESBL BACTERIOPHAGES AGAINST BACTERIAL CLINICAL ISOLATES.....	190
REPORTER REPLICON SYSTEMS FOR THE SCREENING OF POTENTIAL ANTIVIRAL AGENTS AGAINST ZIKA, YELLOW FEVER AND CHIKUNGUNYA VIRUSES	191
COMPOUNDS RELATED TO INHIBITION OF ESCRT-MACHINERY PROTECTS IN VITRO ASSAY AGAINST OROPOUCHE VIRUS INFECTION	192
DETERMINATION THE CLINICAL DIAGNOSIS OF CHIKUNGUNYA, COMPARED TO THE GOLD STANDARD OF LABORATORY DIAGNOSIS BY MOLECULAR BIOLOGY (RT-QPCR) AND ANTIBODY DETECTION (ELISA)	193
GLYCAN RESIDUES ARE ESSENTIAL FOR THE NEUTRALIZING ACTIVITY OF HUMAN IgG1 ANTIBODIES INDUCED BY PRE- EXPOSURE PROPHYLAXIS FOR HUMAN RABIES.....	194
INHIBITION OF THE YIELD OF BRAZILIAN ZIKA VIRUS GENOME BY SYNTHETIC NAPHTHOQUINONES.....	195
HUMAN BOCAVIRUS IN GASTROENETERIC PATIENTS IN BRAZIL, 2010-2013.....	196



CIRCULATION OF CHIKUNGUNYA VIRUS EAST/CENTRAL/SOUTH AFRICAN LINEAGE IN RIO DE JANEIRO, BRAZIL.....	197
EVALUATION OF INNATE LYMPHOID CELLS ACTIVITY AGAINST DENGUE VIRUS INFECTION	198
OROPOUCHE VIRUS DETECTED IN SALIVA AND URINE	199
DETECTION OF HUMAN PAPILLOMAVIRUS L2 GENE DNA FRAGMENTS IN THE VIROME OF ACUTE FEBRILE PATIENTS IN AMAZONAS, BRAZIL	200
PHYLOGEOGRAPHIC STUDY OF DENGUE SEROTYPE 4 IN THE STATE OF AMAZONAS, BRAZIL, 2011 TO 2016	201
PREDICTORS FACTORS FOR CERVICAL CANCER IN HPV UNIMMUNIZED WOMEN: MOLECULAR AND EPIDEMIOLOGICAL STUDY	202
THERAPEUTIC EFFICACY OF SEAWEED CANISTROCARPUS CERVICORNIS AGAINST ZIKA VIRUS	203
DENGUE, ZIKA AND CHIKUNGUNYA INFECTION: SEROEPIDEMIOLOGICAL STUDY OF ARBOVIRUSES IN TANGARÁ DA SERRA – MT, 2018.....	204
CHIKUNGUNYA FEVER IN THE MIDWEST REGION OF BRAZIL AND THE STATE OF MATO GROSSO IN 2018.....	205
ANTIVIRAL EFFECT OF THE N-SULFONATED NAPHTHOQUINONE AGAINST THE ZIKA VIRUS	206
FREQUENCY OF SEXUAL VIRAL AND BACTERIAL TRANSMISSION INFECTIONS IN ANAL BRUSHES SAMPLES OF PARAGUAYANS FEMALE SEX WORKERS BY MOLECULAR METHODS	207
STUDY OF IMMUNOREGULATORY MECHANISMS MEDIATED BY REGULATORY T CELL DURING DENGUE INFECTION IN HUMANS	208
IL10+ MULTIFUNCTIONAL T CELLS ARE ASSOCIATED WITH MILD FORMS OF DENGUE INFECTION IN HUMANS.....	209
EPIDEMIOLOGICAL PROFILE OF PATIENTS DIAGNOSED WITH DENGUE, ZIKA AND CHIKUNGUNYA BY RT-PCR BY LACEN- MT IN 2018	210
MODULATION OF HERV EXPRESSION BY DIFFERENT ARBOVIRUSES DURING INFECTION OF HUMAN PRIMARY ASTROCYTES	211
DROSOPHILA CELLS AS ANTIGEN SUPPORT FOR ZIKA VIRUS DETECTION.....	212
BIOKINETIC STUDIES FOR SCALING UP SPODOPTERA FRUGIPERDA (SF9) CELL CULTURE PRODUCING RABIES VIRUS LIKE PARTICLES (VLPS)	213
VIRTUAL SCREENING OF BRAZILIAN NATURAL PRODUCTS TARGETING NSP2 PROTEASE FROM CHIKUNGUNYA VIRUS.....	214
USUV INFECTION DURING PREGNANCY IN MOUSE MODEL AND ITS CONSEQUENCES TO CONCEPTUSES	215
EVALUATION OF THE KNOWLEDGE ON HIV/AIDS OF AGED PEOPLE LIVING IN SANTA MARIA/RS: AN EPIDEMIOLOGICAL APPROACH.	216
INHIBITORY EFFECT OF ISOLATED SUBSTANCES FROM MARINE ALGA LAURENCIA CATARINENSIS AGAINST CHIKUNGUNYA VIRUS.....	217



RESPIRATORY SYNCYTIAL VIRUS CIRCULATION IN PEDIATRIC PATIENTS: MOLECULAR EPIDEMIOLOGY AND RESISTENCE.....	218
APPLYING SYNDROMIC SURVEILLANCE IN BRAZIL: EPIDEMIOLOGICAL ANALYSIS OF NEGATIVE CASES OF ARBOVIROSES IN THE STATE OF RIO DE JANEIRO.	219
VALIDATION OF TWO TESTS TO RABIES VIRUS NEUTRALIZING ANTIBODY EVALUATION. PRECISION PARAMETER TO SIMPLIFIED FLUORESCENCE INHIBITION MICROTEST (SFIMT) AND RAPID FLUORESCENT FOCUS INHIBITION TEST (RFFIT) ON MICROPLATES.	220
SUSCEPTIBILITY OF DIFFERENT CELL TYPES PRESENT IN THE CENTRAL NERVOUS SYSTEM TO ZIKA VIRUS INFECTION.....	221
MONITORING AND MOLECULAR CHARACTERIZATION OF ARBOVIRUS ISOLATED FROM DIPTERS OF THE BRAZILIAN WEST AMAZON (STATE OF RONDÔNIA).....	222
POS-MORTEN DIAGNOSIS OF HANTAVIROSES AT THE MATO GROSSO STATE DEATH VERIFICATION SERVICE.....	223
INTERACTION CHARACTERIZATION OF RABIES DIAGNOSIS IN THE LABORATORIES NETWORK OF THE STATE OF SÃO PAULO, IN THE CONTEXT OF RABIES CONTROL AND SURVEILLANCE.....	224
EVALUATION OF ANTIHERPES ACTIVITY OF TOTAL ALKALOIDS FRACTION OF <i>Fusaea longifolia</i> (Aubl.) Saff.	225
CYTOKINE ANALYSIS ON THE DEVELOPMENT OF FACTOR VIII INHIBITORS IN HAEMOPHILIA PATIENTS INFECTED BY HEPATITIS C VIRUS (HCV)	226
DETECTION OF HUMAN RESPIRATORY SYNCYTIAL VIRUS IN PEDIATRIC PATIENTS USING A HIGH SPECIFIC RAPID ANTIGEN DETECTION.....	227
MOLECULAR DETECTION OF YELLOW FEVER VIRUS IN HUMAN SAMPLES, CENTRAL WEST, 2018, BRAZIL	228
ANTIVIRAL ACTIVITY OF SAPONIN FRACTIONS FROM <i>QUILLAJA</i> SPP. AGAINST CHIKUNGUNYA VIRUS	229
MODULATION HUMAN CHEMOKINE ASSOCIATED WITH THE DEVELOPMENT OF FACTOR VIII INHIBITORS IN HEMOPHILIA PATIENTS INFECTED BY HEPATITIS C VIRUS (HCV)	230
ANTIVIRAL ACTIVITY OF <i>CHIOCOCCA ALBA</i> (L.) HITCHC. AGAINST MAYARO VIRUS	231
THREE POST-MORTEM DIAGNOSED CASES OF SEVERE ATYPICAL CHIKUNGUNYA FEVER WITH PNEUMOLOGICAL MANIFESTATIONS IN A DEATH VERIFICATION SERVICE	232
CASE REPORT OF COINFECTION ARBOVIRUS DENGUE, CHICKUNGUNA, AND YELLOW FEVER, LEADING TO DEATH.....	233
ANALYSIS OF GENOTROPISM IN HIV-1 SUBTYPES B, BBR AND F1 INFECTED INDIVIDUALS FAILING TO COMBINED ANTIRETROVIRAL THERAPY.....	234
EVIDENCE OF LYMPHOCYTIC CORIOMENINGITIS VIRUS INFECTION IN EMPLOYEES WHO HANDLE ANIMALS AND RODENTS IN RIO DE JANEIRO STATE, BRAZIL	235
HANTAVIRUS SEROPREVALENCE IN A RURAL POPULATION OF SUGARCANE CUTTERS IN THE STATE OF GOIÁS: PRELIMINARY RESULTS.....	236



CARBON PASTE ELECTRODE MODIFIED WITH GOLD NANOPARTICLES AND/OR GRAPHITE POWDER WITH ELECTRODEPOSITED GOLD AND ITS FUNCTIONALIZATION: APLICATION IN ELETROCHEMICAL BIOSENSOR FOR HANTAVIRUSES.....	237
DETERMINATION OF POTENTIAL ANTIGENIC TARGETS OF MAYARO VIRUS GLYCOPROTEIN E2	238
INFECTION OF LYMPH NODES BY RESPIRATORY SYNCYTIAL VIRUS	239
NON-HUMAN PRIMATES CARRY FLAVIVIRUS IN SÃO PAULO STATE- BRAZIL.....	240
BMC INFECTIOUS DISEASES	241
PRODUCTION AND PROTOTYPING OF AN ENZYME-LINKED IMMUNOASSAY FOR DIAGNOSIS AND SURVEILLANCE OF CHIKUNGUNYA	242
RECONSTRUCTION OF THE SPATIAL AND TEMPORAL DYNAMICS OF HEPATITIS B VIRUS GENOTYPE D IN THE AMERICAS	243
IMMUNOBIOLOGICALS.....	244
EVALUATION OF CYTOKINE PRODUCTION AND IMMUNODOMINANCE PROFILE OF T CELL RESPONSE TO ZIKA VIRUS.....	245
STUDY OF THE INTERACTIONS BETWEEN PEPTIDES DERIVED FROM THE YELLOW FEVER VIRUS AND THE YF-17D VACCINE WITH HLA CLASSES I AND II: AN IN SILICO APPROACH ...	246
REFOLDING OF RECOMBINANT EDIII ZIKV PROTEIN GENERATED UNDER HIGH PRESSURE CONDITIONS PRESERVES ANTIGENICITY AND IMMUNOGENICITY IN MICE.....	247
RECOMBINANT EXPRESSION OF ZIKA VIRUS-LIKE PARTICLES (VLPS)	248
GENERATION OF A LIBRARY OF HYBRIDOMAS WITH ANTI-MAYARO VIRUS REACTIVITY.....	249
AN IMMUNOENZYMATIC ASSAY FOR ZIKA VIRUS INFECTION DIAGNOSIS UTILIZING IMMUNOGLOBULIN Y.....	250
DEVELOPMENT OF A ELISA BASED ON PEPTIDE ASSAY (ELISA-PEPTIDE) FOR SPECIFIC DETECTION OF ZIKA VIRUS: PRELIMINARY RESULTS	251
ANTIGENIC AND PHYSICOCHEMICAL CHARACTERIZATION OF HBSAG VLPS	252
NANOMULTILAMELLAR LIPID VESICLES POTENTIALIZE THE IGG ANTIBODY RESPONSES AGAINST ZIKA VIRUS NS1 PROTEIN.....	253
FINANTIAL SUPPORT:.....	253
CROSS-REACTIVE NEUTRALIZING HUMAN SURVIVOR MONOCLONAL ANTIBODY BDBV223 TARGETS THE EBOLAVIRUS STALK	254
GUANOSINE IS NOT PROTECTIVE IN THE CONTEXT OF USUTU VIRUS INFECTION	255
THE MARBURGVIRUS-NEUTRALIZING HUMAN MONOCLONAL ANTIBODY MR191 TARGETS A CONSERVED SITE TO BLOCK VIRUS RECEPTOR BINDING.....	256
PLANT AND INVERTEBRATE VIROLOGY	258
MOLECULAR DETECTION OF HONEY BEE VIRUSES IN APIARIES OF SOUTHERN BRAZIL	259



BACULOVIRUS INFECTION TRIGGERS DIFFERENT CYTOSOLIC DNA SENSING PATHWAYS IN MAMMALIAN CELLS	260
THE INHIBITORS OF APOPTOSIS GENES LIMITS THE IN VITRO HOST RANGE OF CHRYSODEIXES INCLUDENS NUCLEOPOLYHEDROVIRUS	261
ULTRASTRUCTURAL STUDIES OF THE COTESIA FLAVIPES OVARIES AND ITS ENDOSYMBIOTIC POLYDNAVIRUS	262
AN IN-SILICO APPROACH TO VALIDATE THE CAPSID ARCHITECTURE OF NEW PUTATIVE ICOSAHEDRAL VIRUSES: GEMINIVIRIDAE AS CASE STUDY.	263
EMERGENCE AND ADAPTATION OF TOMATO BEGOMOVIRUSES IN BRAZIL: ASSESSING REPLICATIVE AND TRANSMISSION FITNESS	264
ASPECTS OF THE ASSOCIATION BETWEEN LEONURUS YELLOW SPOT ALPHASATELLITE AND BIPARTITE BEGOMOVIRUSES: EFFECTS ON INFECTION AND TRANSMISSION BY BEMISIA TABACI MIDDLE EAST-ASIA MINOR 1	265
MALVAVISCUS YELLOW MOSAIC VIRUS, A BEGOMOVIRUS CARRYING A NANOVIRUS-LIKE NONANUCLEOTIDE AND A MODIFIED STEM-LOOP STRUCTURE	266
COMPOSITION OF BEGOMOVIRUS POPULATIONS IN CULTIVATED AND NON-CULTIVATED HOSTS DETERMINED BY HIGH-THROUGHPUT SEQUENCING	268
THE OVEREXPRESSION OF SLDJ1 PROTEIN IN NICOTIANA BENTHAMIANA LEADS TO DECREASED INFECTION BY TURNIP MOSAIC VIRUS	269
SPECIFIC NUCLEOTIDES IN THE COMMON REGION OF THE BEGOMOVIRUS TOMATO RUGOSE MOSAIC VIRUS (TORMV) ARE RESPONSIBLE FOR THE NEGATIVE INTERFERENCE OVER TOMATO SEVERE RUGOSE VIRUS (TOSRV) IN MIXED INFECTION	270
RSIBR1, AN INOVIRUS THAT CAN MODULATE MOTILITY AND BIOFILM PRODUCTION OF THE PHYTOPATHOGEN RALSTONIA PSEUDOSOLANACEARUM	271
SYSTEMIC INFECTION OF PLANTS BY A GEMCIRCULARVIRUS (FAMILY GENOMOVIRIDAE)	272
INTERCEPTION OF BARLEY STRIPE MOSAIC VIRUS-BSMV: A QUARANTINE VIRUS ABSENT IN BRAZIL DETECTED IN IMPORTED BARLEY GERMPLASM	273
AN ASYMPTOMATIC IFLAVIRUSES COVERTLY INFECTING BRAZILIAN STINK BUGS: MOLECULAR AND ULTRASTRUCTURAL CHARACTERIZATION	274
NOVEL VIRUSES IN SALIVARY GLANDS OF ANOPHELES MOSQUITOES FROM MATO GROSSO, BRAZIL	275
COMPARISON OF PARAMETERS FOR CHRYSODEIXIS INCLUDENS NUCLEOPOLYHEDROVIRUS IN VIVO PRODUCTION	276
VIRAL METAGENOMICS OF HEMATOPHAGOUS INSECTS COLLECTED IN THE COMPLEXO MINERADOR DE CARAJÁS AREA, STATE OF PARÁ	277
NEW VIRUS IN MOSQUITOES FROM CANAA DOS CARAJAS, NORTH BRAZIL.....	278
NOVEL VIRUSES IN MOSQUITOES FROM BRAZILIAN PANTANAL.....	279
CHARACTERIZATION OF SPODOPTERA ERIDANIA NUCLEOPOLYHEDROVIRUS ISOLATE VPN165 AND THE EVOLUTION OF A BACTERIAL CHONDROITIN LYASE HOMOLOG ACQUIRED BY BACULOVIRUSES	280



A CPD-PHOTOLYASE-CONTAINING ALPHABACULOVIRUS INFECTIOUS TO THE PLUSIIINAEAN SOYBEAN LOOPER RACHIPLUSIA NU PRODUCES TETRAHEDRAL OCCLUSION BODIES AND CLARIFIES THE EVOLUTION OF DNA REPAIR GENES IN BACULOVIRUS.....	281
THE VIROME OF WILD ACCESSIONS OF CAPSICUM SPP.: LOW DIVERSITY OF VIRUS SPECIES MAY SUGGEST NEW SOURCES OF RESISTANCE TO PLANT VIRUSES.....	282
HIGH-RESOLUTION METATRANSCRIPTOMIC REVEALS SEVERAL NEW VIRUSES IN CAPSICUM ANNUUM SAMPLES COLLECTED IN ECUADOR.....	283
CONSTRUCTION OF INFECTIOUS CLONE OF CUCURBIT APHID-BORNE YELLOWS VIRUS BRAZILIAN MELON ISOLATE.....	284
TWO NEW VIRUSES NATURALLY FOUND CO-INFECTING LEGUMINOUS FORAGE PLANTS IN BRAZIL, BELONG TO A NEW PUTATIVE GENUS OF THE POTYVIRIDAE FAMILY TRANSMITTED BY WHITEFLY.....	285
PEPPER MILD MOTTLE VIRUS (PMMOV) DETECTED IN IMPORTED QUARANTINE CHILI PEPPER GERMPLASM.....	286
P0 AND P4 FROM CLRDV SHOW SYNERGIST EFFECT WITH VIRUS FROM DISTINCT FAMILIES.....	287
VETERINARY VIROLOGY	288
MULTIPLICATION OF BOVINE HERPESVIRUS TYPE 1 (BOHV-1) AND 5 (BOHV-5) IN DIFFERENT CELL LINES.....	289
CHARACTERIZATION OF THE VIROME IN NASAL CAVITY OF NURSERY PIGLETS.....	290
CORONAVIRUS DETECTED IN BATS FROM PARK OF INSTITUTO BUTANTAN, SÃO PAULO, BRAZIL.....	291
DETECTION OF TESCHOVIRUS A IN DIFFERENT BREEDING STAGES OF A SWINE HERD.....	292
EVALUATION OF RABIES LYSSAVIRUS REPLICATION AND CELL GROWTH IN DIFFERENT CONCENTRATIONS OF N2A CELL LINE.....	293
FIRST DETECTION OF ANTI-OROPOUCHE NEUTRALIZING ANTIBODIES IN SERA SAMPLES FROM SLOTHS (BRADYPUS VARIEGATUS) FROM ALAGOAS STATE.....	294
SURVEILANCE OF INFLUENZA A VIRUS IN DOMESTIC PIGS IN NORTHEAST BRAZIL.....	295
MULTIPLEX REAL-TIME PCR VALIDATION FOR DETECTION OF PCV2A AND PCV2B.....	296
EVALUATION OF THE EFFECTS OF AN HVT-INFECTIOUS BURSAL DISEASE VECTOR AND AN IMMUNOCOMPLEXED VACCINE ON THE IMMUNE SYSTEM AND PRODUCTION PARAMETERS OF COMMERCIAL BROILERS.....	297
EPIDEMIOLOGIC SURVEY OF EQUINE INFECTIOUS ANEMIA VIRUS IN EQUIDAE FROM NORTHEASTERN BRAZIL.....	298
BAT INFLUENZA A VIRUS H18N11 IN BRAZILIAN BATS ARTIBEUS LITURATUS.....	299
NEWCASTLE DISEASE VIRUS REPLICATION IN DIFFERENT CELL SYSTEMS.....	300
IN VITRO CHARACTERIZATION OF DIFFERENT GENETIC LINES OF RABIES VIRUS ISOLATED FROM NON- HEMATOPHAGOUS BATS.....	301



VIROME OF THE NASAL CAVITY OF SWINE PRIOR TO SLAUGHTER	302
DETECTION OF NEUROPATHOGENIC GENOTYPIC VARIANT OF EQUINE HERPESVIRUS TYPE 1 (EHV-1) ASSOCIATED WITH ABORTION AND REPRODUCTIVE PROBLEMS IN BRAZIL: DIAGNOSIS AND CASE DESCRIPTION.....	303
DETECTION OF EQUINE GAMMAHERPESVIRUS 2 IN HORSES.....	304
AMINO ACID VARIATIONS IN PARTIAL HA SEQUENCES OF H1 SWINE PANDEMIC FLU, FROM 2009 TO 2015	305
FELINE IMMUNODEFICIENCY VIRUS (FIV): OCCURRENCE IN NORTHERN REGION OF CEARÁ, BRAZIL	306
FIRST COMPLETE GENOME CHARACTERIZATION OF A BRAZILIAN BEAK AND FEATHER DISEASE VIRUS ISOLATE.....	307
THE PROPOSED AVIAN CHAPPARVOVIRUS: A NOVEL PARVOVIRUS FOUND IN BRAZILIAN WILD BIRDS' FECES BY A METAGENOMIC APPROACH	308
WILD ANIMALS AND THEIR IMPORTANCE FOR THE MAINTENANCE OF RABIES IN THE STATE OF SÃO PAULO – DATA COLLECTION	309
EQUINE INFECTIOUS ANEMIA (EIA) IN DONKEYS, NORTHEAST, BRAZIL.....	310
SUPPRESSION OF STAPHYLOCOCCUS AUREUS BIOFILM FORMATION BY BACTERIOPHAGE VB_SAUM_UFV4 IN A DYNAMIC AND STATIC SYSTEM	311
METAGENOMIC DETECTION OF HEPACIVIRUS IN ORAL AND RECTAL MICROBIOME OF OPOSSUMS FROM CAMPINAS METROPOLITAN REGION, STATE OF SÃO PAULO, BRAZIL ...	312
CHARACTERIZATION OF ROTAVIRUS POSSESSING A DS-1-LIKE VP3 GENE FROM PIGS IN BRAZIL: EVIDENCE FOR ZOOANTHROPONOTIC TRANSMISSION.	313
GENETIC DIVERSITY OF CANINE MORBILLIVIRUS GENOTYPE CIRCULATING IN THE WEST-CENTRAL REGION, BRAZIL.....	314
CHARACTERIZATION OF THE HEMAGGLUTININ PROTEIN GENE OF CANINE MORBILLIVIRUS FROM NATURALLY INFECTED DOGS IN THE STATE OF MATO GROSSO	315
CHARACTERIZATION OF BRAZILIAN GENETIC LINEAGES OF THE RABIES VIRUS COMPATIBLE WITH SAMPLES ISOLATED IN DOMESTIC AND WILD CANIDS AND VAMPIRE BATS IN RT-QPCR ASSAY.....	316
A CONTEMPORARY BRAZILIAN SENECAVIRUS A ISOLATE: IN VITRO CHARACTERIZATION - PARTIAL RESULTS -	317
PHYLOGENY OF CIRCULATING STRAINS OF CAPRINE ARTHRITIS ENCEPHALITIS VIRUS FROM GOATS OF THE SÃO PAULO STATE, BRAZIL	318
GENETIC CHARACTERIZATION OF AVIAN POXVIRUS IN SOUTHERN BRAZIL.....	319
TEMPORAL AND SPACE CHARACTERIZATION OF RABIES IN LIVESTOCK IN THE STATE OF MATO GROSSO BETWEEN 2009 AND 2018.....	320
IN VITRO SUSCEPTIBILITY OF BOVINE CELLS TO SMALL RUMINANTS LENTIVIRUS.....	321
DETECTION OF HOBI-LIKE PESTIVIRUS IN AN OUTBREAK OF RESPIRATORY DISEASE IN CALVES OF SÃO PAULO STATE, BRAZIL.....	322



OCCURRENCE OF GENOGROUP I PICOBIRNAVIRUS IN SHEEP FLOCKS FROM PARANA.....	323
DETECTION OF PICOBIRNAVIRUS IN FECAL SAMPLES FROM PIGS AND PIG FARM WORKERS IN THE WESTERN REGION OF PARANÁ.	324
CRITICAL ANALYSIS OF FACTORS THAT MAY INFLUENCE THE DOMESTIC HERBIVORE RABIES DIAGNOSIS	325
MOLECULAR MODELING AND STRUCTURAL ANALYSIS OF THE NS5B POLYMERASE OF NOVEL HEPACIVIRUS AND PEGIVIRUSES INFECTING HORSES.....	326
DIAGNOSTIC ACCURACY OF LENTZ BODY INCLUSIONS TEST FOR CANINE MORBILLIVIRUS DETECTION.....	327
PCV2a AND PCV2b DETECTION IN DOGS FROM A NON-VACCINATED PCV2 POSITIVE PIGS' HERD	328
IMPROVING INFECTIOUS BRONCHITIS VIRUS ANTIGEN PROCEDURES FOR SEROLOGICAL ASSAYS.....	329
BIOCHEMICAL AND HEMATOLOGICAL PROFILE OF PREGNANT RHESUS MONKEYS (MACACA MULATTA) EXPERIMENTALLY INFECTED WITH ZIKA VIRUS AND TREATED WITH SOFOSBUVIR – A DESCRIPTIVE ANALYSIS.....	330
IMMUNOGENICITY OF AN INACTIVATED VACCINE AGAINST BOVINE ALPHAHERPESVIRUS TYPE 5, ASSOCIATED WITH THE THERMOLABILE ENTEROTOXIN OF ESCHERICHIA COLI.....	331
MOLECULAR INVESTIGATION OF THE PRESENCE OF FELINE PARAMYXOVIRUS RNA IN KIDNEYS OF DOMESTIC CATS FROM CUIABÁ, MATO GROSSO.....	332
THE FIRST REPORT OF CANINE MORBILLIVIRUS INFECTION IN GIANT ANTEATER (MYRMECOPHAGA TRIDACTYLA) IN BRAZIL.....	333
PCR SURVEY OF BOVINE ALPHAHERPESVIRUS 1 DNA IN SEMEN FROM BULLS FROM MATO GROSSO STATE.....	334
CLINICAL AND LIVER HISTOLOGICAL FINDINGS OF A CHRONIC EQUINE HEPACIVIRUS (HEPACIVIRUS A, EQHV) INFECTED HORSE	335
FIRST DESCRIPTION OF THEILER'S DISEASE ASSOCIATED VIRUS (TDAV) IN BRAZIL	336
IDENTIFICATION OF CLADE E AVIPOXVIRUS IN BRAZIL	338
Leonardo Clasen Ribeiro ¹ , Francielle Liz Monteiro ¹ , Domitila Brzoskowski Chagas ¹ , Tamires Ellen Tomio ¹ , Marcelo de Lima ¹ , Geferson Fischer ¹ , Gilberto D'Ávila Vargas ¹ , Silvia de Oliveira Hübner ¹	338
Hélio Gelli Pereira Award	339
FAECAL VIROME ANALYSIS OF WILD ANIMALS FROM BRAZIL	340
PRODUCTION OF YFV AND HIV VIRUS LIKE PARTICLES (VLPS) USING BACULOVIRUS EXPRESSION SYSTEM AND INSECT CELLS.....	341
INFLUENCE OF PHAGE vB_EcoM-UFV13 ON BIOFILM FORMED BY CONSORTIUM P48SEP.	342
PRODUCTION AND PROTOTYPING OF AN ENZYME-LINKED IMMUNOASSAY FOR DIAGNOSIS AND SURVEILLANCE OF CHIKUNGUNYA	343



MORPHOLOGIC AND GENOMIC ANALYSES OF NEW ISOLATES REVEAL A SECOND LINEAGE OF CEDRATVIRUSES.....	344
DEVELOPMENT AND VALIDATION OF REVERSE TRANSCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP) FOR RAPID DETECTION OF ZIKV IN MOSQUITO SAMPLES FROM BRAZIL.....	345
INTERPLAY BETWEEN THE ACTIVATION OF THE KALLIKREIN-KININ SYSTEM AND VIRUS REPLICATION DURING DENGUE VIRUS INFECTION	346

ENVIRONMENTAL VIROLOGY



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HOUSEHOLD-BASED BIODIGESTERS PROMOTE THE REDUCTION OF ENTERIC VIRUSES AND BACTERIAS IN VULNERABLE AND POVERTY RURAL AREA

Maria Célia da Silva Lanna ², Aline Viancelli ³, William Michelin ³, Helen Treichel ⁴, Gislaine Fongaro ¹

¹UFSC - Universidade Federal de Santa Catarina (Florianópolis), ²UFOP - Universidade Federal de Ouro Preto (Ouro Preto), ³UnC - Universidade do Contestado (Concórdia-SC), ⁴UFFS - Universidade Federal da Fronteira Sul (Erechim - RS)

Abstract

The present study evaluated the river water quality improvement by implementation of household-based biodigesters in vulnerability and poverty rural area, in Minas Gerais State-Brazil. Biodigesters were installed for domestic wastewater treatment. Wastewater was collected before and after treatment and the physicochemical parameters and pathogens removal (human adenovirus (HAdV), hepatitis A (HAV) virus, *Salmonella* sp. and *Escherichia coli*) were evaluated; Additionally, river water was sampled before and after the household-based biodigesters implementation, to verify the contamination reduction and the positive impact of domestic wastewater treatment on waterborne pathogen reduction, considering HAdV, HAV, *Salmonella* sp. and *E. coli* quantification. The applicability in real-scale of decentralized treatment systems using household-based biodigesters promoted reduction of 90, 99, 99.99 and 99.999% from HAV, *Salmonella* sp., *E. coli* and HAdV from domestic wastewater, respectively; The river water quality improvement before the wastewater treatment application was highlight in the present study, considering that the reduction of waterborne pathogens in this water in 90, 99.99 and 99.999% of *E. coli*, HAV and HAdV, respectively (*Salmonella* sp. was not detected in river water). In general, this is an important study for encouraging the decentralized sanitation in vulnerable and poverty area, as well in rural sites, considering the positive impact of this implementation on public health.

Keywords: Public health, Waste Treatment, Circular Economy, One Health



MICROBIOLOGICAL EVALUATION OF MAMPITUBA RIVER - TORRES / RS

Jaqueline Rhoden¹, Kelly Concari Posser¹, Janaína Franciele Stein¹, Débora Rech Völz¹, Mariana Henz Kuhn¹, Larissa Schemmes Heinzelmann¹, Caroline Rigotto¹

¹ Feevale - Universidade Feevale (ERS-239, 2755. Novo Hamburgo, RS.)

Abstract

Mampituba River is in the northeast of Rio Grande do Sul, and its waters are used for rice irrigation, fishing, tourism and recreation. The assessment of bathing is performed through Resolution Nº 274/2000 of the National Council of the Environment (CONAMA), and the presence of bacteria such as total coliforms (TC), *Escherichia coli* (FC) and *Enterococcus* spp. (ENT). Other microorganisms are also present in water, such as enteric viruses. *Human Mastadenovirus* (HAdV) belongs to *Adenoviridae* family, with double stranded DNA and absence envelope. Objective of this study was to evaluate the presence of TC, FC, ENT and HAdV-C. Eight points (P) were delimited, P1 is closer from river mouth e P7 from river source and 500ml of water were collected in December 2018, January and February 2019. For bacterial analysis of TC, FC and ENT, 100mL aliquots were tested by Colilert® and Enterolert® (IDDEX) kits. The presence of HAdV was evaluated applying the ultracentrifugation method, with the extraction of genetic material by the commercial kit BioPur® followed by Real-Time Polymerase Chain Reaction (qPCR) targeting the hexon protein of the capsid. All groups of bacteria were positive at all points and collections, while the presence of HAdV-C showed variation. TC established all points in all collection as unfit for recreation, with quantification >2000/100ml. In December, FC above acceptable values at one point (P1) and ENT at two (P1, P5). Furthermore, in 3 points ENT (P5, P6, P7) was higher than FC, and HAdV was only negative at 1 point (P7). In January, 5 points (P3, P4, P5, P6, P7) ENT was highest than FC. FC above acceptable values at 1 point (P1) and ENT at 3 (P1, P4, P5). HAdV was positive in 3 points (P1, P2, P7). In February, ENT established all points as unfit for recreation, in addition to FC above acceptable values at 2 points (P1, P2). In 4 points (P4, P5, P6, P7) ENT was greater than FC, and HAdV was positive at 4 points (P1, P2, P3, P4). At some points it was possible to observe the relationship between the high prevalence of bacteria and virus positivity, as well as the increase of all parameters in the month of February. From the analysis of the samples with different indicators it was possible to observe the low microbiological quality of the water highlighting the presence of viral indicator and the relation with levels of bacteria, being important to emphasize that ENT was more efficient compared to the FC. Financial Support: CNPq, Feevale University.

Keywords: Bacteria, HAdV, Surface water, qPCR



MICROBIOLOGICAL EVALUATION OF WATER AND SAND OF NORTH RIO GRANDE DO SUL BEACHES

Jaqueline Rhoden¹, Kelly Concari Posser¹, Janaína Franciele Stein¹, Débora Rech Völz¹, Mariana Henz Kuhn¹, Larissa Schemmes Heinzelmann¹, Caroline Rigotto¹

¹ Feevale - Universidade Feevale (ERS-239, 2755. Novo Hamburgo, RS.)

Abstract

North coast of Rio Grande do Sul has economy associated with tourist activity. The assessment of water bathing had done by Resolution of the National Environment Council (CONAMA) Nº 274/2000, which uses markers such as total coliforms (TC), *Escherichia coli* (FC) and *Enterococcus* spp. (ENT) to define fecal contamination. Enteric viruses are present in waters and sand too. The *Human Mastadenovirus* (HAdV), is a non-enveloped virus and double-stranded DNA. The aim of this study is to evaluate the presence of TC, FC and ENT, as well as HAdV-C in water and sand samples from four beaches: Torres, Capão da Canoa, Imbé and Tramandaí. The collection had performed in December 2018, January and February 2019, where 500ml of water and 50g of wet and dry sand were collected. Bacteria were evaluated using 100 mL aliquots, tested by the Colilert® and Enterolert® kits. Concentration of water samples was by the ultracentrifugation method, and the sand was eluted with MEM pH 11,5, followed by extraction of the genetic material by the BioPur® kit and Real-Time Polymerase Chain Reaction (qPCR). In water samples, TC showed values above the limits in 4 samples, with quantification >2000/100ml, while FC and ENT were within the limits. HAdV was positive in 75% (3/4) samples in December, with loads from 3.17×10^4 to 4.15×10^4 genomic copies/5 μ L (gc/5 μ L), 50% (2/4) in January ranging from 3.41×10^4 and 1.50×10^5 gc/5 μ L, 25% (1/4) in February with a load of 5.78×10^4 gc/5 μ L. In wet soil, TC, FC and ENT were within the reference values, and in December ENT was higher than the FC value in Capão da Canoa. HAdV was positive in 100% (4/4) samples in December, with loads from 2.93×10^4 to 1.16×10^5 gc/5 μ L, and positive in 25% (1/4) in February, with a load of 6.69×10^4 gc/5 μ L. In dry soil, TC above in two samples, FC and ENT within the values, but with ENT above FC at one point in January. HAdV was positive in 100% (4/4) samples in two months, ranging from 3.68×10^4 to 8.28×10^4 gc/5 μ L in December, 4.36×10^4 to 6.93×10^4 gc/5 μ L in February. In January it was positive in 50% (2/4), with loads of 5.07×10^4 and 3.65×10^4 gc/5 μ L. With bacteriological values above the references it is possible to classify water as unfit for bathing and ENT, because it is more resistant to seawater, can be a more efficient marker when compared to FC. Beyond that, it is also possible to assess that in December there were the largest detections of microorganisms. Financial Support: CNPq, Feevale University.

Keywords: Beach, HAdV, Sand, Water, qPCR



A NEW DSRNA MYCOVIRUS INFECTING THE PHYTOPATHOGENIC FUNGI MYCOSPHAERELLA FRAGARIAE

Lorhan Lima Leal ¹, Johan Manuel Murcia Bermudez ^{1,2}, Flávia de Oliveira Souza ¹, Fernanda Prieto Bruckner ¹, Poliane Alfenas Zerbini ¹

¹ UFV - Universidade Federal de Viçosa (Avenida Peter Henry Rolfs, s/n), ² UFPEL - Universidade Federal de Pelotas (R. Gomes Carneiro, 1 - Centro, Pelotas)

Abstract

Mycoviruses are widely distributed in the major taxonomic groups of filamentous fungi including phytopathogens and can be associated with serious disorders in the natural physiology of the host such as debilitation, hypovirulence, toxin production and morphological changes. In this work we identify a new mycovirus infecting *Mycosphaerella fragariae*, etiological agent of Common Leaf Spot Disease in strawberry. *M. fragariae* was isolated and grown in PDA, and total nucleic acid were extracted from mycelia. After treatment with DNase I and S1 nuclease, five dsRNA elements were detected, which indicated viral infection. Viral particles were purified, and extraction of dsRNA from the purified viral particles showed the same pattern of dsRNA found in fungi mycelia. The dsRNAs were sequenced, the reads were trimmed and quality filtered and the virus genome was assembled using de novo assembly. The contigs obtained ranging between 600 and 2000 nucleotides were selected and submitted to BLAST analysis. The genomic dsRNA1 has 1,829 nucleotides and codes a putative RNA-dependent RNA Polymerase, dsRNA2 has 1,588 nucleotides and codes a putative Coat Protein (CP). Blast analysis showed that RdRp sequence shares 82.81% with amino acid sequence of *Ustilaginoidea virens partitivirus* and CP sequence shares 71.36% with amino acid sequence of *Discula Destructiva virus 1*, both species of *Partitiviridae* family. The dsRNAs 3, 4 and 5 (1073, 937 and 620nt in size) was identified as RdRp and CP defective sequences. According with International Committee on Taxonomy of Viruses (ICTV), the taxonomic criteria for the demarcation of a new species in the genus *Gammapartitivirus* is identity $\leq 80\%$ for the aa sequence of CP and $\leq 90\%$ for the aa sequence of RdRp. Our results suggest that a mycovirus isolated from *M. fragariae* belongs to *Partitiviridae*, being a new specie in the *Gammapartitivirus* genus.

Financial Support: CNPQ, CAPES.

Keywords: Characterization, Fungi, Mycosphaerella, Mycovirus, Partitiviridae



WIDESPREAD DISTRIBUTION OF PROPHAGES SIGNALING THE POTENTIAL FOR ADAPTABILITY AND PATHOGENICITY EVOLUTION OF RALSTONIA SOLANACEARUM COMPLEX GENOMES

Flavia Oliveira Souza ¹, Osiel Silva Gonçalves, Fernanda Prieto Bruckner ¹, Mateus Ferreira Santana ¹, Poliane Alfenas Zerbini ¹

¹ UFV - Universidade Federal de Viçosa (Av. P. H. Rolfs, s/n. Viçosa, MG)

Abstract

Prophages can have a positive or negative effect on the host cell, affecting its lifestyle, genomic diversity and bacterial fitness. However, many basic aspects of how these organisms affect the host cell remain poorly understood. *Ralstonia solanacearum* is a gram-negative plant pathogenic bacterium, encompassing a great diversity of ecotypes regarded as a species complex (*R. solanacearum complex* - RSC). *Ralstonia* genomes have a mosaic structure containing numerous elements, signaling to the potential for its evolution through horizontal gene transfer. In this context, we have made a screening in 120 RSC complete genomes from the NCBI database in order to identify prophage sequences integrated into RSC genomes. In total, 374 prophage-like elements were found in both the chromosome and megaplasmid. These elements encode several genes, including some related to host fitness, virulence factors, antibiotic resistance and niche adaptation that might contribute for RSC adaptability. Putative complete prophages belonging to the families *Inoviridae*, *Myoviridae* and *Siphoviridae*, were found, the most abundant being the members of *Inoviridae* family. Similar prophage-like elements are widespread into the complex at different species and/or geographic origin, suggesting that RSC phages are ancestrally acquired. Also, an analysis of CRISPR-Cas spacer sequences demonstrated the presence of viral sequences that indicate successive infection events during the bacteria evolution. Among the complete prophages, we found 14 novel putative viruses integrated into RSC genomes. These genomes have hallmark proteins from bacteriophages, and might be active. Altogether, our results provide insights about the diversity of prophages in RSC genomes and suggest that these elements may deeply affect the shape of the genome evolution among the strains impacting the virulence and host-range adaptation. Financial support: CAPES, CNPq, FAPEMIG.

Keywords: Prophages, *Ralstonia*, Genome evolution



EXPLORING THE VIRAL DIVERSITY OF SINGLE-STRANDED (SS) DNA VIRUSES IN DAIRY CATTLE RUMEN

Flávia de Oliveira Souza ¹, Fernanda Prieto Bruckner ¹, Rafael Reis de Rezende ¹, Poliane Alfenas-Zerbini¹

¹ UFV - Universidade Federal de Viçosa (Avenida Peter Henry Rolfs, s/n - Campus Universitário, Viçosa - MG, 36570-900)

Abstract

Ruminant animals stand out for their ability to efficiently convert food into milk, meat and derivatives. This efficient use of ingested energy is directly linked to the symbiotic relationship of the microorganisms present in the rumen of these animals. Microorganisms are essential because they can produce enzymes to degrade the food ingested by the animal. It is well known that rumen is not only important for digestion, but it also plays a central role in ruminant growth, high production performance and health in general. Studying and promoting strategies that optimize the functioning of the rumen has been the objective of several studies. There are several studies about rumen microbiome. Recently, interest in rumen virome has been increasing due to the important ecological role of viruses in different environments such as soil and seawater. Studies using the metagenomic approach associated with the advent of high throughput sequencing have been disseminated as a method to investigate the virome associated with various animal species. This approach has proven to be efficient in identifying new as well as previously described viruses from various animals. Here, we used a metagenomic approach associated with an enrichment for circular viral DNA using rolling circle amplification (RCA) to recover ssDNA genomes from ruminal fluid collected from 2 dairy cattles. Analysis of BLASTn and BLASTx revealed genomes of Anelloviridae, *Circoviridae*, *Geminiviridae*, *Inoviridae*, *Microviridae*, *Parvoviridae* and *Smacoviridae*, *Geminiviridae*, *Inoviridae*, *Microviridae*, *Parvoviridae* and *Pleolipoviridae*. Partial sequencing of clones showed sequences of capsid and replication initiator proteins *Microviridae* family. Finally, we recovered three complete virus genomes from the *Microviridae* family using back to back primers. ssDNA viruses are common and ubiquitous in nature and studies are needed to evaluate the impact and the relationship of these viruses with their hosts.

Financial support: CNPq, CAPES, FAPEMIG

Keywords: CATTLE RUMEN, METAGENOMIC, MICROVIRIDAE, ssDNA GENOMES, VIRAL DIVERSITY



THE INVOLVEMENT OF VIRAL SRNAS IN THE CONVERSION OF THE PHYTOPATHOGENIC *RALSTONIA PSEUDOSOLANACEARUM* INTO A COMMENSAL BACTERIUM BY AN INOVIRUS

Rafael Reis de Rezende¹, Renan de Souza Cascardo¹, Poliane Alfenas-Zerbini¹

¹ UFV - Universidade Federal de Viçosa (Avenida Peter Henry Rolfs, s/n - Campus Universitário, Viçosa - MG, 36570-900)

Abstract

In a previous work we isolated and characterized an inovirus *Ralstonia solanacearum* inovirus brasil 1 (RSIBR1), which is capable to infect *Ralstonia* spp., and modulates bacteria pathogenesis. We hypothesized that this strong phenotypic alteration in virus infected bacteria should be modulated by a global pathogenicity regulator coded or induced by virus replication. Several studies have pointed out the role of sRNAs in regulating pathogenicity mechanisms in bacteria. By computational analysis (RNA space) we were able to predict six putative sRNAs, coded by RSIBR1 genome, and sRNAs structures were predicted using mfold. The possible targets of putative sRNAs coded by RS1BR were predicted using IntaRNA and TargetRNA2 and we identified 144 and 145 targets in *R. pseudosolanacearum* chromosome and megaplasmid, respectively. We observed that targets are involved or related with protein folding, phage protein, signal peptide protein, DNA-binding protein, secretion, transmembrane transporter protein, transcription regulator protein and several hypothetical proteins, suggesting that sRNAs can be used by RS1BR1 to convert the plant pathogenic *R. pseudosolanacearum* into a commensal bacterium. The analysis of expression of sRNAs will be performed by Northern blot which, along with a transcriptome analyses of infected bacteria, will help us to better understand the phenotypic alteration induced by RS1BR1 in *Ralstonia* spp.

Financial support: FAPEMIG, CNPq, CAPES, Suzano

Keywords: Inovirus, *Ralstonia pseudosolanacearum*, sRNAs



RELATIONSHIP BETWEEN ZIKA VIRUS INFECTION AND PLUVIOMETRIC PRECIPITATION IN MATO GROSSO, 2016.

Jenniffer Francielli de Sousa Alves¹, ROMERO DOS SANTOS CALÓ¹, Rita Adriana Gomes de Souza¹, Emerson Soares dos Santos¹

¹ ISC-UFMT - INSTITUTO DE SAÚDE COLETIVA - UNIVERSIDADE FEDERAL DE MATO GROSSO (Av. Fernando Corrêa da Costa, nº 2367 - Bairro Boa Esperança. Cuiabá - MT - 78060-900 Fone/PABX: +55(65) 3615-8000 / FAX: +55 (65) 3628-1219))

Abstract

Zika virus (ZIKV) is an arbovirus, transmitted by *Aedes* mosquitoes and its incidence may be related to factors resulting from human activity in the environment, such as deforestation, increasing urbanization, low socioeconomic levels, and also related to natural factors, such as rapid climate change. Taking into account the high incidence of the disease in the state of Mato Grosso, we sought to analyze if the cases of ZIKV infection are correlated with rainfall. This is a descriptive, ecological study. Reported case data comes from the Reporting Disease Information System (SINAN) for 2016, which was made available by the State Health Department of Mato Grosso (SES-MT), for population-based data were used 2016 estimates provided by the Brazilian Institute of Geography and Statistics (IBGE) and rainfall data were collected from the Tropical Rainfall Measuring Mission (TRMM) database, thanks to the 3B42 sensor, with average weekly values per millimeter (mm). For the correlations, we used the data of number of reported cases and the average precipitation by Epidemiological Weeks (SE). It was also used the concept of time lag, with a lag period of 0 to 6 weeks, taking into account the larva development time until the adult mosquito life time. Spearman's correlation coefficient was used and a significance level of 5% and $p \leq 0.05$ was adopted. It was found that the highest proportion of cases was concentrated between the 1st to 13th SE (91%), which includes summer in the state, with high temperatures and greater precipitation volume. It was observed that in only two periods analyzed the correlations were significant, being the 06 weeks of accumulated rainfall that preceded the cases of SE 1 ($r = -0.18$) and the 3 weeks of accumulated precipitation that preceded the SE 4 cases ($r = 0.03$). Although the largest proportion of cases occurred during the rainy season in the state, even with the use of different lag times, the correlations between the variables were weak. Thus, these results suggest that rainfall in the state of Mato Grosso is not a determinant of ZIKV proliferation, but allows the ideal conditions for mosquito proliferation through containers that can serve as breeding grounds.

Financial Support - Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

Keywords: Climate Processes, Determining Factors, Epidemiology, Health Surveillance, Zika Virus Infection



INFECTION RISK ASSESSMENT OF HUMAN MASTADENOVIRUS SPECIES C AND F IN CONTAMINATED WATERS FROM SOUTHERN BRAZIL

Bruna Hoffmeister ¹, Larissa Mallmann ¹, Karoline Schallenger ¹, Bruna Saraiva Hermann ¹, Meriane Demoliner ¹, Viviane Girardi ¹, Juliane Deise Fleck ¹

¹ Feevale - Universidade Feevale (ERS- 239, 2755 Bairro: Vila Nova, Novo Hamburgo - RS, Brazil)

Abstract

In the region of Vale dos Sinos, four streams of great importance are located for the communities that developed around them, which are Pampa stream (A1), Estância-Portão stream (A2), Luis Rau stream (A3) and Schmidt stream (A4). The lack of basic sanitation, industrial and domestic sewage disposal and the irregular urban occupation, make them important objects of study for this region. The absence of resolution to these important environmental and infrastructure issues results in the spread of microorganisms. The purpose of the study was to determine the presence of *Human mastadenovirus* (HAdV) genomes of species C and F, and estimate the risk of infection from exposure to these viruses. HAdV-C causes respiratory tract diseases, while HAdV-F causes enteric diseases. The collections were performed bimonthly in the springs (S), at intermediate points (I) and mouths (M), between 2017 and 2018. A total of 96 samples were concentrated by ultracentrifugation. Viral DNA was extracted with the commercial Biopur[®] kit and quantified by real-time polymerase chain reaction (qPCR) using oligonucleotides flanking the hexon capsid protein region. Also, qPCR was used to calculate quantitative microbial risk assessment (QMRA) that was used to estimate risk of infection when the population are exposure to this contaminated environment. All samples were negative for HAdV-C in their S points, but viral loads of HAdV-F were detected in 72% of the samples, with A1 presenting the highest concentration (1.59×10^8 gc/L). In the I points, 75% of the samples had the HAdV-C genome, and A4 had the highest viral concentration (1.99×10^8 gc/L). Still, on the I point, 75% was positive for HAdV-F, and the highest viral load was found in A3 (1.08×10^8 gc/L). In the M points, 50% of the samples of the stream showed positive results for HAdV-C, A1 presented 3.58×10^8 gc/L and 78% of the samples were positive for HAdV-F, with A3 containing 1.95×10^8 gc/L. The HAdV-C contaminated samples present the average of 6.33×10^{-1} for daily infection risk and in 32% of total samples was estimated the daily infection risk at 9.99×10^{-1} . HAdV-F present the average of 7.14×10^{-1} for daily infection risk and 14% samples were 9.99×10^{-1} for daily risk. The results of qPCR, corroborating the high risk obtained by QMRA, suggest that the waters of the streams are becoming mere open sewers that endanger daily the lives of the surrounding residents.

Developers: FEEVALE, CNPq, FAPERGS E FINEP.

Keywords: Infection risk, HAdV-C, HAdV-F, QMRA, Streams



MICROBIOLOGICAL EVALUATION IN THE COMPOSTING PROCESS FROM A DRY TOILET UNIT

Kelly Concari Posser ¹, Tanise Ferreira Guimarães ¹, Jaqueline Rhoden ¹, Débora Rech Volz ¹, Mariana Henz Kuhn ¹, Larissa Schemes Heinzemann ¹, Caroline Rigotto ¹

¹ Feevale - Universidade Feevale (ERS 239, 2755 - Vila Nova, Novo Hamburgo - RS, 93525-075)

Abstract

In order to increase access to basic sanitation and reduce the environmental impact, the sustainable and low cost technology of dry toilet has been adopted in developing countries. It is an alternative that uses the composting process to treat waste produced before disposal. Thus, it is necessary to verify the efficiency of the process to avoid contamination by pathogenic microorganisms that may be present in this organic matter. The samples of this work were collected in April 2019 in the dry toilet installed at the Arca Verde Institute in São Francisco de Paula - RS, covering all phases of the composting process (7 points). The presence, quantification and infectivity of the enteric viruses represented by the *Human mastadenovirus C* and F serotypes (HAdV-C and F) were evaluated. Also bacteria of the fecal coliforms group (CF), represented by *Escherichia coli* (*E. coli*), total coliforms (CT) and *Enterococci* (ENT). For viral analysis, the extraction of viral DNA by Biopur[®] kit and the amplification of genomic copies by real time polymerase chain reaction (qPCR) were performed. Colilert[®] and Enterolert[®] kits were applied for bacteriological analysis, following manufacture instructions. The samples showed a 17.98% reduction in the CT parameter of the third phase of the process and ENT in the second phase (0.7%). *E. coli* (13.30%) was reduced in the third phase and was absence in the final compost (fertilizer). HAdV - C genomes were detected throughout the process, ranging from 2.16×10^3 to 2.16×10^4 genomic copies (gc) / 5uL, while HAdV - F was present in lower concentrations (average of 4.8×10^2 gc/ 5uL). After performing the integrated cell culture qPCR assay (ICC

- qPCR), samples showed absence of infectivity for HAdV - C and F. It is important to highlight that this is a preliminary study and the evaluated system needs to be better monitored through larger sampling and collection at different periods, in order to verify the safety in the use of the dry toilet and subsequent disposal of the fertilizer. Financial Support: CAPES. FAPERGS. FEEVALE.

Keywords: Basic sanitation, Enterococci, *Escherichia coli*, HAdV, Qpcr



HUMAN MASTADENOVIRUS IN ENVIRONMENTAL WATER SAMPLES DETECTED BY REAL TIME PCR AND IMMUNOCHROMATOGRAPHIC

Daiane Lorenzon¹, Kelly Concari Posser¹, Jaqueline Rhoden¹, Débora Rech Volz¹, Mariana Henz Kuhn¹, Gislaine Fongaro¹, Caroline Rigotto¹

¹ Feevale - Universidade Feevale (ERS 239, 2755, Vila Nova, Novo Hamburgo - RS, 93525-075)

Abstract

Human enteric viruses are largely excreted in feces and current wastewater treatment methods are sometimes not efficient to eliminate viruses. *Human mastadenovirus* (HAdV) are the important causes of acute respiratory tract infections, conjunctivitis, hemorrhagic cystitis and gastroenteritis. The present study aimed to evaluate two commercially available immunochromatographic (IC) kits to identify the presence of HAdV in environmental samples in comparison with real time polymerase chain reaction (qPCR). Environmental water samples were collected at two points in Novo Hamburgo city (sewage and drinking water), before and after treatment every 15 days during 2 months, totaling 16 samples. The samples were concentrated by ultracentrifugation and tested for HAdV with two different IC kits: Bionexia (Biomerix) and Coris (Serion). After concentration, viral DNA was extraction using the Biopur[®] kit and the amplification of genomic copies was performed by qPCR targeting the hexon capsid protein of HAdV serotype C. However, when qPCR was performed the HAdV-C genome was present in 100% (4/4) of raw sewage samples, 75% (3/4) in treated sewage samples, 50% (2/4) in raw water samples and 75% (3/4) in treated water samples. The viral loads in positive samples ranged from 7.47×10^4 to 1.75×10^6 genomic copies/5 μ L, achieving the highest concentration in non-treated sewage samples. From these preliminary study is possible to stressed that the IC kits were unable to detect the presence of viral antigens in the concentration range presented in the analyzed samples. Moreover demonstrated the need for further research in the development of IC kits, enabling its future use as low cost methodology and simple handling possible viral detection method in environmental samples. Financial Support: University FEEVALE, FAPERGS/MS/CNPq/SESRS, CAPES.

Keywords: HAdV, Lateral flow, qPCR, Sewage



WATER AND SEWAGE COLIFAGES AS MICROBIOLOGICAL INDICATORS OF FECAL CONTAMINATION

Júlia Regina Schuch Garcia ¹, Guilherme Correa Schenkel ¹, Nicole Mariele Santos Röhnelt ¹, Fabiana Tais de Souza Hack ¹, Karhen Wiltgen Teixeira ¹, Ana Paula Pustay ¹, Mayara Paula de Borba ¹, Simone Ulrich Picoli ¹

¹ Feevale - Universidade Feevale (ERS-239, 2755, Novo Hamburgo, RS, CEP 93525-075)

Abstract

Introduction: Waterborne diseases are an important public health problem, and it is essential to prove and maintain the water's quality available for human consumption. Such verification is usually done by coliforms, preferably *Escherichia coli*. However, it has been suggested the expansion of indicators used due to the presence of different viruses in the water, including bacteriophages. The quantification of coliphages, viruses that infect *E. coli*, as a parameter of microbiological water quality has already been recognized by the American Public Health Association (APHA), and the presence of such viruses is related to faecal contamination of water resources. **Objective:** With that in mind, the objective of this study was to research coliphages in raw and treated water and sewage samples as indicators of fecal contamination. **Methodology:** To this end, two water samples (one raw and one treated) from one treatment plant (ETA) and two sewage samples (raw and treated) from one treatment plant (ETE) were collected on the same day in sterile flasks and carried under refrigeration. Phage quantification was performed on 1 out of a total of 6 collections that will be performed at ETA and ETE in the municipality of Novo Hamburgo during 2019. The coliphages were screened by agar overlay lysis plate assay using *E. coli* ATCC 13706 as a host. For the tests, 1 mL aliquots of raw water and treated sewage samples were used, while for the raw sewage only 100 µL was used. Besides, the treated water sample was enriched in order to amplify the presence of phages. Three independent experiments were performed, in duplicate, for each water / sewage sample. **Partial Results:** The average coliphage found, expressed in Plaque Forming Units per mL (PFU / mL), was: i) raw sewage = 688; ii) sewage after treatment = 5; iii) raw water = 7; iv) post treatment water = 3. **Conclusion:** The results showed that sewage treatment was very effective in reducing viable phages as it decreased over 99% of the present coliphages. On the other hand, in raw water (pretreatment) a small amount of phage was detected, but surprisingly viable coliphages were found in the treated water sample, and after treatment no coliform bacteria (coliphage hosts) were detected. The presence of coliphages in water considered fit for human consumption corroborates the importance of research related to alternative water quality indicators aimed at maintaining public health. **Financial support:** Feevale University

Keywords: Bacteriophages, Potable water, Water quality



MOLECULAR DETECTION OF OROPOUCHE VIRUS IN MOSQUITOES FROM MATO GROSSO, BRAZIL, 2017

Lucinéia Claudia De Toni Aquino da Cruz ¹, Raquel da Silva Ferreria ¹, Nilvanei Aparecido Neves ¹, Laura Marina Siqueira Maia ¹, Renata Dezengrini Silhessarenko ¹, Marina Atanaka ¹

¹ UFMT - Universidade Federal de Mato Grosso (Cuiabá)

Abstract

Arboviruses represent a public health problem in Mato Grosso every year. We conducted a survey for arboviruses in adult female mosquitoes 19.110 collected in four municipalities of Mato Grosso, Midwestern Brazil, between 2017 and 2018, after Zika and Chikungunya virus introduction and spread in the State. Female pools (n=261) were processed to RNA extraction followed by RT-PCR targeting alphavirus, flavivirus and orthobunyavirus species. Our partial results permitted the identification of three pools of *Culex quinquefasciatus* collected in Caceres, Sinop and Cuiabá positive for Oropouche virus (OROV) in March, June and July, 2017, respectively. Maximum likelihood estimation (MLE) was of 3.25 (IC 4.61-8). Phylogenetic analysis indicated 100% similarity with samples from a study previously carried out in the state, belonging to subgenotype Ia, clustering with other isolates obtained from humans and animals in the Brazilian Amazon, and 99.4% identity with the subgenotype Ic also from northern Brazilian samples. The results corroborate previous findings showing the circulation of this arbovirus in the state.

Financial Support: Mato Grosso State Research Support Foundation

Keywords: Arbovirus, Oropouche, Mosquitoes



RISK OF ZIKA VIRUS INFECTION IN MATO GROSSO MUNICIPALITIES, BRAZIL, 2016.

Jennifer Francielli De Sousa Alves ¹, Romero Dos Santos Caló ¹, Rita Adriana Gomes De Souza¹, Emerson Soares dos Santos ¹

¹ UFMT - UNIVERSIDADE FEDERAL DE MATO GROSSO (Av. Fernando Corrêa da Costa, nº 2367 - Bairro Boa Esperança. Cuiabá - MT - 78060-900 Fone/PABX: +55 (65) 3615-8000 / FAX: +55 (65) 3628-1219)

Abstract

Since 2014, Zika Virus (ZIKV) infection has been spreading rapidly in Brazil. The state that reported the most cases in 2016 was Mato Grosso, with an incidence of 671 cases per 100,000 inhabitants. Thus, a study was conducted to identify priority areas for ZIKV infection control actions. This is an ecological study, with data from reports of cases of ZIKV infection by Epidemiological Weeks, which took place in 2016, in the municipalities of the state of Mato Grosso. Case notification data were extracted from the Reporting Disease Information System (SINAN) and population data were based on 2016 estimates provided by the Brazilian Institute of Geography and Statistics (IBGE). Relative risk (RR) was calculated as the ratio between the number of observed cases and the expected number of cases. To perform the RR calculation, the spatial scanning technique was used with the SatScan software. The periods of the year and the municipalities of the state where the risk for developing the disease was highest were identified. It was found that the highest proportion of cases was concentrated between the 1st to 13th epidemiological weeks (91%), with a decrease in notifications in the following weeks. In the last week of the year (epidemiological week 52) there was an 89% increase in cases compared to the number of cases reported in the previous week. During the scan, 3 clusters were identified, 2 high risk (RR = 11.66 and 6.99) and 1 low risk (RR = 0.03). The high-risk primary cluster (RR = 11.66) was located in the North and Northeast mesoregions of the state of Mato Grosso. The municipalities with the highest RR in the northern mesoregion were Lucas do Rio Verde (RR = 12.22), Nova Mutum (RR = 18.10) and Brasnorte (RR = 8.10). The municipalities with the largest RR of the Northeast mesoregion were Água Boa (RR = 18.16), Nova Mutum (RR = 18.10) and Lucas do Rio Verde (RR = 12.22). In conclusion, the highest rates of the disease were recorded in the period that includes the summer in the state, marked by higher volumes of rainfall, higher relative humidity and high temperatures. The priority areas for health surveillance, which had higher RR, are bordering municipalities, located in the North and Northeast mesoregions of Mato Grosso. In order to control the disease, it is essential that intersectoral actions that transcend the exclusive chemical control actions of the vector are agreed upon.

Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico

Keywords: Zika Virus, Risk, Mato Grosso, Relative risk, Brazil



CONTAMINATION OF THE SOIL FROM A PUBLIC PARK BY HUMAN AND CANINE MASTADENOVIRUSES

Ana Karolina Antunes Eisen ¹, Meriane Demoliner ¹, Kelen Gras De Oliveira ¹, Eduardo Artur Troian ¹, Larissa Mallmann ¹, Micheli Filippi ¹, Paula Rodrigues De Almeida ¹, Fernando Rosado Spilki ¹

¹ FEEVALE - Universidade Feevale (ERS-239 2755 Bairro Vila Nova Novo Hamburgo - RS)

Abstract

Public squares and parks are places often used by adults and children for leisure and recreation, usually accompanied by their dogs. Circulation of these animals in these environments may cause soil contamination by pathogens eliminated through feces, as is the case of enteric viruses of the families *Adenoviridae* and *Parvoviridae*. The goal of this work was to evaluate the presence of *Carnivore protoparvovirus 1* (CPV-1) and different species of *Mastadenovirus* in the soil of a public park with animal recreation area and the sand of the children's playground during a period of 6 months. Soil and sand were sampling bi-monthly. Samples were eluted in Eagle's minimum essential medium. DNA extraction were made with ReliaPrep™ Blood gDNA Miniprep System (Promega®), samples were then analyzed through polymerase chain reaction and later submitted to sequencing by Sanger method and to phylogenetic tree construction. During first three samplings all samples were negative, however after works that were carried out in streets near the park and after some rainy days, next samplings presented 30% (64/216) of positivity for *Human mastadenovirus C* (HAdV-C), 1,4% (3/216) for HAdV-E and still 0,4% (1/216) for *Canine mastadenovirus A* (CAdV-A), no sample was positive for CPV-1. Contamination with viruses of human origin in the park soil may be caused due to exposition of the water pipes in the works that were done in the streets near the park, since that no viral inactivation treatment is done on the water. Rain may have been responsible for spreading these contaminants to the park soil and to the playground sand. Thereby, once again it is noticeable how anthropic actions may be interfering with the environment even indirectly.

Keywords: anthropic actions, children playground, microbial contamination



COMPARISON OF IMMUNOMAGNETIC SEPARATION AND ULTRACENTRIFUGATION AS CONCENTRATION METHODS FOR HUMAN MASTADENOVIRUS IN WATER SAMPLES

Juliana Schons Gularte¹, Roana de Oliveira Hansen², Meriane Demoliner¹, Jacek Fiutowski², Ana Karolina Antunes Eisen¹, Horst-Günter Rubahn², Fernando Rosado Spilki¹

¹ Feevale - Universidade Feevale (ERS-239, 2755 | Novo Hamburgo, RS - CEP 93525-075), ² SDU - University of Southern Denmark (Alsion 2, Sønderborg - 6400 - Denmark)

Abstract

Even though viruses are discarded in large quantities in water resources by domestic sewage releases, they may be present in very low amounts in water samples, making direct analysis and detection a challenge, thus it is often needed concentration of large sample volumes. Therefore, rapid and reliable methods are needed to detect a small number of viral particles, especially infectious, in environmental samples. Immunomagnetic separation (IMS) is a method that concentrates viral particles through the use of an antibody-antigen complex. Paramagnetic particles are coated with a specific antibody for the target pathogen. The pathogen binds to the specific antibody and the antigen-antibody complex can easily be concentrated in a small volume by applying an external magnetic field. The goals of this study were to standardize IMS combined with real-time polymerase chain reaction (qPCR) (IMS-qPCR) to detection of *Human mastadenovirus* (HAdV) in water samples and to compare difference in the viral concentration methods between ultracentrifugation and IMS. Fifteen sites with different kinds of superficial water were sampled in a city of the South of Brazil. The samples were concentrated by ultracentrifugation and IMS. In the IMS step, monoclonal and polyclonal antibodies against HAdV were used to coat the paramagnetic beads. For viral detection, qPCR assays were performed using a specific primer (VTB1) to detected HAdV species F (HAdV-F). No samples that were concentrated by ultracentrifugation showed contamination by HAdV-F. However, in the water samples concentrated by IMS were detected 33% (5/15) of positive samples for each antibody, being only one site positive for both antibodies, totalizing nine different sites with HAdV-F contamination. The rate of detection varied from 1.39E+05 to 3.03E+06 genomic copies/L. Until now, IMS showed to be a concentration step to viral particles more effective than ultracentrifugation. IMS is considered a versatile assay with very high specificity, so special attention needs to be given for this method. The use of IMS-qPCR demonstrated to improve the assessment of HAdV in water resources.

Financial Support: CAPES, CNPq, DCIT – Ministério da Saúde – Brasil, Programa Institucional de Bolsas de Doutorado Sanduíche no Exterior (PDSE) / CAPES (processo nº: 88881.187818/2018-01).

Keywords: Immunomagnetic Separation, Human mastadenovirus , Antibodies



PROSPECTION OF NEW ENTEROBACTER AEROGENES BACTERIOPHAGES FOR BACTERIA CONTROL PURPOSE

Paula Rogovski ¹, Raphael da Silva ¹, Rafael Dorighello Cadamuro ¹, Estêvão Brasiliense Souza ¹, Michelly da Silva Alves ¹, Doris Sobral Marques Souza ¹, Maria Célia da Silva Lanna ², William Michelon³, Aline Viancelli ³, Gislaïne Fongaro ¹

¹ UFSC - Universidade Federal de Santa Catarina (R. Eng. Agrônomo Andrei Cristian Ferreira, s/n - Trindade, Florianópolis - SC, 88040-900), ² UFOP - Universidade Federal de Ouro Preto (R. do Seminário, s/n, Mariana - MG, 35420-000), ³ UnC - Universidade do Contestado (Rua Victor Sopelsa, 3000 - Salete, Concórdia - SC, 89711-330)

Abstract

Enterobacter aerogenes is a gram-negative bacteria with clinical significance as an opportunistic pathogen, responsible for causing hospital-acquired infections in intensive care patients. This species can be found on the urinary, blood, gastrointestinal and respiratory human tract, been especially dangerous for patients on mechanical ventilation. Over the last three decades, this pathogen has been recognized as a multidrug resistant bacteria, increasing the challenges on its elimination and on the infections treatment. Although bacteriophages have been discovered more than one century ago, their use is still very low. Phages can be a good alternative to treat multidrug-resistant pathogens, as *Enterobacter aerogenes*, due the capacity of lyse bacteria, its specific host range, rapid self-proliferation and low intrinsic toxicity. In addition, the advantages of this viruses include the possibility of eliminating the pathogen from the environment, since this enterobacteria has a fecal-oral route and can be spread through the wastewater for example. In the present work the main aim was to isolate *Enterobacter aerogenes* phages from human wastewater for application in environmental bacteria control. The bacteriophage isolation process was not described due to intellectual protection product and process. After 12h at 37°C the plates were analyzed seeking for lysis plate. Two different profile of phages capable of causing lysis in *Enterobacter aerogenes* were found, with two different lysis plate patterns. The first of them caused a significant number of small plates (diameter < 1 mm) while the second one formed a few plates with bigger size (diameter > 8 mm). The first phage presented lysis plates similar to those formed by *E. aerogenes* phages previously described on the putative Siphoviridae family, however other phage infecting the same bacteria was classified on the putative Myoviridae family. This phages have icosahedral capsids, long tails and double-stranded DNA, they are also non- enveloped and hence very stable on the environment. Both phages found presented great power of lysing the bacterial host and favorable features for environmental application, indicating potential for biocontrol of *Enterobacter aerogenes*.

Financial support: CNPq, Embrapa and CAPES.

Keywords: Enterobacteria control, lysis plate, multidrug-resistant pathogens



VIRUSES AND BACTERIA ADSORPTION FROM SWINE WASTEWATER USING *Moringa oleifera* SEED SHELL

Estêvão Brasiliense de Souza ¹, Doris Sobral Marques Souza ¹, Paula Rogovski ¹, Rafael Dorighello Cadamuro ¹, Raphael da Silva ¹, Michelly Alves da Silva ¹, Gislaine Fongaro ¹, Maria Célia da Silva Lanna²

¹ UFSC - Laboratório de Virologia Aplicada, Universidade Federal de Santa Catarina (Florianópolis-SC, Brazil), ² UFOP - Laboratório de Microbiologia e Microbiologia, Universidade Federal de Ouro Preto (Ouro Preto-MG, Brazil.)

Abstract

Moringa oleifera is a plant originally from Asia, but it is found in many countries, including Brazil (common name - “Acácia branca”). It is called “miraculous tree” as a result of the various medicinal properties (in seed, flowers, leaves and roots) that it has, which made its use spread in several countries. Besides its medicinal use, its crushed seed has active coagulating agents with the ability to reduce bacterial contamination and water turbidity. The ***M. oleifera*** seed is composed of a globular and a three winged part, however only its globular part is used in water treatment and the winged parts are discarded. The aim of this study was to evaluate the use of these whole seeds (globular and three winged parts) in reducing the turbidity and pathogens contamination in swine wastewater. Before the tests started, the swine wastewater samples were screening for some bacteria and viruses. HAdV-2 was inoculated into each sample and functioned as a positive control for the viral recovery. Samples were added with different concentrations of ***M. oleifera*** whole seed (10mg/L, 100mg/L and 1000mg/L) and analyzed until 8 h. Every 2 h, 2 ml of each sample were collected and pathogens [***Escherichia coli***, DNA viruses (PAdV and PCV2) and RNA viruses (HEV and RVA) viruses] were quantified in swine wastewater. After 8 h, the bacteria and viruses adsorbed to the seeds were eluted in PBS and quantified by bacterial culture and RT-qPCR, respectively. The results showed that the adsorption pattern among the pathogens studied were different. The higher quantity of ***E. coli*** in the three treatments was measured after 8 h in swine wastewater and it was inversely proportional to the seed quantity added in the samples (1.25g>2.5g>5.0g). ***E. coli*** was detected in the seeds shells after 8 h of adsorption and showed the same proportion observed in wastewater samples (1.25g>2.5g>5.0g). HAdV-2 decayed over time, but did not bind to seeds, in all treatments applied. Increase detection of PAdV and PCV2 were observed after 2 h compared to time-zero of incubation (before adding the seeds), point to an inhibition effect reduction in the samples. The adsorption process (viral and bacterial) from animal effluent are still pioneered studies using ***M. oleifera*** seed shells. These results will support future biomembrane studies for animal effluents hygienization, aiming environmental health by decentralized sanitation. Financial support: Projeto Universal - CNPq n. 420398/2016-3; CAPES.

Keywords: Virus inactivation, environmental health, hygienization, bacteria, biomembrane



BACTERIOPHAGES ISOLATION AGAINST *KLEBSIELLA PNEUMONIAE* AND *PROTEUS MIRABILIS* FOR ENVIRONMENTAL APPLICATION

Rafael Dorighello Cadamuro ², Paula Rogovski ², Raphael da Silva ¹, Estêvão Brasiliense Souza ², Michelly da Silva Alves ¹, Doris Sobral Marques Souza ², Maria Célia da Silva Lanna ³, William Michelon⁴, Aline Viancelli ⁴, Gislaine Fongaro ²

² UFSC - Laboratório de Virologia Aplicada, Universidade Federal de Santa Catarina (Florianópolis-SC, Brasil), ³ UFOP - Laboratório de Microbiologia e Microbiologia, Universidade Federal de Ouro Preto (Ouro Preto-MG, Brasil), ⁴ UnC - Universidade do Contestado, Programa de Mestrado Profissional em Engenharia Civil, Sanitária e Ambiental (Concórdia-SC, Brasil)

Abstract

The indiscriminate use of antibiotics in poultry pigs and poultry chickens had lead to an increase of multidrug- resistant bacteria (MDR) through the years. Bacteriophages are specific viruses to infect bacteria and can be used as an alternative to biocontrol bacteria cells and destabilize biofilm. Phages can be found and isolated from wastewater and other sources of the environment. In this context, the main objective of this study was to isolate bacteriophages which can infect *Klebsiella pneumoniae* and *Proteus mirabilis* from samples of the environment and use that as an alternative against biofilm control. Samples of the environment include wastewater, the effluent of wetland, poultry pig and poultry chicken. The bacteriophage isolation process was not described due to intellectual protection product and process. After 12h of incubation at 37°C in order to optimize replication of bacteria cells and bacteriophages, the plaque lysis plaque were measured, indicating activity of bacteriophages in samples with the capability of lyse the hosts. The plates ranged from 3 to 9 mm in diameter approximately. Phages of *Proteus m.* and *Klebsiella p.* usually belongs to order *Caudovirales*, can belong to family *Podoviridae* and *Myoviridae*, respectively. The phages isolated proved themselves to be biocontrol options to colonies of *Klebsiella pneumoniae* and *Proteus mirabilis*, being necessary to conduct experiments to evaluate the activity against biofilm in controlled and real samples. It's still unknown if one or more of the isolated viruses can infect multiple bacteria such as both *Proteus m.* and *Klebsiella p.*, future experiments may be created to evaluate such aspects to find a phage which can degrade multiple biofilmes and therefore be used in phages cocktails. Financial support: Projeto Universal - CNPq n. 420398/2016-3; CAPES.

Keywords: Bacteriophage, Environmental technology, bacteria control



BRAZILIAN CATTLE BACTERIOPHAGES ISOLATION

Raphael da Silva ¹, Paula Rogovski ¹, Rafael Dorighello Cadamuro ¹, Estêvão Brasiense Souza ¹, Michelly da Silva Alves ¹, Dóris Sobral Marques Souza ¹, Maria Célia da Silva Lanna ², William Michelin³, Aline Viancelli ³

¹ UFSC - Universidade Federal de Santa Catarina (Florianópolis - SC, Brasil), ² UFOP - Universidade Federal de Ouro Preto (Ouro Preto-MG, Brasil), ³ UNC - Universidade do Contestado (Concórdia-SC, Brasil.)

Abstract

According to the Secretary of Health Surveillance annual data, in 2017, *Escherichia coli* and *Salmonella* spp. were the main etiological agents causing human outbreaks of food poisoning in Brazil. Antibiotic therapy has been used in livestock, to prevent the spread of pathogenic bacteria among the host and zoonotic transmission. It has shown to be a useful and efficient method, however, the number of bacterial resistance is increasing. In addition, medicating all the animals (sick and health) is costly and can produce environmental contamination. In this scenario, new methods of decontamination were necessary. One efficient method is the bacteriophage cocktail discovery. Just a small fraction of phage, about 0.0002%, are already known in the biosphere, this shows the importance of studies like this. The aim of this study was to search for new phages that have lytic activity and with a wide host range for different strains of enterobacteria in cattle (as swine and poultry residues). The bacteriophage isolation process was not described due to intellectual protection product and process. Various phage units were isolated on *Salmonella enteritidis*, *Aeromonas allosaccharophila*, and *Shigella sonnei*. Two possible families of viruses (Siphoviridae and Myoviridae) that infect enteric bacteria were isolated in this study. Myoviridae consists in viruses with a contractile tail long (113×16 nm) and relatively thick (80–455×16–20 nm), compared with other tailed virus and bacteriophages, the myoviruses often have larger heads, in an icosahedral capsid, with 152 capsomers, and higher particle weights, the genome is DNA usually double-stranded, genome size range from ~24 kb to ~316 kb. Siphoviridae are bacteriophage with a non-contractile tail (150×8 nm), the head is icosahedra, about 60 nm in diameter, and consist of 72 capsomers, genome (DNA) size range from ~16.5 to ~80 kb. Next-generation sequence pipeline will proceed to determine phage taxonomy. This phage cocktail can be used in bacterial control in an environmental sample in One Health approach (human, animal, and environmental health). Financial support: Projeto Universal CNPq n.420398/2016-3, CAPES.

Keywords: BACTERIOPHAGES, ISOLATION, CATTLE, ENTEROBACTERIA, MULTIDRUG-RESISTANT PATHOGENS



VIRUCIDAL POTENCIAL OF MICROALGAE EXTRACTS CULTIVATED IN SWINE MANURE

Isabella Dai Prá Zuchi ¹, William Michelon ², Aline Viancelli ², Gislaine Fongaro ³, Izabella Thaís Silva ¹

¹ UFSC - Universidade Federal de Santa Catarina (Departamento de Ciências Farmacêuticas, Laboratório de Virologia Aplicada), ² UnC - Universidade do Contestado (Campus Concórdia-SC, Programa de Mestrado Profissional em Engenharia Civil, Sanitária e Ambiental), ³ UFSC - Universidade Federal de Santa Catarina (Departamento de Microbiologia, Imunologia e Parasitologia, Laboratório de Virologia Aplicada)

Abstract

Use of microalgae for swine effluents treatment and for energy purposes has been widely reported in the context of nutritional recycling and biomass valuation, having enormous potential for environmental sanitation purposes and for obtaining biocidal products. The present study aimed to evaluate the virucidal action of microalgae extracts cultivated in swine manure against the herpetic model (Herpes Simplex Virus type 1, KOS strain). For this, microalgae *Chlorella* sp. was obtained from a field-scale lagoon used to remove nutrients from swine wastewater digestate originated from an anaerobic biodigester (Brazilian Agricultural Research Corporation, EMBRAPA, Concórdia, SC, Brazil). After 11 days following inoculation, the growth medium containing the microalgae biomass was harvested via centrifugation. The harvested biomass was immediately frozen (-40 °C) and lyophilized under vacuum. It was performed a liquid-liquid fractionation of the lyophilized biomass using solvents of increasing polarity, such as *n*-hexane, dichloromethane and methanol. Mixtures of the *n*-hexane, dichloromethane and methanol extracts, in different concentrations, and 4×10^4 PFU of HSV-1(KOS strain) in serum-free MEM, were co- incubated for 15 min at 37°C, prior to the dilution of those mixtures to non-inhibitory concentrations of them (1:100). The residual infectivity was determined by viral plaque number reduction assay. Dichloromethane and methanol extracts decreased 100% of HSV-1 infectious capacity at the lowest tested concentration (3.125 µg/mL) when compared to the untreated control. This assay was conducted in order to determine if the microalgae extracts were capable of inactivating the virus in the absence of cells, thereby measuring the virucidal potential of these samples on viral particles and their consequent decreasing in HSV-1 infectious capacity.

Financial support: CNPq

Keywords: Biocidal products, Effluents treatment, HSV, Microalgae



DOE OPTIMIZATION PROCESS OF A T4-LIKE BACTERIOPHAGE USING THE ROTATIONAL CENTRAL COMPOSITE DESIGN (RCCD) METHODOLOGY TO DETERMINE OPTIMAL CARBON SOURCES AND CULTIVE CONDITIONS

Jéssica Duarte da Silva ¹, Roberto Sousa Dias ¹, Adriele Jéssica do Carmo ¹, Maíra Paula Sousa ^{2,1}, Marcella Silva Vieira ¹, Maraisa Marques Marcelino, Bruna Almeida Leão Ayupe ¹, Mirelly Jady Fernandes e Silva ¹, Thainá Iasbick Lima ¹

¹ UFV - Universidade Federal de Viçosa (Avenida Peter Henry Rolfs, s/n - Campus Universitário, Viçosa - MG, 36570-977), ² CENPES/PETROBRÁS - Centro de Pesquisas Leopoldo Américo Miguez de Mello (Av. Horácio Macedo, 950 - Cidade Universitária da Universidade Federal do Rio de Janeiro, Rio de Janeiro - RJ, 21941-915)

Abstract

Phage therapy is the application of virus that kill bacteria, also called phages, to control the bacterial growth. The use of phages to combat bacterial diseases has been used since the description of these viruses, about 1917. However, with the discovery of antibiotics and the relatively inconsistency in the treatment of patients with phage cocktails (probably due to the poor understanding of phage biology that the researchers had in that time), the phage therapy was forgotten. Recently, due to the increase of bacterial strains resistant to the action of antibiotics, phage therapy has been reclaiming its space in the treatment of diseases and environmental problems caused by bacteria. There is still the possibility of using phage enzymes such as depolymerases and lysines, to disrupting bacterial biofilm. When cells are in a biofilm array, they become much more resistant to the action of biocides, and cause major damage in the industrial and hospital area. Despite the full potential of bacteriophages as bacterial growth suppressing agents, little is discussed about the optimization of viral particles for the use of these organisms on a large scale. The aim of this work was to evaluate the progeny of a T4-like phage, the vB_EcoM_UFV09, in seven culture media (LB rich medium and M9 minimal medium with the carbon sources: acetate, lactic acid, pyruvate, glycerol, succinate and glucose) together with the influence of temperature, incubation time, agitation and Multiplicity of infection (MOI) from the Rotational Central Compound Design (DCCR) methodology. The results indicated that the culture parameters influenced the viral production differently in each sources. Furthermore, some of them were insensitive to the environmental stimuli. We believe that this happens due to the overloaded physiological state of the bacterial cell growing in a medium with carbon sources that are more difficult to obtain energy. The determination of the best growth condition for the optimization of vB_EcoM_UFV09 production took into account the economic viability of the carbon source, the stability of the source in the culture medium and the impact of the cultivation parameters on viral progeny production. The practical evaluation of the ideal conditions predicted by the DCCR demonstrated that the model was efficient in determining the optimal cultivation conditions.

Financial support: PETROBRAS, CAPES, CNPq, FAPEMIG

Keywords: Bacteriophage, Central Composite Design (CCD), T4-like phage



EXPANDING THE REPERTOIRE OF AMOEBIA GIANT VIRUSES: ISOLATION AND CHARACTERIZATION OF ORPHEOVIRUS BRASILIENSIS IN VERMOAMEBA VERMIFORMIS

Victória Fulgêncio Queiroz¹, Rodrigo Rodrigues¹, Lorena Silva¹, Fernanda Souza¹, Maurício Lima¹, Danilo Oliveira², Jônatas Abrahão¹

¹UFMG - Universidade Federal de Minas Gerais (Av. Pres. Antônio Carlos, 6627 - Pampulha, Belo Horizonte - MG, 31270-901), ²UFVJM - Universidade Federal do Vale do Jequitinhonha e Mucuri (R. Cruzeiro, 1 - Jardim Sao Paulo, Teófilo Otoni - MG, 39803-371)

Abstract

Since the discovery of the first giant virus in 2003, prospecting studies have promoted the isolation of these viruses from a wide range of environments, such as soil and water samples from rivers, lakes, oceans and sewers. Characterization studies of these isolates sparked several debates and revealed features never before described, expanding the boundaries of the then virosphere. We attempted to isolate new amoeba giant viruses from water samples collected from lagoons in Diamantina city, MG, Brazil. A total of 30 samples were inoculated in *V. vermiformis* monolayers. A total of 19 samples induced cytopathic effect in the cells. Molecular assays revealed the isolation of Kaumoebavirus (in 18 samples) and Orpheovirus (in one sample). We went further in the characterization of the second Orpheovirus isolated in the world, which we named Orpheovirus brasiliensis. The viral particle and cycle were analyzed by transmission (TEM) and scanning (SEM) electron microscopy and by immunofluorescence (IF) assays. We sequenced and analyzed some gene markers to phylogenetically characterize our isolate. SEM images revealed oval shape particles, ranging around 1172nm, presenting a unilateral depression in the particle longitudinal portion. TEM images revealed that Orpheovirus brasiliensis particles present an ostiole in one of the apices, and viral capsid is composed by at least 2 outer layers covering an inner membrane. Asynchronous viral infection induces the formation of a large (about 5,22µm in length) electron-lucent viral factory in the cytoplasm, frequently aside of cell nucleus and surrounded by mitochondria. Synchronous infection was performed to study viral cycle by IF. The viral entry occurs in less than 1 hour, and more than one particle can be incorporated by amoeba. Particle's genome uncoating was observed close to amoeba nucleus and newly-formed virus begin to be observed 8h PI followed by a massive viral release observed from 12h to 24h PI. Particles seem to be released from cells both by exocytosis and lysis. Furthermore, the analysis of DNA Pol δ reveals 12 polymorphisms in comparison to Orpheovirus IHUMI-LCC2, and phylogenetic analyses show that both Orpheovirus cluster with viruses belonging to the putative "Pithoviridae" family. Taken together, our results represent a step-forward in the understanding of the diversity and the biology of Orpheoviruses.

Financial Support: CNPq, CAPES, FAPEMIG, Ministério da Saúde, Pró-Reitoria de Pesquisa da UFMG

Keywords: Orpheovirus, Vermamoeba vermiformis, giant viruses, NCLDV



INFLUENCE OF FAECAL CONTAMINATION FROM THE CAMBORIÚ RIVER ON THE MICROBIOLOGICAL QUALITY OF WATER IN A BIVALVE SHELLFISH PRODUCTION AREA

Doris Sobral Marques Souza¹, Vanessa Moresco¹, Gislaine Fongaro¹, Célia Regina Monte Barardi¹, Robson Ventura de Souza², Luís Hamilton Pospissil Garbossa²

¹ UFSC - Universidade Federal de Santa Catarina (Florianópolis, SC), ² Epagri - Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Florianópolis, SC)

Abstract

The state of Santa Catarina is a major shellfish producer in Brazil, responsible for 95% of the national production of bivalves such as *Crassostrea gigas* oysters and *Perna perna* mussels. A significant part of the shellfish production areas (SPAs) in this state is located close to rivers draining urban areas lacking sewage collection and treatment systems (SCTS). This is a concern since bivalves are filter feeders and may accumulate human pathogens in their tissues, becoming vectors of diseases. One of these SPAs is located ~1.5 km far from the Camboriú River (CR) mouth. The river catchment covers two municipalities with very different profiles: Camboriú, upstream, has agriculture and industry as main activities, an estimated human population of 80,830 and no coverage with STCS; and Balneário Camboriú (BC), downstream, has tourism as its main activity, hosting every year around 3 million tourists during the summer. BC has an estimated baseline population of 138,730, and high STCS coverage, 94%. The aim of this study was to evaluate the prevalence of faecal indicator bacteria and enteric viruses along the CR and analyze the impact of the river discharge on the microbiological quality of water in the adjacent SPA. The levels of Thermotolerant coliforms (TC), Human Adenovirus (HAdV), Hepatitis A virus (HAV), Norovirus (NoV) genogroups I and II were monitored fortnightly during one year at 5 sites: 4 points along the CR and 1 at the SPA. TC were quantified by culture and the enteric viruses by qPCR. A gradient in the TC levels was observed, with the highest results obtained in the most upstream point (geometric mean-GM= $3.9 \cdot 10^4$ MPN.100 mL⁻¹) and decreasing levels were detected in the points located downstream. The TC levels in the SPA were the lowest (GM= 4.5 and 90th percentile = 29 MPN.100 mL⁻¹), within the legal limits for shellfish direct consumption without post-harvesting treatment (Approved area under US criteria). NoV and HAV were not detected, while HAdV was detected exclusively in points within the CR. The genome of this virus was detected in one to three samples among the 24 samples collected per site. The study confirms the faecal contamination of the CR and suggests that the pollution arising from this river does not affect significantly the microbiological water quality in the SPA located in the coast of BC. Modelling studies are needed to further understand the pollution dynamics in this area.

Financial support: CNPq 420398/2016-3 ;406428/2016-6.

Keywords: Adenovirus, Bacteria, Hepatitis A virus, Oyster, Norovirus



EVALUATION OF LYTIC PHAGE POTENTIAL IN DECREASE BIOFILM FORMATION OF ENTEROBACTERIA

Jéssica Duarte da Silva ¹, Roberto Sousa Dias ¹, Marcella Silva Vieira ¹, Adriela Jéssica do Carmo ¹, Maraisa Marcelino Marques ¹, Maíra Paula de Sousa ^{2,1}, Mirelly Jady Fernandes e Silva¹
¹ UFV - Universidade Federal de Viçosa (Avenida Peter Henry Rolfs, s/n - Campus Universitário, Viçosa - MG, 36570-900), ² Centro de Pesquisas Leopoldo Américo Miguez de Mello - CENPES/PETROBRÁS (Av. Horácio Macedo, 950 - Cidade Universitária da Universidade Federal do Rio de Janeiro, Rio de Janeiro - RJ, 21941-915)

Abstract

Phage therapy is the application of virus that kill bacteria – known as bacteriophages - to control bacterial growth. The use of phages to combat bacterial diseases such as dysenteries, typhoid and cholera has been used since the description of such viruses in the late 1910's. However, with the discovery of antibiotics and the relative inconsistency/ineffectiveness of treatments of patients with phage cocktails - probably due to the poor understanding of phage biology by that time -, the phage therapy was left aside. Recently, due to the increase of bacterial strains resistance to the action of antibiotics, the need for alternative methodologies to control the growth of pathogenic bacteria 'gained force', making phage therapy return to be a good alternative for this purpose. While application of bacteriophages for treatment in humans is still being tested, their use in veterinary diseases such as mastitis and environmental problems associated with biofilm formation has been recurrent and with excellent results. The aim of this study was to isolate specific bacteriophages against *Escherichia coli*, *Serratia marcescens*, *Citrobacter freundii* and *Shigella flexneri*, and to evaluate their influence on the growth and biofilm formation of these bacteria. The phages were isolated from samples of sewages from the municipality of Viçosa- MG. We found two specific phages for *E. coli*, one for *S. marcescens*, one for *C. freundii*, and one for *S. flexneri*. The effectiveness of phages in inhibiting bacterial growth was assessed from the 96-well plate growth curve incubated at 37 ° C for 24 hours. Phages were added to the culture medium (approximately 5×10^5 PFU / mL final concentration) containing previously activated hosts and at initial OD of 0.1. Biofilm quantification was performed after the incubation period by Violet Crystal colorimetric method and the absorbance was measured at 590 nm. The results indicate that the phages were able to decrease bacterial growth by approximately 60% and inhibited, on average, 80% of the biofilm formed by the hosts during the evaluated period. Studies of morphological characterization, viral infection kinetics and genome of these phages are being developed, so that they can be effectively used as phage therapy agents in the future.

Financial support: PETROBRÁS, CAPES, CNPq, FAPEMIG

Keywords: Bacteriophages, Biofilm, Enterobacteria



DETECTION OF HUMAN BOCAVIRUS RECOMBINANT STRAINS IN SEWAGE FROM URUGUAYAN CITIES

Salvo M¹, Tort LFL¹, Mir D², Lizasoain A¹, Colina R¹ and Victoria M¹

¹Laboratory of Molecular Virology, & ²Laboratory of Genomic and Bioinformatic, CENUR Litoral Norte, Sede Salto, Universidad de la República, Uruguay.

Abstract

Group A Rotaviruses (RVA) are the main cause of acute gastroenteritis (AG) in children under 5 years of age worldwide. This study aims to detect, quantify and assess the microbial risk of RVA in the watersheds of the Santa Lucia and Uruguay rivers in Uruguay. Monthly sampling (June 2015 to May 2016) were carried out for one year in six points in the watershed of the Santa Lucía River and four in the Uruguay River, totalizing 120 samples. Viral concentration was performed with the adsorption-elution method and detection and quantification of RVA was carried out by quantitative PCR (qPCR). RVA was detected in 41% (49/120) of the analyzed samples for both watersheds, being 42% (20/48) detected in the Uruguay River and 40% (29/72) in the Santa Lucía River. The virus was present in all the analyzed points in both watersheds and in the coldest months of the year in the Uruguay River, however, no clear pattern of seasonality was observed in the Santa Lucía River. The mean concentration for RVA was $1,3 \times 10^5$ genomic copies/L (g.c./L). The microbiological risk assessment shows that Santa Lucía watershed presented the highest risk of infection ($6.41E-01$) and illness ($3.20E-01$) estimated for the point downstream of Florida city in July 2015 meanwhile for Uruguay River, the highest probabilities of infection ($6.82E-01$) and illness ($3.41E-01$) were estimated for the point of drinking water intake in Salto city in August 2015. These results suggest that the RVA contamination of these important rivers negatively impact on their microbiological quality since they are used for recreation and drinking water intake, demonstrating that the disposal of waste from cities located in their riverside confers a constant threat of infection for the general population, especially for children.

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EDETECTION, QUANTIFICATION AND MICROBIAL RISK ASSESSMENT OF GROUP A ROTAVIRUS IN RIVERS FROM URUGUAY

Bortagaray V¹, Tort LFL¹, Girardi V², Lizasoain A¹, Tort LFL¹, Spilki FR², Colina R¹, Victoria M¹
¹Laboratory of Molecular Virology, CENUR Litoral Norte, Sede Salto, Universidad de la República, Uruguay.

²Laboratório de Saúde Única, Universidade Feevale, Novo Hamburgo, Brazil.

Abstract

Human Bocaviruses (HBoV) are mainly associated with respiratory and gastroenteric infections. These viruses belong to the family *Parvoviridae*, genus *Bocaparvovirus* and are classified in four subtypes (HBoV1-4). Recombination and point mutation have been described as basis of parvovirus evolution. In this study, three viral sequences obtained from HBoV positive sewage samples collected in two Uruguayan cities were characterized by different methods as recombinant strains. The recombination event was localized in the 5' extreme of VP1 gene and the parental strains were identified as members of HBoV3 and HBoV4 subtypes. Uruguayan HBoV recombinant strains have a high percentage of similarity among them, suggesting a successful dispersion throughout the country. As far as we known, this study represents the first detection of HBoV recombinant strains in the Americas.

Financial Support: "Comisión Sectorial de Investigación Científica", Project I+D 2014 (ID 287), Universidad de la República, Uruguay.



ENTERIC VIRUSES DETECTION IN ENVIRONMENTAL WATER FROM MIDWEST-BRAZIL AFTER ONE DECADE OF ATTENUATED VACCINE AGAINST ROTAVIRUS INTRODUCTION

Rafael D. Cadamuro¹, Paula Rogovski¹, Estêvão B. de Souza¹, Raphael da Silva¹, Chaline Bonatto²; Helen Treichel³ Gislaine Fongaro¹, Alex M. Machado⁴

¹Laboratory of Applied Virology, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil. ² Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil ³Laboratory of Microbiology and Bioprocesses, Federal University of Fronteira Sul, Erechim, Rio Grande do Sul, Brazil ⁴Federal University of Mato Grosso do Sul, Três Lagoas - MS, Brasil

Abstract

After one decade of attenuated vaccine against rotavirus-A (Rotarix - against RVA-G1 P[8]) in Brazil, RVA (G4), Human Adenoviruses, and Human Norovirus (G2) are circulating in urban water from Midwest-Brazil, indicating a possible tendency to viral succession over time, considering the vaccination absence against norovirus and adenovirus.

Keywords: Rotavirus A-G4, Adenovirus Human, Norovirus, putative viral succession.



INFLUENCE OF PHAGE VB_ECOM-UFV13 ON BIOFILM FORMED BY CONSORTIUM P48SEP

Adrielle Jéssica do Carmo¹, Roberto Sousa Dias², Clara Nogueira Laguardia², Larissa Cristina Araújo², Deborah Romaskevis Gomes Lopes¹, Jéssica Duarte da Silva¹, Maíra Paula de Sousa¹, Sérgio Oliveira de Paula^{2*}

¹Department of Microbiology, Federal University of Viçosa, Av. Peter Henry Rolfs, s/n, Campus Universitário, 36570-900, Viçosa, Minas Gerais, Brazil ²Department of General Biology, Federal University of Viçosa, Av. Peter Henry Rolfs, s/n, Campus Universitário, 36570-900, Viçosa, Minas Gerais, Brazil

Abstract

Control of microbiologically induced corrosion (MIC) is a challenge for the oil exploration sector. MIC is the result of electrochemical reactions, directed by microorganisms, mainly sulphate-reducing-bacteria (SRB), which accumulate on the surface of the ducts forming biofilms. SRB use sulfate as the final electron receptor, leading to production of hydrogen sulfide, compounds highly reactive, corrosive and toxic. The main form of control of SRB is the injection of biocides, however, these chemicals cannot penetrate the biofilm matrix and reach the microorganisms that cause MIC, besides being expensive, requiring continuous application and can generate resistant bacteria. This study evaluated the potential of phage vB_EcoM-UFV13 in preventing biofilm formation by microbial consortium P48SEP, consisting of SRS. Through scanning electron microscopy, chemical analysis and cell count in the biofilm showed the phage's ability to prevent biofilm development, reduced the main biomolecules responsible for biofilm adhesion and stabilization, and the number of living cells. In addition, the presence of the virus was able to alter the gene expression pattern of the analyzed genes, as well as the relative abundance of some proteins related to biofilm formation and cell stress response. This study shows for the first time the phage's ability to prevent the formation of biofilm from SRB, being a promising alternative for the control of biocorrosion in oilfields.



ROTAVIRUS A IN WILD AND DOMESTIC ANIMALS FROM AREAS WITH ENVIRONMENTAL DEGRADATION IN THE BRAZILIAN AMAZON

Bruno de Cássio Veloso de Barros¹, Elaine Nunes Chagas¹, Luna Wanessa Bezerra², Laila Graziela Ribeiro², Jose Wandilson Barboza Duarte Júnior², Diego Pereira², Edvaldo Tavares da Cunha Junior¹, Julia Rezende Silva³, Delana Andreza Melo Bezerra¹, Renato Silva Bandeira¹, Helder Henrique Costa Pinheiro⁴, Sylvania de Fa´tima dos Santos Guerra^{1,2}, Ricardo Jose´ de Paula Souza e Guimarães¹, Joana D'Arc Pereira Mascarenhas¹

¹Evandro Chagas Institute, Ministry of Health, Ananindeua, Pará, Brazil, ²Amazon Metropolitan University Center, Belém, Pará, Brazil, ³University of the State of Pará, Belém, Pará, Brazil, ⁴Federal University of Pará, Belém, Pará, Brazil

Abstract

Acute gastroenteritis is one of the main causes of mortality in humans and young animals. Domestic and mainly wild animals such as bats, small rodents and birds are highly diversified animals in relation to their habitats and ecological niches and are widely distributed geographically in environments of forest fragmentation in some areas of the Amazon, being considered important sources for viruses that affect humans and other animals. Due to the anthropical activities, these animals changed their natural habitat and adapted to urbanized environments, thus representing risks to human and animal health. Although the knowledge of the global diversity of enteric viruses is scarce, there are reports demonstrating the detection of rotavirus in domestic animals and animals of productive systems, such as bovines and pigs. The present study investigated the prevalence of Rotavirus A in 648 fecal samples of different animal species from the northeastern mesoregion of the state of Pará, Brazil, which is characterized as an urbanized area with forest fragments. The fecal specimens were collected from October 2014 to April 2016 and subjected to a Qualitative Real-Time Polymerase Chain Reaction (RT-qPCR), using the NSP3 gene as a target. It was observed that 27.5% (178/648) of the samples presented positive results for RVA, with 178 samples distributed in birds (23.6%), canines (21.35%), chiropterans (17.98%), bovines (14.6%), horses (8.43%), small rodents (6.74%), pigs (3.93%) and felines (3.37%), demonstrating the circulation of RVA in domestic animals and suggesting that such proximity could cause transmissions between different species and the occurrence of rearrangements in the genome of RVA as already described in the literature, associated to the traces of environmental degradation in the studied areas.

BASIC VIROLOGY





PREVALENCE AND INCIDENCE DE DENGUE AND ZIKA IN THE PARTICIPANTS OF THE PROSPECTIVE COHORT STUDY IN SAO JOSE DO RIO PRETO, SP.

Nathalia Zini ¹, Cassia Fernanda Estofolete ¹, Rafael Alves Da Silva ¹, Gislaine Celestino Dutra Da Silva ¹, Ana Carolina Bernardes Terzian ¹, Bruno Henrique Goncalves De Aguiar Milhin ¹, Flora Gandolfi ¹, Marcos Tayar Augusto ¹, Leonardo Cecilio Da Rocha ¹, Rodrigo Sborgi ¹, Pedro Henrique Carrilho ¹, Eliane Aparecida Favaro Pereira ¹, Camila Soares ², Jaqueline Cruz ⁵, Danielle Bruna Leal De Oliveira Durigon ², Edison Luiz Durigon ², Luis Carlos De Souza Ferreira ², Albert Icksang Ko ^{6,5}, Francisco Chiaravalloti Neto ⁷, Mauricio Lacerda Nogueira ¹

¹ FAMERP - Medicine School of The São José do Rio Preto (Avenida Brigadeiro Faria Lima, 5416 - Sao Pedro, Sao Jose Do Rio Preto, Sao Paulo, Brasil), ² ICB - USP - University of Sao Paulo (Avenida Prof. Lineu Prestes, 1374 - Butantan, Sao Paulo, Brasil), ⁵ FIOCRUZ - Institute Goncalo Muniz (Rua Waldemar Falcao, 121 Candeal - Salvador - Ba, Brazil), ⁶ YSPH - Yale School Of Public Health (60 College Street, New Haven, Connecticut, Usa.), ⁷ UPH - USP - University Public Health de Sao Paulo (Avenida Dr. Arnaldo, 715, Sao Paulo, Brasil)

Abstract

Dengue and Zika are arboviruses transmitted to humans through the hematophagous vector bite. Dengue has 4 serotypes (DENV1-4), responsible for annual epidemics in Brazil and in many other countries, considered a challenge for the Public Health System. The emergence and spread of Zika virus (ZIKV) in endemic areas for dengue have raised a concern about how this virus would impact in a population exposed to dengue. In Brazil, Zika has caused different clinical syndromes, ranging from asymptomatic, mild symptomatic and severe forms with neurological disorders and fetal malformations. Seroprevalence studies of arboviruses are important to determine the disease severity, co-circulation of viruses and establishing prevention and control measures. The objective of this study was verifying the prevalence and incidence of anti-DENV and anti-ZIKV antibodies in participants from a prospective cohort, who live in an endemic area for dengue in São José do Rio Preto city, São Paulo, Brazil. Paired samples of peripheral blood from 777 individuals were collected in accordance with the eligibility criteria of the project, one at the time of the study entry and the other in the subsequent year. They were analyzed by IgG ELISA serological tests and data obtained through the sociodemographic questionnaire were analyzed by Launch Epi Info software. Of the 777 analyzed samples, 577 (74.3%) was a reagent for DENV and 80 (10.3%) for ZIKV. In the follow-up year, 665 (85.6%) samples were a reagent for DENV and 227 (29.2%) for ZIKV. When the 777 samples were analyzed in groups matching serological status, the result of the previous antibodies prevalence was 64 (8.2%) ZIKV+/DENV+ (both reagent); 16 (2.1%) ZIKV+/DENV-; 513 (66.0%) ZIKV-/DENV+; and 184 (23.7%) ZIKV-/DENV- (double non-reagent). In the year of follow-up, 199 (25.6%) was ZIKV+/DENV+; 28 (3.6%) ZIKV+/DENV-; 466 (60.0%) ZIKV-/DENV+; and 84 (10.8%) ZIKV-/DENV-. The attack rate of ZIKV was 21.1% and for DENV was 44.0%. Our data has shown a high prevalence of anti-Zika and anti-dengue antibodies in the population. After two major dengue outbreaks, in 2015 and 2016, the high prevalence of anti-Zika antibodies in our study groups suggests DENV and ZIKV co-circulation in non-apparent levels. Our data also highlight the importance of additional studies to verify if the presence of anti-dengue and anti-Zika in the population might provide a protective factor or support more severe manifestations caused by these viruses.

Keywords: Dengue, Zika, Sero-epidemiological Study, Serological diagnosis



HIV-1 TAT MODULATES M1-M2 ACTIVATION PHENOTYPE OF BV-2 MICROGLIAL CELLS.

Douglas Bardini Silveira ¹, Monique Ferrary Américo ¹, Hegger Machado Fritsch ¹, Hernan Francisco Terenzi ¹, Aguinaldo Roberto Pinto ¹

¹ UFSC - Universidade Federal de Santa Catarina (Campus Reitor João David Ferreira Lima, Bairro Trindade, Florianópolis, SC, Brasil.)

Abstract

The HIV-1 transactivator protein (Tat) plays a critical role in viral replication and is related to several pathological features of HIV-1 infection, including a broad spectrum of neurological harms known as HIV-1-Associated Neurocognitive Disorders (HAND). HIV-1 Tat-induced bystander activation of microglia has been documented as a key factor of development and maintenance of HAND. Reactive microglia assumes diverse phenotypes, which are roughly categorized into M1 and alternative M2 activation phenotypes. M1 microglia releases pro-inflammatory cytokines and reactive oxygen species, which are implicated in neuronal damage; M2 microglia acts in clearing debris and repairing neuronal injuries by establishing an anti-inflammatory environment. Therefore, M1-M2 polarization may play a role in determining the potential neurotoxic or neuroprotective activity of microglia in HIV neurodegeneration disorders, although the mechanisms regulating differential microglial activation during the course of HIV-1 infection remain largely unknown. Based on this, the present work aimed to explore the potential M1-M2 phenotypic modulation of microglial cells induced by HIV-1 Tat bystander activation. BV-2 microglial cells were stimulated with recombinant HIV-1 Tat and expression levels of M1-M2 activation markers were assessed through flow cytometry and RT-qPCR. The bystander stimulus of Tat on BV-2 cells resulted in a synergistic overexpression of major M1 markers comprising secreted TNF- α , IL-6 and MCP-1 as well as CD16/32 extracellular receptors and iNOs gene. However, Tat-treated cells did not show detectable changes in expression levels of the canonical M2 markers IL-10, CD206, and Arg-1. These findings suggest that HIV-1 Tat can drive a M1 microglial activation profiling, directly triggering an inflammogenic and neurotoxic response. Overall, a better understanding of how HIV-1 infection and viral protein exposure modulate microglial function during the course of infection could lead to the identification of novel therapeutic targets for both the eradication of HIV-1 reservoir and treatment of neurocognitive impairments. Financial support: CNPq.

Keywords: HIV-1, TAT, Microglial Cells, Neurocognitive Disorders



INFECTION OF ENDOTHELIAL CELLS BY DENGUE VIRUS INDUCED ROS PRODUCTION BY DIFFERENT SIGNALING PATHWAYS, AFFECTING VIRUS REPLICATION, CELLULAR ACTIVATION, DEATH AND VASCULAR PERMEABILITY

Lana Monteiro Meuren ¹, Luiza Rachel Pinheiro De Carvalho ², Yasmin Mucunã Mustafá¹, Michelle Premazzi Papa ¹, Andrea Thompson Da Poian ², Luciana Barros De Arruda ¹

¹ Virologia - UFRJ (Av. Carlos Chagas Filho, 373, CCS - Bloco I, Lab ISS - 048 Rio de Janeiro, RJ - Brasil), ² Bioquímica Médica - UFRJ (Universidade Federal do Rio de Janeiro, Instituto de Bioquímica Médica. Av. Carlos Chagas Filho, 373, CCS, bloco E, sala 18)

Abstract

Increased vascular permeability has been described as one of the factors for dengue disease complication. Reactive Oxygen Species production induces changes in cell physiology and can act as signaling molecule for cell death. Our group had previously demonstrated that infection of endothelial cells with DENV results in the activation of RNA sensors, production of interferon and proinflammatory cytokines, cell death and permeability. We have also reported that DENV infection promoted ROS production, but the signaling associated to this event and its consequences for virus replication and endothelial cell physiology had not been investigated yet. In the present study, we evaluated the role of mitochondrial function and NADPH oxidase activation for ROS production and investigated how these mediators affected brain microvascular cells (HBMEC) infected by dengue virus (DENV). HBMECs were infected with DENV2, at MOI of 1. The oxygen consumption was measured by high resolution respirometry and ROS production by flow cytometry. Virus replication was evaluated by qRT-PCR, flow cytometry and plaque assay. Cell death was evaluated by flow cytometry. Cytokine production was analyzed by qRT-PCR and ELISA. DENV-infected HBMECs showed a decrease in the maximal respiratory capacity and altered membrane potential, indicating functional mitochondrial alteration, what might be related to mitROS production. Indeed, mitROS was detected at later time points after infection, which was dependent on RIG-I activation. Specific inhibition of mitROS diminished virus replication and cell death but did not affect cytokine production. On the other hand, inhibition of cytoplasmic NADPH oxidase-associated ROS production inhibited virus replication, cell death and permeability, and the secretion of inflammatory cytokines, including IL-8, and CCL5. Importantly, the production of cytoplasmic ROS did not depend on virus RNA sensing. These data indicate that DENV replication in endothelial cells induced ROS production by different pathways. Both RIG-dependent mitROS and NADPHox- dependent ROS were important for virus replication and cell death, affecting endothelial permeability; however only the species triggered by cytoplasmic stress affected inflammatory signals, which could further contribute to endothelial activation and vascular lesion associated to DENV infection.

Keywords: DENV, Cell endothelial, ROS, Cell death , Vascular permeability



PHENOTYPE OF VIRUS-LIKE PARTICLES (VLP) AND HPV-POSITIVE CARCINOMA CELL LINES BY ELECTRON MICROSCOPY

Rachel Siqueira de Queiroz Simões¹, Ortrud Monika Barth¹

¹ Fiocruz - Fundação Oswaldo Cruz (Avenida Brasil 4.365 - Manguinhos, Rio de Janeiro, cep: 21040- 900)

Abstract

Papillomavirus express two oncogenes E6 and E7 which are produced in the basal layers of the epithelium under regulation of the E2 gene and disrupts in the differentiation program of the cell cycle. After initial infection, the viral DNA is maintained in episomal form in low copy number. There are several phenotype of antigen-presenting cells (Langerhans cell, Migratory LC, Langerin dendritic-cell populations, dermal macrophages) in the skin which are migratory in the epithelial tissue. Some studies have investigated the ability of the cytokine to inhibit proliferation "*in vitro*" normal keratinocytes and infected with HPV as well as the expression of E6 and E7 oncogenes. The present study reports ultrastructural cell morphology in samples of *bovine papillomavirus* (BPV) virus-like particles (VLP) and describes morphological alterations inside the SiHa and HeLa cell lines by electron microscopy. Few studies have assessed the transmission electron microscopy in different cell lines. For ultrastructural analysis, the specimens were embedded in epoxy resin, fixed in 1% glutaraldehyde and post-fixed in 1% osmium tetroxide. Later steps followed by washes in cacodylate buffer 0.2 M in sodium sucrose 0.7% and distilled water. The dehydration steps were performed. Warts and cells lines were included in epoxy resin and kept at 60°C to complete polymerization. Ultra-thin sections and semi-thin sections were performed. Morphologically, very electron-dense cells were detected by electron microscopy presenting well developed mitochondria and rough endoplasmic reticulum (rER), many vesicles and ribosomes in HeLa and SiHa cell lines. Cellular modifications similar to antigen-presenting cells, many activated mitochondria and vesicle transport well preserved also were observed. Furthermore, the presence of VLP and cellular junctions like desmosomes were also detected in samples de BPV. These morphological alterations suggest a high cellular activity of SiHa and HeLa cell lines can be possible prognostic markers of cervical cancer. One of the main functions of natural killer cells (NK) are cytotoxic activity with ability to destroy virus-infected cells and the ability of NK cells to distinguish infected cells from uninfected cells is related with the presence of destruction inhibitory receptors on their surface are called killing inhibitory receptors (KIR). So more studies about immunology of viral infection are need.

Keywords: cell lines, cellular markers, keratinocytes, ultrastucture, virus like particles



IN VITRO EVOLUTION OF ZIKA VIRUS IN INSECT CELLS

Karina Pinheiro Pessoa ¹, Valdinete Alves do Nascimento ¹, Debora Camila Gomes Duarte ¹, Victor Costa de Souza ¹, Felipe Gomes Naveca ¹

¹ ILMD - Fiocruz - Instituto Leônidas e Maria Deane (Rua Terezina, 476 - Adrianópolis, Manaus - AM)

Abstract

The Zika virus (ZIKV) is an arbovirus, genus *Flavivirus*, *Flaviviridae* family, with single-stranded RNA genome, of positive polarity. Just like the other RNA viruses, ZIKV shows variations in their sequences due to the lack of proofreading activity of the viral RNA polymerase and the high replication rate. Considering the occurrence of mutations as an event that may lead to the selection of variants with altered fitness, this study aimed to evaluate the *in vitro* evolution of ZIKV, analyzing the mutations that occurred during the adaptation process in cultured cells. A positive ZIKV sample denominated BR_AM_ILMD_0305JFMB, was isolated in *Aedes albopictus* C6/36 cells and submitted to successive 33 passages in biological replicates. The complete genome of the BR_AM_ILMD_0305JFMB directly from plasma and from passages P1, P33.1, P33.2, was obtained by capillary sequencing using an ABI 3130 automatic sequencer. Sequencing data were analyzed using the Geneious software v10.2.6 showing that seven mutations occurred in the regions coding for the non-structural proteins, two synonyms mutations in the NS1 and NS2A CDS; three non-synonyms mutations occurred in the NS3 CDS other two in NS5 CDS. To evaluate the impact of these mutations on viral competence, P1 and P33 were titrated by RT-qPCR using the delta-delta Ct method and then inoculated for *in vitro* (C6/36) and *in vivo* (*Aedes aegypti*) fitness assays. For the *in vitro* assay, we evaluated the number of viral RNA copies present in both cells supernatant and cells pellets. The results showed a significant increase in the number of copies of viral RNA in P33 infected cells at 72 hours post-infection. The results of the *in vivo* experiment showed a significantly lower viral copy quantity for P33, in eight days (P-value 0,333) and twelve days post-infection (P-value 0,040), suggesting that the mutations observed in P33 adversely affected the viral replication in *Aedes aegypti*. Further studies should be performed to confirm the effects of the mutations observed in other biological systems both *in vivo* and *in vitro*.

Financial Support: DECIT-MS, CNPq, CAPES.

Keywords: Zika Virus, Evolution, *in vitro*



HUMAN INTERFERON-INDUCED PROTEIN WITH TETRATRIPEPTIDE REPEATS 5 (IFIT5) INHIBIT RABIES VIRUSES

Camila Mosca Barboza ¹, Jaíne Gonçalves Garcia ¹, Raphaela Mello Zamudio ¹, Lucas Matheus Stangherlin ², Maria Cristina Carlan da Silva ², Juliana Galera Castilho ¹, Paulo Michel Roehe ³, Ana Claudia Franco ³, Helena Beatriz de Carvalho Ruthner Batista ¹

¹ IP - Instituto Pasteur (Av. Paulista, 393 - Cerqueira César, São Paulo), ² UFABC - Universidade Federal do ABC (Alameda da Universidade, Bairro Anchieta - São Bernardo do Campo), ³ UFRGS - Universidade Federal do Rio Grande do Sul (R. Sarmento Leite, 500 - Farroupilha, Porto Alegre - RS)

Abstract

Rabies is a fatal zoonotic disease caused by *Rabies lyssavirus* (RABV). Although there are effective vaccines for rabies, this disease results in 60,000 human deaths worldwide every year, being an important social and economic problem. Dogs are the main source of infection but in Brazil, bats have developed an important role in RABV transmission. Due to the absence of an effective cure, there is an urgent need for an alternative antiviral compounds. In this scenario, the Interferon-induced proteins with tetratricopeptide repeats (IFITs) can be highlighted. IFITs are innate immune molecules that confer antiviral defense to the host. There are four members in humans (IFIT1, 2, 3 and 5). Amongst the members, IFIT5 is highly sensitive to various cellular stresses, including those caused by dsRNA, lipopolysaccharides and virus infections. Consequently, one major feature of IFIT5 involves inhibition of virus replication by nucleic acid sensing and possibly translation inhibition. The aim of this study was to evaluate the activity of human IFIT5 against RABV from different genetic lineages. Two distinct RABV isolates recovered from central nervous system (CNS) tissues of different species and considered representative of different RABV genetic lineages were used in the present study. Isolate IP4005/10 is a virus genetic lineage whose natural host is the hematophagous bat *Desmodus rotundus* and isolate IP3629/11 was originated from a domestic dog. The samples were titrated in HEK-293T cells and 100 fifty percent tissue culture infectious doses (TCID₅₀) were determined and inoculated onto V5-tagged humanIFIT5 transfected cell monolayers. Supernants of transfected and infected cells were collected then titrated and compared with the control cell monolayers (untransfected infected cells). Preliminary results indicate a reduction of virus titres when the bat isolate (4005/10) was inoculated onto humanIFIT5 transiently expressing cells, in comparison to the titres obtained with the dog isolate. These results suggest that human IFIT5 has potential antiviral effect against RABV. Additional studies are necessary to a better understanding of the mode of action of this antiviral protein. Financial support: Instituto Pasteur

Keywords: Antiviral , Innate immunity, IFIT5, Rabies virus



RABIES VIRUS ISOLATION IN HUMAN EMBRYONIC KIDNEY (HEK-293T) CELL LINE: AN ALTERNATIVE FOR RABIES DIAGNOSIS AND RESEARCH

Jaíne Gonçalves Garcia ¹, Camila Mosca Barboza ¹, Adriana Candido Rodrigues ¹, Willian de Oliveira Fahl ¹, Juliana Galera Castilho ¹, Maria Cristina Carlan da Silva ², Raphaela Mello Zamudio ¹, Ana Lee Aparecida Francisco ¹, Helena Beatriz de Carvalho Ruthner Batista ¹

¹ IP - Instituto Pasteur (Av. Paulista, 393 - Cerqueira César, São Paulo), ² UFABC - Universidade Federal do ABC (Alameda da Universidade, Bairro Anchieta - São Bernardo do Campo)

Abstract

The Rabies lyssavirus (RABV) is a neurotropic virus that causes encephalitis in all mammals. Despite the recognized stability of RABV, differences among isolates from different species have been identified. The Gold standard method to detect RABV is direct fluorescent antibody test (DFAT). The rabies tissue culture infection test (RTCIT) can be used as potential complementary test of DFAT, it enables propagation of virus and have been valuable for obtaining large quantities of virus for production of vaccine and other studies. The viruses can infect a several cell lines of neural origin and non-neural cells. Several different cell types have been used for virus propagation. The Murine neuroblastoma (Neuro-2A) and the Baby hamster kidney (BHK-21) cell lines are the most commonly used, however not all rabies strains replicate equally in these lines. Human embryonic kidney cell line (HEK-293T) expresses several neuronal proteins and can be an alternative cell line for RTCIT. This study evaluated the susceptibility of HEK-293T for RTCIT and compare with results obtained with Neuro-2A cell line.

Test was performed in 96-well plates, the procedure involved the addition of a brain tissue suspension from different species to a suspension of HEK-293T cells. The plates were incubated in CO₂ incubator at 37°C at different times (24h and 48h) adopting two distinct conditions where the virus was allowed to adsorb for 2h and another plate without virus adsorption. At time the plate was fixed, stained with fluorescein isothiocyanate (FITC) anti-rabies conjugate antibody and examined with a fluorescence microscope. The results show that at 48h post infection with virus adsorption the fluorescent focus can be easily noticed. Our preliminary results suggest that HEK-293T can be an alternative cell line for RTCIT.

Keywords: Cell culture, HEK-293T, Rabies, Virus isolation



ANALYSIS OF THE EFFECT OF ANTIOXIDANT ACTIVITY OF A CARBON-BASED NANOMATERIAL ON ZIKA VIRUS INFECTIONS

Samille Henriques Pereira ¹, marina de souza ladeira ², leticia trindade de almeida ¹, cintia lopes de brito magalhaes ¹, luiz orlando ladeira ², Breno de Mello Silva ¹

¹ UFOP - universidade federal de ouro preto (campus morro do cruzeiro - ouro preto - minas gerais), ²UFMG - universidade federal de minas gerais (belo horizonte - minas gerais)

Abstract

Zika virus (ZIKV) is one of the most important public health arboviruses. The increase of ZIKV cases in recent years has led to a worldwide concern due to the ease of dissemination, difficulty to combat vectors and related serious diseases. It is already known that during the viral infections occurs an increase of reactive species (RS) that leads an imbalance in redox homeostasis that causes biological damage important to viral pathogenesis.

Thereby, to control RS may be a strategy to fight the infection. Some Carbon-based nanomaterial (CBNs), has antioxidant activity due to its high capacity for sequestering the RS. In this sense, this study aimed to evaluate the antioxidant activity of one CBN during ZIKV infection. To evaluate CBN cytotoxicity, a MTT cell viability assay was performed on Vero cells. The antioxidant capacity of CBN was tested from ORAC assay using a standard Trolox curve. The antioxidant potential of CBN was measured by reactive oxygen species assay (ROS), U87MG cells were infected with ZIKV (MOI 1) and treated with CBN for 24 hours, after the incubation the Carboxy-DCFDA probe (Invitrogen™) was added and the reading was performed on the Victor X3 (Perkin Elmer) plate reader with wavelength of 485/535nm. The results showed that for all tested concentrations (up to 12.5 μ M) are not cytotoxic. CBN presented an antioxidant capacity in low concentrations 50, 20, 5 and 10 nM, as expected since in higher concentrations occurs the formation of crystals, which could impair its activity. The reduction of ROS production of 17, 44, 43 and 57% was observed at concentrations 50, 25, 12.5 and 6.25 nM, respectively.

Also, preliminary results have been shown a reduction of virus multiplication in cell treated with this CBN in lower concentrations. These results suggest that this CBN could be used against ZIKV in the context of infections.

Keywords: zika, antioxidant, nanomaterial, carbono



HUMAN BETAHERPESVIRUSES 6 AND 7 SALIVARY SHEDDING IN RENAL TRANSPLANTATION RECIPIENTS: LONGITUDINAL STUDY REVEALS ACTIVE REPLICATION

Jéssica Vasques Raposo ³, Dmitry José de Santana Sarmiento ², Rafaela Barbosa da Silva Pinto, Amanda de Oliveira Lopes ³, Tânia Regina Tozetto Mendoza Tozetto Mendoza ², Paulo Henrique Braz da Silva Braz da Silva ¹, Vanessa Salete de Paula ³

¹ FOUSP - Departamento de Estomatologia - Faculdade de Odontologia da Universidade de São Paulo (Av. Dr. Enéas de Carvalho Aguiar, 470 - Jardim America, São Paulo), ² USP - Laboratório de Virologia do Instituto de Medicina Tropical / Universidade de São Paulo (Av. Prof. Lineu Prestes, 2227 - Butantã, São Paulo - SP), ³ IOC/FIOCRUZ - Instituto Oswaldo Cruz – Fundação Oswaldo Cruz (Av Brasil, 4365, Manguinhos. Rio de Janeiro)

Abstract

Infections remain the most common non-cardiovascular causes of death after kidney transplantation, accounting for approximately 15% to 20% of deaths. In pediatric or adult renal transplant recipients, Human Betaherpesvirus 6 (HHV-6) and Human Betaherpesvirus 7 (HHV-7), also called Roseolovirus, often react after transplantation. The reactivation Roseolovirus in immunocompromised patients has been associated with fever, rash, encephalitis and bone marrow suppression. Although it has been reported that Roseolovirus latency and persistence may occur in the salivary glands, there is few information about salivary excretion of these viruses. Therefore, the aim of this study was to evaluate the active Roseolovirus infection in saliva samples from renal transplant recipients. The monitoring of the viral load and detection mRNA of 32 patients were performed in three different moments: T1: before the transplant; T2: 15 to 20 days after transplant, and T3: 40 to 60 days after the transplant. A duplex qPCR was used to quantification of Roseolovirus (gene). The positive samples were tested by nRT-PCR to mRNA detection. HHV-7 showed a significant increase in viral replication during in T3 (72.9%) compared to the pre- transplant period T1 (25%) (McNemar Test, $p=0.001$). HHV-6 also showed an increase in replication in T2 and T3, but without statistical significance ($p>0.05$). Analysis of the ratio of viral replicative to quantitative (DNA copies / mL) showed that positive cases for viral replication had a higher number of DNA copies ($> 10^6$) when compared to cases without replication for both HHV-6 and for HHV-7 (p

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Keywords: Herpesviruses, qPCR, Renal Transplantation, Roseolovirus, Saliva



THE ROLE OF P53 PROTEIN IN HCMV REPLICATION IN U138 GBM CELL LINE

Gustavo Mocruha Alcantara ¹, Lucas Matheus Stangherlin ¹, Clarissa Ribeiro Reily Rocha ², Maria Cristina Carlan Da Silva ¹

¹ UFABC - Universidade Federal do ABC (São Bernardo do Campo, São Paulo - Brasil), ² UNIFESP - Universidade Federal de São Paulo (São Paulo, São Paulo - Brasil)

Abstract

The human cytomegalovirus (HCMV), belongs to the *Herpesviridae*, *Betaherpesvirinae* subfamily and is a high prevalent infectious agent on the worldwide. In immunocompetent hosts, HCMV primary infection is generally asymptomatic, however in immunosuppressed individuals, life threatening diseases can occur. After primary infection HCMV establishes life time latency in some cellular types, such as CD14+ monocytes in the bone marrow, characterized by the presence of the viral genome, limited gene expression and absence of viral particles production. During lytic infection the viral genes are expressed in a coordinate cascade manner and are classified as immediate early (IE), early (E) and late (L). Numerous studies have associated HCMV with cancer and is assumed that the virus can increase tumor malignancy in a process termed as oncomodulation. In the last few decades, many studies have focused in the relation between HCMV and glioblastoma multiforme (GBM), a highly malignant CNS tumor. The virus can modulate the cell cycle, apoptosis, angiogenesis, cell invasion, and host immune response in tumor cells. The P53 protein, expressed by the gene *TP53*, also called tumor suppressor protein, is required for control of the cell cycle and plays an important role in the HCMV replication on permissive cells. Single point mutations that impair the functions of P53 protein are frequently found in diverse tumors, including GBMs. The role of P53, containing mutations that affect its functions, for HCMV replication in tumor cells is not well understood and therefore we decided to investigate HCMV infection and replication in the U138 GBM cell line with P53 mutated (P53mut). We have produced a cell line in which expression of P53mut is inhibited by shRNA and analyzed HCMV infection and viral gene expression in these cells comparing to parental U138 cells. Our results demonstrated that in both U138 and U138 knockdown cells all classes of genes are expressed (IE (UL123), E (UL44/UL83) and L (UL99)), however inhibition of P53 leads to an increase in their expression levels. This initial data indicates that P53mut is able to inhibit viral gene expression, which may leads to viral persistence in tumor cells, contrary to the effect of P53_{wt} in permissive cells. Further experiments are underway to better understand the role of P53 in HCMV replication in tumor cells.

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP

Keywords: P53, CANCER, GBM, GLIOBLASTOMA, HCMV



THE HOST PROTEIN AP1 IS RELEVANT TO HIV-1 NEF ANTAGONISM AGAINST SERINC5

Cristina Santos da Costa ¹, Érika da Silva Czernisz ¹, Luis Lamberti Pinto da Silva ¹

¹ FMRP USP - Faculdade de Medicina de Ribeirão Preto, USP (Av. Bandeirantes 3900, Monte Alegre, Ribeirão Preto- SP)

Abstract

The HIV-1 is a lentivirus that causes one of the largest viral pandemics. One of the first immune system responses to infection is mediated by cell restriction factors, such as Serinc5 – a recently discovery host transmembrane protein incorporated onto viral particles decreasing its infectivity. The viral accessory protein Nef is responsible for the viral antagonism to Serinc5 and acts by removing Serinc5 from the plasma membrane, avoiding its incorporation on viral progeny. It is known that Nef manipulates the endocytic pathway to change the subcellular localization of Serinc5. However, the cellular components involved in this process are not well elucidated. As Nef utilizes the clathrin adaptor complex protein 1 (AP1) on CD4 and MHC-I downregulation, we hypothesized that AP1 could also be involved on Nef-induced Serinc5 redistribution. Thus, the present study aimed to verify the role of AP1 on Nef antagonism to Serinc5. To test the importance of AP1 on Serinc5 redistribution by Nef, the AP1 subunit g-1 was depleted by siRNA in HeLa cells with transient co-expression of Serinc5 and Nef. Indirect immunofluorescence and confocal microscopy analysis confirmed that Nef expression re-localizes Serinc5 from the cell periphery to intracellular structures. Importantly, in the absence of AP1 Nef effect on Serinc5 is abolished, and Serinc5 was localized mostly on cell periphery. In order to get a homogeneous Serinc5 expression, we generate a cell line with doxycycline inducible expression of Serinc5 fused to HA (HEK_SER5-HA). After determining the minimal required concentration of doxycycline, HEK_SER5-HA cells was transfected with either pIRES-GFP or pNef- IRES-GFP plasmids, and the SER5-HA localization accessed by immunofluorescence. In this way, we confirmed that Nef is able to modify the distribution of Serinc5 in this novel cell line system. To verify if Nef antagonizes the SER5- HA incorporation onto HIV-1 particles, HEK_SER5-HA cells was transfected with pNL4-3 WT or Δ Nef proviral plasmids. After 48h the virus particles were purified by ultracentrifugation. Western blot analyzes revealed a higher incorporation of SER5-HA on HIV-1 Δ Nef than WT, validating the our SER5-HA cell system. We are currently knocking out AP1 in HEK_SER5-HA through CRISPR/Cas9 system to establish the specific function of AP1 in Nef- induced cell surface dowregulation of Serin5 and the consequent impairment incorporation onto budding HIV-1 particles.

Financial Support: CAPES, CNPq, FAPESP.

Keywords: HIV-1, SERINC5, Nef, AP1



IMMUNOMODULATION OF MONOCYTES AND LYMPHOCYTES BY HYDROXYPROPYL-BETA-CYCLODEXTRIN (HP- BCD) AS A POTENTIAL STRATEGY TO CONTAIN HIV-ASSOCIATED CRHONIC IMMUNE ACTIVATION

Bruno Braz Bezzerá ¹, Flavio Lemos Matassoli ¹, Natália Caetana de Souza Santos ¹, James E. K. Hildreth ², Luciana Barros de Arruda ¹

¹ UFRJ - Universidade Federal do Rio de Janeiro (Cidade Universitária - Rio de Janeiro), ² UCD - University of California - Davis (California - EUA)

Abstract

Chronic immune activation is a hallmark of HIV infection and increased frequency of activated monocytes and T lymphocytes are strong predictor of disease progression. Hydroxypropyl-beta cyclodextrin (HP-bCD) is a cholesterol-sequestering drug that inhibit HIV infectivity. Since cholesterol metabolism is also involved in inflammation, we investigated whether treatment of monocytes and CD4⁺T lymphocytes with HP-bCD impacted their response to inflammatory stimuli as proof of concept for anti-HIV therapeutic strategy. We previously demonstrated that treatment of monocytes from HIV-patients with HP-bCD potently inhibited the expression and secretion of TNF- α and IL-10 induced by LPS, by a p38MAPK dependent pathway. Also, transcriptome analysis indicated altered expression of PPAR- γ HP-bCD treated cells. Here, we further investigated the molecular mechanisms associated to inhibition of monocyte activation and evaluated if this drug also impacted CD4⁺T cells activation. Cells from HIV-patients or healthy donors were treated with HP-bCD and the expression of cholesterol and raft-associated proteins were followed. Cholesterol-associated raft was replenished after 48h post treatment, and we chose this time point to stimulate the cells. Monocytes were stimulated with LPS and we followed the TLR4-mediated signal transduction pathways. Corroborating our previous data, HP-bCD treatment resulted in decreased expression of TNF- α , however, production of IL-8 and IFN- α were unaffected, indicating that MyD88 and TRIF adaptor proteins were functional. HP-bCD-treated cells showed reduced phosphorylation of I κ B α , suggesting an inhibition of NF-KB transcription factor. HP- β CD also reduced PPAR γ expression. However, addition of PPAR-agonists did not impact TNF- α production, indicating that this may not be the pathway associated to HP- bCD function. Regarding T cell activation, we initially evaluated PHA-induced expression HLA-DR and CD38, since HIV progressor patients present increased frequency of HLA-DR⁺/CD38⁺ CD4⁺ T cells. Pretreatment of purified T cells with HP-bCD significantly reduced the expression of those markers, without affecting cell viability, indicating that the drug affects unspecific T cell stimulation. Further assays are needed to determine the impact on T cell specific response and on the monocyte-T cells interaction. Still, our data suggest that HP-bCD has an immunomodulatory effect, which may impact HIV pathogenesis and AIDS progression.

CNPq, FAPRJ e CAPES

Keywords: HIV 2, HP-BCD , Monocyte , Lymphocytes, Inflammation



DIFFERENTIAL MODULATION OF TYPE I IFN RESPONSE BY DISTINCT ZIKA VIRUS ISOLATES AND ITS ROLE FOR VIRUS REPLICATION AND DISSEMINATION TO THE CENTRAL NERVOUS SYSTEM

Yasmin Mucunã Mustafá¹, Flavio Lemos Matassoli¹, Michelle Premazzi Papa¹, Sharton Vinícius Antunes Coelho¹, Luan Rocha Lima¹, Lana Monteiro Meuren¹, Ricardo Correia da Silva¹, Pedro Moreno Pimentel Coelho², Luciana Barros de Arruda¹

¹ IMPG - Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro (Avenida Carlos Chagas Filho, 373, CCS. Cidade Universitária. Rio de Janeiro, RJ, Brasil. CEP. 21941-902), ² IBCCF - Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Avenida Carlos Chagas Filho, 373, CCS. Cidade Universitária. Rio de Janeiro, RJ, Brasil. CEP. 21941-902)

Abstract

We have previously demonstrated that ZIKV crosses the blood brain barrier (BBB) and causes neurological syndrome without affecting the barrier integrity. We also developed lethal or nonlethal *in vivo* experimental models based on infection with distinct ZIKV isolates - ZIKVMR766 and ZIKVPE243, respectively. In these models, we observed that the BBB was disrupted at late time points only in mice infected with ZIKVMR766, which succumb to infection. The relevance of type I interferon response for the control of ZIKV infection was clearly evidenced by higher virus replication efficiency in IFNAR-deficient mice (A129 strain), in comparison to wild type (Sv129) mice, when inoculated systemically. But, the role of IFN response for BBB integrity had not been addressed. Here, we investigated whether ZIKVPE243 and ZIKVMR766 interfered in the production and response to type I IFN upon infection of human brain microvascular endothelial cell line (HBMEC), as an *in vitro* BBB model. We observed that infection of HBMEC with ZIKVMR766 induced a higher and faster production of IFN- β , concurrent with increased IRF-3 phosphorylation, in comparison to infection with ZIKVPE243. Infection with both viruses strains significantly inhibited IFN-mediated response, as evidenced with a luciferase/ISRE-reporter HBMEC (lucHBMEC). Accordingly, addition of IFN- β to the cultures did not affect virus replication, nor cell viability, corroborating that ZIKV escapes from this response. Infection with ZIKVMR766 resulted in a more potent inhibition of IFN response, associated to increased degradation of STAT2, and stronger inhibition of STAT1 and STAT2 phosphorylation. On the other hand, when the cells were treated with IFN- β prior to ZIKV infection, ISRE activation was induced and a decrease in viral replication was detected. *In vivo* assays demonstrated that intracerebral inoculation of ZIKV in A129, but not Sv129 promoted BBB disruption, despite virus replication in both mouse strains, indicating the relevance of IFN response for the integrity of BBB. Importantly, ZIKVMR766 showed similar titers in A129 and Sv129, corroborating its higher ability to escape the IFN-mediated response. These data suggest that infected, but not bystander cells are resistant to IFN mediated antiviral response. Therefore, whereas ZIKV-infected BMECs block antiviral IFN responses, allowing virus replication, IFNs produced during infection may restrict virus dissemination to bystander cells and control BBB disruption.

Keywords: ZIKV, IFN response, Blood-brain barrier, HBMEC



INVESTIGATION OF THE ROLE OF VARIATIONS IN E AND NS1 PROTEINS IN ZIKA VIRUS PATHOGENESIS

Iasmim Silva de Mello ¹, Déberli Ruiz Fernandes ¹, Nathalia Dias Furtado ¹, Alexandre Araújo C. dos Santos ¹, Marta Pereira dos Santos ¹, Lidiane S. Menezes Souza ¹, Myrna C. Bonaldo¹

¹ Fiocruz - Fundação Oswaldo Cruz (Rio de Janeiro, RJ, Brasil)

Abstract

Zika virus (ZIKV) is an arthropod-borne virus belonging to the genus *Flavivirus* and in the family *Flaviviridae*. ZIKV is mainly transmitted by *Aedes* spp. mosquitoes. In the recent epidemic, ZIKV has been associated with severe neurological and congenital syndromes. This study aims to correlate amino acid substitutions in the envelope protein (E) and the nonstructural protein-1 (NS1), detected in post-epidemic strains, with the disease's pathogenesis. Infectious clones based on parental virus Rio-U1 (Genbank: KU926309), and two other clones carrying the changes in E and NS1 proteins were constructed using reverse genetics technology. The construct was based on the cloning of fragments that bear the complete ZIKV genome into a low copy number plasmid followed by PCR reactions. The resulting genomic cDNA was transcribed *in vitro* into viral RNA and transfected in Vero cells. The other two clones with alterations in E and NS1 proteins were obtained by site-directed mutagenesis. The IC Rio-U1, IC. MutE and IC. MutNS1 constructs were able to generate infectious particles. RT-PCR confirmed viral recovery from the RNA extract of the culture supernatant. Furthermore, the detection of the E protein was positive by immunofluorescence with the panflavivirus 4G2 antibody for all the variants. The IC. RioU1 virus genomic sequence is identical to its parental virus, Rio-U1. The replication profile of the parental virus is similar to the infectious clone in both Vero and C6/36 cells. The viruses were analyzed by plaque size assay and the phenotype of the IC. Rio-U1 displayed smaller plaques than the parental virus Rio-U1. The plaque phenotype of the IC. MutE virus is similar to the IC. Rio-U1 virus. The IC. MutNS1 virus has similar plaque sizes as the isolate in which the mutation in NS1 was described, Rio-BM1. In summary, our preliminary findings indicate that the infectious clone IC. Rio-U1 exhibits similar viral fitness compared to the parental virus. The methodology of synthetic ZIKV recovery was achieved and will be of great value in evaluating the role of the amino acid changes in E and NS1 proteins in viral fitness and virulence. Financial support: MCTIC / FNDCT - CNPq / MEC-CAPES / MS-Decit. (Grants 426767 / 2018-7 and 88881.130684 / 2016-01) and INOVA-Fiocruz (Grant VPPIS-004-FIO18)

Keywords: Zika Virus, Infectious clone, Reverse genetic, Site-directed mutagenesis



THE PROTEIN COMPLEX MTORC MAY INFLUENCE CHIKV INFECTION IN MURINE DENDRITIC CELLS

Gabriela Fabiano de Souza ¹, Stéfanie Primon Muraro ¹, Mariene Ribeiro Amorim ¹, Aline Vieira ¹, Matheus Cavalheiro Martini ¹, Daniel Augusto de Toledo Teixeira ¹, Renata Sesti Costa ¹, Pedro Manoel Mendes de Moraes Vieira ¹, Carla Cristina Judice ¹, José Luiz Proença Modena ¹

¹ UNICAMP - Universidade Estadual de Campinas (Rua Monteiro Lobato, 255 CEP: 13083-862 Campinas - SP - Brasil.)

Abstract

The Chikungunya virus (CHIKV) is an emerging arbovirus present in tropical and subtropical regions transmitted by the arthropod vector *A. aegypti*. Although CHIKV infection may be asymptomatic, it usually leads to the development of an acute febrile illness, joint pain and swelling. One of the major complications associated with CHIKV are chronic manifestations such as arthralgia and arthritis, these symptoms can last for months to years. The chronic disease resulting from CHIKV infection has similar characteristics to rheumatoid arthritis which there is an imbalance in the function of dendritic cells (DCs). Thus, we believe that the infection, activation and metabolic imbalance of dendritic cells may play an essential role in the pathogenesis of CHIKV and in the development of chronic inflammation in the joints. To analyze the effect of CHIKV on the function and metabolism of DCs in vitro, DCs differentiated from WT C57BL/6's bone marrow were pretreated with rapamycin (5, 10, 100 or 200 ng/mL) and infected with CHIKV (MOI 1) for 3, 6 and 24 hours. We characterize viral replication and expression of genes associated with the immune innate response. Preliminary results suggest that bone marrow-differentiated dendritic cells from wild type mice previously treated with inhibitor of the MTORC 1 and 2 metabolic pathways and infected with CHIKV may contribute to an increase in infection within 24 hours. In addition, we observed changes in the expression of IRF 3, 5 and 7, NFκ-B and RNase L at different concentrations of inhibitor, suggesting an important role of these pathways during CHIKV infection.

Financial support: FAPESP

Keywords: Chikungunya, Dendritic cells, Metabolism, mTOR, Arbovirus



THE ROLE OF INNATE RECOGNITION PATHWAYS IN PLACENTAL CELLS AFTER OROV INFECTION

Stéfanie Primon Muraro ¹, Gabriela Fabiano de Souza ¹, Aline Vieira ¹, Mariene Ribeiro Amorim¹, Matheus Cavalheiro Martini ¹, Daniel Augusto de Toledo Teixeira ¹, Eurico de Arruda Neto ³, Jean Pierre Schatzmann Peron ², Maria Laura Costa do Nascimento ¹, José Luiz Proença Modena¹

¹ UNICAMP - Universidade Estadual de Campinas (Rua Monteiro Lobato, 255 CEP: 13083-862 Campinas - SP - Brasil.), ² USP - Universidade de São Paulo (Av. Prof. Lineu Prestes, 1730 Cidade Universitária - CEP 05508-000 São Paulo - SP - Brasil), ³ FMRP-USP - Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo (Av. Bandeirantes, 3900 CEP 14049-900 Ribeirão Preto – SP- Brasil)

Abstract

The Oropouche virus (OROV) is an arbovirus with potential to cause epidemics in the most populated regions of Brazil. Individuals infected with OROV develop a febrile illness, which can progress to neurological and hemorrhagic complications. Furthermore, an increased incidence of abortion was reported during major OROV epidemics. However, the teratogenic potential of OROV and the pathogenetic mechanisms associated with the hematoplacental barrier breakdown have not yet been investigated. The immunological response and antiviral activity in the placenta are dependent on the expression of pattern recognition receptors (PRRs) and production of type I interferon (IFN), mainly on cytotrophoblast, syncytiotrophoblast cells and placental macrophages. However, some viruses are able to establish long-term placental infections, in part by mechanisms dependent on the antagonism of the type I IFN pathways. Thus, we intend to characterize the mechanisms and pathways by which type I and III IFNs modulate the placental immune response during infection by OROV. Preliminary results show that OROV replicates in human placental lineage and can induce the expression of IFN type I and III genes in addition to interferon regulatory genes such as IRF-1, IRF-3 and IRF-7. These results suggest an involvement of immunological recognition pathways and contribute to the follow-up of the next experiments.

Financial support: FAPESP, CNPq

Keywords: Oropouche, Placenta, Interferon, Arbovirus



CELLULAR ALIX PROTEIN: A HIV INFECTIVITY PROMOTER

Gustavo Peixoto Duarte da Silva ¹, Isadora Alonso Corrêa ¹, Pedro Telles Calil ¹, Luís Lamberti Pinto da Silva ², Luciana Jesus da Costa ¹

¹ UFRJ - Universidade Federal do Rio de Janeiro (Av. Carlos Chagas Filho, 373, Rio de Janeiro), ² USP- RP - Universidade de São Paulo - Ribeirão Preto (Av. Bandeirantes, 3900, Monte Alegre, Ribeirão Preto)

Abstract

Cellular Alix protein plays different roles in cell such as regulating basal autophagy, incorporation of LBPA in late endosomes, plasma membrane and lysosome repair. It's well known that Alix plays a central role in ESCRT machinery and both can be coopted for enveloped viruses budding. Our group have previously demonstrated that Alix can interact with HIV accessory protein Nef, but the role of Alix cooption by Nef at the life cycle of HIV-1 was not fully characterized. HIV Nef plays an essential role increasing viral infectivity and progression to Aids. It has been reported that Nef interacts with different cell partners, however the function related to increased viral infectivity wasn't completely understood. Alix-Nef interaction can mediate downregulation of CD4 receptor from the cell surface and targets CD4 to lysosomal degradation. That interaction occurs by a late domain-like motif in Nef. The aim of this study is to investigate the role of Alix on HIV-1 infectivity and the importance of interaction with Nef in this process. In our assays, we observed that silencing of Alix in Hek293T and HeLa cells by transfection of a pool of siRNA against Alix followed by transfection of these cells with pNL 4-3 (HIV-1 infectious clone) leads to a 4-fold decrease in infectivity of viral progeny produced from these, but yet no impact on viral release was observed. Nevertheless, no impact on viral infectivity was observed on a NL 4-3ΔNef upon Alix silencing. A better characterization of viral progeny produced from Alix knock-down cells by analyses of viral protein contents present in iodixanol gradient fractions showed that although viral particles are released from these cells they are partially or not mature, indicating that Nef and Alix together cooperate to virus processing.

This data confirms that Alix influences the infectivity of HIV-1 viral progeny and it dependent on Nef by a mechanism related to HIV Protease activity. Interestingly, lymphocytic MOLT4 cells silenced for Alix was shown to be less susceptible to HIV-1 infection spread. Alix silenced MOLT4 cells transfected with pNL 4-3 showed a lower expression of viral proteins and lower virus production after 72h, demonstrating a phenotype of lower permissiveness to HIV-1 infection in cells lacking of Alix. Thus, we confirmed that Alix plays an important role during the replicative cycle of HIV-1. Financial support: CNPq; Faperj; Capes.

Keywords: HIV, Alix, Infectivity, Nef, Protease



MOLECULAR, BIOLOGY AND CLINICAL CHARACTERIZATION OF CHIKUNGUNYA VIRUS STRAIN RJ-IB1 FROM RIO DE JANEIRO, BRAZIL.

Vinícius Wakoff Pereira Fonseca ¹, Romário Mattos de Souza ¹, Pedro Antônio Gomes Costa ¹, Marcela Sabino Cunha ¹, Iranaia Assunção Miranda ¹, Luciana Jesus da Costa ¹

¹ UFRJ - Departamento de Virologia, Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro (Av. Carlos Chagas Filho - Cidade Universitária, Rio de Janeiro - RJ, 21941-590)

Abstract

Chikungunya virus (CHIKV), member of alphavirus genus, is the etiological agent of an arthritogenic disease with significant morbidity. Our group isolated and characterized a CHIKV strain (RJ-IB1) from Rio de Janeiro, that clustered with the 1st Brazilian ECSA isolate, from Feira de Santana (BHI3745, 2014). Given the recent introduction of CHIKV in Brazil, little is known about strains viral growth kinetics and pathogenesis. Thus, the aim of this study was to characterize *in vitro* biological behavior and *in vivo* pathogenesis of RJ-IB1. Initially, we performed viral growth kinetics in 4 different cell lines: Hela, HEK293T, HBMEC, and MEF. RJ-IB1 replicated in all cell lines, reaching a replication peak in 24 hpi, ranging between 5×10^5 and 1.5×10^9 PFU/ml, depending on the cell line. In general, RJ-IB1 infectious titers were up to 1 log higher than BHI3745. Wild type SV129, 12 (PN12) or 21 days-old (PN21), were inoculated subcutaneously in the left paw with 10^6 PFU/animal with RJ-IB1 or BHI3745. In PN12 mice, we observed for both isolates a pronounced swelling on the left paw as early as 1 dpi that persisted up to 5 dpi, and also a progressive weight loss during this period. We also observed other clinical symptoms: diarrhea, fecal hypocholia, joint pain, hair loss, restless behavior (possible impact on CNS) and skin erythema, with survival rates ranging between 70% and 80%, for both isolates. From RJ-IB1-infected mice we accessed the viremia (peak of 1×10^7 PFU/ml in 1 dpi) and viral load in brain (peak of 1×10^4 PFU/ml in 3 dpi); left gastrocnemius muscle (peak of 1×10^4 PFU/ml in 3 dpi); and liver (peak of 8.2×10^5 PFU/ml in 1 dpi). Viral load in brain (4×10^3 PFU/ml) and muscle (6×10^4 PFU/ml) was persistent on 6 dpi. In PN21 mice, the paw swelling persisted up to 10 dpi. Viremia reached a peak on 2 dpi in RJ-IB1-infected mice and 3 dpi in BHI3745-infected mice. The weight gain curve was similar and no other clinical symptoms were observed, for neither of experimental conditions, demonstrating the ability of Brazilian CHIKV isolates to establish infection in 3 weeks- old mice. Histological analysis shows reversible and irreversible cell lesions and presence of mononuclear cell infiltrates in muscles and livers. In conclusion, our results show that infection of SV129 mice with an isolate circulating in Brazil displace an apparently higher pathogenicity in those animals, with possible neurological symptoms and intense viral replication, including in CNS.

Keywords: CHIKV, Characterization, RJ-IB1, *in vivo*, *in vitro*



NOVEL QUINOLONE DERIVATIVE COMPOUNDS: BIOTECHNOLOGICAL APPLICATION AS ANTI-MAYARO AGENTS

Leonardo Dos Santos Corrêa Amorim ¹, Letícia Villafranca Faro ¹, Andrew Barcelos Monção Meireles , Vitor Won-Held Rabelo ¹, Luciene Soares Silva ¹, Maria Leonisa Sanchez Nuñez ¹, Aldenise Mont'serrat Rosa Da Silva ¹, Natasha Cristina Da Rocha ¹, Caroline De Souza Barros ¹, Cláudio Cesar Cirne Dos Santos ¹, Maria Cecília B. V. De Souza ¹, Izabel Christina Nunes De Palmer Paixão ¹

¹ UFF - Universidade Federal Fluminense (Rua Outeiro de São João Batista, S/N - Centro, Niterói - Rio de Janeiro)

Abstract

Mayaro virus (MAYV), causative agent of Mayaro Fever, is an arthropod-borne RNA virus enveloped and is classified in the family *Togaviridae*, genus *Alphavirus*. The MAYV has an endemic epidemic enzootic in pan- Amazonian countries surrounding central South America. Until now, there are no vaccines or drugs available for clinical use, so the search for new therapies is an urgent need. Therefore, this study proposes to evaluate the cytotoxicity and antiviral potential of a quinolone derivatives. We studied 8 new substances from quinolone derivatives for the development of drugs against the MAYV. Vero cells were used to evaluate the effect and mechanism of action of these substances. The cytotoxicity effect was evaluated on Vero cells using the MTT method. Our results showed that the CC₅₀ value varied from 659 μ M to 2815 μ M. Next, all compounds were screened for their anti-MAYV activity at concentration of 50 μ M by plaque reduction assay. The most active compound, quinolone derivative 03 (R= *o*-methyl), presented inhibition of plaque formation over than 90% and the value of EC₅₀ was 0,83 μ M. Further, we evaluated the ability of this compound to inactivate the viral particle of MAYV during the replication cycle of the virus (*Time of drug addition assay*). The compound 03 exhibited significant activity in inhibition virus replication in all times of treatment, however the major activity was the beginning of replicative cycle, when cells were infected and treated at the same moment. Moreover, we carried out *in silico* pharmacokinetics and toxicological analysis of this compound using admetSAR2.0 server. Therefore, we identified compounds with high anti-MAYV activity and low cytotoxicity as well as promising theoretical pharmacokinetics, which reinforced their potential to be explored.

Financial support: CNPq , CAPES, FAPERJ, UFF (PROPI)

Keywords: Mayaro, Antiviral, Quinolone derivatives



HIV-1 NEF PROTEIN REGULATES VIRAL PROTEASE ACTIVITY TO INCREASE VIRAL INFECTIVITY VIA ALIX.

Isadora Alonso Corrêa ¹, Pedro Telles Calil Pedro Telles Calil ¹, Gustavo Peixoto Duarte da Silva Gustavo Peixoto Duarte da Silva ¹, Luciana Jesus da Costa Luciana Jesus da Costa ¹

¹ UFRJ - Universidade Federal do Rio de Janeiro (Avenida Carlos Chagas Filho, 373, Centro de Ciências da Saúde)

Abstract

The HIV-1 protease (PR) is an enzyme encoded by the HIV-1 *pol* gene that plays a central role in the virus life cycle by cleaving the viral polyproteins p55^{*gag*} and p160^{*gag-pol*} allowing viral maturation. This process is highly regulated and PR activity could be affected by viral polymorphisms and interactions between PR and other viral proteins. The interaction with its substrate, Gag-Pol, is well described and several studies relate the impact of mutations in Gag on PR activity. Other important interaction partner is the viral protein Nef which is cleaved by viral PR and, in a previous study from our group, was associated with the regulation of the enzyme. However, a clear correlation between Nef and PR activity is lacking. In this study, we used PR mutants, that had already been described to increase (K45I) or decrease (T26S) the enzyme processivity and investigated the influence of Nef on viral phenotype. Virus production and infectivity were measured in supernatants of Hek-293T transfected with HIV-1 wild-type and mutant proviral clones. PRT26S and PRK45I had a deleterious impact on production of mature virions and consequently, viral infectivity due to alterations in viral processing. However, specifically for PRT26S mutant, the absence of Nef rescued virus maturation and infectivity to the levels of the wild-type virus. Nef- deletion in PRK45I mutant lead to a partial recovery. This data confirms the importance of Nef for PR activity and demonstrates for the first time that viral infectivity due to Nef is dependent on PR and Nef association. Nef interacts with cellular protein Alix and this interaction is important to promote viral infectivity. A 3-fold reduction in infectivity of the Nef-deleted PRT26S mutant was observed upon Alix knock-down by siRNA, implicating the Nef and Alix interaction in the regulation of PR activity. Phenotypic assays for several PR inhibitors were performed with PRT26S and PRK45I mutants. The K45I mutation conferred a two-fold increase in IC50% concentration for Tipranavir while T26S mutant showed a greater sensitivity to all PIs tested when compared to the WT. Taking together results clearly demonstrate that the absence of Nef could restore the infectivity of viruses harboring mutations that alter PR processing. In addition, these mutations can directly influence the kinetics and replication rate of these viruses and affect the sensitivity to PIs. Financial support: CNPq; Faperj; Capes.

Keywords: HIV-1, Nef, Protease, Alix



A POSSIBLE ROLE OF RAB27A/B IN OROPOUCHE VIRUS REPLICATION CYCLE.

Juan Oswaldo Concha Casaverde ¹, Cristina Santos da costa ¹, Natalia da Silva Barbosa , Luis Lamberti Pinto da Silva ¹

¹ FMRP - USP - Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo (Ribeirão Preto - São Paulo)

Abstract

The Oropouche virus (OROV) belongs to the Peribunyaviridae family, genus Orthobunyavirus, and is the cause of one of the most important arboviroses in Brazil. OROV is responsible for major outbreaks in different countries of the equatorial region of America, demonstrating a high emerging potential. Despite its relevance, little is known about the molecular aspects of the OROV replicative cycle, and most of the knowledge generated about the genus Orthobunyavirus originates from studies of Bunyamwera virus, the genus prototype virus. The present study aims to elucidate mechanisms used by OROV to release its viral progeny. OROV assembles in virally induced replication compartments derived from Golgi membranes that morphologically resemble multivesicular bodies (MVBs). Because Rab27a and Rab27b are known to be involved in directing MVBs for plasma membrane fusion, we hypothesized that Rab27a and Rab27b may be involved in OROV externalization. To test this hypothesis, confocal immunofluorescence analyses were performed and showed that Rab27a and Rab27b are recruited to intracellular vesicles that are enriched in OROV proteins, which may indicate a possible direct or indirect interaction between these proteins. To further test the involvement of these proteins in OROV production, Rab27a expression was suppressed by siRNA in HeLa cells prior to infection. Expression of OROV proteins was not affected by Rab27a depletion. Interestingly, analysis of the supernatant of these cells 12h post-infection showed a decrease in the percentage of virus released relative to non-silenced control cells, in addition to increasing the amount of infectious virus within cells, suggesting that the presence of Rab27a is important for efficient viral progeny release. To confirm this hypothesis and understand how this possible relationship occurs, more experiments are being performed.

Financial Support: CAPES, FAPESP, CNPq.

Keywords: Oropouche, Rab27a, Rab27b, Orthobunyaviridae, Peribunyaviridae



ZIKA VIRUS INHIBITION BY COPAIBA (*COPAIFERA OFFICINALIS*) OIL NANOEMULSION

Cintia Bittar¹, Tamara Carvalho¹, Marcela Guimarães Landim², Paula Rahal¹, Graziella Anselmo Joanitti², Marília de Freitas Calmon¹

¹ UNESP - Universidade Estadual Paulista "Julio de Mesquita Filho" (Rua Cristóvão Colombo, 2265- Jardim Nazareth- São José do Rio Preto-SP), ² UNB - Universidade de Brasília (Campus Universitário Darcy Ribeiro, Brasília-DF, CEP 70910-900)

Abstract

Since the 2015 outbreak, Zika virus (ZIKV) has spread all over the world. It has become a major global health issue due to the neurological complications related to ZIKV infection such as Guillain–Barré Syndrome and Zika virus Congenital Syndrome. The virus is transmitted by *Aedes* mosquitoes but also by blood transfusion and sexually which allows the transmission of the virus in vector-free environments. So far, there are no vaccines or specific treatments for ZIKV infection, which makes important to develop specific therapies for its treatment. Here we evaluated the ability of a copaiba oil (*Copaifera officinalis*) nanoemulsion to inhibit ZIKV. Copaiba oil nanoemulsion was prepared by ultrasonication and characterized using Zetasizer®. The obtained nanodroplets were homogeneous (Pdl = 0.219) showing hydrodynamic diameter of 120.8 nm and Zeta potential of -29.6 mV. For the *in vitro* assays, first, we defined the highest non-cytotoxic concentration of the copaiba-based nanoemulsion in Vero cells by MTT assay. A concentration of 180 µg/mL was chosen since it maintains 100% cell viability up to 96h after treatment. Vero cells were infected and simultaneously treated with copaiba oil nanoemulsion at the highest non-toxic concentration. After 96h, results were evaluated by plaque assay revealing a viral inhibition of 80%. In order to understand in which steps of the viral life cycle the drug is acting on, we performed time-of-addition experiments and analyzed viral RNA by qPCR after 48h. Preliminary results show that the copaiba oil nanoemulsion has virucidal effect inhibiting 92.5% of virus release. It also showed an effect in post-entry steps inhibiting 99.1% of ZIKV intracellular RNA, when compared to the control. Additional experiments are being performed to confirm the preliminary results and also to understand the mode of action of the copaiba oil nanoemulsion in inhibiting Zika virus infection.

Financial Support: CAPES/CNPq , FAPESP

Keywords: ZIKV, antiviral, OIL NANOEMULSION, copaíba



A NEW (?) PHLEBOVIRUS ISOLATED FROM AMAZONIAN SANDFLIES

Antonio José Leão Cardoso ¹, Eric Fabrício Marialva dos Santos ¹, Karina Pinheiro Pessoa ¹, Dana Cristina da Silva Monteiro ¹, Valdinete Alves do Nascimento ¹, Débora Camila Gomes Duarte ¹, Victor Costa de Souza ¹, Felipe Arley Costa Pessoa ¹, Felipe Gomes Naveca ¹

¹ ILMD-FIOCRUZ/AM - Instituto Leônidas e Maria Deane - FIOCRUZ/AM (Rua Terezina, 476)

Abstract

Sandflies (Diptera: Psychodidae) shows worldwide distribution and approximately 10% of the known species are incriminated as vectors of etiological agents of human illness, mainly parasites of the *Leishmania* genus. Despite the transmission of some medically relevant viruses; sandflies have been treated as neglected vectors of viral diseases. The transmission of arbovirus by sandflies may be observed in urban, periurban, and rural areas, often associated with poverty. The phleboviruses, arboviruses of the *Phlebovirus* genus (family *Phenuiviridae*), may cause from a self-limiting fever (known as sandfly fever) to neurological infections. In the Brazilian Amazon, viruses within this genus were only registered in the state of Pará until now. We conducted an entomological survey in Rio Pardo rural settlement, Presidente Figueiredo municipality, metropolitan area of Manaus, intending to detect viruses in sandflies. Between 2017 and 2018, we use CDC-like light traps, as well as a mechanical aspiration on the base of trees to sandflies capture. Specimens were kept under the cold chain until species identification following entomological keys. A total of 2,468 sandflies were collected, among these, 991 females were distributed in 35 species, generating 460 pools containing from 1 up to 26 sandflies, which were macerated and inoculated into VERO and C6/36 cells. Of those, 35 pools induced cytopathic effect, 21 only in VERO, 11 only in C6/36 and 3 in both. Conventional RT-PCRs were performed for the *Phlebovirus* genus and one pool with 14 female *Lutzomyia* sp., collected in a forest environment, was positive. Preliminary results of nucleotide sequencing and phylogenetic analysis indicate that it may be a new phlebovirus, related to the Uriurana virus, previously isolated in Pará. We tentative denominated the virus isolated in the present study as Rio Pardo phlebovirus (RIOPV). The isolate induced a cytopathic effect in both C6/36 cells and HepG2 cells; thus, suggesting that RIOPV may infect vertebrate hosts. Further experiments are ongoing to fully characterize the RIOPV isolate. In conclusion, our results strengthen the necessity of continuous surveillance of potential humans' pathogens in vectors of the Amazon rain forest.

Keywords: Sandflies, Phlebovirus, Brazil, Amazon

Financial Support: DECIT-MS, Capes, CNPq, Fiocruz

Keywords: Sand flies, Phlebovirus, Brazil, Amazon



HUMAN HERPESVIRUS 6 (HHV-6) AND HUMAN HERPESVIRUS 7 (HHV-7) EXCRETION IN ORAL FLUIDS OF PATIENTS WITH CHRONIC HEPATITIS C

Jéssica Vasques Raposo ¹, Nathalia Alves Araújo de Almeida ¹, Ana Carolina da Fonseca Mendonça ¹, Bianca Leires Marques ¹, Livia Melo Villar ¹, Vanessa Salete de Paula ¹

¹ IOC/FIOCCRUZ - Instituto Oswaldo Cruz (Av. Brasil 4365, Rio de Janeiro)

Abstract

Human Herpesvirus 6 (HHV-6A / B) and Human Herpesvirus 7 (HHV-7), also called Roseoloviruses, belong to the Betaherpesvirus subfamily. Like other herpesviruses, the Roseoloviruses persist in the host after primary infection. Moreover, HHV-6 and HHV-7 are considered opportunistic viruses and co-infections with other viruses are often found and associated with several diseases. In a previous study, the herpesvirus reactivation in patients with chronic Hepatitis C virus (HCV) infection was reported and demonstrated that immune changes that follow HCV might lead to reactivation of other viruses, such as herpesviruses. Due to latency and persistence of Roseoloviruses occur in the salivary glands, the objective of this study was to analyze the risk factors associated with the presence of Roseoloviruses in oral fluids of patients with chronic HCV. Thus, the oral 117 fluids samples were of patients with chronic hepatitis C tested by multiplex qPCR and statistical analyses was performed using R Studio software. The average age of the patients was 54.6 ± 12 years and most of them were (59.9%; 70/117) were female. The presence of Roseoloviruses was found in 47.2% (50/117) to HHV-6 with load viral mean $1,25E+05$ copies/mL and 36.7% (43/117) to HHV-7 with load mean $2,24E+05$ copies/mL. The comparison with co-infections among Roseoloviruses demonstrated that HHV-6 infection is related to HHV-7 infection ($p=0.001$). Furthermore, in patients with high fibrosis levels (F3-F4) there were twice the chance (95% IC=1,30-6.83) of HHV-7 infection when compared to individuals with lower fibrosis levels (F1-F2). Roseoloviruses prevalence was high in oral fluids of patients with chronic HCV. However, more cases are required to confirm whether high fibrosis levels may be associated with increased incidence of HHV-6 and HHV-7 reactivation.

Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

Keywords: Chronic HCV, HHV-6, HHV-7, Roseolovirus, Saliva



POXVIRUS-HOST INTERACTIONS: THE ACTIVATION OF COMPONENTS OF THE HOST'S UNFOLDED PROTEIN RESPONSES (UPR) DURING INFECTIONS BY THE VACCINIA VIRUS STRAINS GUARANI P1 AND PASSATEMPO

Karine Lima Lourenço ¹, Thiago Lima Leão ¹, Flávio Guimarães da Fonseca ¹

¹ UFMG - UNIVERSIDADE FEDERAL DE MINAS GERAIS (Av. Pres. Antônio Carlos, 6627 - Pampulha, Belo Horizonte - MG, 31270-901)

Abstract

Vaccinia virus (VACV) is a member of *Poxviridae* family. Poxviruses multiplication and maturation are closely associated to the endoplasmic reticulum (ER) and their membranes. The RE is able to respond to perturbations through the unfolded protein response (UPR) pathway. This work aims to investigate the effects of infections by zoonotic VACV Brazilian strains, *Guarani P1 virus* (GP1V) and *Passatempo virus* (PSTV), on the activation of UPR pathway sensors in comparison to the VACV *Western Reserve* (WR) prototypical strain. We evaluated, by qPCR, the mRNA levels of genes induced by activation of the UPR pathway in mouse embryo's fibroblasts after infection with GP1V, PSTV or WR viruses. We verified increase in the expression levels of the GADD34 PERK inhibitor after infection with the WR virus. However, this was not observed for GP1V and PSTV. Overall, levels of the ATF4, transcription factor induced by PERK did not increase after infections with any virus. One-step growth curves showed no difference in the multiplication of these viruses in PERK-KO cells when compared to MEF-WT. Activation of ATF6, another sensor of the UPR pathway, was previously confirmed by reporter gene assays; however, the mRNA levels of this protein did not increase after infection with any of the tested viruses. CHOP is another redundant component of the UPR and its transcription up regulation was not observed after infection by the different viruses. CHOP induces the expression of Pdia4, an enzyme that has chaperone and EROI1 oxidoreductase activities. As expected, we detected no increase in Pdia4 transcription after infection. IRE1 is yet another sensor of the UPR, and infected cells treated with the kinase domain inhibitor of this sensor resulted in smaller plaques for all tested VACVs when compared to non-treated cells. XBP1 is a downstream component of the IRE1 UPR arm, and its expression levels increased after infection. As for BIP, a downstream chaperone that is redundant in the UPR pathways, expression levels did not increase after infections and viruses' single cycle curves in the presence of a BIP inhibitor showed no difference in replication compared to the untreated control. Taken together, ours and other results suggest that PERK activation is irrelevant for efficient GP1V, PSTV and WR replication. On the other hand, the ATF6 sensor and the kinase domain of the IRE1 sensor seem to play an important role in the replication of viruses.

Keywords: VACV, UPR, WR, PSTV, GP1V



IDENTIFICATION OF DIFFERENTIALLY EXPRESSED MIRNAS IN HUMAN PROSTATIC CELLS INFECTED WITH ZIKV

Francielly Cristina Machado ¹, Ricardo R F Campos ¹, Paula Rahal ¹, Cintia Bittar ¹, Marília Freitas Calmon ¹

¹ UNESP - Universidade Estadual Paulista "Julio de Mesquita Filho" (Rua Cristóvão Colombo, 2265- Jardim Nazareth- São José do Rio Preto-SP)

Abstract

Zika virus (ZIKV) is a virus transmitted mainly by *Aedes aegypti* mosquitoes. However, recent evidences indicate the occurrence of sexual transmission. Although some studies have indicated testes and prostate as the main organs that collaborate in sexual transmission, little is known about which cell types in these tissues are more susceptible to this virus. In addition, infection with distinct ZIKV strains in some models *in vitro* and *in vivo* has demonstrated that the host's response to infection is strain-dependent. Recent findings suggest that this virus deregulates host miRNA profile and that this is an important event throughout the course of the infection. Herein, we evaluated the susceptibility, the permissiveness and the cellular miRNA profile of human prostatic epithelial cells (PNT1A) to two different strains of ZIKV, a classical African strain, MR766 (ZIKV^{MR766}) and a Brazilian strain, ZIKV^{BR}. So, we infected PNT1A cells with ZIKV strains and performed an indirect immunofluorescence assay for protein envelope; monitored infectious viral particles production and RNA viral copies by plate assay and qPCR, respectively, and analyzed the miRNA cellular profile by PCR array. Our results demonstrated that human prostate cells are susceptible and permissive to ZIKV infection and did not present any imposition regarding infection by distinct strains of this virus. The strains did not differ in the kinetics of replication in prostate cells, but presented differences in miRNA's cell expression modulation. After infection, 16 miRNAs were modulated in prostate cells, a small group of 6 miRNAs were modulated by both strains while a set of 10 miRNAs showed to be modulated exclusively by ZIKV^{BR}. *In silico* analyses predicted that the miRNA upregulated exclusively by the infection by the Brazilian strain may regulate genes and pathways associated to inflammation, immunity, cell survival and cell proliferation. Taken together, our results indicate that prostate may be an important role in the sexual transmission of ZIKV and highlights that different strains of ZIKV may induce a differential host miRNA expression which may influence the differences in the physiopathology presented after the infection by different strains.

Financial support: FAPESP

Keywords: ZIKV, Prostatic cells, miRNA expression.



FULL GENOME CHARACTERIZATION OF GROUP II CONFIRMS THE DICHOTOMY BETWEEN BRAZILIAN VACCINIA VIRUS

Mauricio Teixeira Lima ¹, Felipe Lopes de Assis ², Giliane de Souza Trindade ¹, Jonatas Santos Abrahão ¹, Erna Geessien Kroon ¹

¹ UFMG - Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (Av. Antônio Carlos, 6627 Belo Horizonte, Minas Gerais, CEP 30270-901, Brazil),

² FDA -U.S. Food and Drug Administration (Estados Unidos)

Abstract

Vaccinia virus (VACV), the prototype virus of the genus *Orthopoxvirus* (OPV), shows serological cross-reactivity with other OPV species and was used during the smallpox eradication campaign. Several exanthematic VACV outbreaks have occurred in Asia and South America, affecting mainly the dairy cattle and rural workers. Brazilian VACV (VACV-BR) have been isolated since 1999 and were characterized since then. The VACV-BR belong to at least two distinct clusters, and these groups were referred to as group I (GI) and group II (GII) of the VACV-BR. Here, used VACV isolates Carangola eye 2(CE2) and Serro human 2/2011 (SH2V) previously described as GII members and etiologic agents of outbreaks in humans from Minas Gerais. The viruses were replicated into VERO cells and purified on a sucrose gradient. Viral DNA was extracted and sequenced in a MiSeq-Illumina apparatus with paired-end applications. After sequencing, reads from CE2 and SH2V were *de novo* and read mapping assembled using Geneious software. The gene predictions were performed using Geneious tools. The functional annotations were inferred by BLAST searches against the GenBank NCBI and the genome annotations were then manually curated. The genomes of CE2 and SH2V show 190,567 base pairs (bp) and 185,934 bp encoding 214 and 199 ORFs, respectively. Both genomes showed a very similar G+C content (~33,3%) and 99.62% of identity. Furthermore, we identified 303 single nucleotide polymorphism (SNPs) between CE2 and SH2V genomes. On the other hand, when compared to VACV Cantagalo genome (KT013210) belonging to VACV-BR GI, the isolates show 1.754 SNPs (CE2) and 1.875 SNPs (SH2V). To better understand the evolutionary relationship between VACV-BR and mainly the GII

viruses, we performed phylogenetic analyses. Other GII sequences available in the literature for VACV-BR genes A56R, B5R, C23L and A26L were used. The trees recurrently clustered GII members separately of GI isolates, corroborating all previous analyses. However, the CE2 and SH2V share common ancestor with New York City Board of Health derived isolates in opposite of VACV-BR GI viruses. In summary, our study reported for the first time the full genomic characterization of VACV-BR GII, reinforcing and expanding previous results with these viruses. In addition our study raises new questions and possibilities, to regard VACV-BR evolutionary relationships, origin and the dichotomy of VACV-BR from a new angle.

Financial support: CNPq, CAPES, PRPq-UFMG, FAPEMIG

Keywords: vaccinia virus, genome characterization, group II VACV-BR, Evolution



ILHEUS VIRUS IDENTIFIED IN THE CEREBROSPINAL FLUID OF A PATIENT WITH CEREBRAL HEMORRHAGE IN AN ARBOVIRUS ENDEMIC AREA

Bruno Henrique Gonçalves de Aguiar Milhim ¹, Cássia Fernanda Estofolete ¹, Leonardo Cecílio da Rocha ¹, Elisabete Liso ², Vânia Maria Sabadoto Brienze ², Nikos Vasilakis ³, Ana Carolina Bernardes Terzian ¹, Gislaine Celestino Dutra da Silva ¹, Maurício Lacerda Nogueira ¹

¹ FAMERP - Faculdade de Medicina de São José do Rio Preto (Av. Brigadeiro Faria Lima, 5416 Vila São Pedro. São José do Rio Preto, São Paulo, 15090-000), ² HB - Hospital de Base de São José do Rio Preto (Hospital de Base, São José do Rio Preto, São Paulo, Brazil. 5544 Brigadeiro Faria Lima Ave. Vila São Pedro. São José do Rio Preto, São Paulo, 15090-000), ³ UTMB - University of Texas Medical Branch (Galveston, Texas, USA. 301 University Blvd, Galveston, Texas, 77555.)

Abstract

Ilheus virus (ILHV) is a arbovirus first described in 1944 and isolated from *Aedes* and *Psorophora spp.* mosquitoes during an epidemiological investigation of yellow fever in the city of Ilheus, Bahia State, Brazil. The clinical spectrum of human infections may range from asymptomatic to central nervous system (SNC) involvement, suggestive of encephalitis. Since 2006, we have established an arbovirus surveillance for encephalitis-suggestive cases in the city of São José do Rio Preto, State of São Paulo, Brazil, located in a hyper-endemic area for dengue and Saint Louis encephalitis, Zika, and documented co-infection among various flaviviruses. In September of 2017, a 68-year-old-man was admitted with right hemiplegia, aphasia, dysarthria and deviation of left lip rhyme. During his hospitalization, brain computed angiotomography showed intraparenchymal hemorrhage, with surrounding brain edema, and intraventricular bleeding. Cerebrospinal fluid (CSF) was collected at the onset of symptoms and after 9 and 15 days to analyze the patterns presented. The patient was hospitalized 24 days in UCI, assisted by mechanic ventilation, sedation and vasopressor drugs. Subsequently, the patient developed an urinary infection by multi-drug resistant bacteria in day 20 of hospitalization and died 96 hours later. CSF sample, bacteria culture negative, were submitted to RNA extraction and tested for dengue, Zika and Chikungunya by Trioplex Real-Time reverse transcriptase-polymerase chain reaction (RT-PCR) and other Brazilian arboviruses by Multiplex-nested-RT-PCR primer sets specific for the nonstructural protein 5 gene. The CSF sample tested negative for typical neurotropic viruses such as Rotavirus by Enzyme-Linked Immunosorbent Assay, Enterovirus and Norovirus by the RT-PCR. ILHV was the only arbovirus identified by Multiplex-nested-PCR. The amplicons obtained were sequenced by the Sanger method and Phylogeny of the ILHV isolate was made using a dataset comprised of 401-long nucleotide sequences mapping on the NS5 gene. Our isolate is clustered with isolates sampled in Venezuela in 1997, suggesting the widespread distribution of the virus throughout Latin America. Our observations do not conclusively demonstrate that ILHV infection led to brain hemorrhage and death, however our surveillance program allowed the detection of ILHV circulation in an arbovirus endemic area, suggesting that the population may be at risk for ILHV infection.

Keywords: arboviruses, flavivirus, Ilheus virus, neurologic disorders



IN-DEPTH ANALYSES OF THE REPLICATION CYCLE OF ORPHEOVIRUS EVIDENCED MORPHOLOGICAL CHANGES IN VERMAMOEBEA VERMIFORMES

Fernanda Gil de Souza ¹, Rodrigo Araújo Lima Rodrigues ¹, Erik Vinícius de Souza Reis ¹, Maurício Teixeira Lima ¹, Jônatas Santos Abrahão ¹

¹ UFMG - Universidade Federal de Minas Gerais (Av. Antônio Carlos, 6627, Pampulha - Belo Horizonte - MG - CEP 31270-901)

Abstract

After the isolation of *Acanthamoeba polyphaga* mimivirus (APMV), the study and search for new giant viruses were intensified, thus these viruses have been uncovered in different samples and environments. Most giant viruses are associated with free-living amoebae of the genus *Acanthamoeba*, however others have been isolated, such as Faustovirus, Kaumobavirus and Orpheovirus in *Vermamoeba vermiformes*. Due to this, our knowledge about the diversity, structure, genomics, and evolution of this group of viruses have been expanding. In the present study, we present an in-depth investigation of the replication cycle of Orpheovirus using different microscopy techniques and biological assays with pharmacological inhibitors. We observed through optical and immunofluorescence microscopy morphological changes in *V. vermiformes* during Orpheovirus infection, as well as increased motility. The viral factory formation and viral particles morphogenesis were analyzed by electron microscopy, and we demonstrate mitochondria and membranes recruitment into and around the electron-lucent viral factories. Microscopy analysis coupled with pharmacological inhibitors of membrane traffic revealed that membrane recruitment decrease and affects the viral morphogenesis. The first structure observed during the particles morphogenesis is a crescent-shaped, which extends and is filled by the internal content until the formation of a mature particle. Mature particles are constituted by a layer of fibrils, outer membrane, inner shell, inner membrane and core. We also demonstrate by electron microscopy the formation of defective particles with different formats. Analyses performed under a light microscope revealed that at 12 hours post infection these viruses started their release from the host by exocytosis, as well as increased of viral titer. The results obtained in this study contribute to the understanding of biology, structure and important steps in the replication cycle of Orpheovirus.

Financial support: CNPq, CAPES, FAPEMIG, Ministério da Saúde

Keywords: Orpheovirus, Vermamoeba, Giant viruses, Replication cycle, Microscopy



MOLECULAR CHARACTERIZATION AND PHYLOGEOGRAPHIC ANALYSIS OF THE FIRST COMPLETE GENOMES OF SUBTYPE 2B HEPATITIS C VIRUS IN LATIN AMERICA

Natália Spitz ¹, José Júnior França de Barros ¹, Kycia Maria Rodrigues do Ó ³, Carlos Eduardo Brandão Mello ², Natalia Motta de Araujo ¹

¹ IOC/FIOCRUZ - Laboratório de Virologia Molecular, Instituto Oswaldo Cruz/FIOCRUZ (Avenida Brasil, 4365 Manguinhos - Rio de Janeiro - RJ), ² HUGG - UNIRIO - Hospital Universitário Gaffrée e Guinle - Universidade Federal do Estado do Rio de Janeiro (R. Mariz e Barros, 775 - Maracanã, Rio de Janeiro - RJ), ³ Membro do comitê técnico assessor das Hepatites Vi - Membro do comitê técnico assessor das Hepatites Virais do Ministério da Saúde (Brasília-DF)

Abstract

The Hepatitis C Virus (HCV) has a high genetic diversity, and eight genotypes (1 to 8) with distinct geographic distributions have been described. While HCV genotype 1 is the most prevalent genotype in Latin America, genotype 2 isolates were successful in becoming established and disseminated in different American countries. However, no complete genome sequence of HCV subtype 2b is available from these regions, limiting the contribution of Latin American isolates to phylogenetic and phylogeographic studies. The aim of this study was to determine the first HCV subtype 2b full-length genomes from the Latin America by amplification of large PCR fragments and to reconstruct HCV-2b spatial and temporal dispersion in Brazil. HCV isolates were obtained from serum of two patients from Rio de Janeiro, Brazil. First, the total viral RNA extracted was precipitated and resuspended in a volume of 9.5 μ L. The cDNA was synthesized using SuperScript IV Reverse Transcriptase and a nested PCR was done with Platinum Taq DNA Polymerase High Fidelity. Sequencing was performed using the Sanger method. With this approach, two overlapping amplicons (approximately 5.4 kb and 4.8 kb each) spanning the complete HCV genome sequence were generated. The complete genomes of two isolates named PAT1 (extracted from patient 1) and PAT 2 (patient 2), consisting of 9,318 nt were obtained. Surprisingly, patient 2 had the co-circulation of viral variants containing a 2,022 nt deletion covering most of the E1, E2, p7 and the 5' end of NS2. This deletion was previously associated with advanced age and increased necroinflammatory activity in the liver. In fact, patient 2 is 81 years old and had cirrhosis. Phylogenetic reconstructions of the NS5B region and the complete genome confirmed the classification of PAT1 and PAT2 as HCV-2b. PAT1 and PAT2 clustered into a single phylogenetic cluster along with all Brazilian HCV-2b NS5B sequences, suggesting a single introduction of this subtype into the country. Phylogeographic analysis showed a single entry of HCV-2b in Brazil from the Netherlands between 1977 and 1981. This study provides the first molecular characterization of complete HCV-2b genomes from Latin America and suggests a plausible route of introduction of this subtype in Brazil. These sequences may be used as reference in the diagnosis of HCV-2b, as well as in investigations of the evolution of this subtype on the continent.

Financial Support: CNPq, FAPERJ, FIOCRUZ

Keywords: complete genome, HCV, Phylogeography



GENETIC DIVERSITY AND MOLECULAR EPIDEMIOLOGY OF HIV-1 AMONG THERAPEUTIC FAILURE PATIENTS FROM SANTA CATARINA STATE, SOUTHERN BRAZIL.

Hegger Machado Fritsch ¹, Amilcar Tanuri ², Aguinaldo Roberto Pinto ¹, Tiago Gräf ³

¹ UFSC - Universidade Federal de Santa Catarina (Campus Reitor João David Ferreira Lima, Bairro Trindade, Florianópolis, SC, Brasil.), ² UFRJ - Universidade Federal do Rio de Janeiro (Instituto de Biologia – UFRJ, Av Carlos Chagas Filho, 373, Prédio CCS, Ilha do Fundão, Rio de Janeiro, RJ, Brasil.), ³ IGM - Instituto Gonçalo Moniz (Instituto Gonçalo Moniz - IGM. Rua Waldemar Falcão, 121, Candeal - Salvador, BA, Brasil.)

Abstract

The HIV-1 epidemic in southern Brazil is mostly caused by HIV-1C, with co-circulation of subtype B, F1 and recombinant forms. The Santa Catarina state presents high detection, mortality and incidence rates, with Florianópolis being the second national capital with the worst epidemiological scenario. Although previous reports have aimed to describe the molecular diversity, data collection has focused on the major cities, not describing the population as a whole. In this study we analyzed 3.070 PR/RT gene sequences of patients with therapeutic failure that underwent HIV resistance genotyping tests through the Brazilian Network for HIV-1 Genotyping (RENAGENO) between 2008 to 2017. Sequences were submitted to online subtyping tools and when presenting disagreement between these tools, classification was performed by the construction of phylogenetic trees and the use of bootscanning method. Association between subtypes and discrete variables were measured by Pearson's chi-squared test. Out of 3.070 HIV-1 sequences analyzed, 1.998 (65.1%) were classified as subtype C, 597 (19.4%) as B and 149 (4.8%) as F1. CRF31_BC-like recombination profile was identified in 112 (3.6%) sequences. Unique recombinant forms and CRF with a prevalence $\leq 1\%$ were classified as "others" (N = 214, 7.4% of the total). HIV-1B infection was observed in 23% of the males, while HIV-1C was identified in 69% of the female individuals (p

Keywords: HIV-1, Subtype C, Molecular diversity, Geographical distribution



VERTICAL NATURAL INFECTION IN CULICIDAE FROM MATO GROSSO, BRAZIL

Raquel da Silva Ferreira ¹, Lucinéia Claudia De Toni aquino da Cruz ¹, Nilvanei Aparecido da Silva Neves ¹, Laura Marina Siqueira Maia ¹, Fabio Assis de Campos Junior ¹, Luciano Chaves Franco Filho ², Poliana da Silva Lemos ², Clayton Pereira Silva de Lima ², Marcio Roberto Teixeira Nunes ², Marina Atanaka ¹, Renata Dezengrini Silhessarenko ¹

¹ UFMT - Universidade Federal de Mato Grosso (Av. Fernando Corrêa da Costa, 2367 - Boa Esperança, Cuiabá - MT, 78060-900), ² IEC - Instituto Evandro Chagas (67030-000 - ANANINDEUA / PARÁ / BRASIL)

Abstract

*Vertical infection represents the primary way of insect-specific viruses transmission. This mechanism is responsible for arbovirus maintenance in nature during interepidemic periods. Mato Grosso State (MT) Brazil, presents tropical equatorial climates, which associated to other factors may favor the circulation of several viruses. This study proposed to identify viral natural vertical infection in adult male culicids captured in Cáceres, Cuiabá, Rondonópolis and Sinop, between February 2017 and January 2018. A total of 10,569 specimens were collected and identified as *Stegomyia (Ae.) aegypti* (n=1.139), *Culex (Cx.) quinquefasciatus* (n=9.426), *Culex sp* (n=3) and *Psorophora albigena* (n=1). Specimens were pooled in 267 groups according to species, place and date of collection. These pools were subjected to viral RNA extraction and RT-PCR protocols for 10 flaviviruses, 5 alphaviruses and Simbu serogroup (orthobunyavirus). Positive pools were subjected to 3 passages in VERO cells (alphavirus and orthobunyavirus) or C6/36 cells (flavivirus). Partial results indicate 8 pools of *Cx. quinquefasciatus* positive for Chikungunya (CHIKV), 13 for Mayaro (MAYV), 1 for Oropouche (OROV) and 9 for Zika (ZIKV). Of these, 2 ZIKV were isolated at passage 1 (p1), 1 CHIKV and 2 ZIKV at p2 and 7 CHIKV, 13 MAYV, 1 OROV and 5 ZIKV at p3. 5 pools of *Ae. aegypti* were positive for CHIKV, 1 for Ilheus virus (ILHV), 1 for ZIKV, 1 for dengue 4 (DENV-4) and 1 for Yellow Fever virus (YFV). ILHV was isolated at p1, 1 CHIKV and 1 YFV at p2 and 4 CHIKV, 1 ZIKV and 1 DENV-4 at p3. Also, the extracted RNA of some pools was sequenced on a MinION platform, resulting in the identification of 2 putative novel viruses in pools of *Cx. quinquefasciatus*: one sequence of 461 bp with 53.75% similarity with Negev virus and another sequence of 592 bp with 55.67% similarity with Cordoba virus. Both sequences encode a region between the methyltransferase and the ribosomal methyltransferase RNA of Negevirus taxon, genus Nelorpivirus. Sequencing also resulted in the identification of a putative novel Phasivirus genus, Phenuiviridae family member in an *Aedes aegypti* pool. The segments L (1,047 bp) and S (450 bp) presented 34% and 45.5% similarity, respectively, with those of Phasi charoen-like virus. Insect-specific viruses and arboviruses are frequently classified in the same family of viruses, sharing common ancestors. Metagenomic studies have demonstrated that ISVs comprise a group of surprisingly diverse and ancient viruses.*

Financial support: Capes, FAPEMAT

Keywords: *Aedes aegypti*, arbovirus, *Culex quinquefasciatus*, insect-specifics, mosquitoes



GENE EXPRESSION MODULATION INDUCED BY OROPOUCHE VIRUS INFECTION IN ENDOTHELIAL CELLS

Pierina Lorencini Parise ¹, Daniel Augusto de Toledo Teixeira ¹, Aline Vieira ¹, Mariene Ribeiro Amorim ¹, Fabio Trindade Maranhão Costa ¹, Carla Cristina Judice ¹, José Luiz Proença Modena ¹
¹ IB-Unicamp - Departamento de Genética, Evolução, Microbiologia e Imunologia, Instituto de Biologia, Universidade Estadual de Campinas (R. Monteiro Lobato, 255 - Cidade Universitária, Campinas - SP, 13083-862)

Abstract

Oropouche orthobunyavirus (OROV) is an emerging arbovirus associated with a fever illness called Oropouche fever in the Amazon region of South and Central America. Moreover, OROV can cross the blood-brain barrier and cause central nervous system infection in humans and others vertebrates, such as golden hamster and neonate mice. However, the pathogenic mechanisms associated with the blood-brain barrier breakdown is not fully understood. Thus, to characterize the OROV infection and gene modulation in endothelial cells, we infected human (HBMEC) and murine (BEND3) immortalized endothelial cells with different Multiplicity of Infections (MOIs) and determined the viral load, cell viability and the level of expression of genes related with innate immune response and endothelial adhesion during 1, 4, 12, 24, 48 and 72 hours post infection by focus forming assay, MTT and qRT-PCR, respectively. Immunofluorescence was also performed to analyze the structure of endothelial tight junctions using antibodies against ZO-1 and OROV. Interestingly, although OROV is able to replicate in both endothelial cells, the morphological alterations induced by this infection are different in HBMEC and BEND3 cells. While HBMEC cells are lysed during OROV infection, no death was observed in BEND3 cells until 72 hpi. The OROV infection induced a strong antiviral response in BEND3, with increased expression of TLR7, IRF5, IFN- β , IFIT-1, OAS1L and MX-1. In addition, the OROV replication in BEND3 cells induced a expression of adhesion endothelial factors such as ICAM-1. In contrast, nor Interferon Induced Genes (ISGs), TLR7 and IRF5 nor adhesion endothelial factors were induced in HBMEC cells after OROV infection. Consequently, endothelial Tight-Junctions were not disrupted during OROV infection as seen by anti-ZO-1 staining assay. Thus, we can speculated that OROV is able to antagonize antiviral response in human cells but not in mouse endothelial cells. Financial Support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Keywords: Innate immunity, Virus replication, Peribunyavirus, Tight-Junctions, Endothelial cells



THE ANTIBODY PRODUCTION AND INNATE IMMUNE RESPONSE BY B CELLS ARE ESSENTIAL FOR RESTRICTION OF OROPOUCHE VIRUS PRIME-INFECTION

Daniel Augusto de Toledo Teixeira ¹, Matheus Cavalheiro Martini ¹, Mariene Ribeiro Amorim ¹, Pierina Lorencini Parise ¹, Julia Forato ¹, Gabriela Fabiano de Souza ¹, Stefanie Primon Muraro ¹, Karina Bispo dos Santos ¹, Aline Vieira ¹, Alessandro dos Santos Farias ¹, José Luiz Proença Módena ¹

¹ Unicamp - Universidade Estadual de Campinas (Rua Monteiro Lobato 255)

Abstract

Several arboviruses have emerged or reemerged and dispersed globally in the last years, such as Zika, West Nile and Chikungunya viruses. Brazil has the largest virus diversity in the world and Oropouche (OROV) and Mayaro (MAYV) viruses are pointed as possibly responsible for new outbreaks of arthropod-borne viral diseases in other regions of the world. Oropouche infection is associated with a febrile illness, characterized by symptoms such as rash, photophobia, myalgia, and polyuria. Moreover, some patients have neurologic complications after OROV infection, as encephalitis and meningitis. However, the pathogenic determinants associated with neurological involvement is not fully understood. Thus, the aim of this study was to investigate the role of B cells for protection against neuroinvasion by OROV in a mouse model. For this, we determined morbidity, mortality and viral tropism of 4-5 weeks old C57BL/6 WT mice (n=10), Rag1 KO mice (lacking both mature B and T cells, n=10), μ MT mice (lacking only mature B cells, n=10), and TCRbd mice (lacking only mature T cells, n=10) after infection of OROV by subcutaneous route. Interestingly, while WT and TCRbd mice were resistant to infection, Rag1 KO and μ MT mice were vulnerable and died with signs of neurologic involvement. The viral load determined by focus forming assay showed a liver and brain tropism as soon as three days after infection. In addition, sera harvested from day 6 post- infection could prevent Rag1 KO mice from neurologic disease, while sera from WT uninfected mice were not able to protect Rag1 KO mice. In the end, CD19^{Cre}-MyD88^{fllox/fllox} mice were partially vulnerable to OROV infection. In short, our results suggest that antibody production within 6 days, possibly IgM isotype, and the innate immune response by B cells are essential to restrict OROV replication and neuroinvasion in mice.

Financial Support: São Paulo Research Foundation (FAPESP)

Keywords: Oropouche, B cell, innate immune response, antibody



QUANTITATIVE COMPARISON BETWEEN VERO-76, C6/36 AND BHK-21 CELL LINES USED AS FLUORESCENT FOCUS ASSAY SUBSTRATE FOR FLAVIVIRUS TITRATION.

Egma Marcelin Mayt Huatuco ¹, Enrique Walter Mamani Zapana ¹, Sandra Liliana Landazabal Castillo ¹, Juan Sulca Herencia ¹, Rubén Junior Arancibia Gonzáles ¹

¹ UNMSM - Universidad Nacional Mayor De San Marcos (Av. Universitaria /Calle Germán Amézaga 375. Edificio Jorge Basadre Ciudad Universitaria, Lima 1.)

Abstract

Among research activities in Virology, robust and reliable viral detection and quantification assays are an essential part of the virologist's toolkit. In the case of Flaviviruses, these can be detected and quantified using a variety of virological methods such as Plaque Assays (PA), Reverse Transcription Polymerase Chain Reaction (RT-PCR), Transmission Electron Microscopy (TEM) and 50% tissue culture infective dose (TCID₅₀), each with its own limitations for the detection and quantification of viral genomic particles, viral proteins or intact infectious particles. In this study, we analyzed and compared three different Cell Lines as substrate (Vero-76, C6/36 and BHK-21) for Flavivirus (Dengue Virus, Yellow Fever Virus and Zika Virus) titration in a Fluorescent Focus Assay.

Viruses were adapted independently to each cell line by a certain number of viral passages, each viral strain was subsequently titrated in the three cell lines analyzed by Fluorescent Focus Assay. Titres obtained from the Dengue-2 Virus titration on C6/36, Vero-76 and BHK-21 cell lines were: 6.85x10⁵, 6.26x10⁵ and 5.95x10⁵ respectively. While the Zika Virus titration on Vero-76, C6/36 and BHK-21 cell lines and were 5.61x10⁶, 5.13x10⁶ and 5.01x10⁶ respectively. Finally, titers obtained from the Yellow Fever Virus titration on C6/36, Vero-76 and BHK-21 cell lines were 7.08x10⁷, 639x10⁷ and 6.88x10⁷ respectively. The statistical comparative analysis between the flaviviral titration results demonstrated a trend of higher viral titers on C6/36 and Vero-76

cell lines. These results demonstrate that the quantification of flavivirus by immunofluorescence obtains greater results when using mosquito and kidney cells of green monkey, recommending them as the optimal substrate for flavivirus infection and immunofluorescence detection.

Financed by VRIP-UNMSM

Keywords: Immunofluorescence, Flaviviral Titration, Cell Line, Dengue Virus, Zika Virus



STANDARDIZATION OF FLUORESCENT FOCUS ASSAY AND COMPARISON WITH THE “GOLD STANDARD” PLAQUE ASSAY FOR DENGUE, YELLOW FEVER AND ZIKA VIRUS TITRATION USING VERO-76 AND BHK-21 CELL LINES.

Rubén Junior Arancibia Gonzáles ¹, Harumy Rojas Rojas Chuchón ¹, Katherine Estefany Paz Torres ¹, Egma Marcelina Mayta Huatuco ¹

¹ UNMSM - UNIVERSIDAD NACIONAL MAYOR DE SAN MARCOS (Av. Universitaria /Calle Germán Amézaga 375. Edificio Jorge Basadre Ciudad Universitaria, Lima 1.)

Abstract

Flavivirus is a genus of arthropod-borne viruses belonging to the family Flaviviridae. The flavivirus genome consists of nonsegmented single-stranded positive-sense ribonucleic acid and have enveloped and spherical virus particles that are between 40 and 60 nm in diameter. Flavivirus that emerge globally and cause significant human diseases like encephalitis or hemorrhagic fever. At laboratory level, Plaque Assay is a "Gold Standard" method for Flavivirus titer quantification, which is based on the formation of plaques in a cell monolayer after viral infection. In this investigation a related technique was proposed, the Fluorescent Focus Assay which is performed very similar to Plaque Assay, and is based on the detection of viral proteins expressed by infected cells through fluorescent-labeled antibodies, which does not require agar overlap, and uses only 24-72 hours of infection time to generate results. Three Flavivirus (Dengue-2; Yellow Fever and Zika) were selected in three cell lines used as substrate (Vero-76, C6/36 and BHK-21). Thawing, propagation and maintenance of virus and cell lines was successfully carried out obtaining optimal viral seeds for the quantification tests. Quantitative comparisons between FFA and PA were performed: Means of viral titers of dengue-2, Yellow Fever and Zika viruses using Vero-76 cell line by Plaque Assay were: 5.54×10^5 , 6.79×10^6 and 7.04×10^7 respectively; while the results obtained by FFA were 6.22×10^5 , 7.1×10^6 and 7.49×10^7 respectively. Statistically, the quantification results of three flaviviruses analyzed by FFA were greater (p

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Keywords: Fluorescent Focus Assay (FFA), Plaque Assay (PA), Flavivirus, Viral Titration, Cell Culture



COMPARISON OF IMMUNE RESPONSE IN MICE INTRACRANIALY INFECTED WITH DIFFERENT ZIKA VIRUS ISOLATES.

Natália Natália Lima Pessoa ^{2,1}, Beatriz Beatriz Senra Santos ^{1,2}, Thais Barbara Thais Barbara Souza Silva ¹, Richard Richard JS Barbosa ^{1,2}, Erna Geessien Erna Geessien Kroon ², Marco Antônio Campos ¹

¹ Fiocruz Minas - Fundação Oswaldo Cruz (Avenida Augusto de Lima 1715), ² UFMG - Universidade Federal de Minas Gerais (Avenida Antonio Carlos 6627)

Abstract

Zika virus (ZIKV) is an arbovirus transmitted by mosquitoes of the genus *Aedes*, but vertical and possible sexual transmissions are also reported. Phylogenetic analyses of ZIKV reveal the existence of two major lineages: one includes the African isolates, and the other the Asian and American isolates. ZIKV infection was characterized by causing a mild disease presented with fever, headache, rash, arthralgia, and conjunctivitis, with reports of an association with Guillain-Barré syndrome (GBS), microcephaly and meningoencephalitis. Our objective was to compare the immune response triggered by two Brazilian not mice adapted isolates of ZIKV (PE243 and SPH) when the central nervous system was exposed. For this, 8 week age C57BL/6 wild-type mice and TLR2/9 and iNOS knockout mice were infected intracranially with 400 p.f.u. of PE243, SPH and MR766, an African mice adapted isolate, as positive control. Negative control mice were injected intracranially with C6/36 cell culture supernatant (mock). Mice were observed and weighed daily. C57BL/6 mice infected with MR766 lost weight, had conjunctivitis, paralysis, and died 8 to 12 days after infection and only one mouse survived as described in literature. C57BL/6 mice infected with Brazilian isolates did not die nor showed signs, but mice infected with PE243 gained less weight than control mice, with statistical difference, and mice infected with SPH showed no statistical difference compared to mock. TLR2/9 KO mice infected with MR766 showed the same signs as C57BL/6 mice and died 7 to 9 days after infection, and also only one mouse survived. TLR2/9 KO mice infected with Brazilian isolates did not die nor showed signs but gained less weight than control mice. iNOS KO mice infected with MR766 showed the same signs compared to wild type mice and died at 7 days after infection and no animals survived. iNOS KO mice infected with Brazilian isolates did not die, nor showed signs or lost of weight. We conclude that there was a reduction of weight gain in mice when infected with different Brazilian Zika isolates and TLR2/9 and iNOS in the model used do not impact on infection with ZIKV.

Keywords: zika virus, animal model, brazilian isolates, reduction of weight gain



PRODUCTION OF HUMAN CYTOMEGALOVIRUS UL111A TRANSCRIPTS IN FIBROBLASTS AND GLIOBLASTOMA CELL LINES: IDENTIFICATION OF A NEW TRANSCRIPT

Tainan Cerqueira Neves ¹, Lucas Matheus Stangherlin ¹, Isabela de Godoy Menezes ¹, Maria Cristina Carlan da Silva ^{1,2}

¹ UFABC - Universidade Federal do ABC (São Bernardo do campo, SP, Brasil), ² CAM - University of Cambridge (Cambridge, United Kingdom)

Abstract

The Human Cytomegalovirus (HCMV) belongs to the Herpesviridae family, subfamily *Betaherpesvirinae*, which includes neurotropic viruses with a long replicative cycle and restrict specie specificity. Primary HCMV infection in immunocompetent hosts is commonly asymptomatic due to robust antiviral immune response. On the other hand, the virus can cause devastating diseases in immunocompromised individuals, such as transplant recipients and AIDS patients. In addition, HCMV has been linked to cancers, cardiovascular disease and immune dysfunction. One of the key intriguing features of HCMV biology is the exceptionally large arsenal of virus-encoded proteins capable of counteracting the innate and adaptive host immune defences, allowing the virus to persist throughout the host's life. One group of immunomodulatory proteins expressed by HCMV are host cytokine and chemokine homologues, in particular the human interleukin-10 homologue (hIL-10), called viral IL10 (vIL-10). The vIL-10 coding gene UL111A undergoes alternative splicing producing different isoforms. Classically, the UL111A gene is described as been composed of three exons and two introns, resulting in two transcripts, the cmvIL10, produced by the removal of two introns, coding a 175aa protein which retains the ability to bind and signal through the hIL- 10 receptor and LAcmv-IL10, produced by the removal of the first intron, coding a c-terminus truncated protein of 139aa, impairing its ability to bind to the hIL10-receptor. Five new spliced transcripts were described, but their biological functions during viral infection are not well elucidated. This study aimed to detect the production of UL111A transcripts during HCMV infection in permissive MRC5 primary fibroblasts and semi permissive U138 and U251 glioblastoma (GBM) strains infected with a low passage clinical HCMV strain, TB40e. We report the production of four transcripts previously described in the literature and the identification of a new transcript produced by the UL111A gene. In addition, for the first time, we show the differential expression of UL111A transcripts in permissive and semi permissive infection. Studies of protein expression and analysis of their biological functions in immune cells are under way. These studies can contribute for the understanding of the immunomodulatory role of HCMV IL10 in different states of infection.

Keywords: HCMV, interleukyn 10, cytomegalovirus, cloning, glioblastoma



QUANTIFICATION OF THE UL111A HUMAN CYTOMEGALOVIRUS TRANSCRIPTS IN PRODUCTIVE AND LATENT INFECTED CELLS

Lucas Matheus Stangherlin ¹, Isabela de Godoy Menezes ¹, Ian Groves ³, Helena Beatriz de Carvalho Ruthner Batista ^{2,1}, Emma Poole ³, John Sinclair ³, Maria Cristina Carlan da Silva ^{1,3}

¹ UFABC - Universidade Federal do ABC (São Bernardo do Campo - SP/Brasil), ² Pasteur - Instituto Pasteur (São Paulo - SP/Brasil), ³ CAM - University of Cambridge (Cambridge/United Kingdom)

Abstract

The Human Cytomegalovirus (HCMV) is a highly prevalent member of the Herpesviridae family, Betaherpesvirinae subfamily. The virus establishes asymptomatic lifelong latent infection in the immunocompetent host. However, in immunosuppressed individuals, the virus can reactivate and enter the lytic cycle, causing severe diseases. Many cell types can be infected in the host, however permissiveness to viral replication virus varies according the cell type. During productive or lytic infection, the virus replicates mainly in epithelial cells, endothelial cells, fibroblasts, smooth muscle cells and macrophages. After the control of replication by the immune system the virus persists in a chronic state with low levels of replication and shedding, for long periods of time, in absence of cytopathic effect, mainly in endothelial and epithelial cells. Ultimately, latency is established and is a mode of persistence associated with a profound restricted lytic gene expression in myeloid progenitor cells in the bone marrow. It is well known that the HCMV has a vast arsenal of genes which encodes proteins capable of modulate diverse cell pathways. One of these genes is UL111A which encodes a human interleukin 10 (hIL-10) homologue, termed vIL10. Several transcripts are produced by alternative splicing and denominated from A to G. So far there are no reports regarding the differential expression of UL111A transcripts in cells in different states of infection. Thus, to clarify this matter is of great importance to comprehend the dynamics of viral cycle states transition and the balance between host immune system and the HCMV. Here we investigated by quantitative RT-PCR, the expression of transcripts A, B, E and H, a newfound transcript detected by our group in MRC5 infected cells. The data shows that UL111A transcripts are significantly less expressed in CD14 monocytes when compared to HFF cells. Transcripts E and H were not detected in latent infection, which could indicate that these transcripts are absent or far less expressed during viral latency. Also, in order to lead to viral reactivation, CD14 monocytes were treated with PMA, resulting in a significant increase of all UL111A transcripts. Although the A transcript has been almost exclusively related to productive infection, we detected it at levels as high as B in monocytes Therefore, this work supports the idea of differential UL111A alternate splicing during different phases of HCMV cycle.

Keywords: HCMV, Cytomegalovirus, Alternative splicing, Latency, Interleukin 10



OROPOUCHE VIRUS INFECTION OF HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS IN VITRO

Mariene Ribeiro Amorim ¹, Daniel Augusto de Toledo Teixeira ¹, Stefanie Primon Muraro ¹, Gabriela Fabiano de Souza ¹, Pierina Lorencini Parise ¹, Matheus Cavalheiro Martini ¹, Karina Bispo dos Santos ¹, Júlia Forato ¹, Aline Vieira ¹, Alessandro dos Santos Farias ¹, Renata Sesti Costa ¹, José Luiz Proença Módena ¹

¹ Unicamp - Universidade Estadual de Campinas (R. Monteiro Lobato, 255 - Barão Geraldo, Campinas - SP, 13083-862)

Abstract

Oropouche orthobunyavirus (OROV) is an Amazonian emerging virus with high potential of dissemination for several regions of the world, mainly due to deforestation, climate changes and expansion of population density in the Amazon. The major symptoms associated with OROV infection are headache, myalgia, arthralgia and exanthema. Moreover, hemorrhagic and neurological complications are also frequently associated with OROV infection in humans and other animals. As the infection of monocytes and dendritic cells are usually key events during arboviral infections, we decided to evaluate the viral replication and gene expression modulation in human peripheral blood mononuclear cells (PBMCs) during OROV infection. For this, PBMCs from healthy donors, THP-1 and Jurkat lineages were infected with OROV. Genome and antigenome of OROV were assessed by RT-qPCR and RNA PrimeFlow assay by flow cytometry and immunofluorescence using specific RNA hybridization probes. Productive infection was also evaluated by focus forming units (FFU) assay and the expression of antiviral innate immunity genes was evaluated by RT-qPCR. Interestingly, although OROV was not able to establish a productive infection in human PBMCs, significant levels of viral genome were maintained in a small proportion of these cells (mainly monocytes and B lymphocytes), as demonstrated by RT-qPCR and RNA PrimeFlow. The OROV infection in PBMCs were followed by increased expression of type I and II IFNs and Interferon-stimulated genes (ISGs). Thus, the data indicate that human PBMCs cells are not normally permissive to OROV infection. However, the maintenance of viral genome in lymphocytes and monocytes points that these cells may act as a Trojan horse in specific situations or microenvironments, as observed during immunosuppression in the central nervous system.

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Keywords: Oropouche orthobunyavirus, innate immunity, PBMCs



THE CRISPR/CAS9 COMPLEX AS A NEW ANTIVIRAL THERAPY AGAINST HERPES SIMPLEX TYPE 1

Rafaela Barbosa da Silva Pinto ¹, Jéssica Vasques Raposo ¹, Lauana Ribas Torres ¹, Luciana Rodrigues Carvalho Barros ², Martin Hernan Bonamino ², Elen Mello de Souza ¹, Vanessa Salete de Paula ¹

¹ IOC - Fiocruz - Instituto Oswaldo Cruz (Avenida Brasil, 4365, Manguinhos - Rio de Janeiro - RJ),

² INCA - Instituto Nacional do Câncer (Rua André Cavalcanti, 37, Lapa, Rio de Janeiro, RJ)

Abstract

Herpes Simplex Type 1 is a member of *Herpesviridae* family that affects about 67% of the population of worldwide. The major infection route is the direct contact with lesions and contaminated secretions. The primary infection can be asymptomatic followed by a latency infection in the sensory ganglia. In latency, HSV-1 can be reactivated by factors such as stress, immunosuppression and heat and migrated through the nerves until the peripheric tissue, as corneal tissue. Currently the treatment is the antiviral acyclovir, limited to viral replication only; it can cause renal toxicity and, in some cases, viral resistance. This indicates the need for new antiviral therapies that prevent virus reactivation. The power of CRISPR lies in its simplicity and ease of use, its flexibility to be targeted to any given nucleotide sequence by the choice of an easily synthesized guide RNA, and its ready ability to continue to undergo technical improvements. The aim of this study is to use the CRISPR/Cas9 complex as a new antiviral therapy against HSV-1. The target region chosen, UL39, expresses ribonucleotide reductase protein, essential for virus replication. The CRISPR platform was used to obtain the RNA for the target sequence. The sequence was inserted in the plasmid px 459 from *Streptococcus pyogenes* and into competent bacteria *E. coli*. These bacteria were cloned and the plasmidial DNA isolated. After that, the complex was transfected with lipofectamine to Vero cells in two different moments: before and after the HSV-1 infection, 24h and 48h. Plaque assay, immunofluorescence assay and qPCR were performed to evaluated inhibition of the viral replication. To verify the consequences of the complex in the cells, an MTT assay were made and the cell viability was not affected. The immunofluorescence showed the intracellular CRISPR complex and qPCR showed a decrease in viral load > 90% in cells transfected with CRISPR/Cas 9 anti-HSV-1. Our data demonstrate the utility of using CRISPR/Cas9 system for inhibition of HSV-1 infection.

Financial Support: CNPq

Keywords: Antiviral, CRISPR/Cas9, Herpes, HSV-1



PHYLOGENETIC AND STRUCTURAL ANALYSIS OF THE 5' AND 3' UNTRANSLATED REGIONS OF THE CHIKUNGUNYA VIRUS GENOME

Gabriela Fabiano de Almeida ¹, Maria Eduarda Correa Fonseca de Souza ¹, Ana Carolina Gomes Jardim ², Diego Pandeló José ¹

¹ UFTM - Universidade Federal do Triângulo Mineiro - Campus Universitário de Iturama (Avenida Rio Paranaíba, 1229, Centro - Iturama, MG), ² UFU - Universidade Federal de Uberlândia (Avenida Pará , 1720 - Uberlândia, MG)

Abstract

The genus Alphavirus includes etiological agents responsible for serious human diseases, as the Chikungunya virus (CHIKV). CHIKV is transmitted by *Aedes aegypti* mosquito and can trigger outbreaks of febrile illness showing specific symptoms such as body and joint pains. To date, there is no vaccine against this arbovirus, which has been becoming a serious public health problem in Brazil and other South American countries. Research into the genomic and structural constitution of this virus is becoming increasingly important for the future disease eradication. The present work aimed to perform phylogenetic and structural analysis of the 5' and 3' untranslated regions (UTRs) of the CHIKV genome. For this, fifty sequences of CHIKV complete genome obtained from NCBI were submitted to the phylogenetic reconstructions in the MEGA X software, using the maximum likelihood analysis method with Tamura-Nei substitution model. A thousand replicates were used to test reliability of the tree topology and bootstrap values >60 were considered significant. Bayesian analysis were performed using BEAST v.1.10.1 software package. The prediction of the formation of secondary structures of the 5' and 3' UTR regions were performed using RNAfold WebServer. Phylogenetic analysis of the CHIKV complete genome demonstrated that sequences were grouped according to the virus genotypes West African, East-Central-South African (ECSA), Asian and Indian Ocean, as expected. Sequences from Brazilian CHIKV-infected patients were grouped only in Asian and ECSA clades. Phylogenetic Tree of UTR sequences has shown a similar topography. At least five sequences grouped in the same clade showed differences in the secondary structures of the UTRs. The major secondary structural diversity of these regions was noted inside the Asiatic group. Thus, it was observed a genetic diversity in the UTR regions of sequences grouped in the same clade, resulting in different secondary structures of these regions. It could indicate changes in the virus replicative cycle due to the increase or loss of affinity by some cell factors. However, new in vitro approaches are needed to investigate more details about these interactions. The results present here may, in the future, be useful to elucidate more details about the influence of untranslated regions on the viral replicative cycle.

Financial Support: FAPEMIG

Keywords: Chikungunya, Molecular phylogeny, Mutation, Secondary structure, UTRs



PHYLOGENETIC AND STRUCTURAL ANALYSIS OF THE 5' AND 3' UNTRANSLATED REGIONS OF THE MAYARO VIRUS GENOME

Gabriela Fabiano de Almeida ¹, Maria Eduarda Correa Fonseca de Souza ¹, Ana Carolina Gomes Jardim ², Diego Pandeló José ¹

¹ UFTM - Universidade Federal do Triângulo Mineiro - Campus Universitário de Iturama (Avenida Rio Paranaíba, 1229, Centro - Iturama, MG), ² UFU - Universidade Federal de Uberlândia (Avenida Pará, 1720 - Uberlândia, MG)

Abstract

The genus Alphavirus belongs to Togaviridae family and includes etiological agents responsible for serious human diseases, as the Mayaro virus (MAYV). MAYV is transmitted primarily by Haemagogus genus mosquito and can trigger outbreaks of febrile illness showing specific symptoms such as myalgia, muscle and joint pains. To date, there is no vaccine against this arbovirus, which has been becoming an emergent public health problem in Brazil and other countries from South and Central America. Research into the genomic and structural constitution of this virus is becoming increasingly important for the future disease eradication. The present work aimed to perform phylogenetic and structural analysis of the 5' and 3' untranslated regions (UTRs) of the MAYV genome. Therefore, twenty-eight sequences of MAYV complete genome obtained from NCBI were submitted to the phylogenetic reconstructions in the MEGA X software, using the maximum likelihood analysis method with Tamura-Nei substitution model. A thousand replicates were used to test reliability of the tree topology and bootstrap values >60 were considered significant. Bayesian analysis were performed using BEAST v.1.10.1 software package. The prediction of the 5' and 3' UTR secondary structures formation were performed using RNAfold WebServer.

Phylogenetic analysis of the MAYV complete genome demonstrated that sequences were grouped according to the virus genotypes - genotype D, genotype L and genotype N, as expected. Genotype N presents a unique sequence from Peru. Phylogenetic analyzes of UTRs showed some variations in tree topology compared to the complete genome. Nevertheless, some positions remained the same one, such as the N genotype sequence, which remained isolated in all topologies. UTRs secondary structure analysis have demonstrated close similarity among sequences belonging to the same clade. It could suggest a high degree of conservation of these regions, probably due to their importance in viral replicative cycle. However, sequences belonging to different genotypes have shown greater structural divergence of their UTRs. The results present here may, in the future, be useful to elucidate more details about the influence of untranslated regions on the viral replicative cycle.

Financial Support: FAPEMIG

Keywords: Mayaro, Molecular phylogeny, Mutation, Secondary structure, UTRs



MOLECULAR ANALYSIS OF DENGUE VIRUS TYPE 4 INTRODUCED IN 2012 IN MATO GROSSO, BRAZIL

Janeth Aracely Ramirez Pavon¹, Nilvanei Aparecido da Silva Neves¹, Nayara Zuchi¹, Marcelo A. M. dos Santos³, Leticia B. S. Heinen¹, Steven G. Widen², Sandra V. Mayer², Thomas G. Wood², Nikos Vasilakis², Renata Dezengrini Shlessarenko¹

¹ UFMT - Post-Graduation Program in Health Sciences, Medicine Faculty. Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil. (Cuiabá, Mato Grosso, Brazil), ² UTMB - The University of Texas Medical Branch (Galveston, Texas, USA), ³ LACEN-MT - Central Laboratory of Mato Grosso, State Secretary of Health (Cuiabá, Mato Grosso, Brazil)

Abstract

Dengue virus (DENV-1,-2,-3,-4) is responsible for Dengue Fever, a major public-health concern worldwide. Despite the efforts to control the disease, Dengue incidence increased approximately 30-fold in the past decades. Brazil is the leading American country in annual reports. During 1981 and 1982, DENV-1 and DENV-4 were reported in Boa Vista, Roraima State. DENV-1 outbreaks disseminated cross the country later, in 1986; DENV-2 introduction in Brazil was evidenced in 1990, when the first hemorrhagic febrile cases were detected; DENV-3 autochthonous transmission were reported from 2000 and, after 25 years undetectable, genotypes I and II of DENV-4 were reintroduced in the country in 2008 and 2010, respectively. Between 2010-2018, DENV-1 and DENV-4 were responsible for dengue outbreaks across the country. Brazil reported 1.5 million cases, 1,032 deaths, reaching a incidence rate of 126,7 per 100.000 habitants by the year of 2018. In 2012, the highest incidence rates were recorded in the Northeast (548,2) and Midwest Brazil (483,4); for the state of Mato Grosso (MT) was of 1.609,1. Between October 2011 and July 2012, we sampled 604 patients with acute febrile illness for up to five days suspected of dengue fever in public health institutions of MT. These samples were processed for viral isolation of the four dengue serotypes and yellow fever at LACEN MT. Additionally, serum aliquots were subjected to viral RNA extraction and multiplex semi-nested RT-PCR for flaviviruses, alphaviruses and orthobunyaviruses. In total, from 331 samples positive for DENV in 17 municipalities, 315 were for DENV-4; 217 from patients of the metropolitan area of Cuiabá. We sequenced 26 DENV-4 isolates in an Illumina HiSeq 1000 platform. Phylogenetic analysis based on Envelope gene sequences with another 119 human isolates from previous studies suggested our isolates formed a monophyletic group composed by two distinct lineages within genotype II, closely related with two 2010 isolates from a geographically close region, Boa Vista, Roraima State, which indicates local transmission and spread after initial introduction in North Brazil, and sharing proximity with strains circulating in Venezuela, 2007. Further confirmation of the co-circulation of DENV-4 distinct lineages was made with intrahost variation analysis, which demonstrated a high degree of similarity in the consensus amino acids across samples without insertions or deletions.

Financial support: Capes, CNPq rede pró-centro oeste

Keywords: Arbovirus, dengue outbreak, molecular epidemiology, phylogeny

HUMAN VIROLOGY





YELLOW FEVER VIRUS DETECTION BY RT-QPCR IN Aedes SCAPULARIS MOSQUITO, SÃO PAULO, BRAZIL

Mariana Sequetin Cunha ¹, Nuno Rodrigues Faria ³, Giovana Santos Caleiro ¹, Darlan Silva Candido ³, Sarah Hill

³, Rosa Maria Tubaki ², Regiane Maria Tironi de Menezes ², Juliana Silva Nogueira ¹, Adriana Yurika Maeda ¹, Fernanda Gisele da Silva Vasami ¹, Antonio Charlys da Costa ⁴, Luís Felipe Mucci ²

¹ IAL - Instituto Adolfo Lutz (Av. Dr. Arnaldo, 355, São Paulo, Brazil), ² SUCEN - Superintendência de Controle de Endemias (R. Paula Sousa, São Paulo, Brazil), ³ OX - University of Oxford (Oxford OX1 2JD, United Kingdom), ⁴ IMT - Instituto de Medicina Tropical (Avenida Dr. Enéas Carvalho de Aguiar, 470, São Paulo, Brazil)

Abstract

Yellow fever (YF) is an arboviral disease endemic to tropical regions of Africa and South America caused by the yellow fever virus (YFV), a single-stranded, positive sense 11kb RNA virus member of the *Flavivirus* genus (1). In the Americas YF spread can occur through an “urban cycle” involving transmission between susceptible humans and *Aedes aegypti* mosquitos, and through a “sylvatic cycle”, involving transmission between non-human primates (NHP), mainly *Alouatta* sp., *Sapajus* sp., and *Callithrix* sp.(2), and the canopy-breeding *Haemagogus* sp. and *Sabethes* sp. mosquitoes. Occasional spillover to non- vaccinated humans may occur when these encroach forested areas. Beginning in late 2016 Brazil faced the worst outbreak of Yellow Fever in recent decades. In São Paulo State, the first epizootic events caused by YFV were detected in a previous enzootic area in the North region (São José do Rio Preto and Ribeirão Preto mesorregions). A total of 3716 *culicidae* were collected by Superintendência de Controle de Endemias (SUCEN) in 30 municipalities with ongoing epizootic events and in adjacent cities from June 2016 and March 2017. Mosquitoes were identified and separated into 209 pools (*Aedini* and *Sabethini*) according to specie, and sent to Núcleo de Doenças de Transmissão Vetorial, Adolfo Lutz Institute, for YFV detection by a probe-based RT-qPCR and/or viral isolation in C6/36 cell lines followed by immunofluorescence assay. (IFA). One pool containing 8 female individuals of *Aedes scapularis* mosquitoes collected in José Bonifácio on February 15th 2017 in an agricultural area was positive for YFV. Interestingly, no epizootic event was detected in this city. A full-length genome was obtained using the portable Oxford Nanopore minION technology. Phylogenetic analysis revealed that this YFV belongs to South American genotype 1. However, little is known regarding YFV vector competence on *Aedes* spp mosquitos in Brazil. It is believed that *Aedes scapularis* may act as a bridge vector. More studies must be addressed in order to evaluate different Brazilian *Aedes* species in the YFV cycle.

Keywords: Culicidae, RT-qPCR, Yellow Fever Virus



MOLECULAR EPIDEMIOLOGY OF NOROVIRUS AND CIRCULATION OF THE EMERGENT RECOMBINANT STRAINS GII.P16- GII.4 AND GII.P16-GII.2 IN BRAZIL, 2017-2018.

Sylvia kahwage sarmento ¹, Tulio Machado Fumian ¹, Juliana da Silva Ribeiro de Andrade ¹, Fabio Malta ¹, Marize Pereira Miagostovich ¹

¹ LVCA - Laboratório de Virologia Comparada e Ambiental - FUNDAÇÃO OSWALDO CRUZ (AV. BRASIL, 4365)

Abstract

Norovirus is one of the most important etiological agent related to acute gastroenteritis (AGE) outbreaks worldwide. Norovirus evolves by antigenic drift and recombination, which occurs most frequently at the junction between the non- structural and structural protein coding genomic regions. Here, we describe the molecular epidemiological analysis of norovirus conducted in eleven states from three different Brazilian regions, during a two-year period, between 2017 and 2018. A total of 1,489 fecal samples from AGE cases were analyzed during the period. By using RT-qPCR, norovirus was detected year-round in 520 (34.9%) fecal samples. Among the positive samples, 54 (10.4%) were infected with GI, 459 (88.3%) with GII, and seven (1.3%) were co-infected with GI and GII. The median of Ct (cycle threshold) values detected among GI and GII-positive samples was 28.6 and 20.7, respectively (p

This study was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico and Oswaldo Cruz Institute, PAPES VII (IOC/FIOCRUZ).

Keywords: Norovirus, qPCR, Epidemiology, GII.P16, outbreaks



PREVALENCE OF NOROVIRUS AND OTHER ENTEROPATHOGENS AMONG CHILDREN HOSPITALIZED FOR ACUTE GASTROENTERITIS IN BELÉM, PARÁ, BRAZIL

Hugo Reis Resque ¹, Ian Carlos Gomes de Lima ¹, Edvaldo Tavares da Penha Júnior ¹, Mônica Cristina de Moraes Silva ², Heyde Araújo Tavares ², Cintya de Oliveira Souza ³, Daniela Cristiane da Cruz Rocha ³, Débora de Castro Costa ³, Maria Cleonice Aguiar Justino ¹, Alexandre da Costa Linhares ¹

¹ SAVIR, IEC/SVS/MS - Seção de Virologia, Instituto Evandro Chagas/SVS/MS (Rodovia BR-316, Km 07, S/N CEP 67030-000 - Ananindeua-PA), ² SAPAR, IEC/SVS/MS - Seção de Parasitologia, Instituto Evandro Chagas/SVS/MS (Rodovia BR-316, Km 07, S/N CEP 67030-000 - Ananindeua-PA), ³ SABMI, IEC/SVS/MS - Seção de Bacteriologia e Micologia, Instituto Evandro Chagas/SVS/MS (Rodovia BR-316, Km 07, S/N CEP 67030-000 - Ananindeua-PA)

Abstract

Several studies of gastroenteritis surveillance have been carried out in Belém-PA, Brazil over the years, in order to identify the presence of infections caused by enteric viruses in hospitalized children. However, data available on concomitant infections caused by viral, bacterial and parasitic pathogens are limited. The focus on coinfections is of particular relevance today in view of the increasing importance of noroviruses (*Caliciviridae* family) as a cause of childhood gastroenteritis. In addition, knowledge of mixed infections involving noroviruses will represent a critical factor in the calculation of an adequate sample size during clinical trials with vaccines in development, as well as in regards to the possibility of assessing whether the coinfections might be associated with greater clinical severity. Therefore, the main objective of this study is to determine the prevalence of norovirus among hospitalized children under 6 years of age, in a context of single and mixed infections (association with other enteric viruses, bacteria and parasites) in the period of November 2018 to July 2019. So far, 170 stool samples were collected, from November 2018 to June 2019, and tested for NoV, RVA, AdV, *Cryptosporidium* spp., *Giardia* spp., *Entamoeba* spp., *Salmonella* spp., *Shigella* spp., *E. coli* spp. and *Campylobacter* spp.. Both virological and parasitological detections were carried out using their respective rapid immunochromatographic tests (RIDA®QUICK). Bacteria are detected using both rapid and culture procedures. Of the total of collected samples, 79 (46.5%) presented single infections, 13 (7.6%) presented some kind of coinfection and 78 (45.9%) were negative for all pathogens tested (to date, mostly viral and parasitic agents). Regarding single infections, NoV was the most commonly agent found alone (25.3%), followed by *Giardia* spp. (10%) and RVA (4.1%). Concerning coinfections, the RVA+AdV, NoV+*Giardia* spp. and AdV+*Giardia* spp. associations were the most observed, all found at a rate of 1.8%. One coinfection of RVA+*Salmonella* spp. (0.6%) and a case of triple coinfection of RVA+AdV+*Giardia* spp. (0.6%) were also found. These results will provide a broader knowledge about the etiology of acute childhood gastroenteritis in our region, an important condition for health authorities in order to improve preventive and control actions in the fight against this disease.

Financial Support: Instituto Evandro Chagas/SVS/MS and Takeda Vaccines Inc.

Keywords: Norovirus, Children, Gastroenteritis, Coinfection, Enteropathogens



ROLE OF TAM RECEPTOR LIGAND GAS6 IN THE PATHOGENESIS OF ZIKA VIRUS INFECTION

Leticia Monteiro ¹, João Luiz Silva Filho ¹, Pierina Lorencini Parise ¹, Najara Bittencourt ¹, William Marciel de Souza ², Julia Forato ¹, Daniel Augusto de Toledo Teixeira, Mariene Ribeiro Amorim ¹, Carla Judice ¹, Fabio Costa ¹, José Luiz Modena ¹

¹ UNICAMP - Universidade Estadual de Campinas (Cidade Universitária Zeferino Vaz - Barão Geraldo, Campinas), ² USP - Universidade de São Paulo (Av. Bandeirantes, 3.900 Monte Alegre Ribeirão Preto - SP)

Abstract

Although the mechanisms involved in the Zika virus (ZIKV) infection are not yet fully resolved, TAM receptors have been shown to act as a facilitator of viral entry by associating with the endogenous ligand Growth arrest-specific 6 (Gas6). This study aims to investigate the role of Gas6 in the pathogenesis and severity of ZIKV infection. To this end, we used serum samples from ZIKV-infected patients, both with and without neurological complications, as well as *in vitro* models. Blood samples were obtained from 48 adult patients diagnosed with ZIKV and subsequently characterized as either neurological or non-neurological based on whether the patient developed neurological complications. Monocytes (THP1), human brain microvascular endothelial cells (hBMEC) and PBMCs from healthy donors were infected *in vitro* with the Brazilian strain of ZIKV at different multiplicities of infection. Infected cells were treated or not from the start of infection up to 72h p.i. with warfarin. Warfarin specifically inhibits Gas6 glutamic acid γ -carboxylation, which is an important pathway for Gas6 activation. RT-qPCR and ELISA performed on infected cells revealed that Gas6, TAM receptors and SOCS1 expression were higher in neurological compared to non-neurological patients. However, both showed similar viral load, suggesting a correlation between Gas6/TAM axis signaling and disease severity. Neurological patients also showed decreased IFN β and IFIT1 expression. In cultured monocytes and PBMCs, ZIKV also induced upregulation of Gas6, TAM receptors and SOCS1 as well as downregulation of IFN β and IFIT1 in the early stages of the infection. Interestingly, warfarin inhibited ZIKV-induced Gas6 and TAM receptor expression and treatment restored the anti-viral response. In 72h p.i. no viable viral particles were detected in the supernatant of treated cells, indicating that warfarin can prevent the virus from escape. Together, our results show that Gas6 and TAM receptors expression correlate with disease severity and play key roles in modulating the antiviral response. These results indicate that Gas6 inhibition could be a potential ZIKV therapeutic strategy.

Financial Support: FAPESP.

Keywords: Gas6, Tam receptors, Warfarin, Zika Virus



EPIDEMIOLOGICAL PROFILE OF WOMEN AND RESEARCH OF ARBOVIRUS IN THEIR MILK DONATED TO THE HUMAN MILK BANKS OF CUIABÁ-MT

Marli Eliane Uecker ^{1,2}, Marcus Vinicius Carvalho ³, Ines Stranieri ⁴, Flávia Almeida Ramos ^{1,5}, Natasha Kilsy Rocha Belem ⁵, Flávia Galindo Silvestre Silva ⁵, Gilmar Jorge Oliveira Junior ¹, Patricia Palmeira ⁶, Magda Maria Sales Carneiro-Sampaio ⁶, Vanessa Barbosa Silveira ⁷, Edison Luiz Durigon ⁷, Danielle Bruna Leal Oliveira ⁷, Maria Isabel Valdomir Nadaf ⁸, Olga Akiko Takano ¹

¹ PPG/ISC/UFMT - Programa de Pós-Graduação do Instituto de Saúde Coletiva da UFMT (Av. Fernando Corrêa da Costa, nº 2367 - Bairro Boa Esperança. Cuiabá - MT - 78060-900), ² BLH/HUJM/UFMT - Banco de Leite Humano do Hospital Universitário Júlio Müller/UFMT (R. Luis Philippe Pereira Leite, s/n - Alvorada, Cuiabá - MT, 78048-902), ³ BLH/HG - Banco de Leite Humano do Hospital Geral (Rua 13 de Junho, 2101 - Centro Norte, Cuiabá - MT, 78020-300), ⁴ SES/MT - Secretaria de Estado de Saúde de Mato Grosso (Centro Político Administrativo, Palácio Paiaaguás, Rua D, S/N, Bloco 5, CEP: 78049-902), ⁵ LIBM/HG - Laboratório de Imunogenética e Biologia Molecular do Hospital Geral (Rua 13 de Junho, 2101 - Centro Norte, Cuiabá - MT, 78020-300), ⁶ ICr/HC/FMUSP - Instituto da Criança do Hospital das Clínicas FMUSP (Av. Dr. Enéas Carvalho de Aguiar, 647 - Cerqueira César, São Paulo - SP, 05403-000), ⁷ LV/ICB II/USP - Laboratório de Virologia do Instituto de Ciências Biomédicas II/USP (Av. Prof. Lineu Prestes , nº 1374 – Butantã – São Paulo/SP – CEP 05508-000), ⁸ DP/FM/UFMT - Departamento de Pediatria da Faculdade de Medicina da UFMT (R. Luis Philippe Pereira Leite, s/n - Alvorada, Cuiabá - MT, 78048-902)

Abstract

Introduction: In recent years circulation of arboviruses like dengue, Zika, Chikungunya and yellow fever has caused important epidemics in Brazil, which raises concerns about the possibility of vertical transmission of these viruses through human milk. The objective was to describe the profile of healthy donors and to verify the presence of arboviruses in their milks: dengue, Zika, Chikungunya and yellow fever. **Material and Methods:** a cross-sectional study where samples of 200 donors from the Human Milk Bank (HMB) of Cuiabá-MT were analyzed. The RT-PCR technique was used to analyze the samples. **Results:** The mean age of donors was 27.8 years (SD=6 years, ranging from 15.5 to 41.1 years) and 12.0% were adolescents; (1 to 7 pregnancies), median of deliveries=2 (1 to 5 deliveries), abortion=45 (22.5%), cesarean delivery=107/199

(53.5%), had complications in pregnancy=44/195 (22.0%); they used medication=76/199 (38.2%), reported smoking=8 (4.0%) and denied being a alcoholic=200 (100%). The majority came from Cuiabá (n=142, 71.0%) and race/color brown (n=124, 62.0%); had complete or incomplete superior course (n=111, 55.5%); (n=141, 70.5%), had prenatal care (n=198; 99%), had prenatal care in the Unified Health System (n=109, 54.5%), had prenatal care complete (n=174, 87.0%). Evaluation of nutritional status (n=192) at the beginning vs. end of gestation found: eutrophic 54.1% vs. 35.4%; overweight 28.1% vs. 23.4%; obese 9.4% vs. 16.1% and low weight 8.3% vs. 25.0%. All donors had negative tests for: syphilis (VDRL), hepatitis B (HBsAg), human immunodeficiency virus (HIV). Anemia was observed in 11.7% (n=8/68). The majority reported having a profession=112/199 (56.6%); 29.6% worked at home and 14.1% were students. A total of 200 milk samples were analyzed: 75.5% were collected at the HMB/HUJM and 24.5% at the HMB/HG. The classification of milk type was: mature milk=120 (60.0%); colostrum=53 (26.5%); transition=27 (13.5%). The mean Dornic acidity was 3.1 (SD=1, from 2 to 8). The RT-PCR results



for the dengue, Zika, Chikungunya and yellow fever viruses were all negative. **Conclusion:** Arboviruses were not detected in donated human milk samples probably because they are healthy donors and the low urban circulation of these arboviruses, Cuiabá and other municipalities of Mato Grosso are not in epidemic period, and the occurrence of yellow fever is wild.

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Keywords: human milk, arbovirus, real-time polymerase chain reaction, epidemiology



FREQUENCY OF DRUG RESISTANCE MUTATIONS USED IN THE TREATMENT OF HIV INFECTION OBTAINED BY GENOTYPING TEST, CARRIED OUT IN THE PERIOD 2013 TO 2015 IN THE STATE OF PARÁ

Jennifer Ferreira Viana ¹

¹ UFPA - Universidade Federal do Pará (Av. Perimetral, 2-224 - Guamá, Belém - PA, 66077-830)

Abstract

The need for antiretroviral (ARV) treatment is primary for people living with HIV or AIDS (PLWHA). According to data from the Ministry of Health, about 316 thousand deaths were found in Brazil as a basic cause of HIV/AIDS, from 1980 to 2016. The main objective of this project was to describe the profile of individuals with HIV/AIDS living in the state of Pará regarding the prevalence of drug resistance mutations used in the treatment of HIV infection obtained by genotyping test, seeking to identify virological, epidemiological and laboratory factors that may influence the course of the infection. The data were obtained through the collection of information contained in the reports of patients submitted to the genotyping test, whose results were under the custody of the Central Laboratory of Pará (LACEN). All patients were included, regardless of gender or age, whose samples were sent to LACEN from 2013 to 2015. Information such as sex, age, race, origin, mutations and drug resistance profile were collected. The male gender was predominant with 53.19%. The age group from 36 to 45 years old was the most prevalent with 33.51%, being the majority brown and with elementary education; besides the dominant origin in the metropolitan region, but suggesting possible interiorization. 96.27% of the patients had detectable viral load (VC) and CD4+ T lymphocytes less than 500 cells/mm³. The class of ITRNs and Protease Inhibitors (IP) were more prevalent with the M184V and 41K mutations respectively; and ITRNN third with the 103N mutation more frequent. Since in the class of ITRN the median CV of patients with mutations was 21,886 copies/ml, there was a mean of 2.074 on the change of schemes. As for the drugs used in therapy, those of class ITRN had a higher resistance profile, the class of ITRNN in second place and of PI the class of greater susceptibility. In summary, considering that non-adherence to treatment is the main driver of mutations, the failure in virological control enables the emergence of opportunistic diseases worsening the patient's health status and quality of life. Thus, pharmacological and genotypic monitoring of each patient is necessary. Besides the correct description of ARV according to the resistances found.

Keywords: HIV-1, Mutation, Resistance, Antiretroviral , Genotyping



DETECTION OF COINFECTION WITH CHIKUNGUNYA VIRUS AND DENGUE VIRUS SEROTYPE 2 IN SERUM SAMPLES OF PATIENTS IN STATE OF TOCANTINS, BRAZIL

Robson dos Santos Souza Marinho ¹, Rodrigo Lopes Sanz Duro ¹, Josias Gabriel Gonçalves da Silva ¹, Giulia Luiza Santos ¹, James Hunter ¹, Maria da Aparecida Rodrigues Teles ³, Rafael Brustulin ^{3,4,5}, Flavio Augusto de Padua Milagres ^{5,3,6,4}, Ricardo Sobhie Diaz ¹, Shirley Vasconcelos Komninakis ^{1,2}
¹ UNIFESP - Universidade Federal de São Paulo (Rua: Pedro de Toledo, 781, Vila Clementino, São Paulo 04023-062, Brazil), ² FMABC - Faculdade de Medicina do ABC (Av. Lauro Gomes, 2000 - Vila Sacadura Cabral, Santo André - SP, 09060-870), ³ LACEN/TO - Laboratório Central de Saúde Pública do Tocantins (93, Q. 602 Sul Avenida LO 15, 77, Palmas - TO 77016-330, Brazil), ⁴ UFT - Universidade Federal do Tocantins (Quadra 109 Norte, Avenida NS15, ALCNO-14 - Plano Diretor Norte, Palmas - TO, 77001-090), ⁵ Secretary of Health of Tocantins - Secretary of Health of Tocantins (Tocantins 77453-000, Brazil.), ⁶ USP - Universidade de São Paulo (São Paulo 01246-903, Brazil)

Abstract

Introduction: The co-circulation of Dengue virus (DENV), Zika virus (ZIKV) and Chikungunya virus (CHIKV) increased the risk of coinfections among these arboviruses. Some cases of coinfections between these three arboviruses have been reported in Brazil. Here, we document the detection of coinfections involving CHIKV and DENV serotype 2 in clinical samples obtained during an outbreak of CHIKV in 2017 in state of Tocantins, Brazil. **Material and Methods:** The Central Public Health Laboratory of Tocantins (LACEN-TO) sent to the Laboratory of Retrovirology of the Federal University of São Paulo a total of 102 samples showing symptoms compatible with CHIKV infection that were collected from January to March of 2017 by different health units of state. The RNA was extracted using the QIAamp Viral RNA Mini kit (Qiagen), then were tested for CHIKV, four serotype Dengue virus and ZIKV using the GoTaq[®]Probe1-Step RT-qPCR System (Promega). The processing of all samples included negative and positive controls as well as internal controls (Ribonuclease P, RNase P) to ensure a reliability of the reaction. **Results:** Of the total of 102 samples showing symptoms compatible with CHIKV infection, 52 were confirmed as CHIKV positive. Of this total, 5 were identified as being coinfecting by DENV serotype 2. The other 47 samples were characterized as being monoinfected by CHIKV. The 50 samples that were characterized as being negative for CHIKV, we detected mono-infection by dengue virus serotype 1 (28 cases) and 2 (22 cases). We did not detect Zika virus in the analyzed samples. **Conclusion:** Environmental changes have been occurring over time benefiting the spread and maintenance of vectors for the transmission of arboviruses. The Chikungunya and Zika viruses have significantly expanded their geographical distribution in Brazil, as has the Dengue virus. Therefore, our findings suggest that clinical and epidemiological criteria are not efficient for the differentiation of ZIKV, CHIKV and DENV infection and reinforce the need for a better understanding of the co-circulation of these arboviruses and the possibility of individuals becoming infected simultaneously by these three arboviruses. Coinfections by CHIKV and DENV serotype 2 may play a role not recognized in the pathogenesis, virulence, and clinical expression of Dengue and Chikungunya infections. Thus, Patients with coinfections involving these two arboviruses may present more severe clinical manifestations.

Financial Support: CNPQ / FAPESP

Keywords: Arbovirus, Coinfection, Chikungunya, Dengue, RT-qPCR



DEVELOPMENT OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAYS FOR CONFIRMATORY DIAGNOSIS OF HTLV-1/2 INFECTIONS.

Yago Côrtes Pinheiro Gomes ¹, Ana Claudia Celestino Bezerra Leite ¹, Marco Antonio Sales Dantas de Lima ¹, Abelardo Queiroz Campos Araújo ¹, Marcus Tullius Teixeira da Silva ¹, Otávio de Melo Espíndola ¹

¹ INI - Instituto Nacional de Infectologia Evandro Chagas - FIOCRUZ (Av. Brasil, 4365 - LAPCLIN-NEURO - INI - FIOCRUZ - Manguinhos, Rio de Janeiro - RJ, CEP: 21040-900)

Abstract

Human T-lymphotropic viruses (HTLV) are retroviruses transmitted by unprotected sexual intercourse, blood transfusion, and from mother-to-child by breastfeeding. There are four subtypes described to date. However, only HTLV-1 and -2 are epidemiologically relevant in Brazil, with a mean prevalence of 0.46% among blood donors. The algorithm recommended by the Brazilian Ministry of Health for diagnosis of these infections includes an initial serological screening by ELISA, chemiluminescent immunoassays, and/or particle agglutination tests, followed by confirmation with Western blot (WB), INNO-LIA test, and/or PCR. Although WB and INNO-LIA tests are more sensitive than PCR, they are laborious, expensive, and indeterminate patterns are seen in approximately 30% of the cases, particularly among HTLV-2 carriers. Thus, the use of molecular assays prior to WB or INNO-LIA have been suggested to reduce costs. Therefore, we developed virus-specific loop-mediated isothermal amplification (LAMP) assays with primers designed for the *tax* gene of HTLV-1 and -2. Accuracy of LAMP assays were evaluated with DNA samples from peripheral blood of HTLV-1 (n=80) and HTLV-2 (n=22) carriers. Moreover, analysis of the 5' long terminal repeats (LTR) and *tax* sequences was performed to genotype proviruses and to determine polymorphisms in the hybridization regions of LAMP primers. HTLV-1 samples belonged to the Cosmopolitan Transcontinental subgroup (HTLV-1aA), and 88.75% presented a C7401T substitution, which was within the F3 primer sequence. Analysis of HTLV-2 *tax* sequences identified samples as HTLV-2c, which is predominant in Brazil. This strain displayed the A7819G and A7991G substitutions, which were into the F2 and B3 primer sequences, respectively. However, the performance of LAMP assays was resistant to these variations, and no cross-reactivity was observed between viruses. HTLV-1/2 LAMP tests presented a very good agreement with PCR (Cohen's $\kappa=0.93$), and accuracy was also high (99.01%). In conclusion, HTLV-1/2 LAMP assays described here represent an alternative to conventional or real-time PCR, since they have lower cost, reduced risk of non-specific DNA amplification, and require simpler laboratory structure and less laborious time. Financial support: PROAP/CAPES and FIOCRUZ.

Keywords: confirmatory diagnosis, HTLV-1, HTLV-2, LAMP



MUTATIONS IN HTLV-1 TAX-RESPONSIVE ELEMENTS IN HAM/TSP PATIENTS ARE ASSOCIATED WITH LOWER PROVIRAL LOAD BUT NOT TO DISEASE PROGRESSION

Yago Côrtes Pinheiro Côrtes Pinheiro Gomes ¹, Marcus Tullius Teixeira da Silva ¹, Ana Claudia Celestino Bezerra Leite ¹, Marco Antonio Sales Dantas de Lima ¹, Abelardo Queiroz Campos Araújo ¹, Isaac Lima da Silva Filho ¹, Ana Carolina Paulo Vicente ², Otávio de Melo Espíndola ¹

¹ INI - Instituto Nacional de Infectologia Evandro Chagas - FIOCRUZ (Av. Brasil, 4365 - LAPCLIN-NEURO - INI - FIOCRUZ - Manguinhos, Rio de Janeiro - RJ, CEP: 21040-900), ² IOC - Instituto Oswaldo Cruz - FIOCRUZ (Av. Brasil, 4365 - IOC - FIOCRUZ - Manguinhos, Rio de Janeiro - RJ, CEP: 21040-900)

Abstract

Human T-lymphotropic virus type 1 (HTLV-1) is a *deltaretrovirus* associated with the development of a slowly progressive neurodegenerative disease, denominated HTLV-1-associated myelopathy/Tropical spastic paraparesis (HAM/TSP). This virus presents 5' and 3' long terminal repeats (LTRs) displaying a motif known as Tax-responsive elements (TRE), which resembles the human cAMP responsive element (CRE) and directs provirus expression by CRE-binding transcription factor. TRE is constituted of three conserved domains (A, B and C), imperfectly repeated three times. It is known that mutations within the A and C domains reduce provirus expression *in vitro*, while mutations in the B domain completely abolish TRE promoter activity. Furthermore, mutations in the TRE of bovine leukemia virus, another *deltaretrovirus*, drastically reduce the proviral load (PvL) in a sheep model. Since a high HTLV-1 PvL is a risk factor for the development of HAM/TSP, we decided to evaluate mutations in the 5' LTR of HTLV-1 and their association with PvL in asymptomatic carriers (AC) and HAM/TSP patients. Data from AC (n=29) and HAM/TSP patients (n=45) followed in an open cohort for approximately 12 years showed that PvL presented a significant intra-individual variation over time, ranging between 2.20 to 230 times. Therefore, mean PvL values were used into analysis to reduce cross-sectional bias. Individuals infected with HTLV-1 presenting the canonical TRE, considering ATK-1 strain as consensus, displayed sustained higher PvL. By contrast, the LTR A125G and G174A mutations were observed in 28.38% to 33.78% of the samples and were associated with reduced PvL in HAM/TSP patients. However, it did not influence the rate of disease progression, which was defined by the quotient between the score in the IPEC-1 disability scale and the time of disease duration. Phylogenetic analysis of LTR showed that these mutations were observed in strains from the Latin American subgroup of the HTLV-1 Cosmopolitan Transcontinental (1aA) subtype, particularly from Brazil and Peru. Therefore, polymorphisms in the TRE of the HTLV-1 5' LTR may represent another factor influencing the PvL in HAM/TSP patients. Indeed, elevated PvL in peripheral blood has been correlated with an increased inflammatory activity in the spinal cord and to a poorer prognosis in HAM/TSP. However, this event was not associated with mutations in HTLV-1 TRE. Financial support: PROAP/CAPES and FIOCRUZ.

Keywords: HAM/TSP, HTLV-1, mutation, proviral load, Tax-responsive elements



SUSCEPTIBILITY TO MEASLES AND RUBELLA IN ADOLESCENTS, YOUTH AND ADULTS IN THE MUNICIPALITIES OF BELÉM AND ANANINDEUA.

Maria Izabel de Jesus ¹, Marluce Moraes Marluce Moraes ¹, Dorotéa Silva Dorotéa Silva ¹, Fernanda Sagica Fernanda Sagica ¹, Renato Medeiros Renato Medeiros ¹, Sueli Rodrigues Sueli Rodrigues ¹

¹ IEC - Instituto Evandro Chagas-SVS/MS (Rodovia BR 316 s/nº Bairro Levilândia, Ananindeua-Para. CEP 67030000)

Abstract

Measles and Rubella are exanthematic and contagious viral infectious diseases transmitted by upper respiratory tract, having similar clinical features and likely to evolve to severe complications. There is no specific treatment and both are universally distributed; the prevention is through vaccination. This study aimed to evaluate the status of measles and rubella-specific antibodies in a population aged 15-39 years, identifying the susceptible individuals between 2016 and 2018, in the municipalities of Belém and Ananindeua, Pará, Brazil. The detection of human IgG antibodies against the virus of measles and rubella in blood serum were performed by ELISA method with the SIEMENS laboratory kit, according to manufacturer's instructions. Individuals with non-reactive and inconclusive were considered susceptible to measles and rubella. There were 2220 participants, 1109 of whom lived in the municipality of Belém and 1111 in Ananindeua. In Belém, the age group most susceptible to measles were 15 to 19 years with 22,4% followed by 20 to 29 years with 16,2% and 30 to 39 years with 8,8%. In relation to the rubella virus in Belém it was observed that the susceptible are between the ages of 15 to 19 years; 20 to 29 years with 11,3% and 6,6% respectively. For Ananindeua age groups 15-19 and 20-29 and 30-39 years were vulnerable to measles virus, presenting susceptibility of 21%, 17,7% and 9,5% respectively, but for rubella virus only Age ranges from 15 to 29 years were significant. The risk of measles and rubella outbreaks is concluded in the municipalities of Belém and Ananindeua, pointing to the need for strengthening of state vaccination and decision-making strategies and border monitoring, as immigration of unvaccinated foreigners can lead to reintroduction of measles and rubella to Brazil.

Financial support: IEC/SVS/MS

Keywords: Measles, Rubella, Epidemiology



EPIDEMIOLOGICAL ANALYSIS OF ZIKA AND DENGUE IN CITY OF LARANJAL DO JARI, STATE OF AMAPÁ, BRAZIL USING REAL TIME (RT-QPCR) AND SANGER-BASED SEQUENCING

Rodrigo Lopes Sanz Duro¹, Robson dos Santos Souza Marinho¹, Josias Gabriel Gonçalves da Silva¹, Giulia Luiz Santos¹, Raimundo Nonato Picanço Souto², Ricardo Sobhie Diaz¹, Shirley Vasconcelos Komninakis^{1,3}

¹ UNIFESP - Universidade Federal de São Paulo (Rua Pedro de Toledo, 781, 16 andar, Vila Clementino, São Paulo, SP), ² UNIFAP - Univesidade Federal do Amapá (Rod. Juscelino Kubitschek, km 02 - Jardim Marco Zero, Macapá - AP), ³ FMABC - Faculdade de Medicina do ABC (Av. Príncipe de Gales, 821, Bairro príncioe de Gales, Santo André, SP)

Abstract

Introduction: The city of Laranjal do Jari in Amapá within the Amazon Biome, presenting tropical climate and ideal environment for the circulation, spreading, formation of reservoirs and transmission of arboviruses. The clinical symptoms of these arboviruses are similar and serological cross-reactivity can occur making it difficult to diagnose and thus requiring additional molecular tests. Our goal was to characterize the incidence of Zika (ZIKV) and Dengue virus (DENV) in plasma samples collected in Laranjal do Jari/Amapá. **Material and methods:** 430 plasma samples were collected between 2013 and 2016 in Laranjal do Jari and selected based on the presence of clinical symptoms of infection by arboviroses and sent to the Retrovirology Laboratory at UNIFESP. Viral RNA was extracted from plasma using commercial QiAmp Viral RNA Mini kit according to manufacturer's instructions. They were analyzed to four RT-qPCR protocols using the commercial Promega for ZIKV and DENV results were sequenced by Sanger-based sequencing (SBS). Then, the sequences submitted to blastn tool for confirmation. **Results:** In the present study, 25 (5.81%) of the 430 samples tested were positive for ZIKV or DENV. Out of a total of 430, 15 samples (3,49%) were positive for ZIKV Ct mean 34.9 (35.4 to 39.5) and 10 (2.3%) positive with the Ct mean 30.5 (12,3 to 37,9) for DENV. The analysis by SBS indentified and confirmed all positive ZIKV and Classified DENV for Type 1 or 2, being found two (20%) DENV type 1 and eight (80%) DENV Type 2. **Conclusion:** The low identification of ZIKV and DENV may be associated with the period between symptoms onset and collection, since the patient delays seeking medical assistance, because the symptoms presented in most cases are asymptomatic or low symptomatic which also contributes to the increase of transmission. Thus by knowing that the viremia period of the DENV and ZIKV viruses lasts for approximately five to seven days, the amount of virus in the sample may be compromising the detection of these arboviruses. The Rt-qPCR assisted in the identification of Zika and DENV positive samples and sequencing analysis helped to identify the serotypes of the DENV, characterizing them as type 1 or 2. Currently DENV 2 is circulating in Brazil and causing several epidemics in cities such as São Paulo this year.

Financial Support: CNPQ (Scholarship #141972/2017-3) /FAPESP (grant #2015/19343-0).

Keywords: Zika, DENV, Sequenciamento, Real Time (qPCR), Arbovirus



EPIDEMIOLOGY OF VITAMIN D RECEPTOR POLYMORPHISMS IN THE CITY OF MACEIÓ/ALAGOAS AND THEIR RELATIONSHIP WITH ZIKA AND CHIKUNGUNYA VIRUS INFECTIONS

Giulia Luiza Santos ¹, Rodrigo Lopes Sanz Duro ¹, Robson dos Santos Souza Marinho ¹, Josias Gabriel Gonçalves da Silva ¹, Ricardo Sobhie Diaz ¹, Shirley Vasconcelos Komninakis ^{1,2}

¹ UNIFESP - Universidade Federal de São Paulo (Rua Pedro de Toledo, 781, 16 andar, Vila Clementino, São Paulo, SP), ² FMABC - Faculdade de Medicina do ABC (Av. Príncipe de Gales, 821, Príncipe de Gales, Santo André, SP)

Abstract

Introduction: The Vitamin D Receptor (VDR) is highly expressed by defense cells and the Vitamin D/VDR interaction helps in immune response contributing against infectious diseases. However, VDR polymorphisms may compromise the immune response. Here, we analyze the incidence of VDR polymorphisms in the city of Maceió/Alagoas correlating with Zika and Chikungunya virus infections. **Material and Methods:** The Central Public Health Laboratory of Alagoas sent to the Laboratory of Retrovirology of the Federal University of São Paulo a total of 355 samples of patients showing symptoms compatible with Zika virus (ZIKV) and Chikungunya virus (CHIKV) infection that were collected in 2016 during an outbreak. The confirmation of ZIKV and CHIKV infection was performed by RT-qPCR. Out of total of 355, we performed a partial analysis of 30 samples. The DNA was extracted using the kit QIAamp DNA Blood Mini Kit. Polymerase Chain Reaction (PCR) was established to amplify the region encoding the VDR gene exon 2 (rs2228570), intron 8 (rs1544410) and intron 8/9 (rs7975232). Then, amplified products were sequenced in the ABI Prism 3130 Genetic Analyzer. **Results: Of total 30 samples,** the RT-qPCR revealed that 10% of infection was ZIKV and 86% CHIKV. The incidence of VDR polymorphisms for rs2228570 (exon2) was (C/C) in 80% CHIKV; 6,6% ZIKV; C/T in 100% CHIKV; T/T in 100% CHIKV. For rs1544410 (intron 8) was G/T in 100% CHIKV; G/G 50% CHIKV, 33,3% ZIKV, 16,7% CHIKV and ZIKV. For rs7975232 (intron 8/9) was G/G in 66,6% CHIKV, 16,6% ZIKV; G/A 66,6% CHIKV, 33,3% ZIKV; A/A 80% CHIKV. **Conclusion:** The immunological response is critical for the evolution of the clinical condition in viral infection and one of the factors that modulate the immune system is the Vitamin D/RVD interaction. The receptor function is affected by polymorphisms in Exon 2, Intron 8 and Intron 8/9. Partial analyzes of the prevalence of polymorphisms showed higher frequency in CHIKV infected patients than in ZIKV infected, which may indicate that CHIKV infected are more likely to have polymorphisms than ZIKV infected individuals and this could influence in symptomatology CHIKV infection.

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Keywords: Vit D, Zika, Chikugunya, Polymorphisms, Epidemiology



VIRUCIDAL INFLUENCE OF IMIDAZOLIUM IONIC LIQUIDS AGAINST TO ZIKA VIRUS

Bruna Saraiva Hermann¹, Caroline Rigotto¹, Henri Stephan Schrekker², ANA LUIZA ZIULKOSKI¹
¹ FEEVALE - Universidade Feevale (Laboratório de Citotoxicidade, Feevale. ERS-239, nº 2755. Prédio 3, sala 104. Vila Nova, Novo Hamburgo, RS - Brasil), ² UFRGS - Universidade Federal do Rio Grande do Sul (Instituto de Química, Departamento de Química Orgânica, UFRGS. Avenida Bento Gonçalves, 9500, Campus do Vale, Gabinete E207B, Agronomia, Porto Alegre, RS, Brasil.)

Abstract

The imidazolium ionic liquids molecules (ILs) are molecules with adaptive physical, chemical and biological properties. ILs may present a combination of organic cations of lower symmetry and a variety of organic and inorganic anions in their structure. Despite their interesting molecular characteristics, ILs has still been little explored for their action against viruses. Viruses are given the potential to cause a public health emergency, and the absence of efficacious drugs for most of them suggests the research and development of new drugs. Thus, this study assessed the virucidal influences potential of four ILs against to Zika virus (ZIKV). To determine CC50 (50% cytotoxic concentration), cytotoxicity assays was previous made: MTT reduction and Neutral Red Uptake (NRU). To evaluate the virucidal influence of the ILs, 2 mL of 0.30, 0.60 and 1.25 μM of [C16MImMeS], [C10MImMeS], [C16MImCl] or [C18MImCl] solutions and the viral control were placed in tube and incubated at 37°C water bath for 15 minutes, with 100 PFU of ZIKV. Subsequently, these solutions were transferred to a monolayer of Vero cells cultivated in 24 well plates. After 1 hour at 37°C, MEM 2X with 2% carboxymethylcellulose was added on the wells and incubated for 72 hours. Then, the cultures were stained with violet crystal 0.4%. The assays were performed in two independent experiments. CC50 results for MTT and NRU were (respectively) 1.21 and 0.37 μM [C16MImMeS], 3.55 and 3.50 μM [C10MImMeS], 1.01 and 10.67 μM [C16MImCl], 3.67 and 0.88 μM [C18MImCl] from MTT and NRU assays. The IL [C18MImCl] showed the highest virucidal action with 70% and 50% reduction in 1.25 and 0.60 μM , respectively. Action of 60% and 65% against ZIKV was observed to [C16MImMeS] and [C16MImCl], both at 1.25 μM . The IL [C10MImMeS] only presented reductions below 22%. The ILs imidazole-based molecules with long alkyl chain, evaluated in our study, consist of a hydrophilic charged head group and a hydrophobic tail, giving these molecules an amphiphilic nature. This amphiphilic character favors interaction with biological membranes. This is confirmed by the highest virucidal effect against ZIKV observed for [C18MImCl], with the longest chain, and by the lowest virucidal results for [C10MImMeS], with the shortest chain. In summary, even though the partial virucidal action against the ZIKV observed for ILs, our results demonstrated the potential of this molecules still little exploited against viral agents. CAPES, FEEVALE.

Keywords: Imidazolium salts, virucidal action, ZIKV



ANTIHERPES EVALUATION OF EXTRACTS AND FRACTIONS OF ILEX GUAYUSA LOES. LEAVES

Daniella Cualla Trujillo ^{2,1}, Isabella Dai Prá ¹, Iara Zanella Guterres ¹, Ingrid Vicente Farias ¹, Flávio Henrique Reginatto ¹, Izabella Thaís Silva ¹, Geison Modesti Costa ²

¹ UFSC - Universidade Federal de Santa Catarina (Laboratório de Virologia Aplicada), ² PUJ - Pontificia Universidad Javeriana (Carrera 7 No. 43-82, Bogotá, Colombia)

Abstract

Herpes Simplex Virus type 1 (HSV-1), the main cause of oral herpes virus, is one of the main viral conditions in the world, causing physical and psychological problems to people who suffered it, because virus presence is associated with oropharyngeal, ocular and central nervous system lesions. On the other hand, their resistance to the most common available treatment (acyclovir) make herpetic infections a serious worldwide health problem. In this context, natural products become an important source of active substances to treat different diseases and the use of traditional knowledge allows to find new antiviral agents. *Ilex guayusa* is a colombian amazonian plant used in medical-magical rituals by shamans to treat multiple conditions, such as a stimulator of the nervous, muscular digestive and diuretic systems. The aim of this work was to evaluate the antiherpes activity against Herpes Simplex Virus-1 (KOS strain) of extracts and fractions prepared from leaves of *I. guayusa*. Two extracts were prepared simulating traditional drinks, an aqueous infusion (11:10) and a hydroalcoholic maceration (M2072). It was performed a liquid-liquid fractionation to the maceration using solvents of increasing polarity, such as ethyl acetate (AcOEt), butanol (BuOH) and water (Acu). Both, extracts and fractions, were evaluated against HSV-1 (KOS strain) by plaque reduction assay, and the cytotoxicity of the extracts and fractions was evaluated on VERO cells by sulforhodamine b assay. Results were expressed as 50% cytotoxic concentrations (CC50) and 50% of viral replication inhibitory concentrations (IC50) as well as the selectivity index (SI) of each sample (CC50/IC50), which indicates how promising they are. All the samples showed no cytotoxic effects at maximum tested concentration (2,500 µg/mL). In relation to the anti-HSV-1 activity, both extracts showed antiherpetic activity. However, the aqueous fraction (Acu) was the most active showing SI value >40. The active fraction showed to be enriched in saponins and some polyphenols of high polarity, molecules that may be related to the activity observed.

Financial Support: CNPq

Keywords: Herpes, *Ilex guayusa*, natural products



STUDY OF 17 PATIENTS WITH GUILLAIN-BARRÉ SYNDROME OCCURRED IN CUIABÁ-MT

Flávia Almeida Ramos ^{1,2}, Natasha Kilsy Rocha Belem ², Vanessa Barbosa da Silveira ³, Camila Pereira Soares ³, Débora da Costa Ormond Rosa ⁴, Gilmar Jorge de Oliveira Junior ¹, Flávia Galindo Silvestre-Silva ², Heloíse Helena Siqueira Borges ^{5,6}, Inês Stranieri Stranieri ⁷, Patricia Palmeira Palmeira ⁸, Magda Maria Sales Carneiro- Sampaio Carneiro-Sampaio ⁸, Edison Luiz Durigon ³, Danielle Bruna Leal de Oliveira ³, Maria Isabel Valdomir Nadaf Nadaf ⁹, Olga Akiko Takano Takano ¹

¹ PPG/ISC/UFMT - Programa de Pós-Graduação do Instituto de Saúde Coletiva da UFMT (Programa de Pós- Graduação do Instituto de Saúde Coletiva da UFMT (Av. Fernando Corrêa da Costa, nº 2367 - Bairro Boa Esperança. Cuiabá - MT - 78060-900)), ² LIBM/HG - Laboratório de Imunogenética e Biologia Molecular do Hospital Geral (Rua 13 de Junho, 2101 - Centro Norte, Cuiabá - MT, 78025-000), ³ LVCM/ICB II/USP - Laboratório de Virologia Clínica e Molecular do Instituto de Ciências Biomédicas II/USP (Av. Prof. Lineu Prestes, nº 1374 – Butantã – São Paulo/SP – CEP 05508-000), ⁴ PPG/CAAH/HUJM-EBSERH/UFMT - Programa de Pós- Graduação em Ciências Aplicadas à Atenção Hospitalar/HUJM-EBSERH/UFMT (R. Luis Philippe Pereira Leite, s/n Alvorada, Cuiabá - MT, 78048-902), ⁵ SN/HG - Serviço de Neurologia do Hospital Geral (Rua 13 de Junho, 2101 Centro Norte, Cuiabá - MT, 78025-000), ⁶ DCM/FM/UFMT - Departamento de Clínica Médica da Faculdade de Medicina/UFMT (Av. Fernando Corrêa da Costa, nº 2367 - Bairro Boa Esperança. Cuiabá - MT - 78060-900), ⁷ SES/MT - Secretaria de Estado de Saúde de Mato Grosso (Centro Político Administrativo, Palácio Paiaguás, Rua D, S/N, Bloco 5, CEP: 78049-902), ⁸ ICr/HC/FMUSP - Instituto da Criança do Hospital das Clínicas FMUSP (Av. Dr. Enéas Carvalho de Aguiar, 647 - Cerqueira César, São Paulo - SP, 05403-000), ⁹ DP/FM/UFMT - Departamento de Pediatria da Faculdade de Medicina da UFMT (R. Luis Philippe Pereira Leite, s/n - Alvorada, Cuiabá - MT, 78048-902)

Abstract

Introduction: Guillain-Barré syndrome (GBS) has become the leading cause of acute flaccid paralysis after polio eradication worldwide. With the occurrence of the ZIKV epidemic in Cuiabá, this study investigated some cases hospitalized and/or reported with diagnosis of GBS in Cuiabá-MT, from 2015 to 2018. **Objective:** To report 17 cases of Guillain-Barré Syndrome occurred in Cuiabá-MT, 2015-2018 and perform laboratory tests for ZIKV. **Material and Methods:** Cross-sectional study using data from medical record, notification forms and complemented by telephone interviews. Tests performed: ZIKV IgG(ELISA) and RT-PCR. **Results:** We interviewed 11/17 cases of GBS. Most were males (58.8%), race/color brown (82.4%), mean age 39.2 years (from 1 to 80 years); had completed high school education or higher (n=8/13, 61.5%); received treatment with intravenous immunoglobulin (n=14/16, 87.5%) and all of them progressed to discharge. The median length of hospital stay was 12.7 days (from 3 to 43 days). The GBS Protocol of the Ministry of Health of 2015 was used, and all cases met the essential clinical criteria and at least 3/7 suggestive clinical criteria. The liquor test was compatible with the diagnosis of GBS, with protein increase (n=15/15, media=180 mg/dL) and number of normal cells (n=14/14, media=2.2 cells/mm³). In the GBS period, 52.9% were positive for ZIKV IgG (ELISA) and 41.2% negative; one patient was positive on the collected test and performed 2 years after discharge. In 1/9 CSF tested for ZIKV IgG (ELISA) was positive (11.1%). The patient who presented IgG + serology in the cerebrospinal fluid and serum had also registered in the IgM + for CHIKV. The RT-PCR was negative for all samples tested for four arboviruses: Zika, dengue, Chikungunya



and yellow fever. **Conclusion:** The positive serology for ZIKV IgG during GBS indicates the possibility of the disease being related to the previous infection by this virus. Negative serology rules out this possibility. The RT-PCR in serum and CSF were negative probably because the GBS frame develops outside the viremia period. Of the patients interviewed, it was noticed that after discharge home, patients need continuity of treatment with multiprofessional team until complete recovery, physical and emotional.

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Keywords: Guillain-Barre syndrome, Zika virus, arbovirus, enzyme-linked immunosorbent assay, real-time polymerase chain reaction



MOSQUITO POOL SAMPLES DO NOT INHIBIT ZIKV dPCR DETECTION

Paula Rodrigues Almeida ^{1,2}, MERIANE DEMOLINER ^{1,2}, Ana Karolina Antunes Eisen ^{1,2}, Juliana Schons Gularte^{1,2}, Débora Couto da Rosa ¹, Fernando Rosado Spilki ^{1,2}

¹ Universidade Feevale - Universidade Feevale (ERS 239, 2755, Novo Hamburgo - RS), ² Feevale Techpark - Feevale Techpark (Edgar Hoffmeister, 500, Campo Bom - RS)

Abstract

Since the recent outbreaks occurred in French Polynesia, Yap island and Northeastern Brazil, Zika virus (ZIKV) has become an important pathogen worldwide, causing Guillain Barré Syndrome (GBS) and Congenital Zika Syndrome (CZS). ZIKV is established in Brazil, and is one of the arboviruses under constant surveillance. The influence of host inhibitors could impair detection techniques routinely used in this surveillance. Digital Polymerase Chain Reaction (dPCR) is a technique that allows highly accurate quantification of DNA targets using a Taqman based assay. The goal of this study was to assess the quantification of ZIKV genomic copies in mosquito pools and compare it to culture minimum essential medium (MEM) diluted samples to assess the influence of inhibitors and the effect of low nucleic acid material in RNA extraction, reverse transcription and PCR. Samples from ZIKV MR766 were quantified through dPCR before serial dilution in mosquito pools and MEM. ZIKV was spiked in two negative mosquito pools in dilutions of 1:10 to 1:1000. Pool 1 contained a single mosquito and pool 2 contained 10 mosquitoes. Additionally, ZIKV was diluted in MEM in 1:10 to 1:1000 fold dilutions (sample 3). After dilution, RNA was extracted with TRIzol™ and cDNA was synthesized with Promega GoScript™. Lanciotti et al., 2008 ZIKV assay protocol was applied to dPCR reactions. The ZIKV MR766 stock utilized presented 2094 copies/μL with a precision of 2,29%. Only results with precision under 5% were utilized. MEM diluted samples presented lower genomic copy numbers than mosquito pools 1 and 2 in the same dilution. Dilutions of 1:1000 presented no differences between pools with 1 or 10 mosquitoes. The difference observed in MEM diluted samples could be attributed to reverse transcriptase lower efficiency in samples with low overall RNA concentration, or to individual pipetting variability in any steps, since each dilution was extracted and cDNA was synthesized separately. TRIzol™ RNA extraction method results in high yields and acceptable purity, therefore it is unlikely that this difference results from the extraction method. The results in mosquito pools show that there is no apparent influence of inhibition in this material to dPCR reaction, and the technique has good repeatability and robustness for ZIKV detection in mosquito pool samples regardless of the amount of mosquitoes.

CAPES MCTIC/FNDCT-CNPQ/MEC

Keywords: dPCR, mosquito, RNA, Zika Virus



ANALYSIS OF SPATIAL DIFFUSION OF YELLOW FEVER EPIDEMIC IN STATE OF SÃO PAULO, 2016 TO 2019.

Alec Brian Lacerda ¹, Priscilla Ikefuti ¹, Leila del Castillo Saad ^{1,2}, Francisco Chiaravalloti Neto ¹
¹ FSP-USP - Faculdade de Saúde Pública - Universidade de São Paulo (Av. Dr. Arnaldo, 715 - São Paulo - SP - Brasil - CEP - 01246-904), ² CVE/SES-SP - Centro de Vigilância Epidemiológica "Professor Alexandre Vranjac"/CCD/SES-SP (Av. Dr. Arnaldo, 351 - Cerqueira César, São Paulo - SP, 01246-000)

Abstract

Introduction: Yellow fever (YF) is a viral disease of compulsory notification, of short duration and variable severity, being mild and in some cases severe with high lethality. The Geographic Information System (GIS) has become an important tool for monitoring and fighting diseases, and together, it corroborates the identification of spatial diffusion processes. The spatial diffusion is among the pertinent themes for geographic epidemiology, being a space-time process of spread of a phenomenon in a space over time. The latest epidemic of YF, which began in 2014, recorded hundreds of human and epizootic cases and deaths in Brazil, mainly in the Southeast, extending to the present. **Methodology:** Ecological study of observation of yellow fever virus behavior in the state of São Paulo from the notification of epizootics and human cases, characterized by the probable local of infection (LPI), according to the municipality of occurrence of epizootics and human cases. Quarterly thematic maps with information from municipalities with proven and unproven viral circulation were made to identify the origin of the outbreak and its spatial diffusion. **Results:** A total 626 confirmed human cases and 827 epizootics were registered, most of them in regions without vaccine recommendation. The municipality of Mairiporã had 29% of human cases and 11% of total epizootics during the study period. The beginning of the epidemic was considered in March 2017, when there was greater presence and displacement in the eastern region of the state, which did not contain vaccine recommendation. The municipalities of Amparo and Monte Alegre do Sul were diagnosed as sites of the beginning of the epidemic. **Conclusion:** Epizootics and human cases were related during this period, showing similarity of displacement. Amparo and Monte Alegre do Sul represent the beginning of the outbreak because, from these locations, the yellow fever virus spread throughout the territory of state of São Paulo, reaching the major cities, the coast and moving towards the south of the state, initially representing hierarchical and contagious diffusion, by presenting the jumps between nearby locations. **Financial Support:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Keywords: Brazil, Geographic Information System (GIS), Spatial Diffusion, Yellow Fever



MAYARO VIRUS (MAYV) DETECTION IN PARÁ WESTERN

Cassiano Junior Saatkamp ^{2,1,6,1}, Valdinete Alves do Nascimento Valdinete Alves do Nascimento ⁴, Victor Costa de Souza Victor Costa de Souza ⁴, Jamille Gomes dos Santos Saatkamp Jamille Gomes dos Santos Saatkamp ⁷, João Alberto Coelho João Alberto Coelho ³, Rose Grace Brito Marques Rose Grace Brito Marques ³, Felipe Gomes Naveca Felipe Gomes Naveca ⁴, Regina Maria Pinto de Figueiredo Regina Maria Pinto de Figueiredo ⁵, Luís Reginaldo Ribeiro Rodrigues Luís Reginaldo Ribeiro Rodrigues ¹

¹ UFOPA - Universidade Federal do Oeste do Pará (Campus Tapajós, Rua Vera Paz S/N, Salé, Cep 68040-255, Santarém, PA, Brasil), ² BIONORTE - Programa de Biodiversidade e Biotecnologia da Rede BIONORTE (Campus Tapajós, Rua Vera Paz S/N, Salé, Cep 68040-255, Santarém, PA, Brasil), ³ DIVISA/SEMSA - Divisão de Vigilância em Saúde (DIVISA), Secretaria Municipal de Saúde de Santarém (SEMSA), (AV MOAÇARA, SANTARÉM - PA), ⁴ ILMD/Fiocruz Amazônia - Fundação Oswaldo Cruz - Instituto Leônidas e Maria Deane (Rua Terezina, 476 - Adrianópolis, Manaus - AM, 69057-070), ⁵ FMT-HVD - Fundação de Medicina Tropical Heitor Vieira Dourado (Av. Pedro Teixeira, s/n - Dom Pedro, Manaus - AM, 69040-000), ⁶ UEPA - Universidade do Estado do Pará (Av. Plácido de Castro, 1399 - Aparecida, Santarém - PA, 68040-090), ⁷ LabSantos - Laboratório Santos (Tv Sete de Setembro, 786 C Santarem PA)

Abstract

The Mayaro virus (MAYV) is transmitted by arthropod vectors and is classified as an arbovirus of the genus *Alphavirus*, family *Togaviridae*. It was isolated in Brazil in 1955 and since then, several outbreaks have occurred in several regions, including Pará state. Participants (n = 49) came from the cities of Itaituba in 2016 and Alenquer in 2017, Pará do Oeste, and blood samples were collected, which were sent to the Zoonoses Control Center of Santarém-PA to search for agents. Molecular diagnosis for MAYV was performed by detection of viral RNA in human serum samples. RNA was extracted using the QIAamp Viral RNA Mini Kit (QIAGEN). Firstly, it was realized the molecular search for Dengue virus, with negative results in 100% of the analyzed samples. Submitting the same samples to RT-qPCR for MAYV virus identification, 4 (8.2%) (threshold cycle, Ct, between 34.0 - 36.9) were positive, in which two samples were from Itaituba and two from Alenquer. In 1978, an outbreak of Mayaro fever occurred in Belterra city, with 55 confirmed cases, whose region is the same of the present study. Due to clinical characteristics similar to other arboviruses, the diagnosis is not defined in several cases, thus requiring laboratory confirmation. These findings serve as a warning for underreporting cases of MAYV and other arboviruses that present dengue-like clinical symptoms in ParáWestern.

Financial Support: State of Amazonas Research Support Foundation – FAPEAM (www.fapeam.am.gov.br, call FAPEAM/SUSAM-SES-AM/MS/CNPq nº 001/2013 – PPSUS)

Keywords: MAYARO, VIRUS, PARA, RT - PCR, qPCR



INFLUENCE OF THE REPLACEMENT RADICAL OF IMIDAZOLIC IONIC LIQUIDS ON CYTOTOXICITY AND ANTIVIRAL ACTIVITY AGAINST MAYARO VIRUS

¹Daiane Metz Krajeski ¹, Fernando Jardim ¹, Karoline Schallenberger ¹, Giovanna Marx Machado ¹, Henri Stephan Schrekker ², Ana Luiza Ziulkoski ¹, Caroline Rigotto ¹
¹ Feevale - Universidade Feevale (Novo Hamburgo- RS), ² UFRGS - Universidade Federal do Rio Grande do Sul (Porto Alegre- RS)

Abstract

The design of antiviral drugs associated with the advance in the understanding of the structural biology of viruses has provided new strategies for the development of compounds capable of acting against different etiological agents. Currently, viral infections caused by arboviruses such as Mayaro (MAYV) require attention, mainly due to their high spread and transmission. This arbovirus is associated with a disturbing disease with disabling joint conditions. In view of this, the evaluation of new antivirals is necessary, since there are no specific pharmacological treatments. Based on the literature, ionic liquids (LIs) such as imidazoles are of great interest due to their satisfactory chemical properties. Allied to this, they express in their structure constituents that can be used to optimize physicochemical properties and interact with enzymes and receptors. Cytotoxicity and antiviral activity of three LI (SI16MImCl, BTA2CMImCl and BTACMImCl) were evaluated (differing from the substituent radical). To this end, mitochondrial functionality assay was performed by reducing the methyl tetrazolium salt MTT in VERO cells. Cell cultures were exposed to serial dilutions at concentrations from 0.312 to 20 μ M. After incubation time, the MTT test was performed and the cytotoxic concentration was calculated for 50% of the cells (CC50). Also, the potential antiviral activity of LI against MAYV was evaluated by the plaque reduction assay. The non-toxic concentration range obtained in the cytotoxicity evaluation was 3.51 μ M for SI16MImCl with a cytotoxic effect observed in about 99% of cells at 20 μ M concentration. For the other LIs it was not possible to estimate because there was no cytotoxicity at the highest concentration tested. The compounds BTA2CMImCl and BTACMImCl showed a reduction in the number of lysis plaques at all concentrations evaluated when compared to the viral control, however the compound BTA2CMImCl was more effective, since it inhibited viral replication around 30% in four concentrations evaluated. In contrast, SI16MImCl presented a pro-viral effect at the two highest concentrations tested. It is concluded that SI16MImCl is more cytotoxic and does not show significant viral inhibition, whereas BTA2CMImCl and BTACMImCl have cytotoxicity below 5%. Although they have not present an antiviral effect, were capable to inhibit 30% of viral replication. Financial support: CAPES, FEEVALE, CNPq.

Keywords: Arbovirus, Rational planning, MTT, Plaque assay, Cell viability



EVALUATION OF ANTIVIRAL ACTIVITY OF TWO IMIDAZOLIC ION LIQUIDS AGAINST CHIKUNGUNYA VIRUS

Daiane Metz Krajeski ¹, Karoline Schallenger ¹, Fernando Jardim ¹, Henri Stephan Schrekker ², Ana Luiza Ziulkoski ¹, Caroline Rigotto ¹

¹ Feevale - Universidade Feevale (Novo Hamburgo- RS), ² UFRGS - Universidade Federal do Rio Grande do Sul (Porto Alegre- RS)

Abstract

Brazil is currently facing a major manifestation of arboviral epidemics in virtually every region of its territory. CHIKV is responsible for Chikungunya fever, a disease that causes severe joint pain and can be disabling. There is no specific pharmacological treatment for this viral agent, bringing forward the need for the planning and development of new antivirals. Imidazole ionic liquids (ILs) are substances consisting of an imidazole ring with cationic nucleus and anion- associated substituent radicals, which due to their structural characteristics, interact easily as biological systems, making them promising compounds for the pharmaceutical industry. Following cytotoxicity evaluation and prior viral titration, the antiviral activity of two ILs JCZ 107 ([PhC₃(oMIm)]Cl) and JCZ 108 ([PhC₃MIm]Mes) were evaluated by the plaque reduction assay. For this, VERO strain cells were previously cultured in 12-well plates at a density of 3.5x10⁵ per well. Cells were inoculated with viral suspension and after adsorption, were exposed to serial concentrations from 87.5 to 700µM of both compounds followed by 48 hours incubation. After that they were fixed with 4% formaldehyde solution and stained with Violet Crystal for counting of plaques. Compound JCZ 107 showed a reduction in the number of lysis plaques in all tested concentrations, achieving the maximal reduction of 20% at 300µM concentration. Compound JCZ 108 had an inhibitory effect of about 15% at the concentration of 87.5 µM , but on the other hand, the highest concentration tested (700µM) showed a slight pro-viral effect. At other concentrations the inhibitory effect remained around 10%. Conclusion: Compound JCZ 107 showed inhibitory effect at all concentrations evaluated. Compound JCZ 108 had a subtle pro-viral effect at the highest concentration evaluated, while the others had an inhibitory effect. However, it is not sufficient to indicate them as antiviral agents. It is noteworthy that these results are preliminary, and more tests are needed to better characterize these compounds. Financial support: CAPES, FEEVALE, CNPq

Keywords: Plaque assay, arbovirus, cell culture, Imidazole



MOLECULAR EPIDEMIOLOGY AND SURVEILLANCE OF CIRCULATING ADENOVIRUS IN BRAZILIAN PATIENTS WITH GASTROENTERITIS, 2008-2011

Ellen Viana Souza ¹, Talita Gonçalves Aires de Queiroz ¹, Roberta Salzone Medeiros ¹, Yasmin França Viana Pires de Souza ¹, Lais Sampaio de Azevedo ¹, Rodrigo Sanz-Duro ², Robson dos Santos Souza Marinho ², Shirley Vasconcelos Komninakis ^{2,3}, Maria do Carmo Sampaio Tavares Timenetsky ¹, Adriana Luchs ¹

¹ IAL - Instituto Adolfo Lutz (Av. Dr. Arnaldo, 355 - Pacaembu, São Paulo - SP, 01246-000), ² UNIFESP - Universidade Federal de São Paulo (Rua Sena Madureira, n.º 1.500 - Vila Clementino - São Paulo - SP - CEP: 04021-001), ³ FMABC - Faculdade de Medicina do ABC (Av. Príncipe de Gales, 821, Bairro Príncipe de Gales, Santo André/SP - CEP: 09060-650)

Abstract

An accurate understanding of Human Adenovirus (HAdV) prevalence in acute gastroenteritis is important for control and preventive measures, especially in the post-rotavirus (RVA) vaccine era. In Brazil, limited data are available regarding the contribution of HAdV in gastroenteric patients and there is a gap in the understanding of the molecular epidemiology of HAdV. The aims of the present study were to investigate the frequency of HAdV infections in patients with gastroenteritis during a 4-year period (2008-2011) and conduct molecular characterization of positive strains. A total of 2901 fecal samples negative for both, RVA and Norovirus, were selected and tested for HAdV by PCR. Positive HAdV samples were sequenced to genotype characterization. HAdV was detected in 120 cases (4.1%); median age of 2.6 years. Gender was found to not play a role in HAdV infection. HAdV infection was observed throughout the year and no consistent seasonal pattern was identified. Detection rate not significantly varied according to the year. HAdV-F41 was the most frequent genotype detected (63.3%, 76/120), followed by HAdV-F40 (10.8%, 13/120), HAdV-C2 (8.3%, 10/120) and HAdV-C1 (7.5%, 9/120). Together, HAdV-F41 and -F40 were responsible for more than half (74.1%) of the HAdV positive cases obtained here. Other genotypes, including HAdV-C5 (3.3%, 4/120), HAdV-C6 (0.8%, 1/120) and HAdV-A12 (0.8%, 1/120) were also identified. Species D (not typed) was detected in 5.2% (6/120) of the cases. Children ≤ 5 years exhibited higher positivity rate, reinforcing that HAdV is an important pathogen in childhood diarrhea. The data presented here confirmed the epidemiological role of species F in gastroenteritis etiology, with a HAdV-F41 predominance. Nevertheless, HAdV has not currently a major epidemiological impact in Brazil after the RVA vaccine introduction. The methodology used was proven to be suitable for the identification of a wide range of species (A, C, D and F) and genotypes (F40, F41, A12, C1, C2, C5 and C6); however, the detection of "non-enteric" HAdV in stool (i.e. A, C and D) could not necessarily be associated with diarrheic symptoms, as HAdV can exhibit a lingering shedding in feces after previous infections in other organs. HAdV screening should be considered as differential diagnosis in Public Health Laboratories. This study has the potential to contribute to the clinical definition and significance of HAdV infections in Brazil.

Keywords: Gastroenteritis, diarrhea, enteric adenovirus, molecular characterization, surveillance



DETECTION OF FLAVIVIRUS RNA IN CEREBROSPINAL FLUID OF CHILDREN WITH NEUROLOGICAL SYMPTOMS IN MINAS GERAIS

Ana Paula Correia Crispim ¹, Paula Eillanny Silva Marinho ¹, Aline Almeida Bentes ^{2,3}, Isabela Guedes ², Sara Tavares ², Alice Martins Alvarenga ^{2,2}, Aline Batista ², Rafaela Pezzopane ¹, Guilherme Frota ¹, Talitah Candiani ², Roberta M Castro Romanelli ³, ERNA GEESIEN KROON ¹

¹ UFMG - Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (Av. Antônio Carlos, 6627 Belo Horizonte, Minas Gerais, CEP 30270-901, Brazil), ² HIIPII - Hospital Infantil João Paulo II (Alameda Ezequiel Dias, 345 - Centro, Belo Horizonte - MG, 30130-110), ³ FM, UFMG - Departamento de Pediatria, Faculdade de Medicina, Universidade Federal de Minas Gerais (Av. Prof. Alfredo Balena, 190 - Santa Efigênia, Belo Horizonte - MG, 30130-100)

Abstract

Brazil is an endemic country for arboviruses, such as those caused by viruses of the family *Flaviviridae*, genus *Flavivirus*, including the species *Zika virus* (ZIKV), which, like *Dengue virus* (DENV), is transmitted mainly by the bite of mosquitoes of the genus *Aedes*. Infection in humans is often asymptomatic and may lead to symptoms such as fever, headache, myalgia, arthralgia and skin rash. In more severe cases, neurological symptoms are reported due to central nervous system (CNS) involvement, such as the development of meningoencephalitis. This study aimed to perform the detection of flaviviruses in cerebrospinal fluid (CSF) from patients with neurological symptoms in a children's hospital located in Belo Horizonte, Minas Gerais. CSF samples were collected during 2018 and 2019 and stored at -70 ° C. From them, RNA was extracted and cDNA synthesized by reverse transcription. cDNA was used as a template for the polymerase chain reaction (PCR) targeting DENV, ZIKV, yellow fever virus, West Nile virus, Saint Louis encephalitis virus and other viruses commonly found causing CNS infection as human herpes virus 1, 2 and 3, enterovirus (ENTV) and chikungunya virus. 181 CSF samples were tested, of which nine were positive for ZIKV (5%) and five were positive for ENTV (2.8%). Of the 181 samples, so far, 56 CSF samples were tested for DENV and 13 were positive (23.3%), four of which positive for DENV-1 (7.1%), two positive for DENV-2 (3,6%), three positive for DENV-3 (5.4%), two were positive for DENV-4 (3.6%) and two samples showed a co-infection between DENV-1 and DENV-3 (3.6%). The results corroborate other studies that demonstrate these viruses as causing CNS infection, especially DENV which had a higher prevalence, higher than ENTV which is clinically more accepted as causing infections in CNS. Positive samples for ENTV were collected in September and October 2018, which corroborates the seasonality already described for infections of these viruses. The positivity for ZIKV evidenced its circulation during the collection period, a fact contrary to what was disclosed by the Ministério da Saúde. This information can be used for epidemiological purposes and to aid in clinical decision making, which reinforces the importance of doing work like this.

Financial support: CAPES, CNPq, FAPEMIG, DECIT-MS, PRPG-UFMG

Keywords: flavivirus, CEREBROSPINAL FLUID, NEUROLOGICAL SYMPTOMS, enterovirus, zika virus



DYNAMIC PROTEOMICS OF MAYV-INFECTED AEDES AEGYPTI CELLS

Anna Fernanda Pinheiro de Vasconcellos Brum de Souza ¹, Samuel Coelho Mandacaru ¹, Athos Silva de Oliveira¹, Wagner Fontes ¹, Sebastien Charneau ¹, Renato Oliveira Resende ¹

¹ UnB - Universidade de Brasília (Campus Universitário Darcy Ribeiro - Asa Norte 70910 - Brasília - Distrito Federal - Brasil)

Abstract

Mayaro virus (MAYV) is an arbovirus that belongs to the genus *Alphavirus*, family *Togaviridae*. Currently, MAYV circulation is almost restricted to Latin America. Besides being mainly transmitted by *Haemagogus spp.*, mosquitoes of other genera are also efficient MAYV vectors, such as *Aedes aegypti*. This fact potentially elevates MAYV dissemination. Previous inefficient strategies for vector control have highlighted the need to better understand the molecular aspects of virus-vector interaction in an attempt to develop molecular tools in order to prevent viral spread. Since proteins are key regulators of cellular processes, a proteomic analysis of *A. aegypti* Aag-2 cells during MAYV infection was performed to elucidate key aspects of the virus-vector interaction and the viral replication. Following MAYV confirmation by RT-PCR and growth kinetics, the MOI=1 was selected when infecting Aag-2 cells. MAYV-infected Aag-2 cells in biological triplicate were collected at 0h (non-infected), 12hpi and 48hpi, separately. After samples preparation and quantification, label-free tryptic peptides of each triplicate were injected into the liquid chromatograph nano-UPLC-Dionex 3000 system coupled to the Orbitrap Elite™ hybrid ion trap-orbitrap mass spectrometer. The raw data were aligned with Progenesis Q1 and were processed using Peaks for protein identification (with a merge *A. aegypti* and MAYV database from UniProt). Functional annotation of identified proteins and their interaction were verified with Blast2Go. A decrease in total host cell protein abundance was identified, while the amount of MAYV proteins increased over the 48h of infection. Therefore, we suggest the up regulated *A. aegypti* proteins may have an important role for viral replication, such as: transcription factor HCFC1, chaperones, synaptobrevins, e1 enolase phosphatase, ATP synthase, among others. Moreover, when evaluating the secretion pathways used by Aag-2 cells, it was observed that unconventional secretion pathways are activated to the detriment of the conventional pathways during MAYV infection. The data from the present study, more than being consistent with data from previous proteomic studies about arboviruses, also point out candidate proteins for future biological validation experiments. Financial support: FAP-DF, CNPq, CAPES, Finep.

Keywords: PROTEOMICS, AEDES AEGYPTI, MAYV, INFECTION, MASS SPECTROMETRY



MOLECULAR CHARACTERIZATION OF HEPATITIS C VIRUS QUASISPECIES IN LIVER TISSUE OF PATIENTS WITH HEPATOCELLULAR CARCINOMA

Ana Luíza Leal ¹, Maria Dirlei Ferreira de Souza Begnami ^{3,2}, Antônio Hugo José Fróes Marques Campos ², Natalia Motta de Araujo ¹

¹ FIOCRUZ - Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Laboratório de Virologia Molecular (Av. Brasil 4365, Manguinhos, Rio de Janeiro, RJ, Brasil), ² A C Camargo Cancer Center - A C Camargo Cancer Center (Rua Tamandaré, 764, São Paulo-SP, Brasil), ³ Hospital Sírio-Libanês - Hospital Sírio-Libanês (Rua Dona Adma Jafet, 91, São Paulo-SP, Brasil)

Abstract

Hepatocellular carcinoma (HCC) is the third most common cause of cancer mortality, with an estimated 780,000 deaths each year worldwide. In Brazil, most cases of HCC are associated with chronic hepatitis C virus (HCV) infection. HCV has an extremely high genetic variability and circulates as closely related genetic variants, called quasispecies. HCV genotypes and Core protein mutations have been associated with increased oncogenic potential. Additionally, the differential microenvironment of malignant hepatocytes may influence viral evolution and select variants with distinct pathogenic properties. The aims of this study are (i) to compare the genetic diversity of HCV quasispecies from tumor and adjacent non-tumor liver tissues; (ii) to investigate the occurrence of HCV quasispecies compartmentalization in malignant hepatocytes. A total of 12 (six tumor and six non-tumor) cryopreserved liver tissue samples from six patients diagnosed with HCC due to chronic HCV infection were obtained. HCV-RNA was extracted from tissue samples and the complete Core genomic region (573 bp) was amplified by reverse transcription followed by nested PCR. Viral genotypes were determined by PCR-direct sequencing and phylogenetic analysis. All patients were positive for HCV-RNA in both tumor and adjacent non-tumor tissues. HCV genotypes were determined in 5 patients: 1a (3/5, 60%), and 3a (2/5, 40%). No difference in HCV genotypes was observed between tumor and non-tumor paired samples. Interestingly, 4/5 (80%) patients showed several nucleotide variations throughout the Core region between tumor and adjacent non-tumor tissues. Molecular cloning of the PCR products is in progress to determine whether these nucleotide substitutions are exclusively found in tumor tissue, which may be associated to HCC development.

Financial Support - CNPq

Keywords: hepatocellular carcinoma, hepatitis C virus, quasispecies, liver, cancer



ANTI-CANCER DRUG DELIVERY BY A LYTIC EUKARYOTIC VIRUS EXPRESSED IN PLANTS

Jonas Rafael Siqueira Ribeiro ¹, Matheus Alves Pereira Cavalcante ¹, Tatiana Domitrovic ¹

¹ UFRJ - Universidade Federal do Rio de Janeiro (Avenida Carlos Chagas Filho 373, Cidade Universitária, Rio de Janeiro.)

Abstract

Virus-like particles (VLPs) are produced from heterologous expression of viral proteins, preserving the structural and functional characteristics of viruses without, however, being infectious. Many VLPs are nanoparticles applied as biotechnological tools for vaccine development and intracellular delivery of drugs or therapeutic genetic material. N ω V is a single-stranded RNA (ssRNA) insect virus, belonging to the *Alphatetraviridae* family. The expression of the N ω V capsid protein in *N. benthamiana* leads to assembly of VLPs that exhibit a maturation process, which involves a structural transition and auto cleavage of the capsid proteins generating a lytic peptide, non-covalent associated with VLP. After maturation, the N ω V VLP is able to penetrate cellular membranes. These features make the N ω V VLP a promising biotechnological tool for drug delivery. The objective of this study is to evaluate the ability of N ω V VLPs to encapsulate Doxorubicin (Dox), an anthracycline capable of binding to RNA, often used for the treatment of different kinds of cancers. We seek to develop a new Dox carrier system targeted to tumor cells, avoiding Dox severe side effects, such as cardiotoxicity observed in patients using the drug. First, we evaluated the particle stability against temperature, pH and different Dox concentrations using native agarose gel electrophoresis. We observed a band corresponding to the intact particle up to 0.3 mg/mL Doxorubicin concentration at physiological pH (7.4). To test Dox incorporation by VLPs, we incubated different Dox concentrations with N ω V VLPs. We then performed size exclusion chromatography, monitoring the absorbance of the Dox molecule (at 480 nm) and capsid proteins (at 280 nm). We observed a VLP concentration dependent decrease in the peak corresponding to the free Dox molecule, suggesting the incorporation of the drug by the particles. As next steps, we will evaluate the effect of the drug carried by VLPs on tumor lineage cells.

Keywords: Virus-like particle, Plant, Drug delivery, Doxorubicin, Nanoparticle



ANTIHERPETIC EVALUATION OF PLANTS BELONGING TO THE GENUS BACCHARIS

Isabella Dai Prá Zuchi ¹, Maria Beatriz De Oliveira Rabelo ², Alessandra Caroline Montes Frade ², Priscilla Rodrigues Valadares Campana ², Fernão Castro Braga ², Rodrigo Maia de Pádua ², Geraldo Wilson Afonso Fernandes ³, Izabella Thaís Silva ¹

¹ UFSC - Universidade Federal de Santa Catarina (Departamento de Ciências Farmacêuticas, Laboratório de Virologia Aplicada), ² UFMG - Universidade Federal de Minas Gerais (Departamento de Produtos Farmacêuticos, Faculdade de Farmácia), ³ UFMG - Universidade Federal de Minas Gerais (Departamento de Biologia Geral, Instituto de Ciências Biológicas)

Abstract

Natural products represent an important source of biologically active substances, performing a key role in research and development of new antiviral medicines, for example, for mucosa and skin infections caused by Herpes Simplex Viruses (HSV). Nowadays, the antiherpes drugs are restricted, and the appearance of resistant virus strains to the first-line treatment (acyclovir) has made it difficult to manage these infections. Some metabolites of plants belonging to the genus *Baccharis*, such as trichothecenes, flavonoids and terpenes, are well known for their inhibitory effects of viral infection. The aim of this study was evaluate the anti-HSV-1 (KOS strain) activity of extracts obtained from the leaves of sixteen different species of the genus *Baccharis*. The cytotoxicity was evaluated on VERO cells by Sulforhodamine B assay and the antiviral action was evaluated by plaque reduction assay. The extracts obtained from the *B. reticularia* and *B. altimontana* leaves suppressed 100% of the viral replication (at 50 µg/mL), whereas the *B. calvescens* inhibited 99,35% of the viral infection at the same concentration. For the cytotoxic potential, the CC50 value obtained for *B. reticularia* was 442.3 µg/mL, for *B. altimontana* was >500 µg/mL and *B. calvescens* was 385.8 µg/mL on VERO cells. The results obtained by this screening provided evidences that the *B. reticularia*, *B. altimontana* and *B. calvescens* species have a low cytotoxicity and a high potential on viral inhibition, being necessary more studies about their antiviral mechanism of action, and such experiments are in progress in our research group.

Financial support: FAPEMIG and CNPq

Keywords: Antiherpes, Baccharis, Herpes simplex viruses, HSV, Natural products



DEVELOPMENT OF AN IMMUNOCOMPETENT ANIMAL MODEL FOR THE STUDY OF HETEROTYPIC DENGUE VIRUS INFECTION

Rúbens prince dos santos Alves ^{1,2}, Annie Elong Ngono ², Sujan Shresta ², Luis Carlos de Souza Ferreira ¹

¹ USP - Universidade de São Paulo (Avenida Prof. Lineu Prestes 1374), ² LJI - La Jolla Institute for Immunology (9420 Athena Circle La Jolla, CA 92037)

Abstract

Dengue is the main arboviruses that affect humans and despite its high epidemiological importance, there are no effective drugs or a completely safe and widely available vaccine capable of preventing viral infection when used in endemic areas. The lack of an adequate animal model for dengue virus (DENV) infection is often mentioned as a major obstacle to better understanding DENV pathogenesis in humans, consequently delaying the development of globally efficient vaccines and antiviral drugs. The development of such murine model capable of reproducing the signs observed in humans has been a long-standing challenging, mostly because DENV clinical isolates do not readily replicate or cause pathology in immunocompetent mice. Aiming to develop a WT mice model we used a described non-adapted naturally neurovirulent DENV2 strain (JHA1) in a sublethal intravenous infection in B6 WT mice. Viral burden analysis demonstrated once again that JHA1 is neurotropic and causes encephalitis in immunocompetent mice. Infection of CD8KO B6 mice resulted in high viral loads and full lethality, demonstrating the critical role of CD8⁺ T cells in the control of dengue virus (DENV) infection in this model. Furthermore, in order to develop a heterotypic model for secondary DENV infection, we characterized the immune response elicited by DENV3 infection in B6 WT, describing and validating five antigen epitopes derived from the E, NS2B, NS3 and NS4B proteins of DENV3. Wherein, DENV3-primed memory CD4⁺ T cells were able to protect against DENV2 (JHA1) infection. The results describe a new WT mouse model that emphasizes the scenario of endemics areas – i.e. more than one DENV serotype can co-circulate in a given period of time. Therefore, this work may provide significant insights into the development or evaluation of a novel preventive vaccine strategy against DENV.

Keywords: Dengue, mice, model, T cells, vaccine



MORPHOGENETIC ANALYSIS OF THE SLEV INFECTION MECHANISMS AND ITS EFFECTS IN A MURINE MODEL OF PLACENTAL DEVELOPMENT

Aline Freitas de Paula Melo ¹, Marina Alves Fontoura ¹, Rebeca de Paiva Froes Rocha ¹, Lais Durco Coimbra ¹, Mariana Bortoletto Grizante ¹, Rafael Elias Marques ¹, Murilo de Carvalho ^{1,2}

¹ LNBIO/CNPEM - National Biosciences Laboratory, Brazilian Center for Research in Energy and Materials (Polo II de Alta Tecnologia - R. Giuseppe Máximo Scolfaro, 10000 - Bosque das Palmeiras, Campinas), ² LNLS/CNPEM

- Brazilian Synchrotron Light Laboratory, Brazilian Center for Research in Energy and Materials (Polo II de Alta Tecnologia - R. Giuseppe Máximo Scolfaro, 10000 - Bosque das Palmeiras, Campinas)

Abstract

The proper formation of the placenta in mammals is crucial to the embryo development. The transient and dynamic nature of this organ mediates the interaction between dam and conceptuses. Even subtle modifications in this environment can affect embryonic development. TORCH agents and mosquito-borne viruses such as Zika virus (ZIKV) are amongst the pathogens able to cross the placenta barrier eventually reaching the embryo. ZIKV-related virus, such as St. Louis Encephalitis virus (SLEV), not only can cause severe neurological disease in humans, but also could overcome the placental barrier. [REM1] The present study aims to determine whether SLEV interacts with the placenta and cause congenital malformations in murine model.[REM2] Placental explants were harvested from FVB/NJ pregnant mouse females at 7.5, 8.5, 10.5, 12.5 and 13.5 days post coitum (dpc) and infected *in vitro* with SLEV (BeH 355964). Tissue and culture supernatants were collected at 48, 96, 144, and 196h post infection (pi) for histological analyses and viral load assay, respectively. Our results showed that the 7.5 dpc explants were permissive for viral replication up to 196h pi with roughly linear increase in viral titer along time. On the hand, placental explants of more advanced stages either maintain SLEV at lower titers or undetected after 48h pi. For *in vivo* experiments pregnant mouse females were retro-orbitally infected at 5.5 dpc. Placentas were harvested at 10.5 dpc for viral titer assays and histological analysis. SLEV were detected by plate lysis assay in all placentas from infected dams. Embryos were morphologically documented and compared to embryos from Mock pregnant females. Around 30% of the embryos from infected dams exhibited signs of several degrees of malformations, from subtle alterations to severe restrictions in intrauterine growth likely caused by SLEV infection. Altogether, our *in vitro* data points out that SLEV is capable of infecting mouse placentas up to 7.5 dpc. It is likely able to cross placental barrier and impair embryo development. Conversely, placentas from more advanced gestational periods suggest the restriction of virus replication. Our *in vivo* results seem to support these data, since SLEV was capable to infect immature placentas, eventually leading to embryo malformations. In near future, we intent to investigate SLEV infection in pregnant mouse females at 6.5 dpc to 13.5 dpc.

Keywords: Placenta, SLEV, Saint Louis Virus, Placental Development



ZIKA VIRUS TRANSMISSION THROUGH MICE BITE, A CASE REPORT

MARIANE TALON DE MENEZES ¹, Renato Santana de Aguiar ^{1,2}, Raíssa Rilo Christoff ¹, Luiza Higa ¹, Paula Pezzuto ¹, Liane Jesus Ribeiro ¹, Orlando Costa Ferreira Júnior ¹, Amilcar Tanuri ¹, Patrícia Garcez ¹

¹ UFRJ - universidade federal do rio de janeiro (Ilha do Fundão Avenida Carlos Chagas Filho, Bl L, 373 - Cidade Universitária da Universidade Federal do Rio de Janeiro), ² UFMG - Universidade Federal de Minas Gerais (Av. Pres. Antônio Carlos, 6627 - Pampulha, Belo Horizonte - MG)

Abstract

Zika virus (ZIKV) is an enveloped single-stranded RNA flavivirus that was first identified in Uganda (1947), and has now spread throughout Asia, Western Pacific and Americas. The majority of ZIKV infectious results in mild symptoms such as fever, cutaneous rash and conjunctivitis. However, in rare events ZIKV infection can access nervous systems, causing paralytic nervous system damage, such as Guillian-Barré syndrome, meningoencephalitis, acute myelitis and Congenital Zika Syndrome (CZS). ZIKV transmission occurs through *Aedes* mosquitoes bites, sexual intercourse, mother to-child transmission and blood transfusions. Here, we present the first description of ZIKV transmission through infected mice bite event. The patient is a research student here included as co-author that conduct ZIKV experimental infections using all protective equipment's in safety areas of mice manipulation. The patient described a bite event during manipulation of mice previously infected with the PE Brazilian ZIKV strain. The onset symptoms started 12 days after bite event, such as cutaneous rash, itch and headache. The infection was initially confirmed through RT-qPCR, IgM and IgG serology tests and PRNT. To confirm the route of transmission, the whole viral genome of the human and mice sample were sequenced, as well the virus strain (PE) inoculated in mice. The virus genome was generated using short amplicon strategy using primers covering the whole viral genome and sequenced on Illumina platform. The maximum likelihood phylogenetic reconstruction demonstrates that human patient, mice virus and ZIKV PE strain grouped together in one single clade belonging to Asian genotype with no significant genetic divergence. A Variant Calling demonstrates some exclusive viral variants in non-structural proteins fixed in human or mice samples compared with initial inoculated PE strains, suggesting virus adaptation and compartmentalization in these different organisms.. This is the first description of ZIKV transmission through mice bite with active viruses in animal saliva and human disease manifestation.

Financial support: CNPq

Keywords: zika, virus, transmission



RESPIRATORY VIRUSES IN SECONDARY LYMPHOID TISSUES

Ronaldo Bragança Martins ¹, Ítalo Araujo Castro ¹, Daniel Macedo de Melo Jorge ¹, Joel Del Bel Pádua ¹, Lucas Matias Ferreri ², Fernando Chahud ¹, Daniel Perez ², Eurico Arruda ¹

¹ FMRP - Faculdade de Medicina de Ribeirão Preto (Avenida Bandeirantes, número 3900, CEP 14049900), ² UGA - The Poultry Diagnostic and Research Center, University of Georgia (College of Veterinary Medicine 501 D.W. Brooks Drive Athens, GA 30602)

Abstract

Studies by several groups, including ours, have reported detection of common respiratory viruses by real-time PCR in tonsillar tissues from children with chronic adenotonsillar diseases. With that in mind, we assessed the presence of respiratory virus in secondary lymphoid tissues other than tonsils obtained from adults who died of causes unrelated to respiratory viruses at necropsies done at the Pathology Department, University of São Paulo School of Medicine, Ribeirão Preto, Brazil. We have already tested palatine tonsil, lymph nodes (cervical and mediastinal), spleen, Peyer's patches, and bone marrow collected at necropsies done on 6 patients (four males) with 59 years of age in average, within a maximum of 8 hours post mortem. Tissue samples were placed in Trizol reagent for molecular tests, and fixed in 4% paraformaldehyde for paraffin embedding for later histology studies. By real-time PCR we detected rhinovirus (RV), influenza A (Flu-A), and respiratory syncytial virus-B (RSV-B) in three different cases. One patient had palatine tonsils simultaneously positive for RV and Flu-A, plus RV in a cervical lymph node and in the spleen. Sequencing of the 5' UTR of RV genome confirmed RV-C specie in palatine tonsil and spleen specimens. In another patient we detected RV and Flu-A genomes in palatine tonsil,

Flu-A in cervical and mediastinal lymph nodes, and in a Peyer's patch. A third case had RSV-B detected in the spleen. Immunohistochemical analysis revealed the presence of RV and Flu-A antigen throughout the epithelial surface of palatine tonsils, and for RV antigen in the parenchymal zone of cervical lymph node and spleen, and also for Flu-A in cervical and mediastinal lymph nodes, and in Peyer's patches. Next-generation sequencing detected all eight segments of the Flu-A genome in one of the cases studied, revealing it to be H1N1 similar to USA 2018 lineages. This preliminary study indicates that lymphoid infections by respiratory viruses are systemic in nature.

Keywords: respiratory viruses , secondary lymphoid tissues, post mortem, rhinovirus, influenza



EVALUATION OF ANTIVIRAL EFFECT OF HYBRID COMPOUNDS CHLOROQUINE-SULFADOXINE AGAINST ZIKV

Audrien A. Andrade de Souza ¹, Lauana R. Torres ¹, Lyana R. P. Lima ¹, José Junior F. de Barros ¹, Vanessa S. de Paula ¹, Maria da Gloria Bonecini de Almeida ², Ana Maria B. de Filippis ¹, Luiz Carlos S. Pinheiro ³, Nubia Boechat ³, Elen M. de Souza ¹

¹ IOC/FIOCRUZ - Instituto Oswaldo Cruz/FIOCRUZ (Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - RJ, 21040- 900), ² INI/FIOCRUZ - Instituto Nacional de Infectologia Evandro Chagas/FIOCRUZ (Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - RJ, 21040-360), ³ FARMANGUINHOS/FIOCRUZ - Instituto de Tecnologia em Fármacos/FIOCRUZ (Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - RJ, 21040-900)

Abstract

The Zika virus (ZIKV) that belongs of the genus *Flavivirus* and *Flaviviridae* family is the etiological agent of Zika fever and is associated with congenital syndrome. However as the virus reaches the fetus is still unclear, it is possible that the transmission of ZIKV occur from sexual transmission. Despite the emerging severity caused by the ZIKV infection, there still no specific treatment for this disease. In this study we evaluated the antiviral effect of the inedited hybrid compounds Chloroquine-sulfadoxine (QC7-QC15), that have the 7-chloroquinoline and arylsulfonamide moieties separated by a distinct ligand which is not found in the individual molecular structures of the precursor drugs Chloroquine and Sulfadoxine. Cultures of cervix human cells were ZIKV infected and treated with non-toxic concentrations (3-50 $\mu\text{M}/\text{mL}$) of the compounds for 48 hours and the antiviral effect was assessed by Plaque assay, immunofluorescence, and RT-qPCR. All treatments with compounds at the concentration of 12 $\mu\text{M}/\text{mL}$ showed 100% of reduction of infectious viral particles, except for compounds QC10 and QC11, which reduced only 24 and 50%, respectively. Among the tested compounds, QC12 and QC13 were most promising demonstrating 100% of infectious particle reduction with 6 $\mu\text{M}/\text{mL}$. The evaluation of percentages of the infected cells confirmed reduction of infection by immunofluorescence as well as the quantification of viral load by RT-qPCR. Together, the results obtained demonstrate promising antiviral activity and reinforce the continuity of this study using animal model.

Keywords: Antiviral effect, hybrid compounds Chloroquine-sulfadoxine, ZIKV



EVALUATION OF THE SENSITIVITY OF REALSTAR® HEV RT-PCR KIT 2.0 WITH THREE COMMONLY USED EXTRACTION METHODS

Felipe Loponte Saback¹

¹ Altona - Altona Diagnostics Brasil (Rua São Paulino 221, Vila Mariana, São Paulo, SP)

Abstract

Introduction: Hepatitis E virus (HEV) is a pathogen that causes hepatitis worldwide. A substantial increase in acquired HEV cases is observed across Europe where HEV genotype 3 infections, originating from animal reservoirs, are predominant and have become a common cause of acute viral hepatitis. The aim of this study is to show the high sensitivity of the RealStar® HEV RT-PCR Kit 2.0 for HEV genotypes 1 to 4. **Methods:** Serial dilution of the 1st WHO International Standard for Hepatitis E Virus, genotype 3a (PEI code: 6329/10) in HEV negative human EDTA plasma were tested. Probit analysis was done with StatsDirect statistical software. The LoD was confirmed for the genotypes 1, 2 and 4 (1st WHO International reference panel for Hepatitis E Virus genotypes (PEI code: 8578/13). Three different extraction methods (QIAamp Viral RNA Kit, EasyMag extraction and MagNaPure 96) were used. Additionally, 5 different real-time PCR instruments (LightCycler® 480 Instrument II; CFX96™ Real-Time PCR Detection System; ABI Prism® 7500; Rotor-Gene® Q5/6; VERSANT® kPCR Molecular System AD,) were compared with respect to equivalent performance.

Results: Depending on the extraction method, the Limit of Detection varied between 35 IU/ml to 49 IU/ml. The LoDs were confirmed for all genotypes. **Conclusion:** The RealStar® HEV RT-PCR Kit 2.0 allows sensitive detection and quantification of HEV RNA in human EDTA plasma, independent of the extraction methods and real-time PCR instruments used.

Keywords: HEV, RealStar, Sensitivity



HUMAN TROPHOBLASTS 3D CULTURES FOR ZIKV AND HHV-2 INFECTIVITY AND ANTIVIRAL STUDIES

Lauana Ribas Torres ¹, Lyana R. P. Lima ¹, Audrien Alves Andrade de Souza ¹, Vanessa Salete de Paula ¹, José Junior França de Barros ¹, Ana Maria Bispo de Filippis ¹, Nubia Boechat ³, Luiz Carlos S. Pinheiro ³, Elen Mello de Souza ¹

¹ IOC/FIOCRUZ - INSTITUTO OSWALDO CRUZ- FUNDAÇÃO OSWALDO CRUZ (Av. Brasil, 4.365 - Manguinhos Rio

de Janeiro - RJ - Brasil Cep: 21045-900), ³ FAR/FIOCRUZ - Instituto de Tecnologia em Fármacos-Fundação Oswaldo Cruz (Av. Brasil, 4.365 - Manguinhos Rio de Janeiro - RJ - Brasil Cep: 21045-900)

Abstract

In 2015 there was an outbreak of infants with microcephaly and other neurological disorders born from mothers who were affected by Zika fever in Brazil. It is not clear how the virus reaches the fetus, but the placenta represents an important route of transmission whose functional unit is chorionic villus, specifically trophoblastic cells. The aim was to establish 3D cultures of human trophoblasts lineage (Jeg-3) to evaluate infectivity of ZIKV and HHV-2 and antiviral activity of synthetic compounds. Initially, 2D cultures of Jeg-3 with the multiplicity of infection (MOI) 1; 5 and 10 of ZIKV were infected per 48 hours. The results revealed an infection kinetic showing viral load of $3,7 \times 10^6$ copies/mL at MOI 1, $9,7 \times 10^7$ copies/mL at MOI 5 and $2,8 \times 10^8$ copies/mL at MOI 10. The infection with HHV-2 at MOI 0,001; MOI 0,01 and MOI 0,1 per 48h lead to about $4,2 \times 10^3$; $1,5 \times 10^6$; $3,3 \times 10^8$ viral copies/mL, respectively. 3D Cultures of Jeg-3 cells were carry out by Greiner Bio-One® Nano3D Biosciences Technology. The spheroids were ZIKV infected at high multiplicity of infection (MOI 10) and low HHV-2 multiplicity of infection (MOI 0,001) for further coinfections assays. Preliminary data in 3D system, exhibited viral load of $1,8 \times 10^5$ ZIKV copies/mL and $2,6 \times 10^3$ HHV-2 copies/mL, demonstrated viral susceptibility. For antiviral activity assays, the spheroids were ZIKV infected (MOI 10) and treated with a hybrid molecule of Chloroquine-sulfadoxine (QC- 6 μ M/mL) and Nitaxozanide (NTZ- 25 μ g/mL) drug. The results revealed a viral load reduction of $2,1 \times 10^5$ viral copies/mL in untreated cultures to $1,8 \times 10^5$ and 6×10^4 viral copies/mL in NTZ and QC treated cultures, respectively. The results of ZIKV and HHV-2 antiviral activity were Plaque assays confirmed. The 3D experimental model represents a great tool to explore biological aspects of congenital transmission by ZIKV and HHV-2. In addition, it is an excellent model for identifying potential antiviral compounds.

Keywords: congenital transmission, trophoblasts, ZIKV, HHV-2, antiviral activity



PARVOVIRUS B19 INFECTION DURING PREGNANCY: A CASE REPORT

Arthur Daniel Rocha Alves ¹, Elisabeth de Souza Neves ², Bianca Balzano De La Fuente Villar ², Marcelo Alves Pinto ¹, Luciane Almeida Amado ¹

¹ LADTV/IOC/Fiocruz - Laboratory of Technological Development in Virology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (Rio de Janeiro, RJ, Brazil), ² IFF/Fiocruz - Fernandes Figueira Institute, Oswaldo Cruz Foundation (Rio de Janeiro, RJ, Brazil)

Abstract

Parvovirus B19 (B19V) can be transmitted to the fetus, which may result in an adverse fetal outcome. The prevalence of B19V infection during pregnancy is estimated to be 1% to 5%, increasing up to 13% in epidemic periods. In this study, we report three cases of B19V infection in immunocompetent pregnant during an epidemic period, from October to December 2018, in Rio de Janeiro. Serum and placental tissue samples were obtained to investigate B19V infection. Real-time PCR and serological tests were performed to B19V-DNA and anti-B19V IgM/IgG detection, respectively. Patient 1, a 33-year-old at 23 gestational weeks was diagnosed with mirror syndrome in view of maternal anasarca and proteinuria. Fetal morphological ultrasound revealed the presence of pericardial effusion, ascites, and subcutaneous edema. During the investigation of congenital infection, anti-B19V IgG was detected. One week later, the ultrasound revealed fetal death. B19V-DNA was detected in both serum and placenta tissue with a viral load of 2.7×10^4 and 3.2×10^{10} IU/mL, respectively. Patient 2, a 40-year-old, had a history of two previous abortion without a known cause. During prenatal care, it was detected HTLV and anti-B19V IgM and IgG. The fetus had a hydrops related to anemia, revealed by Doppler ultrasonography, and a mirror syndrome was diagnosed. At 13 gestational weeks, the patient had an abortion. B19V-DNA was detected in serum and in placenta tissue with a viral load of 8.1×10^4 and 8.4×10^{10} IU/mL, respectively. Patient 3, a 39-year-old at 10 gestational weeks had maculopapular rash and arthralgia. During infectious disease investigation, anti-B19V IgM and IgG were detected. B19V-DNA was present in patient serum with a viral load of 4.8×10^5 IU/mL. Fetal anemia and morphological abnormalities were absent. Two weeks later, a second serum sample was collected and it became negative for B19V-DNA. At 39 gestational weeks, a healthy male infant was born. Placenta tissue was collected and B19V-DNA was not detected. Mother and child are currently doing well 2 months after delivery. Our data showed two adverse pregnancy outcomes during B19V infection, as fetal anemia and nonimmune hydrops fetalis, and the risk seemed to be increased when B19V infection occurs during the second trimester. These findings highlight that management of B19V infection in pregnant is important because immediate diagnosis and transfusion in hydropic fetuses could decrease the risk of fetal death.

Keywords: Parvovirus B19 infection, diagnosis, pregnancy, hydrops fetalis, Real time PCR



EVALUATION OF THE ANTI-RABIES VIRUS RIBONUCLEOPROTEIN POLYCLONAL IgG ANTIBODY IN DIRECT RAPID IMMUNOHISTOCHEMISTRY TEST FOR THE RABIES DIAGNOSIS

Bruno Stuart de Castro ¹, Fernanda Guedes Luiz ¹, Gabriela Koike ¹, Elaine Raniero Fernandes ¹, Iana Suly Santos Katz ¹, Sandriana dos Ramos Silva ¹

¹ IP - Instituto Pasteur (Av. Paulista, 393, Cerqueira Cesar, São Paulo-SP)

Abstract

Rabies laboratory diagnosis plays an essential role in disease surveillance, however, the use of the direct fluorescent antibody (DFA) test, assay recommended, in developing countries can be still quite limited. A novel diagnostic assay, the direct rapid immunohistochemistry test (dRIT), has been reported to have a diagnostic sensitivity and specificity equal to the DFA test while offering advantages in cost, time and interpretation. Most previous studies have evaluated the dRIT using monoclonal antibody cocktails. In this work, we aimed to evaluate the anti-ribonucleoprotein polyclonal IgG for *Rabies virus* (RABV) detection by dRIT. For this, horse hyperimmune serum against RABV ribonucleoprotein (RNP) was used for purification of the polyclonal IgG by ionic exchange chromatography on QAE sephadex A-50 (GE Healthcare) followed by immunoaffinity chromatography column. The purity of IgG obtained was analyzed by 10% SDS-PAGE (under reducing and non-reducing conditions). The purified IgG concentration was estimated by methods absorbance at 280 nm. The unlabeled specific IgG preparation was biotinylated using biotin protein labeling kit (Sigma-Aldrich), according to the manufacturer's instructions. The affinity of biotinylated anti-RNP IgG was evaluated by western blot. The diagnostic specificity and sensibility of antibody were tested by dRIT in positive (n=22) and negative (n=20) CNS samples for rabies suspects of different animal species (bovine, cat, dog, equine and bat), previously analyzed by DFAT. As results, the purified IgG contained one band of molecular weights ranged from 250 to 150 kDa under non-reducing conditions, and two bands, one at approximately of 52-58 kDa (H- chain) and another band at 22-29 kDa (L-chain) under reducing conditions, showing electrophoretic pattern compatible with horse IgG. The biotinylated IgG recognized RNP by western blot. The analyses of samples by dRIT revealed that the biotinylated anti-RNP IgG obtained 100% of diagnostic specificity and sensibility for RABV antigen detection. In conclusion, our results demonstrate that the biotinylated anti-RNP polyclonal IgG may be used as a diagnostic reagent for rabies using dRIT. Thus, this essential diagnostic reagent could be readily available and have a cost reduction helping in the epidemiological surveillance in developing countries This work represents an important step forward in efforts to diagnosis of rabies.

Keywords: Rabies, Antibody, Diagnosis, Immunohistochemistry



FERRITIN, ERYTHROCYTE SEDIMENTATION RATE AND C REACTIVE PROTEIN IN PATIENTS WITH CHIKUNGUNYA VIRUS INDUCED CHRONIC POLYARTHRITIS

Maíra Sant Anna Genaro ^{1,2}, Micheli Said Marchi, Matheus Yung Perin ², Isabelle S. Cósso ², Fábio Alexandre Leal dos Santos ¹, Fábio Assis de Campos Júnior ¹, Renata Dezengrini Silhessarenko ¹
¹ UFMT - Universidade Federal de Mato Grosso (Faculdade de Medicina – UFMT – Bloco CCBSI Av. Fernando Corrêa da Costa, Nº 2367 - Boa Esperança, Cuiabá-MT 78060-900), ² UNIC - Universidade de Cuiabá (Rua: Manoel Jose de Arruda nº 3.100 – Bairro: Jardim Europa - Cuiabá - MT, 78065-900)

Abstract

Chikungunya virus is a global emergent arthritogenic alphavirus transmitted by antropophilic *Stegomyia* mosquitoes related to acute febrile illness and chronic arthralgia in nearly 57% of infected patients. This study was developed to identify possible biomarkers to monitor the chronic articular disease activity. Between June, 2017 and December, 2018, clinical data obtained in trimestral clinical follow ups of patients meeting the criteria established by standard protocols to define Chikungunya chronic articular disease: i. involvement of four or more joints for six weeks or more and ii. infection confirmed by E gene detection by RT-PCR (< 5 days of symptoms) or ELISA anti-CHIKV IgM and IgG (> 5 days of symptoms) were analyzed. Patients were classified according the disease activity score (DAS 28). Erythrocyte sedimentation rate (VHS) and their serum levels of ferritin and C reactive protein (PCR) were measured in the first medical appointment. From 93 patients, 85 (91.4%) are women with median age of 51.9 years, 7.49 affected joins and median DAS 28 of 3.95. Median levels of PCR (9.15 mg/L), VHS (25.7 mm/1sth) and stratified ferritin (191.5 ng/dl for men and 138 ng/dl for women) were considered normal according to reference values. Among then, 13 (14%) patients presented elevated PCR, 12 (92.3%) were women and all with age >50 years. VHS levels were increased in 30 (32.3%) female patients; 4 (4.3%) had concomitant increased VHS and PCR levels. Also, 9 (9.7%) women presented increased ferritin, all >50 years of age; 3 (3.2%) of these patients presented concomitant VHS (n=2) or PCR (n=1) increase. These median values were not significantly elevated in the study population. Conclusion: No significant increase in median levels of inflammatory biomarkers in the presence of moderate chronic joint disease (DAS 28) was observed in the study population. VHS increase correlated with DAS 28 because this variable is used to calculate this index. Additional studies are necessary to elucidate if the combination of these tests may be useful to monitor the progression of chronic articular disease induced by CHIKV. The funding was provided by FAPEMAT.

Keywords: arbovirus, arthritogenic alphavirus, inflammatory biomarkers, pathogenesis, public health



CLINICAL-EPIDEMIOLOGICAL PROFILE OF PEOPLE LIVING WITH HIV-1 SUBMITTED TO VIRAL GENOTYPING TEST IN THE PERIOD FROM 2013 TO 2015, IN THE STATE OF PARÁ

Jennifer Ferreira Viana ¹, Alexandre Augusto Bentaberry Rosa ¹, Aline Cecy Rocha de Lima ¹, Maria Amélia de Oliveira da Costa ^{1,2}, Rubens Einar Corrêa Dantas ^{1,2}, Ednelza da Silva Graça Amoras ¹, Vânia Nakauth Azevedo ¹, Antonio Carlos Rosário Vallinoto ¹, Rosimar Neris Martins Feitosa ¹

¹ UFPA - Universidade Federal do Pará (Rua Augusto Corrêa, 01. Guamá. CEP 66075-110. Belém - Pará), ² LACEN/PA - Laboratório Central do Pará (Av. Augusto Montenegro, 524 - Parque Guajará, Belém - PA, 66823- 010)

Abstract

The human immunodeficiency virus (HIV) weakens the immune system of the virus carrier, reducing the amount of CD4+ T lymphocytes. The ability of this virus to mutate against antiretroviral resistance (ARV) is a major public health problem. The objective of this study was to describe the profile of people living with HIV-1, obtained by genotyping tests, performed from 2013 to 2015, and to correlate the clinical, epidemiological and laboratory information. This was a cross-sectional, retrospective descriptive study, which included patients living with HIV-1 and residing in the state of Pará. All the information obtained was edited and quantified in an Excel spreadsheet. A total of 189 genotyping reports were obtained, the most prevalent being the male gender and age group from 36 to 45 years (33.7%), as well as the brown race (34.92%) and elementary school (...). The majority of patients (63%) were diagnosed with HIV-1 between the years 2000 and 2009, followed by the years 2010 to 2014 (19.0%). In this context, 75.1% changed their therapeutic regimen, 39.2% men and 36% women. Of this total, 86.8% performed from 1 to 5 changes of schemes and only 4.2% performed more than 6 changes, however, about 9% of no information was found in the reports. The most common reasons found in this study that led to the changes were: therapeutic failure and/or intolerance (40%), followed by abandonment of treatment (6.9%), non-adherence (3.7%), pregnancy (3.2%), pregnancy associated with therapeutic failure or intolerance (1.1%), adverse reactions (1.1%) and discontinuation of one of the drugs (0.5%), in addition, 80 (42.3%) reports were found without information about the reasons that led to the changes. When drugs and sex were related in all drug classes, male prevalence was observed for the presence of mutations (ITRN - 51.3%, ITRNN - 38.1% and protease inhibitors - 51.9%) in detriment of female with 47.1% (n=89). However, equality was observed in the absence of mutation for both sexes in all classes. A high rate of exchange of therapeutic regimens was observed, probably generating an increase in expenditure for the purchase of medicines. It also shows the need for better awareness of adherence to therapy and proper completion of patient information. Financial Support: Fadesp.

Keywords: HIV-1, Genotyping, Epidemiology, Resistance, Antiretroviral



CYTOKINES PRODUCTION AND GENETIC VARIABILITY OF HEPATITIS B VIRUS (HBV): INFLUENCE ON THE COURSE OF INFECTION IN PATIENTS WITH ACUTE, CHRONIC AND OCCULT HEPATITIS B

Camilla Rodrigues de Almeida Ribeiro ¹, Vinícius da Motta de Mello ³, Bruna da Silva Baptista ³, Natália Spitz Toledo Dias ¹, Oscar Rafael Carmo Araújo ¹, Iury Amancio Paiva ², Lia Laura Lewis Ximenez ³, Luzia Maria de Oliveira Pinto ², Vanessa Salete de Paula ¹

¹ Fiocruz - Laboratório de Virologia Molecular, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (Av. Brasil 4365 Manguinhos - Rio de Janeiro - RJ), ² Fiocruz - Laboratório de Imunologia Viral, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (Av. Brasil 4365 - Manguinhos - Rio de Janeiro - RJ), ³ Fiocruz - Ambulatório de Hepatites Virais, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (Av. Brasil 4365 - Manguinhos - Rio de Janeiro - RJ)

Abstract

Several viral HBV factors, including viral load, genotype, genome mutations and cytokine production have been reported to be associated with different risks of progression of liver disease. The aim of this study was to investigate the influence of genetic variability of HBV and cytokine production in association with the progression of hepatitis B virus infection in acute and chronic conditions. All samples (n=57) of the study were tested for the presence of HBV DNA by real-time and nested-PCR, positive samples were purified, sequenced and genotyped for phylogenetic tree construction and mutation search. The cytokines (IL-35, IL-6, IL-17A, IFN- γ) were detected by ELISA. Four genotypes were found (A, D, E and F) and the isolates obtained were mostly of genotype A, subgenotype A2. We analyzed 65 mutations in the pre-S/S gene region of these 44 were found. The C48G mutation was found only in acute individuals, this mutation may be involved in the reactivation of HBV during an immunosuppression in patients with resolved infection, which in some cases could lead to severe acute hepatitis, fulminant hepatic failure and death. The F141L mutation that may play a vital role in the pathogenesis of hepatocarcinoma as it induces cell proliferation and transformation has been found only in acute individuals and important mutations such as C105T, C7A and G145K/R that also are involved in increased risk of hepatocarcinoma have been found to be more prevalent in chronic individuals. Genotype A subgenotype A2 showed the highest number of mutations and no mutation was found in genotype D. IL-6 levels (p=0.052) were higher in acute patients, this cytokine would be involved in viral elimination and protection against chronicity. In chronic patients the levels of IFN- γ and IL-17A were higher in comparison to the acute patients, these cytokines would be modulating pro-inflammatory effectors and inducing hepatocellular damage, respectively.

Financial Support: IOC – Fiocruz

Keywords: HEPATITIS B, CYTOKINES, GENETIC VARIABILITY, Disease progression, Mutations



PRODUCTION OF YFV AND HIV VIRUS LIKE PARTICLES (VLPS) USING BACULOVIRUS EXPRESSION SYSTEM AND INSECT CELLS

Roberta Corrêa¹

¹ UnB - Universidade de Brasília (Campus Universitário Darcy Ribeiro)

Abstract

The Yellow Fever Virus (YFV) is a member of the Flaviviridae genus of single stranded RNA viruses that infects humans and are transmitted by mosquitoes. YFV is an endemic virus in Brazil and is a major threat to the public health, especially in low income areas. This virus encodes a polyprotein that is cleaved, producing the three structural proteins that make up the infective virions and seven non-structural proteins. The envelope protein (E) is the most important structural antigen because it binds to cellular receptors and is responsible for the fusion of viral envelope and host membrane. In this work, we constructed four different recombinant baculoviruses containing the E protein gene of YFV and used them to infect insect cells in conjunction with other previously constructed recombinant baculoviruses containing the Human Immunodeficiency Virus GAG protein gene. The aim of this work is the generation of possible chimeric Virus Like Particles (VLPs) which may serve as antigens for vaccination and antibody detection. *The YFE gene was amplified by PCR from a cDNA clone containing the whole YFV genome and cloned in a transfer vector for the construction of four recombinant baculoviruses using the Bac- to-Bac® system (ThermoFisher). Insect cells were infected with the different recombinant baculoviruses and E protein expression and VLP formation was demonstrated analyzed by a combination of western blotting, immunofluorescence microscopy, electron microscopy and low pH membrane fusion assays. The expression of the recombinant proteins (E and GAG) was confirmed by western blot showing the presence of the expected bands using specific antibodies. The immunofluorescence microscopy showed the distribution of the recombinant proteins on the cytoplasm and cell membrane projections. The electron microscopy exhibits the expected size of both E and GAG VLPs and the low pH membrane fusion assay showed the formation of syncytia which is expected since the E protein has membrane fusion activity on the surface of the VLPs. As we have now both E and GAG VLPs correctly expressing the proteins, *in vivo* immunological assays in mammals will be conducted to assess the efficacy of the VLPs as a possible vaccine against YFV.*

Financial Suport: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

Keywords: Baculovirus, Flavivirus, Yellow fever virus, VLP



SURVEILLANCE AND VIRAL DETECTION PROGRAM OF YELLOW FEVER IN NONHUMAN PRIMATES IN METROPOLITAN REGION OF RECIFE - PE

Daniela Bandeira Anastácio ^{2,1}, Daniel Friguglietti Brandespim ¹, George Dimech ², Claudenice Pontes ², Vanuska Valença ², Silvana Leal ², WELLINTON TAVARES DE MELO ², Maria Alice Varjal³, Rita de Cássia Carvalho Maia ¹

¹ UFRPE - Universidade Federal Rural de Pernambuco (Rua Dom Manoel de Medeiros, Dois Irmãos, Recife. PE.), ² SES/PE - Secretaria Estadual de Saúde de Pernambuco (Rua Dona Maria Augusta Nogueira, 519, Bongi - Recife-PE - CEP: 50751-530), ³ Fiocruz PE - Instituto de Pesquisas Aggeu Magalhães (Av. Prof. Moraes Rego, s/n Cidade Universitária, Recife - PE, 50670-420)

Abstract

Yellow Fever (YF) is an acute, non-contagious febrile illness caused by an arbovirus of the Flaviviridae family, which remains endemic or enzootic in some countries in Africa and South America. The World Health Organization estimates that 47 countries are endemic or have endemic areas of Yellow Fever. Transmission can occur through two cycles, one urban and one sylvatic, involving non-human primates (NHP), mainly of the genera *Cebus*, *Alouatta* and *Callithrix*, and mosquitoes of the genera *Haemagogus* and *Sabethes*. The Sylvatic Yellow Fever (SYF) reemergence in the extra-Amazon region in 2007 revived the concern of health authorities about the expansion of viral circulation areas in Brazil. Due to the need for early detection of the virus in the sentinel NHP population, as well as the implementation of control measures to prevent the human population, this study evaluates the implementation of the SYF Surveillance Program in the Recife Metropolitan Region (RMR). In the evaluated period (2017-2018) there was an increase in the number of notifications of epizootic events after the first year of implementation of the Program. The highest number of notifications was from the *Callithrix* genus, and so far only one positive primate for YF has been detected in the state of Pernambuco, suggesting a possible contamination of the sample or trafficking of wild animals from areas with viral circulation. Recife was the municipality with the highest number of notifications in the period evaluated. Given the data acquired during the evaluation of the Surveillance Program, we note the need for greater commitment of the Municipal Health Secretariats in the process of rescue and notification of dead NHP, in order to promote timely surveillance of Yellow Fever in the State of Pernambuco. Surveillance of epizootic diseases is a strategy of great importance for early detection of Yellow Fever Virus, since NHP serve as sentinels and their infection precedes the occurrence of YF in humans.

Keywords: Health Surveillance, Primates, Epizooties, Arboviruses



HEV PREVALENCE AND SEROCONVERSION AMONG KIDNEY TRANSPLANTED PATIENTS IMMUNOSUPPRESSED BY TACROLIMUS

Andreza Salvio Lemos ¹, Camilla Rodrigues de Almeida Ribeiro ¹, Caroline Cordeiro Soares ¹, Rafael Brandão Varella ³, Flavia Savassi-Ribas ², Tereza Cristina Simão Wagner ², Jaqueline Mendes De Oliveira ¹, Marcelo Alves Pinto ¹, Vanessa Salete De Paul ¹

¹ IOC - Instituto Oswaldo Cruz (Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - RJ, 21040-900), ² HESFA - UFRJ - Hospital Escola São Francisco de Assis – Universidade Federal do Rio de Janeiro (Av. Pres. Vargas, 2863 - Centro, Rio de Janeiro - RJ, 20210-030), ³ UFF - Centro de Ciências Médicas, Instituto Biomédico – Universidade Federal Fluminense (R. Prof. Hernani Melo, 101 - São Domingos, Niterói - RJ, 24210-130)

Abstract

Hepatitis E virus is the most recently described human hepatitis virus described and presents 4 different genotypes that can infect humans, each one with different ways of transmission and infection courses. HEV genotypes 3 and 4 (HEV-3, HEV-4) have been associated to persistent or chronic infection in immunocompromised or immunocompromised persons worldwide. Among an immunosuppressed population susceptible to HEV-3 infection, the kidney transplanted people highlights as an increasing group in Brazil. This specific patients are mostly immunosuppressed by Tacrolimus, a calcineurin inhibitor which is known to stimulate HEV replication in solid body receptors, contributing to the development of chronic and persistent hepatitis. Thus, this study aimed to investigate and monitor the prevalence and evolution of HEV infection in tacrolimus immunosuppressed renal transplanted patients. Therefore, 250 plasma samples were collected from 50 kidney transplanted patients during the first year of immunosuppression in a follow-up study. Samples were serologically tested for anti-HEV IgG antibodies at two specific moments after the start of the immunosuppressive treatment: one and twelve months. For comparison to immunocompetent prevalence, 50 plasma samples from blood donors were collected and tested as well. Also, all samples were tested for HEV-RNA detection by a previously optimized RT-qPCR technique. Seroprevalence of 16% (8/50) was found at the first time of collection (1 month after transplant) and a seroprevalence of 20% (10/50) was found at the end of the follow-up period of time (12 months after transplant).

Seroprevalence among blood donors was 6% (3/50), a considered to be average prevalence among immunocompetent people. No sample was positive for HEV-RNA detection. These results show the seroconversion of two patients during the monitoring after one year of immunosuppression. The prevalences were compared to those transplanted from other countries and considered to be high. Seroconversion events reinforce the importance of continuous monitoring of this special group.

Keywords: hepatitis e, HEV, kidney transplant, tacrolimus, seroconversion



CARDIOVASCULAR FINDINGS IN CHIKUNGUNYA CHRONIC ARHTROPATHY PATIENTS DURING AN EPIDEMIC IN CENTRAL- WESTERN BRAZIL

Lucas Silva Dias ¹, Maíra Sant Anna Genaro ^{1,2}, Gilmar Antônio Coelho Damin ¹, Micheli Said De Marchi ², Matheus Yung Perin ², Juliano Rasquin Slhessarenko ¹, Renata Dezengrini Slhessarenko ¹
¹ UFMT - Universidade Federal do Mato Grosso (Cuiabá, MT), ² UNIC - Universidade de Cuiabá (Cuiabá, MT)

Abstract

Chikungunya virus (CHIKV) leads to chronic arthritogenic disease in 57-80% of infected patients. Mato Grosso, Midwestern Brazil experienced a large outbreak of Chikungunya fever during 2016-2018. In the present study, we describe the cardiovascular findings of 31 patients classified according the Ministry of Health diagnostic criteria as having Chikungunya- induced polyarthrits, i.e. acute infection confirmed by CHIKV E gene RT-PCR (< 5 days of symptoms) or anti-IgM and IgG capture ELISA (> 5 days of symptoms) and, arthralgia in four or more joints for more than six weeks. Patients were allocated into two groups; GI: 11 (35.5%) with comorbidities (systemic hypertension, other heart disease or type 2 diabetes), mostly women in a proportion of 9:1 and mean age of 50 years and GII: 20 (64.5%) without concurrent chronic diseases, women in a proportion of 10:1 and mean age of 62 years. Disease activity score 28 (DAS 28) showed high (GI: 5.2) and moderate (GII: 5.0) disease activity whereas Clinical Disease Activity Index (CDAI), was moderate for both groups (20.2 – 20.45). Patients were subjected to C reactive protein (CRP), troponin T, N-terminal natriuretic peptide (NT-proBNP), eletrocardiogram (ECG), echocardiogram (Echo), torax X-ray and cardiac magnetic nuclear resonance (MRI). Patients did not reported any cardiovascular complain during examination. Inflammatory and cardiovascular exams presented adequate results according to age and sex references, except for the Echo and MRI. MRI accused 12 (38.7%) patients, six of them with precedent cardiovascular comorbidities, with cardiac lesions characterized by pericardial effusion (41.7%), delayed contrast enhancement (41.7%) and myocardial fibrosis (16.6%). Statistical analysis did not revealed positive correlation among cardiovascular lesions and chronic articular disease ($p=0.47$; $p=0.60$); a correlation was found only when analyzing mean age and presence of comorbidities ($p=0.0124$). These results may reflect the relatively low number of patients included in this study associated to the existence of previous cardiovascular lesions in these meadle age CHIKV-induced chronic articular disease patients.

Keywords: chronic arthropathy, heart lesions, cardiac magnetic ressonance, CHIKV, arbovirus



PREVIOUS CHIKV EXPOSURE INDUCES PARTIAL CROSS-PROTECTION AGAINST SECONDARY MAYV INFECTION IN MICE

Marcilio Jorge Fumagalli ¹, William Marciel de Souza ¹, Luiza Antunes de Castro Jorge ¹, Renan Villanova Homem de Carvalho ¹, Luiz Gustavo Nogueira de Almeida ¹, Benedito Antônio Lopes da Fonseca ¹, Luiz Tadeu Moraes Figueiredo ¹

¹ USP-RP - Universidade de São Paulo (Av. Bandeirantes 3900, Vila Monte Alegre, Ribeirão Preto - SP)

Abstract

Chikungunya virus (CHIKV) and Mayaro virus (MAYV) are evolutionary closely related members of the Semliki Forest virus antigenic complex, classified into *Alphavirus* genus from *Togaviridae* family. These viruses can cause disease in humans, including symptoms as sudden fever and joint involvement that can persist for long periods. MAYV infection represents a growing concern for public health, which has caused sporadic and geographic limited outbreaks in regions of CHIKV circulation. Previous studies have shown that cross-protection between different alphaviruses can be mediated *in vitro* by broadly neutralizing antibodies specific to conserved epitopes. Given the close phylogeny, symptoms similarities and serological relationship, the aim of this study is to evaluate the cross-protective immunity developed by CHIKV exposure to subsequently MAYV infection. Therefore, we have pre-exposed 6 weeks old immunocompetent mice (C57BL/6) to 1×10^6 PFU of CHIKV by intraperitoneal infection and after 4 weeks they were challenged with MAYV by subcutaneous footpad inoculation. We observed a partial reduction in disease severity and in viral tissue burden, which suggests the development of low cross-neutralizing antibodies against MAYV. Furthermore, a partial reduction of inflammatory monocytes recruitment was observed in the footpad and ankle, which correlates with the reduction of histological score and paw edema development. We are currently evaluating the influence of CHIKV pre-existing immunity against MAYV. In summary, our data suggests a relevant cross-protection between CHIKV and MAYV, which may be important to patient management in the case of MAYV emergence in areas of CHIKV circulation.

Keywords: Antibodies, CHIKV, Cross-protection, MAYV, Mice



RECONSTRUCTING THE DISSEMINATION DYNAMICS OF THE MAJOR HIV-1 SUBTYPE B NON-PANDEMIC LINEAGE CIRCULATING IN BRAZIL

Ighor Leonardo Arantes Gomes ¹, Myuki Alfaia Esashika Crispim ², Mônica Nogueira da Guarda Reis ³, Mariane Martins de Araújo Stefani ³, Gonzalo Bello

¹ IOC - Instituto Oswaldo Cruz (Rio de Janeiro, Brazil), ² HEMOAM - Fundação de Hematologia e Hemoterapia do Amazonas (Manaus, Brazil.), ³ IPTSP-UFG - Instituto de Patologia Tropical e Saúde Pública - Universidade Federal de Goiás (Goiânia, Brazil)

Abstract

The HIV-1 subtype B spread in the Americas from a founder strain probably introduced in the island of Hispaniola (shared by Haiti and the Dominican Republic) Most current subtype B infections are driven by dissemination of a pandemic clade (BPANDEMIC) that spread worldwide from North America; but other ancestral non-pandemic subtype B variants (BCAR) are also detected in the Caribbean region. Such variants of the HIV-1 subtype B accounts for a significant fraction of HIV infections not only in several Caribbean islands, but also in northeastern South American countries and the Northern Brazilian states of Roraima and Amazonas. A comprehensive dataset of HIV-1 subtype B *pol* sequences sampled in Amazonas and Roraima between 2007 and 2017 was used to reconstruct the phylogeographic and demographic dynamics of the major HIV-1 subtype B non-pandemic Brazilian lineage, designated as BCAR-BR-I. The analyses revealed that its origin could be traced to one of many viral introductions from French Guiana and Guyana into northern Brazil that probably occurred in the state of Amazonas around the late 1970s. The BCAR-BR-I clade was rapidly disseminated from Amazonas to Roraima, and the epidemic grew exponentially in these Northern Brazilian states during the 1980s and 1990s, coinciding with a period of economic and fast population growth in the region. The spreading rate of the BCAR-BR-I clade, however, seems to have slowed down since the early 2000s, despite the continued expansion of the HIV-1 epidemic in this country region in the last decade.

Financial Funding: CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) FAPERJ (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

Keywords: HIV-1, Subtype B, non-pandemic, Brazil, Phylodynamics



GENOMIC SURVEILLANCE REVEALS HIDDEN DIVERSITY OF CHIKUNGUNYA VIRUS CIRCULATING IN THE METROPOLITAN REGION OF RIO DE JANEIRO

Filipe Romero Rebello Moreira ¹, Mariane Talon de Menezes ¹, Clarisse Salgado ⁵, Tiago Gräf ⁶, Adriana Melo ², Cristiane Lamas ², Raphael Rangel ², Nuno Faria ⁷, Sergian Cardozo ², Ana Tereza Ribeiro Vasconcelos ⁴, Amilcar Tanuri ¹, Renato Satana de Aguiar ³

¹ UFRJ - UNIVERSIDADE FEDERAL DO RIO DE JANEIRO (Avenida Carlos Chagas Filho, 373. Ilha do Fundão 21.941-617 Rio de Janeiro, RJ, Brasil), ² UNIGRANRIO - UNIVERSIDADE DO GRANDE RIO (Rua Professor José de Souza Herdy, 1160 - Jardim Vinte e Cinco de Agosto, Duque de Caxias - 25071-202, RJ, Brasil), ³ UFMG - UNIVERSIDADE FEDERAL DE MINAS GERAIS (Av. Pres. Antônio Carlos, 6627 - Pampulha, Belo Horizonte - 31270-901, MG, Brasil), ⁴ LNCC - LABORATÓRIO NACIONAL DE COMPUTAÇÃO CIENTÍFICA (Av. Getúlio Vargas, 333 - Quitandinha, Petrópolis - 25651-075, RJ, Brasil), ⁵ LUMC - LEIDEN UNIVERSITY MEDICAL CENTER (Albinusdreef 2, 2333 ZA Leiden, Países Baixos), ⁶ FioCruz - BA - FUNDAÇÃO OSWALDO CRUZ - BAHIA (R. Waldemar Falcão, 121 - Candéal, Salvador - 40296-710, BA, Brasil), ⁷ Oxford - OXFORD UNIVERSITY (Oxford OX1 2JD, Reino Unido)

Abstract

Chikungunya virus (CHIKV) is an alphavirus (family: *Togaviridae*) that causes a febrile illness best characterized by a strong and debilitating arthralgia, the Chikungunya fever. Since 2016 CHIKV is the most prevalent arbovirus circulating in the state of Rio de Janeiro, with all characterized genomes grouping in a monophyletic clade that cluster with a sequence from the Sergipe state (SE) in the ECSA (East-Central-South African) genotype, indicating a single introduction in the region. As the CHIKV epidemic continued to develop through the course of 2018 and 2019, we decided to put efforts on genomic surveillance in order to detect new introductions and recognize possible variants that may be under positive selection, explaining the high current estimated prevalence of about 40% in the population. We screened by Real-Time PCR 179 clinical samples collected between 2017 and 2018, from which 6.1% are positive for ZIKV, 3.9% for DENV and 48.6% for CHIKV. Among the later, we were able to sequence 34 partial genomes (82% coverage on average) and a maximum likelihood phylogenetic analysis revealed that 30 sequences grouped within the previously established Rio de Janeiro clade. Differently, the remaining four sequences clustered in a monophyletic clade near sequences from the Paraíba state (PB), indicating an independent and previously uncharacterized introduction of CHIKV in the state of Rio de Janeiro. These results cast doubts about the current comprehension about the arrival of the virus in the state, as well as the timing of this event.

Financial Support: CNPQ / CAPES

Keywords: Chikungunya, Phylodynamics, Molecular Epidemiology, Genomic Surveillance, CHIKV



INFLAMMATORY CHEMOKINES IN THE SERUM AND CEREBROSPINAL FLUID OF HTLV-1-INFECTED INDIVIDUALS: INSIGHTS INTO THE DEVELOPMENT OF HTLV-1-ASSOCIATED MYELOPATHY

Nicole Lardini Freitas ¹, Flávia Souza dos Santos ¹, Rafael Carvalho Torres ², Álvaro Luiz Bertho ³, Ana Claudia Celestino Bezerra Leite ¹, Marco Antonio Sales Dantas de Lima ¹, Marcus Tullius Teixeira da Silva ¹, Abelardo Queiroz Campos Araújo ¹, Otávio Melo Espíndola ¹

¹ INI - FIOCRUZ - Instituto Nacional de Infectologia Evandro Chagas - FIOCRUZ (Av. Brasil, 4365 - LAPCLIN- NEURO - INI - FIOCRUZ - Manguinhos, Rio de Janeiro/RJ - CEP: 21040-900), ² IBCCF - UFRJ - Instituto de Biofísica Carlos Chagas Filho - UFRJ (Av. Carlos Chagas Filho, 373 - CCS Bloco G Sala G1-019 - Cidade Universitária - Rio de Janeiro/RJ - CEP: 21941-902), ³ IOC - FIOCRUZ - Instituto Oswaldo Cruz - FIOCRUZ (Av. Brasil, 4365 - Plataforma de Citometria de Fluxo - IOC - FIOCRUZ - Manguinhos, Rio de Janeiro/RJ - CEP: 21040-900)

Abstract

Human T-lymphotropic virus type 1 (HTLV-1) infection is related to the development of a slowly progressive neurodegenerative disease that is characterized by lower limb weakness, known as HTLV-1-associated myelopathy/Tropical spastic paraparesis (HAM/TSP). The disease results from death of neurons in the spinal cord due to indirect damage promoted by inflammatory factors released during cytotoxic responses of CD8+ T-cells against infected T-cells. Thus, chemokines may play a role in attracting these cells to the central nervous system (CNS). Therefore, 13 inflammatory chemokines were assessed in serum and cerebrospinal fluid (CSF) samples of HTLV-1 asymptomatic carriers (AC) (n=13) and HAM/TSP patients (n=21). In addition, the expression of chemokine receptors was evaluated in CD4+ T-cells from peripheral blood. Patients with HAM/TSP had increased serum levels of chemokines CXCL9, CXCL10, and CXCL11, which are ligands of the CXCR3 receptor. Conversely, CXCL9 and CXCL11 were at negligible levels in the CSF of these patients, while CXCL10 was elevated and positively correlated with CSF leukocyte counts. The chemokines CCL2, CCL3, CCL4, and CCL17 were also at higher levels in the CSF of HAM/TSP patients compared with AC. Then, the probable direction of cellular trafficking in response to a concentration gradient was defined by calculating the ratio between chemokine levels in CSF and serum. Results showed that CCL2, CXCL8, and CXCL10 were at higher concentration in CSF than in serum of both AC and HAM/TSP patients. In addition, infected and uninfected CD4+ T-cells, identified by staining of HTLV-1 Tax protein, expressed distinct patterns of the chemokine receptors CCR4, CCR5 and CXCR3. Most of Tax(+) CD4+ T-cells expressed CCR4. In addition, these cells presented an upregulated expression of CXCR3 in HAM/TSP patients compared with AC. Indeed, Tax(-) CD4+ T-cells of HAM/TSP patients also displayed higher rates of CXCR3. Taking these data together, CXCR3 may play a central role in directing the migration of infected and inflammatory CD4+ T-cells from peripheral blood into the CNS of HTLV-1-infected individuals in response to CXCL10. It is possible that cell signaling mediated by CCL2 and CXCL8 is also involved in the development of HAM/TSP by attracting innate mononuclear cells into the CNS, such as monocytes and NK cells. Financial support: PROAP/CAPES and INOVA-FIOCRUZ.

Keywords: CXCL10, CXCR3, HAM/TSP, HTLV-1, Tax protein



MOLECULAR CHARACTERIZATION OF OCCULT HEPATITIS B VIRUS IN PATIENTS WITH HEPATITIS C BEFORE TREATMENT WITH ORAL DIRECT-ACTING ANTIVIRALS (DAAS)

Nathália Alves Araujo de Almeida ¹, José Junior José Júnior França de Barros ¹, Natalia Natália Spitz Toledo Dias ¹, Letícia Letícia Bomfim Campos ¹, Marcia Amendola Marcia Maria Amendola Pires ², Carlos Eduardo Brandão Carlos Eduardo Brandão Mello ², Vanessa Vanessa Salete de Paula ^{1,2}

¹ Fiocruz - Instituto Oswaldo Cruz- Fiocruz (Laboratório de Virologia Molecular), ² HUGG - Hospital Universitário Gaffrée e Guinle (Ambulatório de Gastroenterologia)

Abstract

Hepatitis B virus (HBV) infection is a worldwide public health problem, even with the existence of an effective vaccine. Coinfection for HBV and hepatitis C virus (HCV) in patients with chronic hepatitis is common in vulnerable populations, mainly due to the similar form of transmission. Occult hepatitis B virus (OBI) infection is one of the most challenging complications in the field of viral hepatitis. The OBI frequency in Brazil is 3.5%, this context may develop of severe liver disease, such as cirrhosis and hepatocellular carcinoma and even death. Host factors and viral factors of OBI induction may be associated with mutations, especially in S protein and co-infection with other viruses how the HCV. OBI is determined by the absence of HBsAg and presence of HBV DNA in liver or serum of infected patients. The clinical and virology relevance of OBI in patients treated with new direct-acting antivirals (DAA) for hepatitis C virus (HCV) infections is currently a hotly debated topic. In cases where HBsAg is not detected, DAA treatment is often initiated without examination for the presence of HBV DNA. In this sense, the objective of the study was to identify the presence of OBI and HBV genotypes in patients with chronic HCV before treatment with the new DAA. OBI was investigated in 128 patients with HCV without HBsAg prior to the application of DAA that did not respond to pegylated interferon and ribavirin (PEG-INF and RBV) treatment by real-time PCR (qPCR), nested PCR of the S region and mutational analyzes of genes. The incidence of OBI was 8.5% (11/128), in which the mean age of the patients was 65.6 years patients developed ALT and AST flare-ups and all were anti-HB-positive, with viral loads ranging from $(8.5 \times 10^2$ to 1.49×10^8 copies/mL), with genotypes 1 and 3 of HCV. Three patients were identified as HBV two A1 and one A2 genotypes without surface protein mutations. The OBI rate among the study population was high when compared to the general population. Therefore, screening for OBI should be a routine practice in patients undergoing treatment with DAA. Amino acid substitutions have not been observed, however, further investigations are required for mutational analysis.

Keywords: Occult hepatitis B, direct-acting antivirals, hepatitis C vírus



DEVELOPMENT OF A MULTIPLEX REAL-TIME PCR WITH HIGH RESOLUTION MELTING (HRM) ANALYSIS FOR THE SIMULTANEOUS DETECTION OF SIX HUMAN HERPESVIRUSES.

Bruna de Moraes Guedes dos Santos ¹, Marco Antonio Sales Dantas de Lima ¹, Abelardo Queiroz Campos Araújo

¹, Otávio Melo Espíndola ¹

¹ INI - FIOCRUZ - Instituto Nacional de Infectologia Evandro Chagas - FIOCRUZ (Av. Brasil, 4365, LAPCLIN - NEURO - INI - FIOCRUZ - Manguinhos, Rio de Janeiro / RJ - CEP: 21040-900)

Abstract

Sporadic cases of encephalitis and meningitis in both immunocompetent individuals and immunosuppressed patients are commonly associated with infections by herpes simplex virus (HSV) 1 and 2, varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human herpesvirus (HHV-6). Currently, PCR is recognized as the gold standard method for the diagnosis of viral infections in the central nervous system, and the development of high-resolution melting (HRM) analysis has allowed the establishment of multiplex assays. Thus, our aim was to develop a multiplex real-time PCR with HRM analysis for the simultaneous detection of HSV-1/2, VZV, EBV, CMV and HHV-6. Initially, primers from the literature were tested *in silico* for the formation of primer-dimers and/or hybridization with human DNA, in order to avoid non-specific amplification. The specificity of selected primers was assayed with commercially available human and viral DNA controls. Subsequently, amplicons from reactions with primers selected for each virus were cloned into plasmids to construct amplification controls. Optimal temperature for the annealing/extension step was tested in a range between 60°C to 70°C, and the performance of individual and multiplex PCR assays were determined with standard curves produced with 10-fold serial dilution of plasmids. The temperature of 70°C was chosen because reactions carried out with lower temperatures developed primer-dimers and/or non-specific amplification. Clear identification of each virus was possible since their amplicons had distinct melting temperatures (T_m): HSV-1, 92.08°C; HSV-2, 91.70°C; VZV, 82.08°C; EBV, 88.17°C; CMV

85.25°C; HHV-6, 79.65°C; with a low inter-assay variation (standard deviation of ±0.2°C). Moreover, rates of amplification efficiency in individual and multiplex reactions were between 93% and 105%. In conclusion, this PCR with HRM analysis represents an innovative and cost-effective assay for the detection of multiple human herpesviruses. However, further investigations are needed to determine its accuracy in the diagnosis of neuroinfections by herpesvirus. Financial support: FIOCRUZ.

Keywords: human herpesviruses, real-time PCR, high resolution melting



THE RISK FACTORS ASSOCIATED IN THE HIGH FREQUENCY OF MUTATIONS L91M IN CORE PROTEINS HEPATITIS C VIRUS (HCV) OF RIO DE JANEIRO

Nathália Alves Araujo de Almeida ¹, Letícia Letícia Bomfim Campos ¹, Kycia Kycia Maria Rodrigues do Ó ³, Marcia Amendola Marcia Maria Amendola Pires ², Carlos Eduardo Brandão Carlos Eduardo Brandão Mello ², José Junior José Júnior França de Barros ¹, Vanessa Vanessa Salet de Paula ¹

¹ Fiocruz - Instituto Oswaldo Cruz- Fiocruz (Laboratório de Virologia Molecular), ² HUGG - Hospital Universitário Gaffrée e Guinle (Ambulatório de Gastroenterologia), ³ MS - MEMBRO DO COMITÊ TÉCNICO ASSESSOR DAS HEPATITES VIRAIS DO MINISTÉRIO DA SAÚDE (BRASÍLIA - DF - BRASIL)

Abstract

Approximately 71 million people worldwide are infected with the hepatitis C virus (HCV), and infections with HCV genotype 1 that still predominate (in 48% of HCV infected people). In 60–80% of cases, infection results in the chronic liver disease, 10 to 25% progress to cirrhosis and hepatocellular carcinoma (HCC), the second most common cause of cancer death worldwide after the lung cancer. HCV infection is associated with at least half of the HCC cases. In patients with hepatitis C, treatment with the direct- acting antivirals (DAAs) of the second generation results in the sustained virological response (SVR) in 98- 99% cases. Unfortunately, due to the high cost and limited availability of DAAs, clinical practice in low to moderate income countries still relies on the pegylated interferon and ribavirin (PEG-IFN/RBV) therapy. The objective this study was to estimate the frequency of L91M mutations and associated risk factors in treated and untreated HCV patients. We analyzed 163 samples of serum genotypes, 80 genotype 1a and 83 genotype 1b. HCV-RNA extraction, partial amplification of the HCV core region (354bp) was performed by RT-PCR and to assess risk factors associated with HCV-RNA statistical analysis was done using R Studio software. The presence of the mutation L91M was found only in 1b patients, with a frequency of 39,8% (65/163). Individuals treated with PEG-IFN / RBV were 6.7 times more likely (CI95% 1.9-29.5) to have a mutation compared to untreated individuals (NAIVE), demonstrating the impact of this mutation on response to interferon- based treatment. Another important associated factor was in cirrhotic individuals with more chance than non-cirrhotics (95% CI 4.2-86.7), this can be a serious complication as the development of HCC is correlated with cirrhosis. Others risk factors were associated with: Individuals with platelet count that is low (95% CI 3.9-19.2), high glucose level (95% CI 4.9-32.1), diabetics (95% CI 4.6- 20.2), and females (95% CI 1.03-14.3). The study highlights the importance of these mutations for the therapeutic response to Interferon treatment and the clinical progression of HCV, especially their identification in cases of cirrhosis, since the development of HCC is closely linked to fibrogenesis.

Keywords: Hepatitis C virus, mutation, treatment, cirrhosis



EVALUATION OF ZIKA VIRUS MARKERS DETECTION IN DIFFERENT SAMPLES FROM PREGNANT MONKEYS UNDER SOFOSBUVIR TREATMENT

Catarina Eugenia de Lima Menezes ^{1,2}, Noemi Rovaris Gardinali ^{1,2}, Sheila Maria Barbosa de Lima ², Tatiana Kugelmeier ³, Márcia Cristina Ribeiro Andrade ³, Thiago Moreno Lopes e Souza ⁴, Patrícia Cristina da Costa Neves ², Jaqueline Mendes de Oliveira ¹, Marcelo Alves Pinto ¹, Juliana Gil Melgaço ^{1,2}

¹ IOC - Instituto Oswaldo Cruz (Rio de Janeiro, Brasil), ² Biomanguinhos - Instituto de Tecnologia em Imunobiológicos (Rio de Janeiro, Brasil), ³ ICTB - Instituto de Ciência e Tecnologia em Biomodelos (Rio de Janeiro, Brasil), ⁴ CDTs - Centro de Desenvolvimento Tecnológico em Saúde (Rio de Janeiro, Brasil)

Abstract

The NS5B polymerase inhibitor sofosbuvir (SOF), currently used for the treatment of chronic hepatitis C virus (HCV) infection, has been shown to be effective against Zika virus (ZIKV), a mosquito-borne flavivirus. Recently, SOF therapy was shown to prevent the vertical transmission of ZIKV to *in vivo* mice models. Congenital Zika syndrome (CZS) is the most severe complication related to maternal-fetal transmission. Considering the evaluation of an antiviral treatment, detection of viral RNA and soluble antigens produced are essential to assess the therapy efficacy on viral elimination, mainly on pregnant women. Here, we aimed to investigate the period of ZIKV detection in blood, urine, saliva and vaginal fluid samples using RT-qPCR to assess viral RNA and an *in house* immunoblot assay to assess viral envelope protein in pregnant dams SOF treated (SOF-t) (n=3) and nontreated (NT) (n=4). Pregnant macaques (under the first gestational trimester) were inoculated with 7log₁₀ PFU of ZIKV. The antiviral therapy consisted of 5mg SOF/kg/day administered subcutaneously during 15 days, from the second day post infection (dpi). Our results showed a slightly decrease of RNAemia in blood (log₁₀ copies/mL[mean]=2.25 (SOF-t) vs 1.19 (NT)), as well as a shortened period of viral RNA detection in blood (dpi[mean]=8 (SOF-t) vs 4 (NT)), and in vaginal fluid (dpi[mean]=18 (SOF-t) vs 8 (NT)) in monkeys treated with SOF. However, the difference was not observed in urine (dpi[mean]=11 (SOF-t) vs 13 (NT)) and in saliva samples (dpi[mean]= 5 (SOF-t) vs 4 (NT)) on viral RNA period detection during SOF therapy. Moreover, using the *in house* immunoblot assay, 14,8µg/mL of Pan-flavivirus antibody was sufficient to detect Zika virus envelope (ZIKVenv) protein in human samples (plasma ZIKV positive at viral loads of 1-6log₁₀ copies/mL), and in one saliva sample from a pregnant monkey up to 10dpi, even in the absence of viral RNA detection. Therefore, these are preliminary results, and additional assays are in progress. In conclusion, our findings showed a positive effect of SOF therapy, by decreasing RNAemia and shortening the detection period of viral RNA in different samples on pregnant monkeys. Besides, our immunoblot assay may be promising for screening test detecting flavivirus antigens using non-invasive samples, such as saliva. Financial Support: Faperj, CNPq, International Development Research Centre – Canadá, Biomanguinhos, Instituto Oswaldo Cruz.

Keywords: Zika, Sofosbuvir, Macaca Mulatta, Viral Markers



INTERLEUKIN 27 PROMOTES DIVERGENT EFFECTS ON HIV-1 REPLICATION IN PERIPHERAL BLOOD MONONUCLEAR CELLS THROUGH BST-2/TETHERIN.

Jairo Ramos Temerozo ^{1,2}, Pedro Lourenço Camara Ferreira ¹, Livia Gobbo ¹, Leandra Linhares Lacerda ^{3,2}, Bruno Cister-Alves ¹, Marcelo Ribeiro Alves ^{4,2}, Rubem Figueiredo Sadok Menna-Barreto ¹, Dumith Chequer Bou-Habib^{1,2}

¹ LPT-IOC/Fiocruz - Laboratory on Thymus Research - Oswaldo Cruz Institute/Fiocruz (Manguinhos - Rio de Janeiro - RJ - Brasil), ² INCT-NI - National Institute of Science and Technology on Neuroimmunomodulation (Av. Brasil, 4365 - Manguinhos - Rio de Janeiro - RJ - Brasil), ³ LIL/UFRJ - Laboratory of Immunobiology of Leishmaniasis (Department of Immunology, Paulo de Goes Institute of Microbiology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil), ⁴ IPEC/Fiocruz - HIV/AIDS Clinical Research Center, Evandro Chagas National Institute of Infectology/Fiocruz (Manguinhos - Rio de Janeiro - RJ - Brasil)

Abstract

Interleukin (IL)-27 is able to inhibit HIV-1 replication in peripheral blood mononuclear cells (PBMCs), macrophages and dendritic cells. Here, we identify that IL-27 can produce opposing effects on HIV-1 replication in PBMCs and that the HIV-1 restriction factor BST-2/Tetherin is involved in both the inhibitory and enhancing effects on HIV-1 infection induced by IL-27. IL-27 inhibited HIV-1 replication when added to cells before or immediately after infection and promoted the prototypical BST-2/Tetherin-induced virion accumulation at the cell membrane of HIV-1-infected PBMCs. BST-2/Tetherin gene expression was significantly upregulated in the IL-27-treated PBMCs, with a simultaneous increase in the number of BST-2/Tetherin⁺ cells, and the silencing of BST-2/Tetherin reduced the anti-HIV-1 effect of IL-27. However, IL-27 also enhanced HIV-1 production when added later to infected cells, and this effect was prevented by BST-2/Tetherin gene knockdown, which also permitted IL-27 to function again as an HIV-1 inhibitory factor. These contrasting roles of IL-27 were associated with the dynamic of viral production since the addition of IL-27 to antiretroviral drug-treated cells infected for four days, as well keeping cells under agitation to avoid cell-to-cell contact, prevented the enhancement of virus replication. Our findings may impact the potential therapeutic use of IL-27 and other soluble mediators that induce BST-2/Tetherin expression for HIV-1 infection.

Keywords: HIV-1, IL-27, BST-2, Tetherin, PBMCs



INJURY IN MULTIPLE ORGANS OF FATAL CASES DENGUE IN CHILDREN: VIRAL DETECTION AND PROFILE OF CYTOKINES.

Leandro Junqueira Moragas ¹, Natalia Gedeão Salomão Natalia Gedeão Salomão ¹, Felipe Alves Felipe Alves ¹, Lucca Siqueira Lucca Siqueira ¹, Kissila Rabelo Kissila Rabelo ², Ronaldo Mohana-Borges Ronaldo Mohana-Borges

³, Fernando Rosman Fernando Rosman ⁴, Marciano Viana Paes Marciano Viana Paes ¹

¹ LIPMed FioCruz - IOC - Laboratório Interdisciplinar de Pesquisas Médicas - Instituto Oswaldo Cruz (Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - RJ, 21040-900), ² UERJ - Laboratório de Ultraestrutura e Biologia Tecidual - Universidade do Estado do Rio de Janeiro (R. São Francisco Xavier, 524 - Maracanã, Rio de Janeiro - RJ, 20550-170), ³ UFRJ - Laboratório de Genômica Estrutural - Instituto de Biofísica Carlos Chagas Filho - Universidade Federal do Rio de Janeiro (Av. Carlos Chagas Filho, 373 - Cidade Universitária da Universidade Federal do Rio de Janeiro, Rio de Janeiro - RJ, 21941-170), ⁴ HMJ - Anatomia Patológica do Hospital Municipal Jesus, Rio de Janeiro, Brasil (R. Oito de Dezembro, 717 - Vila Isabel, Rio de Janeiro - RJ, 20550-200)

Abstract

Dengue is an important arbovirus today due to its high mortality and morbidity rates. In most cases, symptoms are self-limiting, but some cases may develop into severe forms and death. The pathogenesis is not yet fully elucidated, but it is known that liver and lung are the main targets of the virus, causing painful hepatomegaly and increased liver transaminases and hemorrhage, edema and thickening membrane in the lung. This study aims to investigate liver, lung, tracheal and tongue changes caused by DENV in three fatal cases of dengue (IgM+) in children (8 to 12 years) hospitalized in Municipal Jesus Hospital (Rio de Janeiro) from 2008 to 2012. In the liver was observed areas of hepatocytes necrosis, macrovesicular and microvesicular steatosis associated with bleeding areas, dilatation of sinusoidal capillaries, mononuclear infiltrates in sinusoidal capillaries and in the portal space. At lung was showed diffuse areas of septal thickening, edema, and hemorrhage with the presence of mononuclear infiltrates and hyperplastic alveolar macrophages. Focal tracheitis with squamous metaplasia and necrosis in the mucosa and bleeding in the tracheal lumen. Tongue was also observed hemorrhages and hyperplastic lymphoid follicle. NS3 viral protein was detected in hepatocytes, Kupffer cells, circulating macrophages and endothelial cells in liver tissue; alveolar macrophages and type II pneumocytes in the lung; endothelial cells and monocytes in the tracheal lamina propria; and in macrophages around the muscle bundle below the tongue epithelium, showing its replication in these places. TNF α expression was observed in macrophages, Kupffer cells and portal space endothelial cells and in the centrilobular vein endothelium. In addition to a quantitative increase in TCD8 + lymphocytes in the portal space and pulmonary capillaries. The expression of RANTES, VCAM 1 and VEGF R2 was shown in sinusoidal capillary endothelial cells and portal space suggesting a change in vascular permeability in liver tissue. These results will contribute to the investigation of the main cells involved in dengue pathogenesis, elucidating the mechanisms involved during dengue infection in children.

Financial Support: Programa de Excelência Acadêmica - CAPES

Keywords: dengue fever, children, cytokines, pathogenesis, ns3



DEVELOPMENT OF AN ARBOVIRAL LUMINEX SEROLOGICAL ASSAY BASED ON NON-STRUCTURAL PROTEINS

Robert Andreato Santos¹, Jessica Vanhomwegen², Damien Hoinard², Mônica Josiane Rodrigues de Jesus¹, Séverine Matheus², Jean-Claude Manuguerra², Luís Carlos de Souza Ferreira¹

¹ USP - Universidade de São Paulo (Instituto de Ciências Biomédicas 2, Avenida Professor Lineu Prestes, 1374, sala 118, Laboratório de Desenvolvimento de Vacinas.), ² IP - Instituto Pasteur (25-28 Rue du Dr Roux, 75015 Paris, França)

Abstract

Dengue (DENV) and Zika (ZIKV) are the most evident arboviruses in the current international scenario. As these two viruses are transmitted by *Aedes* mosquitoes and co-circulate in the same geographic region, the generation of accurate epidemiological data in endemic areas, such as Brazil, is extremely difficult. This fact is a consequence of the high similarity among the clinical symptoms of the diseases as well as the extensive cross-reaction observed in serological tests. The availability of sensitive, specific and low-cost serological tests, especially for ZIKV, is still limited in the country. Thus, the aim of this work was to develop a multiplex serological test based on the Luminex platform with the use of a recombinant form of the non-structural protein 1 (Δ NS1) of DENV1-4 and ZIKV. DENV1 Δ NS1 coupling to magnetic beads was optimized and validated in the Luminex platform against a DENV1 positive serum panel. The amount of antigen, dialysis steps and three different sera dilutions were evaluated. ROC curves performances were used as criteria to select coupling parameters of each Δ NS1 protein with the respective bead batch. DENV1-positive samples showed cross-reaction with the ZIKV protein, indicating that optimal conditions need to be achieved to differentiate specific antibodies. However, no cross-reaction was observed when we evaluated ZIKV primary positive sera. These results indicate that the multiplex Luminex-based serological detection platform can differentiate DENV and ZIKV. **Financial support:** FAPESP 2016/23560-0, 2016/20045-7; CAPES: 88881.130787/2016-01 and CNPq: 440409/2016-0.

Keywords: Diagnosis, Serology, Dengue, Zika, Multiplex



DEMYELINATING DISEASES ASSOCIATED TO CHIKUNGUNYA VIRUS INFECTION IN RIO DE JANEIRO/BRAZIL: ACUTE DISSEMINATED ENCEPHALOMYELITIS AND TRANSVERSE MYELITIS

Alice Laschuk Herlinger ¹, Fabrícia Lima Fontes-Dantas ², Mariane Talon de Menezes ¹, Marcelo Calado de Paula Tôrres ¹, Liane Ribeiro ¹, Richard Araújo Maia ¹, Cláudia Rêgo ², Amanda Dutra de Araújo ², João Paulo da Costa Gonçalves ², Fernanda Rueda-Lopes ³, Cláudia Cristina Ferreira Vasconcelos ⁴, Osvaldo Nascimento ³, Orlando C. Ferreira ¹, Amilcar Tanuri ¹, Soniza Vieira Alves-Leon ^{2,5}, Renato Santana Aguiar ^{1,6}

¹ LVM/IB/UFRJ - Laboratório de Virologia Molecular, Instituto de Biologia, Universidade Federal do Rio de Janeiro (Av. Carlos Chagas Filho, 373 - CCS, Bloco A - Cidade Universitária - CEP 21941-970 - Rio de Janeiro/RJ), ² LabNet/ICB/UFRJ - Laboratório de Neurociências Translacional, Instituto Biomédico, Universidade Federal do Rio de Janeiro (Av. Carlos Chagas Filho, 373 - CCS, Bloco K - Cidade Universitária - CEP 21941-970 - Rio de Janeiro/RJ), ³ HUAP-UFF - Hospital Antonio Pedro da Universidade Federal Fluminense (Av. Marquês do Paraná, 303 - Centro CEP 24033-900 - Niterói/RJ), ⁴ PPGNEURO/UNIRIO - Programa de Pós-Graduação em Neurologia da Universidade Federal do Estado do Rio de Janeiro (Av. Pasteur, 296 - Urca - CEP 22290-240 - Rio de Janeiro/RJ), ⁵ HUCFF/UFRJ - Hospital Universitário Clementino Fraga Filho da Universidade Federal do Rio de Janeiro (Rua Prof. Rodolpho Paulo Rocco, 255 - Ilha do Fundão - CEP 21941-590 - Rio de Janeiro/RJ), ⁶ DGEE/ICB/UFMG - Departamento de Genética, Ecologia e Evolução, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (Av. Antônio Carlos, 6627 - Pampulha - CEP 31270-901 - Belo Horizonte/MG)

Abstract

Chikungunya (CHIKV; *Flavaviridae*, *Togaviridae*) fever common symptoms are similar to other arbovirus. However, its neurological manifestations, although less common, seem to be rising in recent years. Immune-mediated demyelinating diseases, such as acute disseminated encephalomyelitis (ADEM) and transverse myelitis (TM), have already been reported postinfection; however with only few isolated cases associated to CHIKV described. Herein we describe five confirmed cases of ADEM and TM associated to CHIKV infection. Patients were evaluated by neurologists in healthcare units from Rio de Janeiro/RJ-Brazil between July 2016 and June 2019, and submitted to magnetic resonance imaging (MRI) in order to provide accurate diagnosis. Blood, cerebrospinal fluid and/or urine samples were used for arbovirus infection diagnosis, which included serological and molecular tests for Zika, Dengue and CHIKV. Serological diagnosis was performed by ELISA (IgG and IgM), and positive cases were further confirmed by plaque reduction neutralization test (PRNT). Also, qPCR was performed in order to identify viral genome. Among the 270 evaluated patients, five were diagnosed with either ADEM or TM following CHIKV infection. Patients median age was 45 years-old (range: 16-61), 60% being males. The most common symptoms were fever, arthralgia and myalgia. As revealed by ELISA, CHIKV-specific IgM was detected in all five neurological patients; indicating acute CHIKV infection, which was further confirmed by PRNT. On the other hand, CHIKV genome was detected in only two of these patients. Of note, neither IgM nor viral genome for any other tested arbovirus was detected. The present work shows a high prevalence of neurological cases of CHIKV in Rio de Janeiro, which is unprecedented, as only scarce cases have been reported so far. Further viral genome and exome analyses will allow us to determine whether viral or host characteristics, or a combination of both, are driving such neurological manifestations associated to CHIKV infection. In spite of that, the clinical mimicry with other demyelinating



diseases obliges the inclusion of arboviruses among the differential diagnosis of ADEM and acute TM.

Financial support: CAPES, FAPERJ, CNPq, DECIT/RENEZIKA

Keywords: ADEM, Arbovirus, Chikungunya, Demyelinating diseases, Transverse myelitis



PREVALENCE AND MOLECULAR CHARACTERIZATION OF HUMAN BOCAVIRUS IN ACUTE GASTROENTERITIS CASES IN BRAZIL, 2016 - 2017.

Fábio Correia Malta ^{2,1}, Sylvia Kahwage Sarmiento ¹, Maria Angelica Arpon Marandino Guimarães ², Rafael Brandão Varella ³, Marize Pereira Miagostovich ¹, Tulio Machado Fumian ¹

¹ LVCA - Laboratório de Virologia Comparada e Ambiental - FUNDAÇÃO OSWALDO CRUZ (AV. BRASIL, 4365), ² UFRJ - Faculdade de Medicina - Universidade Federal do Rio de Janeiro. (R. Prof. Rodolpho Paulo Rocco, 255 - Ilha do Fundão, Rio de Janeiro - RJ, 21941-590), ³ UFF - Departamento de Microbiologia e Parasitologia - Universidade Federal Fluminense. (R. Prof. Hernani Melo, 101 - São Domingos, Niterói - RJ, 24210-130)

Abstract

Acute gastroenteritis (AGE) is considered a major cause of morbidity and mortality in children under five years old, mainly in low-income countries. Human Bocavirus (HBoV) is an emerging virus that belongs to the *Parvoviridae* family, genus *Bocaparvovirus*, and divided in four genotypes 1-4. HBoV was first described in 2005, and has been detected worldwide, especially in pediatric patients with respiratory and gastrointestinal infection. Co-detection of HBoV with other gastroenteric viruses has been widely reported. Here, we describe the prevalence and the viral load of HBoV in fecal samples of the Brazilian children up to two years old with AGE. Positive samples were molecularly characterized by sequencing of VP1/2 overlap region, and genotypes were assigned using BLAST. Between January 2016 and December 2017, a total of 886 fecal samples were collected in ten Brazilian states from three different regions. Multiplex real-time quantitative PCR assay, targeting the UTR/NS1 genes junction, was used to detect and quantify HBoV DNA from samples. Other gastroenteric viruses, as rotavirus A, norovirus GI and GII, adenovirus, astrovirus and sapovirus were also detected by qPCR or RT-qPCR. Among fecal samples analyzed in 2016 (n=478), 73 (15.27%) were positive for HBoV. Single infection represented 16.4% (n=12), and co-infection was detected in 83.6% (n=61) of HBoV-positive samples. The viral load ranged from 1.3×10^3 to 2.6×10^9 genome copies per gram (GC/g) of stool. Phylogenetic analysis identified the presence of HBoV-1 in 31.5%, HBoV-2 in 39.7% and HBoV-3 in 28.7% among the positive samples. In 2017, it was analyzed 408 fecal samples, and HBoV was detected in 37 samples (9.1%), being 8 (21.6%) single detection, and 29 (78.4%) co-infection with other gastroenteric viruses. The viral load of HBoV ranged from 1.6×10^2 to 9.6×10^8 GC/g stool and the genotypes detected were HBoV-1 (62.2%), HBoV-2 (32.4%) and HBoV-3 (5.4%). Our data demonstrate the circulation of HBoV 1-3 in the Northeast, Southeast and South of Brazil in the years 2016 and 2017 and highlight the importance of surveillance of HBoV causing acute gastroenteritis as single or co-infection pathogen.

This study was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico and Oswaldo Cruz Institute, PAPES VII (IOC/FIOCRUZ).

Keywords: Human Bocavirus, qPCR, acute gastroenteritis, Molecular characterization, Brazil



MOLECULAR INVESTIGATION OF ENTERIC VIRUSES IN THE ETIOLOGY OF THE CENTRAL NERVOUS SYSTEM VIRAL INFECTIONS IN CEREBROSPINAL FLUID SAMPLES FROM THE HOSPITAL DE BASE OF SÃO JOSÉ DO RIO PRETO - SP, 2016- 2017.

Leonardo Cecílio Rocha ¹, Cassia Fernanda Estofolete ¹, Bruno Henrique G. de Aguiar Milhim ¹, Marcos Tayar Augusto ¹, Antonio Charlys da Costa ³, Ester Cerdeira Sabino ³, Mariana Sequetin Cunha ², Mauricio Lacerda Nogueira ¹, Adriana Luchs ², Ana Carolina Bernardes Terzian ¹

¹ FAMERP - Faculdade de Medicina de São José do Rio Preto (Av. Brigadeiro Faria Lima, 5416, Vila São Pedro, São José do Rio Preto, SP), ² IAL - Instituto Adolfo Lutz (Av. Dr. Arnaldo, 355, Cerqueira César, São Paulo, SP), ³ USP - Universidade de São Paulo (Av. Dr. Enéas de Carvalho Aguiar, 470 Jardim America, São Paulo - SP)

Abstract

Enteroviruses (EVs) are associated with a wide spectrum of human diseases ranging from febrile disease to central nervous system (CNS) syndromes. Recently, gastroenteric viruses have also been associated with CNS neurological disorders. The aim of the present study was to screen cerebrospinal fluid samples (CSF) for EV and gastroenteric viruses (Rotavirus-RVA, Norovirus-NoV and Classical Astrovirus-AstV) in order to ascertain the diagnostic in CNS infection cases with unknown etiology. This is a retrospective study conducted with convenience clinical samples collected from patients attended at the reference Hospital de Base of São José do Rio Preto-SP during 2016-2017. A total of 289 CSF samples were tested for the presence of EV and NoV by real-time (q)RT-PCR, AstV by conventional RT-PCR, and RVA by ELISA. NoV, RVA and AstV were not detected. EV infection was detected in 5.9% of samples (17/289). The region encoding EV VP1 was used in semi-nested RT-PCR directly from positive clinical samples for strain characterization. In parallel, positive EV clinical samples were inoculated in RD and HEp 2 cell lines to prove virus infectivity, and potentially identify EV strains that failed to direct molecular methods. A total of five samples (5/17) were successfully amplified and sequenced in order to determine the genotype. Three different serotypes were identified: Echovirus 3 (E3) (1/5), Coxsackie virus A6 (CVA6) (1/5) and Coxsackie virus B4 (CVB4) (3/5). CVB4 are commonly associated to EV infections and CSF was the most common source of its detection. However, in Brazil, limited data are available regarding CVB4 incidence and its association with CNS infections. E3 was the most commonly detected EV in 1970. Nevertheless, after the 90s, this serotype has become very uncommon, and there are no reports of its detection in Brazil. CVA6 has recently emerged as a major cause of hand, foot and mouth disease worldwide. The CNS infection surveillance proposed in the present study offered an opportunity to identify for the first time in Brazil CVA6 and E3 serotypes. Identifying the circulating EVs can help to elucidate the enteroviral biodiversity, improving our understanding of their potential health burden, and enabling a prompt response in case of outbreaks. This study emphasizes the need to intensify laboratory studies on EV-associated CNS infections in Brazil.

Keywords: Enteroviruses, Gastroenteritis, Neurological Disorders



PREVALENCE AND GENOTYPING OF HUMAN PEGIVIRUS-1 (HPGV-1) IN BLOOD DONORS AND HCV AND HIV INFECTED INDIVIDUALS FROM RIO DE JANEIRO, BRAZIL.

Jéssica Gonçalves Pereira ¹, José Junior França de Barros ¹, Carlos Eduardo Brandão ², Vanessa Salete de Paula¹, Caroline Cordeiro Soares ¹

¹ IOC - Instituto Oswaldo Cruz (Avenida Brasil, 4365 - Manguinhos / Pavilhão Helio e Peggy Pereira - LVM), ² HUGG - Hospital Universitário Gaffrée e Guinle (R. Mariz e Barros, 775 - Maracanã, Rio de Janeiro - RJ, 20270- 001)

Abstract

Human pegivirus-1 (HPgV-1), formerly known as GB virus C, is a member of the Flaviviridae family of single-stranded, positive-sense RNA viruses and has genomic similarity to hepatitis C virus (HCV). However, unlike HCV, HPgV-1 is lymphotropic and establishes a subclinical infection. Several studies reported that HPgV-1 infection is associated with delayed HIV disease progression and in patients with chronic hepatitis C and HIV coinfection, HPgV-1 RNA was associated with significantly lower ALT and AST levels and an improvement in cirrhosis-free survival, suggesting a beneficial effect of HPgV-1 infection on chronic hepatitis C. To better understand the impact of HPgV in co-infections, it is needed to know epidemiological characteristics of this virus. The aim of this study was to determine the prevalence and genotypic distribution of HPgV-1 in blood donors and HCV and HIV patients attended at a hospital in Rio de Janeiro. A RT-PCR assay for specific amplification of 5'UTR region of viral genome was performed in 236 serum samples. The samples were classified into four groups: 56 samples from HCV/HIV coinfecting, 60 from HCV mono-infected, 60 from HIV mono-infected and 60 blood donors. All positive samples were submitted to direct sequencing for genotyping and molecular characterization. The overall prevalence of HPgV-1 was 15.7% (37/236). Among HCV/HIV coinfecting group, HPgV-1 prevalence was 14.3% (8/56), coinfection with HCV (8.3%; 5/60), HIV (28.3%; 17/60) and blood donors (11.6%; 7/60). 35 positive samples were successfully sequenced. Phylogenetic analysis revealed the presence of genotypes 2a (22.8%), 2b (57.1%), 3 (8.5%) and 1(8.5%). Our findings demonstrate that the higher frequencies of HPgV-1 were found in HIV and HCV/HIV coinfecting individuals. Circulating HPgV-1 genotypes described here, have already been reported in Brazil. This is the first data about HPgV-1 infection in HCV and HCV/HIV patients in Rio de Janeiro city. This study intends to contribute with insights about epidemiological characteristics and impact (if there is any) of HPgV-1 in the natural course of HCV and/ or HIV infection.

Financial Support: CNPq

Keywords: Human Pegivirus-1, HIV/HCV coinfection, prevalence, genotype



IDENTIFICATION AND GENOTYPICAL CHARACTERIZATION OF ROTAVIRUS IN ISOLATES OF COPROSCOPIC ANALYSIS OF HIV-INFECTED ADULT INDIVIDUALS IN PORTO VELHO - RONDÔNIA

Annemarie Gracielly de Souza Loeschke ¹, Núcia Cristiane da Silva Lima ², Dara Nayanne Martins Campos ², Luana da Silva Soares ³, Joana D'arc Pereira Mascarenhas ³, Renato da Silva Bandeira ³, Najla Benevides Matos ²

¹ PGBIOEXP - Programa de Pós-Graduação em Biologia Experimental (Porto Velho - RO), ² FIOCRUZ/RO - Fundação Oswaldo Cruz - Rondônia (R. da Beira,7671 - Lagoa, Porto Velho - RO, 76812-245), ³ IEC - Instituto Evandro Chagas (Av. Alm. Barroso, 492 - Marco, Belém - PA, 66093-020)

Abstract

AIDS provokes progressive destruction in the immune system providing opportunistic infections development. Thus, this study aimed to characterize the Rotavirus (RoV) of feces from HIV-infected adult individuals in Porto Velho-RO. RNA extraction was done with Trizol[®], and then the genotyping was performed to determine G and P segments using RT-PCR– Nested and polyacrylamide gel. The phylogenetic analysis was conducted using the BioEdit Sequence Alignment Editor and for assembly and alignment were employed the programs Geneious and Mafft. Of the 150 fecal samples identified, 9.3% (14/150) were RoV positive. Epidemiological data shown that 92.9% (13/14) of individuals discharge of their waste in black pits and all patients RoV positive reported diarrhea. The median of CD4+ cells was 372 cell/mm³ and viral load ranged from undetectable to 273,424 copies/mm³. The most prevalent genotypic profiles were G12P[8] with 28.5% (4/14), followed by G3P[8] and GntP[8], both with 21.4% [3/14] G1P[8], G12 P[nt], G3P[nt] and GntP[nt] genotypes were identified in 7.14% [1/14] of the isolates. Analyzing VP7 protein phylogeny, it could be observed that RoVs from RVA / Human-wt/BRA/RO-68/2015 / G3P[8]; RVA / Human-wt/BRA/RO-152/2016/G3P[8]; RVA/Human-wt/BRA/RO-138/2015/G3P[8]; RVA/Humanwt-/BRA/RO-151/2016/G3P[8] had similarity with American (MF997040), Spanish (KT006919.1), Brazilian - Amazonas (KX469407.1), and closely related viruses of animal origin equine (DQ981479.1). Analyzing VP4 protein from RVA/Human-wt/BRA/RO-26/2014/G12P[8]; RVA/Human-wt/BRA/RO-68/2015/G3P [8]; RVA/Human-wt/BRA/RO- 27/2014/GNTP [8]; RVA/Human-wt/BRA/RO-28/2014/G12P [8] and RVA/Human-wt/BRA/RO-64/2015/G12P [8], similarity with Italian P[8] virus could be observed. (KU048603.1; KU048603), American (MF469404.1; MF469127.1) and close relationship with Japanese virus (KX931951.1), as well as the American equine virus (MF997038.2). Thus, this study provides data that can assist in the planning of actions aimed at improving health care. Financial Support: CNPq; FAPERO; Research Program for SUS - PPSUS; INCT-EpiAmO.

Keywords: Diarrhea, HIV, Rotavirus Infections



DIFFERENT TYPES OF RAW MEAT ACQUIRED IN COMMERCIAL ESTABLISHMENTS CONTAMINATED WITH ROTAVIRUS A AND MASTADENOVIRUS

Ana Karolina Antunes Eisen ¹, Kelen Gras De Oliveira ¹, Meriane Demoliner ¹, Maria Eduarda De Moraes Guerra ², Raíssa Gasparetto ², Matheus Beltrame Padilha ², Vanessa Mendonça Soares ², Juliano Gonçalves Pereira ², Mário Celso Sperotto Brum ², Andréia Henzel ¹, Carolina Kist Traesel ², Fernando Rosado Spilki ¹

¹ FEEVALE - UNIVERSIDADE FEEVALE (ERS-239, 2755 Novo Hamburgo - RS), ² Unipampa - Universidade Federal do Pampa (BR 472 - Km 585 - Caixa Postal 118 - Uruguaiana, RS - CEP 97501-970)

Abstract

Enteric viruses are pathogens that cause gastroenteritis and are often found contaminating foods of animal origin, since that are transmitted through fecal-oral route and remain viable on environment for long periods as of in hands of manipulators. Thus, meat are products strongly prone to enteric viruses contamination due to many handling that this products are exposed, since fridge to sales conter butchery, when hygienic care are not properly attended. The goal of this work was to evaluate presence of *Mastadenovirus* from different species (AdV) and *Rotavirus A* (RV-A) in different types of raw meat samples from 18 commercial establishments of Uruguaiana – Rio Grande do Sul (Brazil). One hundred and thirty samples of 6 types of meat (ground beef, chicken drumstick, pork rack, pork leg, bovine and swine chop) were sampled, from March to April of 2018. About 1 g of each sample was prepared with 1 mL of Eagle's minimum essential medium (MEM), the eluted was used in RNA extraction with TRIzol[®] and in DNA extraction through kit Mini Spin Plus (Biopur[®]). Polymerase chain reaction (PCR) was used to the analysis, in the case of AdV a Nested-PCR was done. Both VP6 and DNA polymerase genes were the targets regions used to amplify and detect RV-A and AdV, respectively. At the end of the reaction, an agarose gel electrophoresis was performed and the results were visualized under UV light. Of the 130 samples 38 (29%) were positive for RV-A and 7 (5%) for AdV. Concerning to the establishments, of the 18 that were sampled only 4 (22%) did not show any contaminated raw meat. These contaminations demonstrate precarious handling of these foods, as the presence of RV-A indicates contamination of human origin. The presence of these pathogens also indicates the possibility of other enteric pathogens such as bacteria. Consumption of raw meat contaminated with these viruses is therefore a health risk and may cause gastroenteric infections.

Financial support: CAPES, CNPq, Feevale

Keywords: RV-A, AdV, Food contamination, Enteric viruses



STUDY OF HUMAN ASTROVIRUSES IN THE IMPLEMENTATION OF EPIDEMIOLOGICAL SURVEILLANCE NETWORK OF CASES OF CHILDHOOD GASTROENTERITIS: PREVALENCE, CO-INFECTION AND MOLECULAR CHARACTERIZATION.

Juliana Da silva ribeiro de andrade ¹

¹ Fiocruz - Fundação Oswaldo Cruz (Avenida Brasil, 4365, Mangunhos. Rio de Janeiro - RJ)

Abstract

The family *Astroviridae* are composed by small (≈ 28 to 35 nm) star-shaped nonenveloped icosahedral viruses with a nonsegmented single-stranded positive-sense RNA genome. The classical human astroviruses (HAstV) are classified into eight serotypes (HAstV1–8). Furthermore, genetically distinct human astroviruses (MLB1-3 and VA1-5) have been described in stool samples from patients with acute gastroenteritis. Although astrovirus represents one of the main viral agent for gastroenteritis, there is a lack of information about its circulation and prevalence. The recent finds of genetically different human astroviruses, highlights the diversity of these viruses, suggesting that the data of astroviruses circulation is underestimated, emphasizing the importance of surveillance and characterization of these viruses in acute gastroenteritis cases. In order to demonstrate the impact of human astroviruses in co-infection cases with other pathogens such as viruses, bacteria and parasites, this work proposes to study the prevalence and molecular characterization of HAstV in stool samples from acute gastroenteritis cases. These samples were received at the Laboratory of Comparative and Environmental Virology (LVCA) for elucidation of acute gastroenteritis cases from children under five years of age living in the Northeast, Southeast and South of Brazil, 2013. In order to detect HAstV, we performed a viral nucleic acid extraction with an automatized system followed by quantitative polymerase chain reaction (qPCR) using the TaqMan Array Card (TAC) platform. Our results confirm that, 22 out 300 of stool samples analyzed presented co-infection with HAstV and other pathogens. The detected HAstV strains were characterized using genomic sequencing and compared by phylogenetic analysis with sequences available in the database (GenBank). This is the first study of co-infection of astroviruses with other clinically important pathogens in cases of gastroenteritis in these regions.

Financial support: Faperj; IOC-Fiocruz.

Keywords: Gastroenteritis, Human Astrovirus, Viruses



EPIDEMIOLOGICAL TRANSITION OF HEPATITIS A VIRUS IN BRAZIL: NEW CHALLENGES

Mariana da Silva Cardoso ¹, Luciane Almeida Amado ¹, Andreza Soriano Figueiredo ¹, Maria de Lourdes Aguiar Oliveira ¹, Anne Louise Parada Faria ¹, Sarah Beatriz Salamene Salvador ¹, Fernando Cesar Ferreira ¹, Maria Cristina Ferreira Lemos ³, Denise B. Arduini ³, Oswaldo Gonçalves Cruz ⁴, Jaqueline Mendes de Oliveira ¹

¹ LADTV/FIOCRUZ - Laboratório de Desenvolvimento Tecnológico Em Virologia – Instituto Oswaldo Cruz/Fundação Oswaldo Cruz (Av. Brasil 4365, Manguinhos, Rio de Janeiro, RJ), ² IOC/FIOCRUZ Laboratório De Virologia Comparada E Ambiental - Instituto Oswaldo Cruz/Fundação Oswaldo Cruz (Av. Brasil 4365, Manguinhos, Rio de Janeiro, RJ), ³ SMS/RJ - SECRETARIA MUNICIPAL DE SAÚDE (Rua Afonso Cavalcante 455, Cidade Nova, RJ), ⁴ PROCC/RJ - PROGRAMA DE COMPUTAÇÃO CIENTÍFICA FUNDAÇÃO OSWALDO CRUZ (Av. Brasil 4365, Manguinhos, Rio de Janeiro, RJ)

Abstract

In Brazil, the overall incidence of hepatitis A virus (HAV) infection has shown a downward trend throughout the last ten years, from 11.7 to 0.6 cases per 100,000 inhabitants. Improved sanitary and living conditions led to a decrease of HAV exposure in early childhood and, in the absence of universal vaccination, an accumulation of susceptible individuals. Recently, independent outbreaks in São Paulo and Rio de Janeiro illustrated the impact of increased susceptibility of the general population.

The present study describes the investigation of a large hepatitis A outbreak in an around 14,000 inhabitant neighborhood in the city of Rio de Janeiro, with hundreds of clinical cases notified between July 2017 and February 2018. In order to identify potential sources of infection, water samples have been assessed in different points, concentrated according to ISO-15261- 1:2017, and screened by qualitative RT-nested PCR. A phylogenetic tree using the BLAST database and MEGA7 software was constructed, including serum/plasma samples from hepatitis A patients and asymptomatic blood donors from the same area, obtained during the outbreak.

The phylogenetic analysis of the VP1-2A region revealed a 100% homology among HAV-IA infected patients, and two HAV subgenotypes (IA and IB) circulating in the environment (groundwater, tap and drinking water) showing high nucleotide similarity (92.6% to 96.9%) with HAV isolates from past waterborne outbreaks in Rio de Janeiro and other Brazilian states. Of note, the majority of those persons affected by the outbreak in Rio de Janeiro were young adults (20 – 29 years) of both genders, with a higher proportion of men (72.6%). Surprisingly, there were four asymptomatic blood donors infected by HAV-1A and 1B. This study highlights the changing epidemiology of hepatitis A in Brazil and reinforces the need for vaccination of risk groups.

Financial support: IOC and CVSLR, Fiocruz, MS.

Keywords: Epidemiology , Hepatitis A, genotypes



GENOMIC INVESTIGATION OF HAV OUTBREAKS INTO EPIDEMIOLOGICAL AND ENVIRONMENTAL SURVEILLANCE FRAMEWORK: A VALUABLE MODEL FOR MONITORING WATER-BORNE VIRAL DISEASES.

Tamires Bomfim Santos Pereira ¹, Maria de Lourdes Aguiar Oliveira ¹, Mariana Silva Cardoso ¹, Eric Lopes Gama¹, Gentil A.B.M.Vasconcelos ¹, Maria Cristina Ferreira Lemos ², Jaqueline Mendes de Oliveira ¹

¹ LADTV, IOC, FIOCRUZ - Laboratory of Technological Development in Virology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil (Av Brasil, 4365 Manguinhos, Rio de Janeiro), ² SMSRJ - Health Surveillance Department, Municipal Health Secretariat, Rio de Janeiro, Brazil. (Rua Afonso Cavalcanti, 455 Cidade Nova, Rio de Janeiro)

Abstract

Despite the global decrease in hepatitis A incidence, a growing frequency of outbreaks has been reported worldwide, especially in low endemic areas. These events are usually related to contaminated food and/or water and mostly affect youth/adults, increasing disease burden and economical impact. The aim of this study was to investigate a hepatitis A outbreak in Rio de Janeiro (July, 2017-February, 2018), under a phylogenetic framework. Epidemiological information, clinical and environmental samples were collected by the surveillance teams. After concentration, viral RNA was extracted, submitted to nested RT-PCR and Sanger sequencing (VP1-2a region, 468bp). Phylogenetic relationships were reconstructed by ML (PhyML) and Bayesian MCMC methods (BEAST1.8.4), with a GTR+G+I nucleotide substitution model. Beast runs were performed using the uncorrelated lognormal relaxed molecular clock model and a time-aware GMRF Bayesian skyride coalescent tree prior. All human cases were classified as HAV1A and showed 100% nucleotide similarity. Although this subtype was also identified in a local water-well and a natural beach shower, human and environmental sequences grouped into distinct clades in MCMC tree. HAV1B was detected in two confiscated lots of commercial bottled-water (initially supposed, but further excluded as a source of infection), and in an inactivated water well at the manufacturing area, located in other state county. These sequences grouped into the same clade, apart from all other clinical/environmental representative HAV1B sequences, suggesting a local contamination of products. Our findings revealed the circulation of HAV1A and HAV1b in RJ's surroundings. In complement to classical epidemiological approaches, phylogenetic analyses added valuable information to identify/confirm presumed outbreak sources and to elucidate relationships between viruses circulating among humans and in other settings. However, for investigation of HAV outbreaks, traceability would be notably improved by sequencing other genomic region(s) and/or increasing the VP1-2A sequencing fragment. Furthermore, additional phylodynamic/phylogeographic analyses will be carried out to complement these initial findings.

Financial support: IOC and CVSLR, Oswaldo Cruz Foundation, Brazilian Ministry of Health

Keywords: ENVIRONMENTAL SURVEILLANCE , EPIDEMIOLOGICAL SURVEILLANCE , HEPATITIS A, MOLECULAR EPIDEMIOLOGY, PHYLOGENETICS



IN VITRO ANTIVIRAL ACTIVITY AGAINST CHIKUNKUNYA VIRUS FROM A NATURAL PRODUCT OF THE BRAZILIAN BROWN SEAWEED DICTYOTA MENSTRUALIS

Priscilla Oliveira Esteves ¹, Mariana Cavalcante Oliveira ^{1,4}, Caroline de Souza Barros ^{1,4}, Izabel Christina de Palmer Paixão ^{1,4}, Valéria Laneuville Teixeira ^{4,3}

¹ UFF - Universidade Federal Fluminense (Alameda Barros Terra, s/n - Centro, Niterói - RJ, 24020-150), ² UFF - Instituto de Biologia, Departamento de Biologia Celular e Molecular – Laboratório de Virologia Molecular e Biotecnologia Marinha, Universidade Federal Fluminense (Niteroi, RJ, Brazil), ³ UFF - Instituto de Biologia - Departamento de Biologia Marinha – Laboratório ALGAMAR (RJ), ⁴ UniRio - Laboratório de Biologia e Taxonomia de Algas (LABIOTAL), Programa de Pós-graduação em Biodiversidade Neotropical, Instituto de Biociências, Universidade Federal do Estado do Rio de Janeiro (Avenida Pasteur 458, Urca, Rio de Janeiro, RJ, Brasil), ⁵ UFF - Programa de Pós-graduação em Ciências e Biotecnologia, Instituto de Biologia, Universidade Federal Fluminense (Niterói, Rio de Janeiro, Brazil)

Abstract



IN VITRO ANTIVIRAL ACTIVITY AGAINST CHIKUNKUNYA VIRUS FROM A NATURAL PRODUCT OF THE BRAZILIAN BROWN

SEAWEED *Dictyota menstrualis*

Esteves PO^{1,2,4}, Oliveira MC^{1,4}, Barros CS^{1,4}, Cirne-Santos CC^{1,4}, Paixão ICPN^{1,4}, Teixeira VL^{2,3}

¹Instituto de Biologia, Departamento de Biologia Celular e Molecular – Laboratório de Virologia Molecular e Biotecnologia Marinha, Universidade Federal Fluminense (UFF), Niteroi, RJ, Brazil; ²Instituto de Biologia - Departamento de Biologia Marinha – Laboratório ALGAMAR, UFF; ³Laboratório de Biologia e Taxonomia de Algas (LABIOTAL), Programa de Pós- graduação em Biodiversidade Neotropical, Instituto de Biociências, Universidade Federal do Estado do Rio de Janeiro, Avenida Pasteur 458, Urca, Ri de Janeiro, RJ,Brasil. ⁴Programa de Pós-graduação em Ciências e Biotecnologia, Instituto de Biologia, Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil.

Chikungunya virus (CHIKV) infection has become one of the most challenging reemergent infections caused by a virus and a still specific anti-viral treatment and a major public health problem in recent years. Since no vaccines are available, and no drugs have effectively treated recent cases of infection, our group evaluated products from *Dictyota menstrualis* for their antiviral potential, alone and in combination with Ribavirin. We first evaluated the compounds cytotoxicity at high concentrations, and then evaluated the inhibition of CHIKV replication by crude extracts and acetylated crude extracts and their fractions at 20 µg/mL. The F-5 and FAc-1 fractions, rich in cyclic diterpenes with aldehyde groupings, inhibited CHIKV replication by >96%, with inhibition behaving in a dose-dependent manner and EC50 values of 0.90 (F-5) and 0.73 (FAc-1) µg/mL. To observe the mechanism of action, we performed the virucidal assay where F-5 showed a strong effect inhibiting up to 88% CHIKV an infectivity 20 µg/mL. In the adsorption assay we evaluated that Fc-A1 had 66% effect also at 20 µg/mL concentration. Associating F-5 and FAc-2 with Ribavirin at suboptimal dosages produced a strong synergistic effect that completely inhibited viral replication and in time of addition experiment we observed that the inhibitory effect of the F-5 remained greater than 85% in addition to the compound for up to 16 hours post infection. Our results indicate these natural products have excellent inhibitory potential against CHIKV replication.

Financial support: CNPq , CAPES, FAPERJ, UFF (PROPPi)

Keywords: antiviral, chikungunya, natural products, brown seaweed, dictyota



DEVELOPMENT OF A SELF-ASSEMBLING NANOVACCINE FOR ZIKA VIRUS

Marianna Teixeira de Pinho Favaro ¹, Aléxia Adrienne Venceslau Brito Carvalho ¹, Mônica Josiane Rodrigues de Jesus ¹, Lennon Ramos Pereira ¹, Robert Andreatta Santos ¹, Rubens Prince dos Santos Alves ¹, Luís Carlos de Souza Ferreira ¹

¹ USP - Universidade de São Paulo (Av Lineu prestes 1374)

Abstract

The development of innovative vaccination strategies is a major priority since the recent Zika Virus (ZIKV) outbreak. Amongst all vaccination strategies, nanovaccines have emerged as a promising approach to improve the efficiency of subunit vaccines, aiming to protect and deliver selected antigens in a more efficient way, mimicking the antigen display of a viral particle. For that purpose, we have employed Self Assembling Protein Nanoparticles (SAPN), a strategy that consists in modifying the antigen protein sequence by adding peptide tags that promote self-assemble in adequate physicochemical conditions. When added to the protein, some of these peptides are highly cationic and may promote cell entry by acting as Cell Penetrating Peptides (CPP), thus enhancing the cellular immune response. The non-structural protein 1 (NS1) of ZIKV was modified by the inclusion of the tags and produced in *E. coli* BL21(DE3) strain and purified by affinity chromatography. We successfully generated a nanovaccine of approximately 100 nm when assessed by Dynamic Light Scattering (DLS), which assumed different sizes depending on the buffer composition. The novel protein retains its antigenicity and, despite the modifications, is recognized by both murine and human ZIKV+ sera in ELISA assays. In addition, the modified protein proved to be more thermally stable than the recombinant protein without the tag fusion. The produced nanovaccine was evaluated in a 3 doses immunization regimen using C57/BL6 mice. The results indicate that the NP-NS1 induced high IgG titers following parenteral administration with aluminum hydroxide (alumen). The results indicate that ZIKV nanovaccines are a promising and innovative vaccination strategy that may be assessed to induce immunological responses.

Financial support: CAPES, CNPq and FAPESP (2016/20045-7 and 2018/08199-4)

Keywords: Nanovaccine, Subunit vaccine, vaccine, Zika vírus



DRUG RESISTANCE VARIANTS DETECTED IN HIV-MULTIEXPERIENCED PATIENTS USING NGS

SHIRLEY VASCONCELOS KOMNINAKIS ^{1,2}, Rodrigo Lopes Sanz-Duro ², Robson dos Santos Souza Marinho ², Giulia Luiza Santos ², Juliana Galinskas ², Danilo Dias ², Ricardo Sobhie Diaz ²

¹ FMABC - Faculdade de Medicina do ABC (Avenida Príncipe de Gales, 821, Santo André/SP), ² UNIFESP - Universidade Federal de São Paulo (Rua Pedro de Toledo, 781- 16º andar - Vila Clementino/SP)

Abstract

Introduction: Combination Antiretroviral Therapy (cART) may lead to emergence of HIV drug-resistant viral populations because of the selective pressure of drugs. Currently, there is no consensus on the importance of HIV-1 Minority Drug Resistance Mutations (HIV-1 MDRM). The aim of this study was to evaluate the occurrence of major and HIV-1 minority resistance mutations in Protease (PR) and Reverse Transcriptase (RT) genes in HIV treatment-multiexperienced patients. **Material and Methods:** 150 HIV treatment-multiexperienced patient plasmas, collected between 2013-2015, with viral load $\leq \log_3$ copies/mL and T CD4+ ≤ 500 cells/mm³, were sequenced to evaluate PR and RT regions by NGS using Miseq Desktop. The mutations were characterized according to Stanford (<https://hivdb.stanford.edu>). **Results:** The median viral load was 4.81 (3.70-6.07 log₁₀ copies/mL) and the mean of T CD4 was 170 (3-471 cells/mm³). All were receiving NRTI, 44.9% NNRTI and 57.8% PI. The mean cARTs used and the treatment time of the group was 6.39 combinations (range from 5 to 13) and 6.81 years (4 to 14). The average reads obtained in Miseq run with Q30 > 90% was 300.000. The major NRTI resistance mutations with clinically importance were observed in this major codons 184 (68.7%), 65 (10.7%), 70 (4%), 74 (10%) and TAMs 41 (19.3%), 67 (19.3%), 70 (16.7%), 210 (12%), 215 (28%), 219 (8.7%). The major NNRTIs were 101 (9%), 103 (48%), 106 (2.7%), 181 (8%), 188 (7%), 190 (16%), 230 (2.7%). The major PIs resistance mutations with clinically importance were observed in the codons 30 (1.3%), 33 (7.3%), 46 (17.3%), 47 (3.3%), 50 (4.7%), 54 (6.7%), 76 (0.67%), 82 (14%), 88 (2%), 90 (8%). The minority resistance mutations were observed to TAMs 41 (12.2%), 67 (26%), 70 (13.2%), 215 (18.6%), 219 (12.9%); NNRTI were in the codons 100 (20%), 101 (10.4%), 103 (19%), 188 (11.1%); to PIs were in the codons 46 (17.3%), 47 (12.5%), 54 (12.5%), 82 (11.1%), 84 (10%). **Conclusions:** There was a high frequency of major resistance mutations for NRTIs and NNRTIs as observed in the last 10 years. Failure to cART increases circulation and transmission of resistant viral variants and is mainly related to adherence to cART, impairing 90-90-90 UNAIDS strategy. In patients failing the cART there is a high frequency of HIV-1 MDRM compromising future regimens. Next-gen can identify HIV-1 MDRM, otherwise cannot be observed by current gold standard Sanger method.

Financial Support: FAPESP (grant#2012/21577-1).

Keywords: HIV-1, Next-gen, resistance mutations, adherence, cART



IDENTIFICATION AND GENOTYPIC CHARACTERIZATION OF NOROVIRUS ISOLATED FROM COPROSCOPIC ANALYSIS OF INFECTED ADULTS BY HIV IN PORTO VELHO, RONDÔNIA.

Annemarie Gracielly de Souza Loeschke ¹, Núcia Cristiane da Silva Lima ², Dara Nayanne Martins Campos ², Jones Anderson Monteiro Siqueira ³, Yvone Benchimol Gabbay ³, Renato da Silva Bandeira ³, Najla Benevides Matos ²

¹ PGBIOEXP - Programa de Pós-Graduação em Biologia Experimental (Porto Velho - RO), ² FIOCRUZ/RO - Fundação Oswaldo Cruz - Rondônia (R. da Beira,7671 - Lagoa, Porto Velho - RO, 76812-245), ³ IEC - Instituto Evandro Chagas (Av. Alm. Barroso, 492 - Marco, Belém - PA, 66093-020)

Abstract

Lymphocytes infected by HIV can make the individual susceptible to opportunistic viral infections, as occurs with Norovirus (NoV). Thus, it was performed the identification and characterization of NoV obtained from isolated coproscopic samples of infected adult individuals by HIV in Porto Velho-RO. RNA extraction was done with Trizol[®] and samples were amplified by RT-PCR using JV12/JV13 primers to amplify the polymerase region from the groups I and II. Phylogenetic analysis was conducted by BioEdit Sequence Alignment Editor and for the assembly and alignment were used the programs Geneious and Mafft. Of 150 faecal samples analyzed, 3.3% (5/150) were positive for NoV. Regarding the epidemiological profile, all NoV+ patients reported antiretroviral treatment and discharge of their waste in black pits, without diarrhoea report. The median CD4+ cell count was 529 cells/mm³ and the viral load ranged from undetectable to 120,880 copies/mm³. The genotypic profiles found were GII.Pe; GII.P17-GII17 and GIIPg-GII.1. Sample Hu/ Br/2014/GII.Pe/PortoVelho12 from the polymerase region shown similarity with the Sydney virus – GII.Pe-GII.4 (KY486271; KY486271) and sample Hu/Br/2016/GII.Pg/GII. 1/PortoVelho148 exhibited similarity with the Russian viruses - GII.Pg-GII.1 (KF895875), Belgian - GII.Pg-GII.12 (GQ845370.2) and Australian - GII.g-GII.12 (GQ845370). Samples Hu/Br/2014/GII.P17/GII.17/PortoVelho15 and Hu/Br/2016/GII.P17/GII.17/PortoVelho164 shown similarity with Japanese viruses GII.P17-GII.17 (LC037415.1), GII.P17-GII.17 (AB983218.1) and GII.17 (KU561249), and Chinese GII.17 (KU757046.1). Sample Hu/Br/2016/GII.Pg/GII.1/PortoVelho148 capsid analysis presented similarity with viruses described in Pará-Brazil: GII.Pg/GII.1 (KX353840) and GII.Pg/GII. 1 (KX353841), and with viruses identified in Belgium: GII (JF697289) and GII (JF697290). Samples Hu/Br/2014/GII.P17/GII.17/PortoVelho15 and Hu/Br/2016/GII.P17/GII.17/PortoVelho164 were similar to Chinese virus GII.17 (KU757046) and Japanese viruses GII.P17-GII.17 (AB983218.1), and GII.17 (KU561249). By this way, this study provides data that can assist in the planning of actions aimed at improving health care. Financial Support: CNPq; FAPERÓ; Research Program for SUS - PPSUS; INCT-EpiAmO.

Keywords: Diarrhea, HIV, Norovirus Infections



PREVALENCE AND CHARACTERIZATION OF NOROVIRUS INFECTIONS IN PERNAMBUCO, NORTHEAST BRAZIL

Klarissa Miranda Guarines ¹, Severino Jefferson Ribeiro da Silva ¹, Renata Pêsoa Germano Mendes ¹, Jurandy Júnior Ferraz de Magalhães ^{1,3}, Lindomar José Pena ¹

¹ Fiocruz Pernambuco - Oswaldo Cruz Foundation (Campus da UFPE - Av. Prof. Moraes Rego, s/n - Cidade Universitária, Recife - PE, 50670-420), ² UFPE - Agreste Academic Center, Federal University of Pernambuco (Av. Campina Grande, s/n - Km 59 - Nova Caruaru, Caruaru - PE, 55014-900), ³ UPE - University of Pernambuco (Campus Serra Talhada)

Abstract

Norovirus (NoV) is currently the leading cause of non-bacterial gastroenteritis worldwide, affecting people of all ages. It represents approximately one-fifth of all diarrhea cases. Due to the lack of a reliable cell culture system, knowledge about the epidemiology and impact of the disease caused by NoV was difficult for a long time. With the advent of molecular biology, studies about NoV biology have become more common and easier, and epidemiological studies as well. In Brazil, there are some studies about NoV distribution, but almost none in the Northeast region. Also in this area of the country, there is a lack of the clinical symptoms description. We aimed to describe, for the first time, the prevalence of NoV on Pernambuco state and the main characteristics of its distribution. For that, we analyzed 1135 stool samples received at Central Laboratory of Pernambuco state, Brazil (LACEN/PE), from 2014-2017. For each patient characterization, their individual notification form was analyzed. NoV was detected by enzyme immunoassay (EIA) in 125 (11.01%) samples. Most of the individuals of the study were vaccinated against Rotavirus and came from Pernambuco capital, Recife. Analyzing the patients' gender, we observed that 55 individuals (44%) were female and 70 (56%) male. Their ages ranged from 5 days to 87 years, and the group most affected by NoV infection (88%) was children under three years. Complete clinical information was available for 88 out of 125 NoV-positive patients. All patients had diarrhea, and the majority had vomit episodes ($n=60$; 68.68%). Most patients did not have bloody stools, 79 (89.77%). 46 (52.27%) individuals had fever, with temperatures ranging from 37.9°C to 39.9°C (mean 38,175°C). NoV was detected mainly on autumn season, corresponding to months March to June. Together, our study provided important information about the clinics and epidemiology of NoV infection in tropical settings. In Brazil, there is no epidemiological surveillance system yet, and all NoV data comes from epidemiological studies. These studies are important and necessary in the country. Here, we showed that this virus continues to circulate, contributing to prove the country distribution. Our project was the first to establish the prevalence and characterize the patients with NoV in a long study period in Pernambuco. We hope to help the government and health professionals to a better disease management and control strategies.

Financial Support: CAPES and FACEPE.

Keywords: Norovirus, Epidemiology, Gastroenteritis



ANTIVIRAL SCREENING OF XANTHENODIONES AGAINST MAYARO VIRUS

Luciana de Souza Fernandes ¹, Milene Lopes da Silva ¹, Roberto Sousa Dias ¹, Róbson Ricardo Teixeira ¹, Sérgio Oliveira de Paula ¹

¹ UFV - Universidade Federal de Viçosa (Avenida Peter Henry Rolfs, s/n - Campus Universitário, Viçosa - MG, 36570-977)

Abstract

Arboviruses are diseases caused by arthropod-borne viruses widely distributed worldwide. They represent a major public health problem, especially in tropical countries such as Brazil, which has favorable conditions for the development and spread of various arboviruses. *Mayaro virus* (MAYV) belongs to *Togaviridae* family and *Alphavirus* genus, transmitted by *Haemagogus* and *Aedes* mosquitoes. The viral infection in humans results in a febrile illness called mayaro fever. Once sporadic and incidental, this disease has spreaded over urban areas as anthropic occupation advanced throughout forested areas. Although non-lethal, MAYV can result in a long-term debilitating arthralgia. Symptoms associated to this disease are similar to other febrile illnesses, which may impair correct diagnosis. Since there is no specific treatment available to MAYV infection, vector monitoring and control are mandatory to eradicate the disease. Heterocyclic compounds containing a pyranosidic nucleus fused on both sides to cyclohex-2-enones rings are called 1,8-dioxooctahidroxantones or xanthenodiones. These molecules have antifungicidal, anti-inflammatory, antibacterial, leishmanicidal and antitumor activity. Twenty-four xanthenodione-derived compounds were evaluated for their antiviral potential against MAYV. Cell culture assays were performed to evaluate the cytotoxicity and antiviral capacity of the compounds. Cell viability was measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. From the cytotoxicity assays it was possible to calculate the 50% inhibitory concentration of cell viability (IC₅₀) for each compound. Antiviral screening was performed on virus-infected Vero cells and 8 compounds were able to maintain cell viability above 50% and were considered inhibitors of viral infection. Given the absence of vaccines and specific treatments for mayaro fever, it is necessary to search for new candidate molecules to fight the virus. Xanthenodiones have proven to be a promising class of molecules for future testing. This study was financially supported by CAPES.

Keywords: Alphavirus, Antivirals, Arboviruses, Mayaro virus, Xanthenodiones



DEVELOPING A QUICK R\$1 TEST TO DIAGNOSE ZIKA IN HUMANS AND MOSQUITO SAMPLES

Severino Jefferson Ribeiro da Silva ¹, Marcelo Henrique Santos Paiva ^{2,1}, Duschinka Ribeiro Duarte Guedes ¹, Larissa Krokovsky ¹, Fábio Lopes de Melo ¹, Adalúcia da Silva ¹, Klarissa Miranda Guarines ¹, Renata Pêssoa Germano Mendes ¹, Jurandy Júnior Ferraz de Magalhães ^{3,1}, Constância Flávia Junqueira Ayres ¹, Lindomar José Pena ¹

¹ Fiocruz Pernambuco - Oswaldo Cruz Foundation (Campus da UFPE - Av. Prof. Moraes Rego, s/n - Cidade Universitária, Recife - PE, 50670-420), ² UFPE - Agreste Academic Center, Federal University of Pernambuco (Av. Campina Grande, s/n - Km 59 - Nova Caruaru, Caruaru - PE, 55014-900), ³ UPE - University of Pernambuco (Campus Serra Talhada)

Abstract

The rapid spread of Zika virus (ZIKV) represents a global public health problem, especially in countries that circulate several vector mosquito vectors and favorable conditions for virus transmission, such as Brazil. Moreover, in countries where there is a circulation of other arboviruses, such as Dengue (DENV) and Chikungunya (CHIKV), the clinical diagnosis of ZIKV infection becomes extremely difficult once the symptoms are very similar. Currently, the reverse transcriptase reaction followed by quantitative polymerase chain reaction (qRT-PCR) is the gold standard method detection of ZIKV in mosquito samples and human samples. However, the technique presents high cost and limitations for Point-of-care (POC) diagnostics. In this context, the aim of this work was to develop and validate a diagnostic platform based on the reverse transcriptase technique followed by isothermal loop-mediated amplification (RT-LAMP) for detection of ZIKV in human samples and mosquito samples. Initially, was determined the ability of RT-LAMP to detect ZIKV in *Aedes aegypti* mosquitoes and serum, urine, saliva and semen under controlled conditions. In addition, the specificity was assayed by testing the cross-reactivity with other arboviruses currently circulating in Brazil, including ZIKV, DENV (1 to 4), yellow fever virus (YFV) and CHIKV. To evaluate the analytical sensitivity, ZIKV strain PE243 was 10-fold serially diluted. Subsequently, the validation of the RT-LAMP test was performed with 60 samples of *A. aegypti* and *Culex quinquefasciatus* mosquitoes. Lastly, the value per reaction was calculated based on the cost of all the reagents. The same methodology was used to evaluate the ability of RT-LAMP to detect ZIKV in human samples. Regarding the results, the RT-LAMP assay was highly specific for detection of ZIKV in 20 minutes without RNA extraction or pretreatment was up to 10,000 times more sensitive than qRT-PCR for detection of ZIKV in mosquito samples. The RT-LAMP had a sensitivity of 100%, specificity of 91.18 %, and overall accuracy of 95.24%, highlighting the potential of RT-LAMP for detection of ZIKV. The cost per sample was one Brazilian Real (R\$ 1), which is considerably lower than qRT-PCR. We have developed a low cost, POC diagnostic platform based on the RT-LAMP assay to detect ZIKV in mosquito samples and human samples collected at the epicenter of the Zika epidemics in Brazil. The test is a robust, simple, fast and inexpensive tool for detection of ZIKV.

Keywords: Diagnostic, ZIKV, Point-of-care, Brazil



PREVALENCE AND MOLECULAR CHARACTERIZATION OF HTLV-1/2 INFECTION AMONG PATIENTS WITH HEMATOLOGICAL DISEASES IN MANAUS, AMAZONAS.

Hygor Halysen Figueiredo ^{3,4}, Diana Mota toro ², Jose Pereira de Moura Neto ², Victor Souza ⁵, Marlon Araujo Nascimento ¹, Maria Edilene Martins de Almeida Martins de Almeida ⁵, Valdinete Alvez do Nascimento, Felipe Gomes Naveca ⁵, gemilson Soares Pontes ^{1,4,3}

¹ INPA - Instituto Nacional de Pesquisa da Amazônia (Av. André Araújo, 2.936 - Petrópolis - CEP 69.067-375 - Manaus - Amazonas), ² UFAM - Universidade Federal do Amazonas (Av. General Rodrigo Octavio Jordão Ramos, 1200 - Coroado I, Manaus - AM, 69067-005), ³ HEMOAM - Fundação de Hemoterapia e Hematologia do Amazonas (Av. General Rodrigo Octavio Jordão Ramos, 1200 - Coroado I, Manaus - AM, 69067-005), ⁴ UEA - Universidade do Estado do Amazonas (Av. Darcy Vargas, 1.200 - Parque Dez de Novembro, Manaus - AM, 69050-020), ⁵ ILMD-Fiocruz Amazônia - Fundação Oswaldo Cruz- Instituto Leônidas e Maria Deane (Rua Terezina, 476 - Adrianópolis, Manaus - AM, 69057-070)

Abstract

HTLV-1/2 infection has a worldwide distribution with prevalence rates varying according to the population group. High prevalence has been observed among patients with hematological diseases around the world. However, in the Amazon region, there are no epidemiological studies regarding the characterization of HTLV-1/2 infection among patients with hematological disease. The main goal of this study was to estimate the prevalence and molecularly characterize the HTLV- 1/2 infection in patients suffering from hematological diseases assisted at the HEMOAM Foundation, Manaus. A total of 306 patients were submitted to the serological diagnosis of HTLV-1/2 infection. HTLV-1/2 seroreactivity was confirmed by real- time PCR (qPCR). Amplification, sequencing and phylogenetic analysis of the LTR region were performed for molecular characterization of HTLV-1/2 infection. The serological analysis revealed one individual positive for HTLV-1/2 (0.36%) and two with an indefinite pattern. However, the molecular diagnosis performed by qPCR confirmed the positivity for only a 29- year-old male patient carrier of sickle cell anemia. This patient had been submitted to multiple blood transfusions over the years. The phylogenetic analysis clustered the sample within subtype HTLV-2c, since it shared over 99% of genetic similarity with the HTLV-2c 5'LTR region sequences available in the Genbank database. This study demonstrated the spread of HTLV- 2c infection to new geographic areas within the Brazilian Amazon region. The emergency of the HTLV-2c infection across new urban areas of Brazil brings to attention the need for an expanded form of public health surveillance on HTLV-1/2 infection, especially in the context of blood transfusion. Financial support: This study was partially supported by CAPES

Keywords: epidemiology, HTLV-1/2, phylogeny, prevalence, blood transfusion



EVALUATION OF RESPIRATORY SYNCYTIAL VIRUS (RSV) MOLECULAR DETECTION WITH VIASURE FLU-A, FLU-B & RSV REAL-TIME PCR DETECTION KIT (CERTEST BIOTEC), IN HOSPITALIZED INFANT PATIENTS WITH RSV RELATED BRONCHIOLITIS

Luciano Kleber de Souza Luna ¹, Raí André Silva Watanabe ¹, Jéssica Santiago Cruz ¹, Vitória Rodrigues Guimarães Alves ¹, Caroline Baldi ², Luana Claudino de Melo ², Nancy Bellei ¹

¹ UNIFESP - Universidade Federal de São Paulo (Rua Pedro de Toledo, 781, 04039-032, São Paulo - SP), ² Biomédica - Biomédica Equipamentos e Suprimentos Hospitalares Ltda (SIA Trecho 03 - Lotes 625/695 - Sala 230C, 71200-030, Brasília - DF)

Abstract

Respiratory syncytial virus (RSV) is the major cause of acute respiratory infection (ARI) in children and the most important etiological agent of acute viral bronchiolitis, mainly in risk groups (premature, bronchodysplastic and congenital heart disease), with great impact on morbidity and mortality. Diagnostic methods are important to confirm RSV infection, together with clinical symptoms that alone can overlap with other viral ARI related diseases, such as influenza A and B (Flu A/B). To circumvent this problem, we evaluated the VIASURE Flu-A, Flu-B & RSV Real-Time PCR Detection Kit (CerTest Biotec, Spain), a commercial multiplex kit that can detect those three viruses in one tube, in comparison to a well established *in house* RSV on-step real-time RT-qPCR as gold standard. We selected 49 nasal swab samples from 33 hospitalized RSV positive and 5 negative patients under 2 years old with bronchiolitis, from a prospective follow up study of RSV viral load conducted at Sao Paulo University Hospital. We used threshold cycle values (Ct) as a semi-quantitative measure. Thus, we selected 29 RSV positive (14 with Ct < 30, 15 between 30 to 40) and 20 negative (7 RSV clearance, 13 true negative) samples to test. Detection with VIASURE kit followed the manufacture instructions. Overall, VIASURE kit detected 26 of 29 HRSV positive samples. In comparison to the gold standard, sensitivity and specificity were 89.66% and 100%, with positive and negative predictive values of 100% and 86.96, respectively (qui square test, $p^2=0.9922$), with regression line slope of 0.96 ± 0.01 (p

Financial Support: CAPES, Biomédica.

Keywords: RSV, bronchiolitis, real-time RT-qPCR, VIASURE RT-qPCR Kit



RAPID SELECTION OF ZIKA VIRUS VARIANTS UPON SERIAL PASSAGES IN MOUSE BRAIN

Rafael Rosa ¹, Barbara Nayane Souza ¹, Severino Jefferson Ribeiro da Silva ¹, Larissa Krokovsky ¹, Lindomar José Pena ¹

¹ IAM - Fundação Oswaldo Cruz-Fiocruz, Instituto Aggeu Magalhães (Recife)

Abstract

Zika virus (ZIKV) is an arbovirus that represents a serious public health in several countries, including Brazil. Vector control is the main strategy for prevention of the disease since vaccines and antivirals are not yet available. One of the major obstacles to the development of vaccines is the lack of a suitable animal model that recapitulates the disease observed in humans. Currently, mice with a genetic deficiency in the IFN pathway have been widely used in studies with ZIKV, since wild type mice do not succumb to the disease. However, the main limitation of such models is that they do not allow a comprehensive study of immunity to ZIKV due to a weak or missing line of defense, which is critical for antiviral response. Thus, the objective of this study was to adapt ZIKV in mice with the hope of selecting a virulent strain for immunocompetent mice. Because serial brain passage in Swiss mice was not successful, we did 10 serial passages in 21-day-old A129 mice inoculated intracerebrally. Upon brain passages, animals showed increased expression of signs of the disease. Neurological manifestations were increasingly evident as motor incoordination and state of stupor. Plaque assay studies identified two plaque size variants at passage 7 (p7) and 10 (p10) when compared to the parental strain. The parental virus (p0) showed large plaque phenotype whereas the p7 virus had a pinpoint plaque phenotype. The p10 virus was unable to plaque. These results indicate that intracerebral passage of ZIKV in mice rapidly selects for ZIKV phenotypic variants. The genetic basis associated with these phenotypical changes and the impact in ZIKV virulence is under investigation.

Keywords: Zika, A129, mice, adaptation



PROCEDURE STANDARDIZATION OF SEMEN SAMPLES FOR DETECTION OF ZIKV USING REAL-TIME PCR

Noely E. Ferreira ¹, Midiã Silva Ferreira ¹, Karin Rottengatter ², Lucy Santos Vilas Boas ¹, Vivian Helena Lida Avelino da Silva ¹, Maria Cássia Jacintho Mendes Correa ¹, Philippe Mayaud ³, Tania Regina Tozetto-Mendoza ¹

¹ IMT-USP - Instituto de Medicina Tropical - Faculdade de Medicina da Universidade de São Paulo - FMUSP (Rua: Dr. Enéas Carvalho de Aguiar, 470 - São Paulo/SP - Brasil), ² GmbH - Altona Diagnostics Brasil LTDA (Rua São Paulino 221 - Vila Mariana - São Paulo - SP - Brasil), ³ LSHTM - London School of Hygiene and Tropical Medicine, Faculty of Infectious and Tropical Diseases, (UK - London)

Abstract

Introduction: The presence of ZIKV in genital fluids of men or women poses important questions regarding transmission of the virus and potential congenital transmission and neurological fetal sequelae. There is a need to determine which sample preparation methods would lead to optimized detection of ZIKV in the most cost-effective manner. Prior pilot experimental work was carried out at the Virology laboratory of IMT in a limited number of human semen specimens spiked with a culture-grown WHO reference strain of ZIKV. To verify if the different preparations of the semen specimens (total semen, diluted semen and seminal plasma) could interfere in the performance of detection the ZIKV by real-time PCR.

Methods: We used spiked different semen specimen with ZIKV culture obtained from ZIKV BR/2015 strain. Subsequently, the different semen specimen were aliquoted into two equal parts of 500 µl each, which were spiked with ZIKV culture with high and low viral load. We used 140 µl of each preparation. In addition, 6 µl of RealStar® Internal Control was mixed in lysis buffer and nucleic acids extraction was performed, according to the manufacturer's instructions (QIAamp® Viral RNA Mini Kit). Finally, it was eluted with 60 µl of elution buffer. The PCR was performed by using ZIKV RealStar® ZIKV RT-PCR kit 1.0. The viral load was measured using the WHO standard curve.

Results: The procedure using seminal plasma and semen 1:1 were more efficient, because we were able to detect viral loads earlier than in other protocols. Both semen 1:1 and seminal plasma presented media value Ct of 32, while total semen was Ct of 35. Notably, there was a reduction in the amplification with the use of total semen, due to the presence of interfering substances. The qPCR performance was assessed as expected: slope -3,55; R²=1; Efficiency 90%.

Conclusion: We have shown that some dilution techniques (over neat sample) for semen led to increased detection rates and lower Ct values (i.e. better sensitivity). There is a need to extend the number of observations and range of samples to determine the validity of these findings.

Keywords: SEMEN, ZIKV, DETECTION, REAL-TIME PCR, DIAGNOSTIC TEST



MAYARO FEVER: REPORT OF CHRONIC ARTHRITIS CASES IN MATO GROSSO, MIDWESTERN BRAZIL, 2018

Matheus Yung Perin ¹, Maíra Sant'Anna Genaro ^{1,2}, Renam Mansur Urt Bumlai ², Isabelle Silva Cosso ¹, Micheli Said Marchi ¹, Amilcar Sabino Damazo ², Renata Dezengrino Silhessarenko ²

¹ UNIC - Universidade de Cuiabá (Avenida Manoel José de Arruda, 3100), ² UFMT - Universidade Federal de Mato Grosso (Avenida Fernando Corrêa da Costa, 2367)

Abstract

Mayaro Virus (MAYV) is an arthritogenic alphavirus prevalent in the South and Central America. It is estimated that 1% of dengue-like fever cases in these regions is caused by MAYV. Human infection might be asymptomatic or progress to acute febrile disease, in some cases accompanied by long-term arthritis. The present study reports five patients from Várzea Grande, metropolitan region of Cuiabá, Mato Grosso, identified in January, 2018 (n=4) and February, 2018 (n=1) as positive for MAYV in the acute phase of disease by RT-PCR and nucleotide sequencing. All patients presented acute febrile symptoms with pronounced arthritis in more than four joints accompanied by skin rash. Two patients had elevated C reactive protein and erythrocyte sedimentation rate associated to moderated joint pain; therapy with disease-modifying antirheumatic drugs (DMARD; methotrexate [MTX] and/or Hydroxychloroquine [HCQ]) was introduced at this point. Three patients continued with regular trimestral clinical follow ups, one discontinued the treatment and returned only after 17 months, without biochemistry results and showing high level of articular pain; HCQ was reintroduced. The other two kept the medical following with reduced pain activity and normal inflammatory tests after one year using both MTX and HCQ. Histopathological analysis of the synovial biopsies from two patients (the first one after 3 months and the other after 7 months of the infection) both revealed leukocyte infiltration, especially of macrophages and lymphocytes, both characteristic of chronic inflammatory process. The macrophages had several cytoplasmic vacuoles suggesting phagocytic activity due to the presence of viruses. Viral RNA was not detected in these biopsies. One patient with intolerance to MTX suspended this drug (after 13 months), however the symptoms returned. MAYV chronic articular infection probably triggers a persistent inflammatory articular process with satisfactory response to DMARD.

Keywords: molecular diagnostic, arthritis, alphavirus, chronic arthropathy, mayaro vírus



INVESTIGATION OF 2'-METHYLCYTIDINE ANTIVIRAL PROPERTIES AGAINST ILHÉUS VIRUS INFECTION IN VITRO AND IN VIVO

Ana Carolina De Carvalho ^{1,2}, Rafael Elias Marques ^{1,2}

¹ CNPEM - Brazilian Center for Research in Energy and Material (R. Giuseppe Máximo Scolfaro, 10000 - Bosque das Palmeiras, Campinas - SP, 13083-970), ² UNICAMP - University of Campinas (Cidade Universitária Zeferino Vaz - Barão Geraldo, Campinas - SP, 13083-970)

Abstract

INTRODUCTION & OBJECTIVES: Neglected diseases pose a significant threat worldwide, negatively impacting the economy of developing countries and quality of life of populations at risk. Ilhéus virus (ILHV) is a neglected arthropod-borne flavivirus, closely related to St. Louis Encephalitis, West Nile and Japanese Encephalitis viruses. Although ILHV circulates in Latin America and infection may lead to severe neurological disease, the mechanisms of disease development are still poorly understood, and no vaccines or treatments are available. We established *in vitro* and *in vivo* models of ILHV infection and initiated the investigation of 2'-methylcytidine (2'CMC) antiviral properties against ILHV, and whether this candidate compound can lead to protection against infection and disease. **METHODOLOGY & RESULTS:** Cytotoxic and effective concentrations (CC₅₀ and EC₅₀) were determined *in vitro* through cell viability and viral load reduction assays. VERO cells were cultured in 96-wells plate, inoculated with ILHV and treated with 2'CMC or DMSO (vehicle) in concentrations from 100µM to <0,2µM for 24h. Supernatant was collected for quantification of viral load through plaque assay and cell viability was assessed through MTT assay. Cytotoxicity wasn't observed up to 100µM 2'CMC and the EC₅₀ obtained was 29,6µM, indicating a safe selectivity index that corroborates literature. Immunodeficient A129 mice inoculated subcutaneously with ILHV and treated with 2'CMC solution (80mg/Kg/day) showed increased survival when compared to vehicle-treated mice, which developed neurological disease symptoms and died 3 days post-infection. **CONCLUSION:** Treatment with 2'CMC *in vitro* in concentration as low as 30µM significantly reduced the viral load of ILHV without toxicity and administration of 2'CMC *in vivo* resulted in prolonged survival in a highly susceptible model. We conclude that 2'CMC has antiviral properties against ILHV and is protective against ILHV-induced disease. Thus, 2'CMC should be considered a candidate treatment for ILHV infection. Time-of-drug-addition experiments and characterization of antiviral activity are being performed too and should allow us to better understand 2'CMC mechanism of action against ILHV.

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Keywords: Ilhéus virus, therapeutic strategies, 2'CMC, flavivirus infection, antiviral



RETROSPECTIVE STUDY OF HUMAN RABIES CASES CONFIRMED BY LABORATORY DIAGNOSIS AT THE INSTITUTO PASTEUR, SÃO PAULO, BRAZIL, IN THE PERIOD 2003-2016

Maria Carolina Camillo Schweiger ¹, Tamires Santos de Arruda ¹, Yasmin Machado Freitas ¹, Enio Mori ¹, Karin Corrêa Scheffer ¹, Karen Miyuki Asano ¹, Willian de Oliveira Fahl ¹, Helena Beatriz de Carvalho Ruthner Batista ¹, Keila Iamamoto Nogi ¹

¹ IP - Instituto Pasteur (Avenida Paulista, 393 - Cerqueira César - São Paulo/SP - CEP: 01311000)

Abstract

In Brazil, the number of rabies human cases transmitted by dogs has decreased, while a considerable increase in transmission by wild animals has been observed over the years. Thus, the knowledge of the spatial and temporal distribution of rabies cases in humans and animals, can contribute to the development of measures for prevention, surveillance and disease control. The aim of this study was to evaluate available data of human rabies cases diagnosed at Instituto Pasteur from São Paulo (IP-SP), Brazil, in the period 2003-2016. The data were collected from diagnostic request forms, the computer system data (Inforaiva). The data were analyzed descriptively considering some epidemiological variables. A total of 58 human rabies positive cases were diagnosed at IP-SP, of these 71% were men, 24,5% were children between 0 to 10 years old and the same percentage for adults of 31 a 40 years old. Furthermore, 77,6% of agressions occurred in the Northeast Region and the Maranhão state presented the highest occurrence, with 71%. In the rural area more human rabies cases (38.0%) occurred than in the urban area (7%). The most frequent route of transmission was the bite with 53,4% and the most commonly reached places were hands (11%) and feet (11%), presenting in most cases single and deep wounds (20%). The most reported symptoms were hyperthermia (26.6%), followed by paresis/paralysis (24.4%). During this period, the bat had greater importance in the transmission of human rabies and were responsible for 28.8% of the positive cases. The genetic and antigenic characterization tests showed a prevalence of variant 3 (38%). From the *antemortem* laboratory tests performed, PCR was highlighted with 72.7% positivity of the hair follicle. Spring was the period with the highest number of cases (34,5%). It is important to note that among the analyzed datasets, a high frequency of uninformed data was observed, ranging from 17,9 to 45.2%. The number of human rabies cases diagnosed in the IP-SP declined in the considered period. On the other hand, a greater number of human rabies transmitted by bats was observed, suggesting a tendency of change in the epidemiological profile in relation to the animal species involved in the transmission.

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Keywords: HUMAN RABIES, LABORATORY DIAGNOSIS, RABIES LYSSAVIRUS, EPIDEMIOLOGY



RETROSPECTIVE STUDY OF HUMAN RABIES CASES CONFIRMED BY LABORATORY DIAGNOSIS AT THE INSTITUTO PASTEUR, SÃO PAULO, BRAZIL, IN THE PERIOD 2003-2016 ESBL BACTERIOPHAGES AGAINST BACTERIAL CLINICAL ISOLATES

Júlia Regina Schuch Garcia ¹, Nicole Mariele Santos Röhnelt ¹, Fabiana Tais de Souza Hack ¹, Ana Paula Pustay ¹, Mayara Paula de Borba ¹, Karhen Wiltgen Teixeira ¹, Simone Ulrich Picoli ¹
¹ Feevale - Universidade Feevale (ERS-239, 2755, Novo Hamburgo, RS, CEP 93525-075)

Abstract

Introduction: Antibiotic resistance is currently a major health challenge, leading to increased morbidity and mortality, financial losses, and the need for new approaches to prophylaxis, treatment and control of these microorganisms. The phage therapy consists in the use of viruses that infect and kill bacteria, and may be an alternative for such a problem. **Objective:** To evaluate the lytic capacity of *Klebsiella pneumoniae* ESBL's bacteriophages against different bacterial clinical isolates of the same species. **Methodology:** The ability of a phage to infect other bacterial samples of the same species was performed by susceptibility testing. For the assay 10 isolates of *K. pneumoniae* ESBL and 5 wild isolates of the same species were used. The method consisted of forming a 3 mL layer of semi-solid Tryptic Soy Broth with 100 µL of the host bacterial inoculum on a Tryptic Soy Agar layer. Afterwards, 10µL aliquots of the bacteriophages were deposited on the layer and incubated in a greenhouse (35°C / 24h). Two independent assays were performed and the bacteriophage with host bacterium of origin (where the phage was previously identified) was used as positive control. The bacteria's susceptibility to the phage was given by the visualization of lysis plates at the aliquot deposition site. **Results:** Phages infected 90% of *K. pneumoniae* ESBL and 60% of wild *K. pneumoniae*. **Discussion:** Determining the host range that a phage can infect is important to indicate its usefulness for bacterial control purposes, since some viruses infect many bacterial isolates belonging to the same species, others can target different species (multipurpose host variety). Such a range of action would presumably lead to less failure due to the greater likelihood of phage binding to the host. The susceptibility test performed showed the action of phages on more than half of wild *K. pneumoniae* and in large part of *K. pneumoniae* ESBL isolates. **Conclusion:** These findings may suggest that the phages are the same, that the hosts themselves may be genetically very close, or that the phage does have a good infection ability against other *K. pneumoniae*. Thus, a better understanding of the results will require molecular characterization studies of these phages. Financial support: Feevale University

Keywords: Bacteriophages, *Klebsiella pneumoniae*, Phage therapy



REPORTER REPLICON SYSTEMS FOR THE SCREENING OF POTENTIAL ANTIVIRAL AGENTS AGAINST ZIKA, YELLOW FEVER AND CHIKUNGUNYA VIRUSES

Rafaela Sachetto Fernandes ¹, Andre Schutzer de Godoy ¹, Laura Helena Vega Gonzales Gil ², Glaucius Oliva ¹

¹ IFSC/USP - Instituto de Física de São Carlos, Universidade de São Paulo (Av. João Dagnone, 1.100 - Jd. Santa Angelina, São Carlos, 13563-120, SP, Brazil), ² IAM-Fiocruz - Instituto Aggeu Magalhães, Fundação Oswaldo Cruz (Av. Prof. Moraes Rego, s/n - Cidade Universitária, Recife, 50670-420, PE, Brazil)

Abstract

Arboviruses belonging to the *Flaviviridae* and *Togaviridae* families cause infections that are either asymptomatic or results in a febrile illness that can evolve to severe symptoms, such as hemorrhage, articular/neurological disorders and shock. To date, there is no specific treatment for any arboviruses-associated diseases. The flaviviruses Zika and Yellow fever, and the togavirus Chikungunya are some of the emerging arboviruses for which there is an urgent need for effective antiviral therapy. Antiviral drug discovery requires the development of reliable biological assays such as replicon cell-based assays. Replicons are a self-replicative subgenomic systems in which the genes encoding viral structural proteins are replaced with a reporter gene, such as fluorescent proteins or luciferases. Inhibitory effects in viral RNA replication are assessed by measuring the reduction of fluorescent or luminescent signals. The objective of this study was to develop and characterize a Zika virus (ZIKV) subgenomic replicon expressing the *Renilla* luciferase (Rluc) for antiviral screening. The Rluc replicon plasmid containing the T7 promoter and the neomycin resistant gene (Neo) at the 5' and 3' end of the cDNA sequence (GenBank KU321639.1), respectively, was constructed by engineering an IRES-Neo cassette into pUC57_ZIKVRep plasmid. Replicon RNA was *in vitro* transcribed and used to transfect BHK-21 cells. In parallel, a replicon cell-based assay was established and used to evaluate the inhibitory effects of different compound series in the viral replication of Yellow fever virus (YFV) and Chikungunya virus (CHIKV). The BHK-21 cell lines expressing the YFV and CHIKV reporter replicons, BHK-21-YFV17D-LucNeolres and BHK-21-T7-GLuc-nsP-CHIKV-99659, respectively, were developed by the group of Profa. Laura Gil at the Instituto Aggeu Magalhães (IAM/Fiocruz – PE) and used to test the antiviral activity of CIBFar compounds. Replicon cell lines were treated with compounds at 200 μ M and those that inhibited the luciferase activity in $\geq 80\%$ were assayed to determine their effectiveness in a dose-dependent manner (EC₅₀). The cytotoxicity (CC₅₀) was then evaluated by a cell proliferation-based MTT assay. Compounds LQBO03 and CPI1091 inhibited the viral replication of YFV and CHIKV, respectively, at a low micromolecular range and displayed a low cytotoxicity demonstrating that reporter replicon systems are a relevant tool in antiviral drug discovery.

Financial support: FAPESP.

Keywords: arboviruses, CIBFar, drug discovery, luciferase, replicon system



COMPOUNDS RELATED TO INHIBITION OF ESCRT-MACHINERY PROTECTS IN VITRO ASSAY AGAINST OROPOUCHE VIRUS INFECTION

Alexandre Borin Pereira ¹, Karina Bispo dos Santos ², Rebeca de Paiva Froes Rocha ^{1,2}, Lais Durço Coimbra ^{1,2}, Simon Bernhard Cämmerer ², José Luiz Proença Modena ², Rafael Elias Marques ¹
¹ CNPEM - Centro Nacional de Pesquisa em Energia e Materiais (R. Giuseppe Máximo Scolfaro, 10000), ² UNICAMP - Universidade Estadual de Campinas (Cidade Universitária Zeferino Vaz - Barão Geraldo, Campinas)

Abstract

Introduction: Oropouche virus (OROV) is the etiologic agent of Oropouche Fever, a disease that affects people from northern Brazil. OROV infection may cause fever, headache, nausea and could affect the central nervous system. There is neither a vaccine nor specific treatment against infection. OROV requires the ESCRT machinery in a host cell to complete its replication cycle. We hypothesized that ESCRT inhibition could be explored in the development of potential treatments. **Methods:** We established an in vitro assay in Vero cells to test a selection of compounds that inhibit the ESCRT machinery for protection against infection. Compounds were provided by Dr. Simon B. Cämmerer. We sought to establish values for CC50 and IC50 for each compound, through MTT and plaque assays to measure viral load. **Results:** We performed assays with 30 compounds to find 7 compounds with antiviral activity within tested concentrations (25 μ M, 12,5 μ M, 6,25 μ M, 3,125 μ M). One compound presented both antiviral and cytoprotective in our testing conditions. Two compounds were markedly cytoprotective, maintaining the monolayer within more than 80% of cell viability. **Conclusion:** further assays might be conducted to characterize the action of the hit compounds on cellular machinery, and check if they are acting in the ESCRT machinery.

Financial Support: CAPES/CNPQ; FAPESP.

Keywords: HTS, Oropouche, SCREENING



DETERMINATION THE CLINICAL DIAGNOSIS OF CHIKUNGUNYA, COMPARED TO THE GOLD STANDARD OF LABORATORY DIAGNOSIS BY MOLECULAR BIOLOGY (RT-QPCR) AND ANTIBODY DETECTION (ELISA)

Andre Frederico Martins ^{1,4}, Hury Hellen Souza De Paula ¹, Raphael Rangel Das Chagas ¹, Paulo Sergio Cerqueira Rangel ¹, Renato Santana De Aguiar ², Cristiane Da Cruz Lamas ^{1,3}, Sergian Vianna Cardozo ¹

¹ UNIGRANRIO - Universidade do Grande Rio (Rua Professor José de Souza Herdy, 1160 - Jd 25 de Agosto, Duque de Caxias - RJ CEP 25071-202), ² UFRJ - Universidade Federal do Rio de Janeiro (Av. Pedro Calmon, 550 - Cidade Universitária, Rio de Janeiro - RJ, Laboratório de Virologia Molecular.), ³ FIOCRUZ/IFF - Fundação Oswaldo Cruz (Av. Rui Barbosa, 716 - Flamengo, Rio de Janeiro - RJ, CEP 20021-140), ⁴ HUGG - Hospital Universitário Gaffreé e Guinle (Rua Mariz e Barros, 775 - Maracanã, Rio de Janeiro - RJ CEP 20270-004)

Abstract

Chikungunya virus (CHIKV), transmitted by the *Aedes (Stegomyia)* mosquitoes, has spread globally and constitutes a serious threat to various tropical but also temperate areas of the world. We aimed to determine the clinical diagnosis of CHIKV, compared to the gold standard of laboratory diagnosis by molecular biology (RT-qPCR) and antibody detection (ELISA). The participants of the study were patients seeking medical care at the Emergency Department located in a general hospital in Duque de Caxias, Rio de Janeiro, Brazil, from January to June 2018. Patients eligible for the study had a clinical diagnosis of CHIKV or of another arboviral disease, regardless of gender and age. Clinical information, duration of symptoms and routine physical examination were recorded. The visual analogue scale was used to assess pain intensity. A single whole blood sample was collected on the day of recording and serum samples were stored at -80°C. RNA extraction was performed with the QIAamp® Viral Mini Kit (QIAGEN, Valencia, CA, USA). Screening for CHIKV was performed using the RT- qPCR. XGEN kits (Biometrix, Brazil) were used to detect the presence of CHIKV-specific IgM and IgG antibodies. In the study, 172 blood samples were collected from patients suspected of an arboviral disease; of these, 94 (54.6%) had a clinical diagnosis of CHIKV and 78 (45.4%) were clinically categorized as other arboviruses. Patients suspected of CHIKV presented moderate/severe arthralgia (93/94, 98.9%) and arthritis (77/94, 81.9%) as the most frequent features. All blood samples were tested by RT-qPCR, and 92 (53.5%) were positive for CHIKV. Of these positive samples, 65/92 (70.6%) presented a clinical suggestive of CHIKV and 27/92 (29.4%) had clinical features suggestive of other arboviruses. The mean duration of symptoms in the CHIKV PCR positive patients was 2.27 days. The sera of 80/172 (46.5%) which were negative in RT-qPCR assay were tested for the presence of IgM and IgG anti-CHIKV antibodies. A total of 18/80 (22.5%) serum samples tested positive for IgM, while 16/80 (20.0%) tested IgG positive. Of the samples that tested positive for IgM antibodies, 12/18 (66.6%) also positive for IgG. Six of 18 (33.3%) tested positive to IgM only, corresponding to the acute phase of CHIKV. Patients who were positive only for IgM had a mean DOS of 6 days. The acute phase of CHIKV was diagnosed in 98/172 (57.0%) according to PCR results and the exclusively IgM positive sera.

Financial Support: FAPERJ; CNPq.

Keywords: chikungunya, molecular biology, diagnosis, arbovirus, serology



GLYCAN RESIDUES ARE ESSENTIAL FOR THE NEUTRALIZING ACTIVITY OF HUMAN IgG1 ANTIBODIES INDUCED BY PRE- EXPOSURE PROPHYLAXIS FOR HUMAN RABIES

Gabriela Koike¹, Bruno Stuart de Castro¹, Iana Suly Santos Katz¹, Fernanda Guedes Luiz¹, Elaine Raniero Fernandes¹, Sandriana dos Ramos Silva¹

¹ IP - Instituto Pasteur (Av. Paulista, 393, Cerqueira Cesar, São Paulo-SP)

Abstract

Rabies virus-specific neutralizing antibodies confer protection after passive immunization. However how the *Rabies lyssavirus* (RABV) specific antibodies neutralize the virus is not fully understood. Previous studies have suggested that virus neutralization probably involves more binding between the antibody and the viral epitope. Because antibody effector functions are critically dependent on carbohydrate modification of antibodies, we aimed to evaluate the glycosylation patterns both neutralizing and non-neutralizing IgG induced by pre-exposure prophylaxis for human rabies. Specific IgG were purified by immunochromatography method from human serum with or without neutralizing activity induced by vaccination (Verolab, 2.5 IU, via IM). The purity and avidity were analyzed by SDS-PAGE and indirect ELISA using NH₄SCN, respectively. The N-linked oligosaccharide chain of purified IgG antibodies was evaluated by a lectin enzyme-linked immunosorbent assay using the lectins: *ConA*, *S. nigra*, *T. vulgaris*, *E. cristagalli*, *U. europeus* and *W. floribunda*. The neutralizing activity of purified IgG and neutralizing IgG deglycosylated by PNGase F enzyme were analyzed by rapid fluorescent focus inhibition test. The purified IgG showed electrophoretic pattern compatible with human IgG. In addition, both antibodies recognize RABV, however neutralizing IgG have a higher avidity (ED₅₀=2.0) than non-neutralizing IgG (ED₅₀=1.0). Moreover, neutralizing IgG were more glycosylated (e.g. galactose, mannose and fucose residues) than non- neutralizing IgG. However, deglycosylated IgG lost its neutralizing activity. Our results suggest that the glycosylation of the antibody is important for neutralizing the RABV, since neutralizing IgG has different glycosylation profile when compared to non-neutralizing IgG. In addition, further research is needed and encouraged to better evaluate the differential glycosylation patterns between antibodies.

Keywords: RABV, Vaccine, Antibody, glycosylation, Neutralization



INHIBITION OF THE YIELD OF BRAZILIAN ZIKA VIRUS GENOME BY SYNTHETIC NAPHTHOQUINONES

Rosa, S. R. S¹, Marinho, R. S. S¹, Vieira, C. B¹, Pinto, A. M. V.¹

¹ UFF - Universidade Federal Fluminense (R. Prof. Hernani Melo, 101 - São Domingos, Niterói - RJ, 24210-130 - Instituto Biomédico) Instituto Biomédico, Departamento de Microbiologia e Parasitologia, Programa de Pós-Graduação em Microbiologia e Parasitologia Aplicadas, Universidade Federal Fluminense (UFF), Niterói, RJ, Brazil

e-mail: stephanie.rangel@hotmail.com - Tel.: (21) 981026121

Abstract

Introduction: Zika virus (ZIKV) is an emerging mosquito-borne belonging to *Flaviviridae* family has emerged as a potential global threat to human health due central nervous system malformations such as microcephaly, severe neuroimmunopathology, fetal abnormalities and Guillain-Barre syndrome. In the absence of vaccines and effective vector borne control ZIKV, it is necessary continuous research looking for promising compounds with anti-ZIKV activity.

Objective: The aim of this study was to select synthetic compounds to be used as standard compound in antiviral RNAi assay. **Material and methods:** The cytotoxic effect of naphthoquinones RV10, RV15 and RV19 was assayed with MTT method: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, in Vero cell in 96-well microplates at 37 °C under a 5 % CO₂ atmosphere. After 24 h cells were treated with different concentrations of compounds (3.125 - 200 μM) in triplicate. An antiviral screening was performed in Vero cell grown in 24 well microplates inoculated with 200 P.F.U ZIKV. After one hour for virus adsorption at 37 °C with 5% CO₂ atmosphere, different concentrations of compounds (3.125 - 100 μM) was added following reincubation for 72 h at 37 °C with 5% CO₂ atmosphere. ZIKV inhibition was determined by qRT-PCR. **Results:** All compounds showed similar CC₅₀ results (RV10: 250 ± 6,4 / RV15: 236 ± 1,6 and RV19: 255 ± 1,8). The antiviral **EC₅₀** for RV10: 6,25μM ± 1,2; RV15: 50μM ± 4,7μM; RV19: negative. **Conclusion:** According the results, we will use the naphthoquinone RV10 as standard compound in the evaluation antiviral activity of RNAi.

Keywords: Zika virus, one step qRT-PCR, antiviral.

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Keywords: Zika virus , one step qRT-PCR, antiviral



HUMAN BOCAVIRUS IN GASTROENTERIC PATIENTS IN BRAZIL, 2010-2013

Roberta Salzone Medeiros ¹, Laís Sampaio de Azevedo ¹, Ellen Viana Souza ¹, Talita Gonçalves Aires de Queiroz ¹, Yasmin França Viana Pires de Souza, João Leandro de Paula Ferreira ¹, Gabriela Bastos Cabral ¹, Maria Isabel de Oliveira ¹, Maria do Carmo Sampaio Tavares Timenetsky ¹, Adriana Luchs ¹

¹ IAL - Instituto Adolfo Lutz (Av Dr Arnaldo, 355, São Paulo, SP, Brasil)

Abstract

Human Bocaviruses (HBoV) have been detected in human respiratory and gastrointestinal infections worldwide. Four genotypes of HBoV (HBoV1–4) have been described; HBoV-1 is associated with respiratory tract infections while HBoV-2, -3, and -4 genotypes are considered as entero-pathogenic although the exact role largely remains unclear. In Brazil, limited data are available regarding the contribution of HBoV in gastroenteric patients. The aims of the present study were to investigate the frequency of HBoV infections in patients with gastroenteritis during a 4-year period (2010-2013) and conduct molecular characterization of positive strains. A total of 2752 fecal samples negative for both, RVA and Norovirus, were selected and tested for HBoV by conventional PCR. Positive HBoV samples were sequenced to genotype characterization. Phylogenetic analysis was applied in order to confirm assigned genotypes. HBoV was detected in 38 cases (1.4%); median age of 2.0 years. Gender did not play a role in HBoV infection. Detection rate did not significantly vary according to the year: 1.3% (11/869) in 2010, 1.3% (9/693) in 2011, 1.1% (8/672) in 2012 and 1.9% (10/518) in 2013. Phylogenetic analysis of the partial VP1 nucleotide sequences identified the presence of HBoV-1 (94.7%, 36/38) and HBoV-2 (5.3%, 2/38) species, both clustering with strains detected worldwide. A suggestive seasonal pattern of HBoV infection was observed, being more commonly during winter and drier months. Children ≤ 5 years exhibited higher positivity rate, suggesting that HBoV could play an important role in childhood diarrhea. Overall, the detection of HBoV appears to partially contribute to the overall detection gap for enteric infections. Therefore, HBoV infection has not currently a major epidemiological impact in Brazil after the RVA vaccine introduction. In particular, the HBoV-1 detection in stool samples must be carefully analyzed, once could not necessarily be associated with diarrhetic symptoms, as HBoV-1 can exhibit a lingering shedding in feces after previous respiratory infections. HBoV screening should be considered as differential diagnosis in Public Health Laboratories, in order to improve the knowledge of HBoV infections and their role in gastrointestinal diseases. This study has the potential to contribute to the clinical definition and significance of HBoV infections in Brazil.

Keywords: Gastroenteritis, diarrhea, human bocavirus, molecular characterization, surveillance



CIRCULATION OF CHIKUNGUNYA VIRUS EAST/CENTRAL/SOUTH AFRICAN LINEAGE IN RIO DE JANEIRO, BRAZIL

JOILSON XAVIER ^{1,2}, Marta Giovanetti ^{2,1}, Vagner Fonseca ¹, Julien Thezé, Tiago Gräf ⁴, Allison Fabri ², Jaqueline Goes ⁴, Marcos Mendonça ², Maria Mares-Guia ², Stephane Tosta ¹, André Abreu ⁹, Wanderson Oliveira ⁹, Carlos Albuquerque ⁸, Alexandre Chieppe ⁷, Tulio de Oliveira ⁵, Patrícia Brasil ⁶, Guilherme Calvet ⁶, Nuno Faria³, Ana Maria Bispo de Filippis Bispo de Filippis ², Luiz Carlos Junior Alcantara ^{2,1}

¹ UFMG - Universidade Federal de Minas Gerais (Belo Horizonte - MG), ² IOC-FIOCRUZ - Instituto Oswaldo Cruz - Fundação Oswaldo Cruz (Rio de Janeiro - RJ), ³ Oxford University - University of Oxford (Oxford, UK), ⁴ IGM- FIOCRUZ - Instituto Gonçalo Moniz-FIOCRUZ (Salvador - BA), ⁵ KRISP - KwaZulu-Natal Research Innovation and Sequencing Platform, University of KwaZulu-Natal (South Africa), ⁶ IEC - Instituto Nacional de Infectologia Evandro Chagas (Rio de Janeiro, Brazil.), ⁷ SVRJ - Superintendência de Vigilância do Estado (Rio de Janeiro, Brazil.), ⁸ OPAS - Organização Pan-Americana da Saúde/Organização Mundial da Saúde (Brasília, Brazil.), ⁹ SVS- MS - Secretaria de Vigilância em Saúde, Ministério da Saúde (Brasília, Brazil.)

Abstract

The emergence of chikungunya virus (CHIKV) has raised serious concerns due to the virus' rapid dissemination into new geographic areas and the clinical features associated with infection. To better understand CHIKV dynamics in Rio de Janeiro, we generated 11 near-complete genomes by means of real-time portable nanopore sequencing of virus isolates obtained directly from clinical samples. Our phylogenetic reconstructions indicated the circulation of the East-Central-South-African (ECSA) lineage in Rio de Janeiro. Time-measured phylogenetic analysis combined with CHIKV notified case numbers revealed the ECSA lineage was introduced in Rio de Janeiro around June 2015 (95% Bayesian credible interval: May to July 2015) indicating the virus was circulating unnoticed for 5 months before the first reports of CHIKV autochthonous transmissions in Rio de Janeiro, in November 2015. These findings reinforce that continued genomic surveillance strategies are needed to assist in the monitoring and understanding of arbovirus epidemics, which might help to attenuate public health impact of infectious diseases.

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Keywords: CHIKUNGUNYA, GENOMIC SURVEILLANCE, PHYLOGENETICS, RIO DE JANEIRO



EVALUATION OF INNATE LYMPHOID CELLS ACTIVITY AGAINST DENGUE VIRUS INFECTION

Iracema Luisa Quintino de Carvalho ¹, Marcela Helena Gonçalves Pereira ¹, Helton da Costa Santiago ¹

¹ UFMG - Universidade Federal de Minas Gerais (Av. Pres. Antônio Carlos, 6627 - Pampulha, Belo Horizonte - MG. Brasil. CEP: 31270-901)

Abstract

Dengue is the most prevalent arbovirus in the world with 390 million cases annually. Caused by *Dengue virus 1-4* (DENV), its clinical manifestations are classified as dengue without warning signs (DWS-), with warning signs (DWS+) or severe dengue (SD). Literature data suggests that DWS+ and SD are related to the induction of an immunopathological disease caused by an exacerbated inflammatory response, which leads to important increase in vascular permeability. Previous analyzes of our group during in vitro infection of PBMCs from non-immune volunteers have shown that cytokines such as IFN- γ , TNF- α and IL-10 are produced at very early times of infection (4 hours) indicating an active participation of cells of the innate immune system during antiviral response. However, it is poorly understood the role of innate immunity in development of the different clinical presentations of dengue. In order to verify if there are differences in innate immunity between individuals who evolved with DWS- of those who developed DWS+/SD, PBMCs of seronegative volunteers (SN) (n=8), and PBMCs of volunteers previously affected by DWS- (n=8) or DWS+/SD (n=8) were in vitro infected with DENV-1 (MOI=0.1) during 8 hours and 7 days. The expression of IFN- γ and IL-10 by innate lymphoid cells (ILC) were evaluated by flow cytometry. After 8 hours of infection a significantly higher number of ILCs IL-10 producing were detected in SN group comparing to DWS- (p=0.0023) and DWS+ (p=0.0092) groups. On the other hand, DWS+ group presents a higher number of IFN- γ producing cells than DWS- (p=0.0186), but not SN group (p = 0.3450). After 7 days of infection ILCs of SN group showed a significant increase (p=0.0256) of the number of IL-10 producing cells, differing from those observed for DWS- and DWS+ groups. A significantly higher number of IFN- γ producing cells were observed in the DWS- group when compared to the SN group (p=0.0101), with no significant difference between DWS- and DWS+ (p=0.3417). Interestingly it was also observed that 7 days post infection the basal amount of ILCs producing IFN- γ is higher in the groups previously exposed to the infection than the seronegative group, regardless of the presence of in vitro infection. Together these results indicate that the innate immune system can be altered by a previous infection by DENV suggesting that there are differences in its activation underlying different clinical forms of this disease.

Financial Support: CNPq, CAPES, FAPEMIG.

Keywords: Dengue, Flow cytometry, Immunology, Immunopathology, Innate lymphoid cells



OROPOUCHE VIRUS DETECTED IN SALIVA AND URINE

Valdinete Alves do Nascimento¹, Dana Cristina da Silva Monteiro¹, Victor Costa de Souza¹, Felipe Gomes Naveca¹

¹ ILMD - Fiocruz - Instituto Leônidas e Maria Deane (Rua Terezina, 476 - Adrianópolis, Manaus - AM)

Abstract

The Oropouche virus (OROV) is an arthropod-borne virus of the *Peribunyaviridae* family, genus *Orthobunyavirus*, transmitted to humans mainly by the bite of infected *Culicoides paraensis*. Oropouche viral infections can result in acute febrile syndrome and exanthematous illness, with symptoms frequently related to other viral infections. Outbreaks caused by OROV were reported in several Brazilian states including Amazonas, Acre, Pará, Mato Grosso, as well as in other South America countries. Considering the known endemicity of OROV in the Amazon region, this study aimed to investigate the presence of this virus in distinct biological samples from patients suspected of arboviruses infection. Between November 2015 and July 2016, a total of 759 acute-phase specimens, from patients presenting symptoms suggestive of acute viral infection, were collected and tested for Zika virus (ZIKV), chikungunya virus (CHIKV), and dengue virus (DENV). Among these, 347 were positive for ZIKV by RT-qPCR, three were positive for CHIKV, and five were positive for DENV. All negative samples were submitted to OROV detection by an RT-qPCR protocol previously developed by our group, targeting the S segment. We detected 11 OROV positive serum samples, which were processed for the nucleotide sequencing on ABI 3130 genetic analyzer. Serum, saliva, and urine were collected for five out of the 11 OROV positive patients, which allowed us to test these body fluids for OROV. Therefore, these samples were processed like described for serum. One female patient, whose samples were collected five days after the symptom's onset, presented positive saliva and urine, with CTs of 31 and 26 respectively. To further confirm the OROV presence in these body fluids, we submitted all the genomic segments to RT-PCR, followed by nucleotide sequencing. Partial CDS sequencing was successful for both the S, M and L segments. The trace files were initially analyzed using the Geneious software v10.2.2 for quality check, trimming, and contig assembly.

Species identification was performed using an ICTV reference dataset and maximum-likelihood phylogenetic reconstruction with PhyML-SMS. The detection of OROV in one patient's urine and saliva confirms that this virus sheds into additional body fluids than serum. Longitudinal studies, with a more significant number of patients, are necessary to evaluate the potential use of different body fluids to OROV detection.

Financial support: DECIT, CAPES, CNPq, Fiocruz

Keywords: Oropouche, Arthropod-borne virus, Urine, Saliva, Detection



DETECTION OF HUMAN PAPILLOMAVIRUS L2 GENE DNA FRAGMENTS IN THE VIROME OF ACUTE FEBRILE PATIENTS IN AMAZONAS, BRAZIL

Victor Costa de Souza ¹, Valdinete Alves do Nascimento ^{1,3}, Andre de Lima Guerra Corado ^{1,3}, Dana Cristina da Silva Monteiro ^{1,2}, Felipe Gomes Naveca ¹

¹ ILMD / FIOCRUZ - Leônidas and Maria Deane Institute (Rua Teresina, 476. Adrianópolis.), ² UFAM - Federal University of Amazonas (Av. Rodrigo Otávio), ³ IOC / FIOCRUZ - Oswaldo Cruz Institute (Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - RJ)

Abstract

The metagenomic approach is an efficient technic for detection of infectious agents, mainly for those considered non-usual. Both pre-processing and sample handling are crucial to preserving the viral infection agent, facilitating the amplification by molecular assays and the detection by bioinformatics pipelines. Between February and June 2016, we investigated the viral etiology of acute febrile illness in patients attended in a sentinel hospital during the emergence of Zika virus (ZIKV) in Manaus, Amazonas, Brazil. Initially, all patients were tested for ZIKV, dengue virus (DENV), chikungunya virus (CHIKV), Mayaro virus (MAYV), and Oropouche virus (OROV). Negative samples were eligible for a viral metagenomics protocol, employing quasi- random amplification, NGS, and bioinformatics analysis. Briefly, eight patients had equal amounts of serum and urine mixed before filtration and treatments with nucleases. Subsequently, samples had the total nucleic acid extracted with the ReliaPrep kit (Promega), followed by the production of double-stranded cDNA and unbiased whole transcriptome amplification with WTA2 (Sigma). The libraries were prepared with NexteraXT and sequenced with MiSeq V2 (1 x 300bp) single-end run at the National Institute of Amazonian Research (INPA), according to the manufacturer's instructions. Initially, a total of 8 Human papillomavirus (HPV) reads were found in one sample with by Kaiju 1.6.3, among the 2 million reads that were generated for this library. Thus, we used the map-to-reference tool in Geneious 10.2.6 to assembly 10 reads against an HPV reference sequence, which generated a 600bp contig. A BLASTn search of this contig returned the HPV type 107 as the closest match. Therefore, a new assembly was performed, resulting in a 633bp contig with 17 reads. A Clustal Omega alignment made with 50 complete HPV sequences available on GenBank, and the sequence obtained in this study, was used for phylogenetic analysis with FastTree 2.1.5, using the generalized time-reversible (GTR) model with discrete gamma distribution. The HPV sequence obtained in the present study clustered with HPV type 107 with 98% of support. We are now developing a specific PCR strategy to characterize this virus fully.

Keywords: Virome, Metagenomic, Human papillomavirus, HPV, Amazonas



PHYLOGEOGRAPHIC STUDY OF DENGUE SEROTYPE 4 IN THE STATE OF AMAZONAS, BRAZIL, 2011 TO 2016

Dana Cristina da Silva Monteiro ^{1,2}, Marineide Souza da Silva ¹, Valdinete Alves do Nascimento ², Victor Costa de Souza ², Regina Maria Pinto de Figueiredo ³, Felipe Gomes Naveca ²

¹ UFAM - Universidade Federal do Amazonas (Av. Rodrigo Otávio), ² ILMD - Instituto Leônidas e Maria Deane, Fiocruz Amazônia (Rua Terezina, Adrianópolis), ³ FMT-HVD - Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (Av. Pedro Teixeira)

Abstract

Dengue is considered one of the major public health problems in the world, and 3 billion people live in areas with a risk of disease. In Brazil, the first laboratory record of dengue virus (DENV) occurred in 1982, with the identification of serotypes 1 and 4. Since then, outbreaks of the disease have been reported almost every year. Although DENV-4 was one of the first identified in the country, its circulation was only registered again in 2008, when it reemerged in the state of Amazonas, northern region of Brazil. Therefore, in order to identify the phylodynamics of DENV-4 that circulated in Amazonas State, we performed the present study with samples collected between 2011 and 2016. Firstly, DENV-4 positive samples were submitted to PCR for complete amplification of the envelope gene (1485pb). For further phylogenetic analysis, a DENV-4 dataset encompassing nucleotide sequences and metadata, including information about place of origin; year of isolation and GenBank accession number was analyzed. Genotyping was conducted by phylogenetic analyzes using the maximum likelihood (ML) method implemented in the PhyML (3.0) software. A total of 36 samples of DENV-4, collected in eight municipalities (Borba, Itacoatiara, Manacapuru, Manaus, Maúes, Novo Airão, São Gabriel da Cachoeira and Tefé), were sequenced and analyzed, all belonging to genotype II. Phylogeographic analyzes performed with the BEAST software (v1.10.1) support at least two independent DENV-4 introductions in the state of Amazonas. The first clade includes sequences obtained in Roraima in 2010 and the Amazonas in 2011, and the second 36 sequences identified in this study, as well as other sequences from Amazonas and Roraima States. Furthermore, our data also suggest the spread beginning from Manaus to the other municipalities inside the Amazonas State. We estimated the evolutionary rate and mean dispersion of DENV-4 in Brazil, as 1.145×10^{-3} and 175.98 [95% range HPD 147.69 - 201, 436] Km/year, respectively. The data obtained in this study contribute to the strengthening of dengue surveillance in the state of Amazonas, especially regarding the dissemination of DENV-4 GII.

Keywords: Dengue, Phylodynamics, DENV-4 GII, Phylogenetic, Surveillance



PREDICTORS FACTORS FOR CERVICAL CANCER IN HPV UNIMMUNIZED WOMEN: MOLECULAR AND EPIDEMIOLOGICAL STUDY

Rachel Siqueira de Queiroz Simões¹, Ortrud Monika Barth¹

¹ Fiocruz - Fundação Oswaldo Cruz (Avenida Brasil 4.365 - Manguinhos, Rio de Janeiro, cep: 21040-900)

Abstract

Cervical cancer is the most common cancer in developing countries induced by Papillomaviruses. These non-enveloped viruses belong to the *Papillomaviridae* family presenting a closed circular double-stranded DNA genome of approximately 8 kb. More than 200 HPV genotypes and several HPV types, associated to particular diseases as oral lesions (Heck's disease, oropharyngeal carcinoma, laryngeal papillomas), anogenital warts (Bowenoid papulosis, Buschike-Lowenstein tumor), Epidermodysplasia verruciformis (plane warts, Pityriasis-like plaques, squamous cell carcinomas of sun-exposed skin) have been described. The development of vaccines against HPV use recombinant DNA technology, as some viral particles have the ability to self-assembling into virus-like particles (VLP) independently of the viral genome. The aim of the present study was to evaluate the prevalence of HPV in sexually active women. The participants were randomly selected and interviewed about demographic and socio-economic characteristics related to cervical cancer. A cross-sectional study was conducted from 2014 to 2016 with women from the Manguinhos Complex community in Rio de Janeiro city, who spontaneously accessed gynecology ambulatory. Cervical samples collected with a cytobrush were analyzed by PCR amplification of L1 ORF, the most conserved region. HPV-DNA positive samples were detected by consensus (MY09/MY11), Nested PCR (GP5+/GP6+) and high-types specific primers (HPV16/18/31/45). In order to evaluate the viral DNA quality, swab samples collected were amplified by β -globin PCR primers (PC04/GH20). Restriction fragment length polymorphism (RFLP) assay patterns for mucosal HPVs were used for genotyping. Chi-square test was used to analyze the risk factors associated with HPV infection. The population study understood 100 women, 15 to 75 years aged and presenting normal cytology. Prevalence of 20% positive samples of cervical HPV-DNA was confirmed. HPV-18 was the most prevalent genotype (8%). About 16% reported being smokers and 3% drug users. Of all the participating women, 27% used alcoholic, 40% reported having had at least one abortion, 15% used oral contraceptives, while 71% did not use any type of condom (p

Keywords: cervical cancer, epidemiology, human papillomavirus, predictors factors, vaccine



THERAPEUTIC EFFICACY OF SEAWEED *CANISTROCARPUS CERVICORNIS* AGAINST ZIKA VIRUS

Caroline de Souza Barros ¹, Claudio Cirne-Santos ¹, Rafaela Gomes ¹, Max Willian Gomes ¹, Ana Carolina Morgon Tavares de Oliveira ¹, Carla Eponina Carvalho Pinto ¹, Valeria Laneuville Teixeira ^{1,2}, Izabel Christina de Palmer Paixão ¹

¹ UFF - Universidade Federal Fluminense (Outeiro de São Joao Batista s/n, Centro Niteroi RJ), ² UNIRIO - Universidade Federal do Estado do Rio de Janeiro (Avenida Pasteur 458, Urca, Ri de Janeiro, RJ, Brasil)

Abstract

Zika virus (ZIKV) is an arbovirus that shown to be sexually transmitted and cause severe congenital defects such as microcephaly. However, there are no FDA-approved therapies or vaccines against ZIKV infection. Studies with the marine brown alga *Canistrocarpus cervicornis* showed antiviral potential. Hence, the aim of this work was to evaluate the anti-ZIKV activity of a marine dolastane isolated from brown alga *C. cervicornis* and its crude extract. Vero cells were used to antiviral assays and treated with different concentrations of *C. cervicornis* extract or dolastane. The inhibitory effect was evaluated by the plaques reduced assay inhibition of the viral plaques and time of drug addition (TOA) cells were treated at different times up to 24 hours post infection. Inhibition of caspases 3 and 7 at 10 μ m of the dolastane in Jurkat cells was also evaluated, using ribavirin as control. To evaluate the therapeutic efficacy of the extract two months old male dexamethasone- immunosuppressed BALB/c mice were infected intraperitoneally with 1 \times 10⁵ PFUs of ZIKV. The infected animals were divided into two groups and treated orally with *C. cervicornis* extract (200mg/kg, n=5) or saline (n=5) once a day. *C. cervicornis* extract and dolastane tested in Vero cells were able to inhibit ZIKV in a dose dependent manner, showing EC50 of 3.3 μ g/mL and 0.95 μ M, respectively. In TOA we observed that both the extract and the dolastane were able to inhibit the replication of ZIKV at different times of addition post infection being still efficient if added after 16 hours of post treatment declining soon after. Dolastane was able to inhibit caspases 3 and 7 in ZIKV infected cells by 95%, while ribavirin inhibited 68% at the same concentration. These results demonstrated that the two substances tested have potent activity against ZIKV *in vitro*. Thus, the therapeutic efficacy test was performed in which *C. cervicornis* extract also showed antiviral activity by keeping testicles within normal range in adult dexamethasone-immunosuppressed mice on the other hands, untreated animals showed alterations such as testicular degeneration, interstitial cell hyperplasia and presence of macrophages in the testicles. Our data demonstrate that *C. cervicornis* could be considered for future treatment of ZIKV infection.

Financial support: CNPq, CAPES, FAPERJ, UFF (PROPI)

Keywords: Arbovirus, ZIKA, *Canistrocarpus cervicornis*, Seaweed



DENGUE, ZIKA AND CHIKUNGUNYA INFECTION: SEROEPIDEMIOLOGICAL STUDY OF ARBOVIRUSES IN TANGARÁ DA SERRA – MT, 2018

Dandára Thaís de Oliveira Ferreira ¹, Lavinia Schüler Faccini ², Giovanni Vinícius Araújo de França ³, Ana Cláudia Pereira Terças Trettel ⁴, Marina Atanaka ¹, Viviane Karolina Vivi Oliveira ¹

¹ UFMT - Universidade Federal de Mato Grosso (Av. Fernando Corrêa da Costa, 2367 - Boa Esperança, Cuiabá - MT, 78060-900), ² UFRGS - Universidade Federal do Rio Grande do Sul (Av. Paulo Gama, 110 - Farroupilha, Porto Alegre - RS, 90040-060), ³ MS - Ministério da Saúde (Zona Cívico-Administrativa, 70058900 - Brasília, DF - Brasil), ⁴ UNEMAT - Universidade do Estado de Mato Grosso (Jardim Industrial 78300000 - Tangará da Serra, MT - Brasil)

Abstract

Brazil is an endemic country for dengue (DENV) arbovirus and zika (ZIKV) and chikungunya (CHIKV) that emerged in the country between 2014 and 2015. In 2017 in the municipality of Tangará da Serra the incidence of dengue, zika and chikungunya cases was respectively 303, 29 and 31 cases for every 100 thousand inhabitants. The objective of the study was to identify the infection profile of dengue, zika and chikungunya arboviruses in the municipality of Tangará da Serra - MT in 2018. A cross-sectional study was conducted with 596 residents in the urban area of Tangará da Serra, which were analyzed demographic, health and biological factors. 596 blood samples were collected for rapid testing to detect antibodies to DENV, ZIKV and CHIKV. The study was conducted between January and March 2018, when there were home visits and the survey participants were interviewed for demographic and socioeconomic data, as well as blood samples were collected. For detection of antibodies to dengue, zika and chikungunya viruses, a rapid test was performed from whole blood collected by intravenous puncture. Statistical measures were used to describe the sociodemographic and health characteristics of the population, and the seroprevalence of arboviruses contemplated in this study was also calculated from them. The global prevalence for sex, age, education, race and marital status for dengue was around 12.8%, for zika the numbers remained between 7.0 and 7.2%, while for chikungunya the overall prevalence was 2.2%. For dengue virus, the highest prevalence was found in males (14.1%), age group 41-54 years (14.6%), incomplete higher education (17.0%), black race (22.4%) and single marital status (15.1%). The prevalence of Zika was concentrated in female participants (7.2%), age ≥ 55 years (7.8%), complete elementary level (11.9%), white race (8.7 %) and divorced or separated marital status (21.4%). For chikungunya the prevalence was 2.6% for males, 2.7% for 18-27-year-old, 7.1% for illiterate, 2.6% for black race, 2.6 % for race/color white and 3.6% for single individuals. Regarding the prevalence of the arboviruses found in the population of Tangará da Serra, considering the sociodemographic profile, this was different in several respects from the prevalence found in other studies considering the same variables. Further studies are needed to help understand the dynamics of transmission of these arboviruses in the municipality. Financial Support: Ministry of Health (Brazil).

Keywords: Arboviruses, Communicable diseases, Mosquito Vectors, Seroepidemiologic Studies



CHIKUNGUNYA FEVER IN THE MIDWEST REGION OF BRAZIL AND THE STATE OF MATO GROSSO IN 2018

Dandára Thaís de Oliveira Ferreira ¹, Viviane Karolina Vivi Oliveira ¹, Marina Atanaka ¹

¹ UFMT - Universidade Federal de Mato Grosso (Av. Fernando Corrêa da Costa, 2367 - Boa Esperança, Cuiabá - MT, 78060-900)

Abstract

Chikungunya is a febrile illness characterized by its sudden onset and severe joint pain. Following its first outbreak in 1952 in Tanzania, the Chikungunya virus spread globally. In 2014, the first cases of Chikungunya in Brazil in the North and Northeast regions were reported. In 2018, the analysis of the incidence rates of probable cases of chikungunya fever showed that the Midwest and Southeast region had the highest incidence rates. Standing out among the Federative Units is Mato Grosso, a state that appeared frequently among those with the highest number of cases of the disease in the respective year of 2018. The objective of the research was to present the incidence rates of chikungunya fever in the state of Mato Grosso during the year 2018. The study was conducted from a documentary analysis of 2018 epidemiological bulletins on the monitoring of dengue, chikungunya fever, and zika virus fever in Mato Grosso state in 2018. Of the 64 epidemiological bulletins published by January 2019, 35 were analyzed. As inclusion criteria established for data analysis, epidemiological bulletins should be available for public consultation on the Ministry of Health's web page and address chikungunya fever monitoring. We describe in this paper the number of probable cases of chikungunya fever in the Midwest region of Brasil and the incidence rate of probable cases of chikungunya fever in the state of Mato Grosso (cases / 100,000 inhabitants) from data from each epidemiological week (EW). In 2018, from epidemiological week 3 to EW 16, the Midwest region had the highest number of likely cases of chikungunya fever (13,526 cases; 18.8%) compared to the total of the country. The number of probable cases of chikungunya fever in the Midwest region increased gradually during 2018 from 603 probable cases registered in EW 3 to 13,862 probable cases in EW 52. The incidence rates of probable cases also increased gradually with each from EW 3 to EW 52, the probable case incidence rate jumped from 17 probable cases per 100,000 inhabitants to 387.6 probable cases per 100,000 inhabitants of the state of Mato Grosso. The epidemiological investigation conducted by health surveillance showed through epidemiological bulletins how Chikungunya fever has taken great proportions, specifically in the state of Mato Grosso, Federative Unit that stood out in higher incidence rates of probable cases according to data from Epidemiological Weeks. Financial support: CNPQ.

Keywords: Arboviruses, Communicable diseases, Chikungunya Fever, Neglected Diseases



ANTIVIRAL EFFECT OF THE N-SULFONATED NAPHTHOQUINONE AGAINST THE ZIKA VIRUS

Claudio Cesar Cirne Santos ¹, Caroline de Souza Barros ¹, Paulo Anastácio Furtado Pacheco ¹, Daniel Gonzaga ², Davi Rodrigues Rocha ^{1,1}, Fernando de Carvalho da Silva ¹, Vitor Francisco Ferreira ¹, Izabel Christina de Palmer Paixão ¹

¹ UFF - Universidade Federal Fluminense (Outeiro de São João Batista S/n, Centro, Niterói, RJ), ² UEZO - Centro Universitário Estadual da Zona Oeste (Rio de Janeiro, RJ)

Abstract

Zika Virus (ZIKV) a member of the family Flaviviridae is a human pathogen of global significance. ZIKV has become a public health problem with increases in numbers of cases and a strong association between ZIKV outbreaks and the spread of cases of Guillain-Barré Syndrome and microcephaly. In this study we evaluated the compound N-sulfonated Naphthoquinone against ZIKV using vero cells and neurons cell culture. These purified cultures of retinal neurons were firstly described by Adler et al. (1984). In this work, they featured that almost 100% of the cells possess a neuronal identity using techniques such as phase-contrast microscopy, lectin staining and electron microscopy. For caspase 3 and 7 assays we used the Muse™ Caspase-3/7 kit to evaluate simultaneous parameters of apoptotic status based on activation of Caspase-3/7 and cell plasma membrane permeabilization and cell death using Muse™ Cell Analyzer. The compound tested in vero cells inhibited ZIKV replication in a dose-dependent manner at low concentrations, with EC50 value of 0.92 μ M and a selective index (SI) of 320. In neurons cells we observed significant results with EC50 of 1.1 and SI of 213 showing to be promise. In addition we demonstrate that the compound had a strong synergistic effect with ribavirin and in time of addition experiment (TOA) we observed that the inhibitory effect remained more than 80% in addition to the compound for up to 12 hours post infection. Interestingly the compound was able to inhibit ZIKV by caspase 3 and 7 activity and cell-viability assays using the EC50 concentration. Our promising results suggest that this compound is excellent candidate to further studies for the development of novel anti-ZIKV agents.

Financial support: CNPq, CAPES, FAPERJ, UFF (PROPI)

Keywords: Arbovirus, ZIKA, N-SULFONATED NAPHTHOQUINONE, Antiviral effect



FREQUENCY OF SEXUAL VIRAL AND BACTERIAL TRANSMISSION INFECTIONS IN ANAL BRUSHES SAMPLES OF PARAGUAYANS FEMALE SEX WORKERS BY MOLECULAR METHODS

Alfonzo Tania Mabel¹, Valenzuela Adriana¹, Graciela Giménez¹, Cardozo Fátima¹, Bernal Laura¹, Mongelos Pamela¹, Kasamatsu Elena¹, Castro Amalia¹, Rodriguez Isabel¹, Paez Malvina¹, Laspina Florentina¹, Medina Graciela⁵, Aguilar Gloria³, Deluca Gerardo⁴, Picconi Alejandra², Mendoza Torres Laura Patricia¹

¹ IICS-UNA - Instituto de Investigaciones en Ciencias de la Salud (Dr. Cecilio baez c/ Dr. Gaspar Villamayor (Campus San Lorenzo, Paraguay)), ² ANLIS - Administración Nacional de Laboratorios e Institutos de Salud "Dr. Carlos Malbrán" (Avda. Velez Sarsfield 563, Bs.As. Argentina), ³ PRONASIDA - Programa Nacional de control de VIH Sida (MSPyBS) (Avda. Venezuela c/ Florida (Asunción, Paraguay)), ⁴ IMR, UNNE - Instituto de Medicina Regional. Universidad Nacional del Nordeste, Resistencia. Argentina (Avda. Las Heras 727 (Resistencia, Chaco. Argentina)), ⁵ IPS - Instituto de Previsión Social (Avda. Santísimo Sacramento (Asunción, Paraguay))

Abstract

ually transmitted infections (STIs) are public health issue worldwide, female sex workers (FSW) are considered to be among high-risk populations for the acquisition of STIs. There is few information about STIs in the anal canal, so the objective of the y was to determine the frequency of viral and bacterial STIs in samples of anal brushing of 149 FSW from the Central, guazú and Amambay Department of Paraguay, by molecular methods (PCR). The frequency of at least one anal STI was 4% (IC95% 52.07% - 68.31%). Among bacterial anal STIs the frequency for *Neisseria gonorrhoeae* was 7.38% (3.74% - 12.83%), *Chlamydia trachomatis* was 4.70% (IC95% 1.91% - 9.44%) and for *Ureaplasma urealyticum*, as for *Mycoplasma genitalium* was % (IC95% 0.02% - 3.68%) each. In addition, 3.36% (95% CI 1.10% - 7.66%) of STIs were Herpes Simplex virus positives, and the est frequency was Human papilloma virus (HPV) infection with 51.7% (IC95% 43.36% - 59.93%) in all women. The highest uency of STIs was seen in women aged between 20 and 30 years. Among the 149 women, 50% reported having anal sex, 9% of them had more than 7 clients per week and 62.4% reported using condoms. No significant differences were observed ween the presence of anal STIs and the sociodemographic characteristics and risk factors associated with this population. In clusion, the results suggest that the introduction of tests for the detection of STIs in the anal canal in populations at-risk is ential to help strengthen the detection, clinical management and surveillance of STIs, with a view to reduce their incidence he country.

Keywords: Anal STIs, Viral infections, FSW, risk factor's



STUDY OF IMMUNOREGULATORY MECHANISMS MEDIATED BY REGULATORY T CELL DURING DENGUE INFECTION IN HUMANS

Marcela Helena Gonçalves Pereira ¹, Maria Marta Figueiredo ², Camila Pereira Queiroz ¹, Tércia Vasconcelos Barros ³, Adriana Mafra ⁴, Lilian Martins Oliveira Diniz ¹, Último Libânio da Costa ⁵, Kenneth John Gollob ⁶, Lis Ribeiro Valle Antonelli ², Helton Costa Santiago ¹

¹ UFMG - Universidade Federal de Minas Gerais (Belo Horizonte/MG), ² FioCruz - Instituto Rene Rachou - Fundação Oswaldo Cruz (Belo Horizonte/MG), ³ H. Sta Casa - Hospital Santa Casa (Belo Horizonte/MG), ⁴ H.M. Odilon Behrens - Hospital Metropolitano Odilon Behrens (Belo Horizonte/MG), ⁵ SMS-MG - Secretaria Municipal de Saúde/MG (Belo Horizonte/MG), ⁶ AC CCC - A. C. Camargo Câncer Center (São Paulo/SP)

Abstract

Introduction: Individuals with dengue most often presents as a self-limiting or asymptomatic disease (mild dengue), but others may develop complications such as the presence of alarm signs and hemorrhagic manifestations (ws+/severe dengue) that require hospitalization. There is little information on the regulation of immunopathology developed in dengue. Regulatory T cells (Tregs) control inflammation both by producing anti-inflammatory cytokine, like IL10, and by direct inhibition of effector cells mediated by immunoregulatory molecules such as CD200, GITR, PD1, LAG3, among others. Therefore, we aimed to investigate the presence of these Tregs in different clinical forms of dengue infection.

Methods and Results: PBMCs of dengue patients during defervescence and convalescent phases were cultured in the presence of ENV or NS3 peptide libraries of DENV. Regulatory molecules were evaluated in Teff cells (CD4+CD45RA-CD27-) and we found that although DENV infection increased the frequency of Teff cells expressing regulatory molecules (GITR, LAP and PD1) but was similar among different dengue classifications in critical phase. Although there are no differences in the frequency of Tregs cells (CD4+CD25hiCD127lowFoxP3+) between dengue groups, we found that regulatory mechanisms are impaired in the ws+/severe group. Regarding the regulatory molecules on Treg cells, while expression of CTLA4, LAP3 and PD1 by Tregs are similar between dengue groups, Tregs from mild patients showed higher levels of CD200, CD226 and GITR when compared with ws+/severe. Noteworthy is a population of ENV-specific Tregs producing high levels of IL10 in mild dengue, but not in ws+/severe individuals. The ENV-specific IL10+ Tregs were GITR+ and expressed Helios, a marker of natural Treg. These ENV-specific IL10+ Tregs do not express Ki67 or Tbet, proliferation markers, and cells compromised with the Th1 profile. In addition, using epitope mapping from DENV1 ENV peptide library we were able to identify the peptide that induces IL10 production by nTregs GITR+.

Conclusion: Teff cells appears to be minor role on different clinical evolution of dengue. Tregs from ws+/severe individuals displayed important deficient in activation markers, suggesting dysfunctional phenotype. The presence of IL10 from nTregs GITR+ cells was associated with the mild presentation of dengue, suggesting that these regulatory mechanisms are important to limit the immunopathology of dengue.

Footnote: CNPq, CAPES and FAPEMIG

Keywords: Anti-inflammatory cytokines, Dengue, Peptides library , Regulatory molecules, Regulatory T cells



IL10+ MULTIFUNCTIONAL T CELLS ARE ASSOCIATED WITH MILD FORMS OF DENGUE INFECTION IN HUMANS

Marcela Helena Gonçalves Pereira ¹, Maria Marta Figueiredo ², Camila Pereira Queiroz ¹, Tércia Vasconcelos Barros ³, Adriana Mafra ⁴, Lilian Martins Oliveira Diniz ¹, Último Libânio da Costa ⁵, Kenneth John Gollob ⁶, Lis Ribeiro do Valle Antonelli ², Helton da Costa Santiago ¹

¹UFMG - Universidade Federal de Minas Gerais (Belo Horizonte / MG), ²FioCruz - Instituto Rene Rachou - Fundação Oswaldo Cruz (Belo Horizonte / MG), ³Hosp. Sta Casa - Hospital Santa Casa (Belo Horizonte / MG), ⁴H.M. Odilon Behrens - Hospital Metropolitano Odilon Behrens (Belo Horizonte / MG), ⁵SMS-MG - Secretaria Municipal de Saúde (Belo Horizonte / MG), ⁶AC CCC - A. C. Camargo Câncer Center (São Paulo / SP)

Abstract

Dengue can be classified in dengue without warning signs (mild dengue) or cases that require hospitalization like dengue with warning signs or hemorrhagic dengue (ws+/severe). Severe dengue manifestations are thought to be the result of uncontrolled immune response activation. IL10-producing multifunctional T cells have been implicated in favorable evolution of many infectious diseases contributing to efficacious immune response while limiting immunopathology.

PBMCs from dengue patients, during defervescence and convalescence phases, were cultured in the presence of ENV or NS3 peptide libraries of DENV. Mild dengue patients displayed higher levels of IFN γ , TNF and IL12p70 in plasma at critical phase of infection. Interestingly, frequencies of ENV- and NS3-specific CD4+ or CD8+ T cells single producers of IFN γ were not different when compared mild dengue and ws+/severe dengue patients during critical and convalescence phases, but TNF or IL10 were higher in patients with mild dengue when compared to ws+/severe patients in a time and antigen dependent manner. NS3-specific CD4+ T cells producing IFN γ /IL10 were increased in mild dengue presentation when compared to ws+/severe dengue patients in critical phase. Patients with mild dengue presented increased frequencies of ENV-specific and NS3-specific CD8+ T cells producing IFN γ /IL10 compare to ws+/severe dengue during critical period. In addition, NS3- specific CD8+ T cells, from mild dengue, producing high levels of IFN γ /TNF and IFN γ /TNF/IL10 when compared to ws+/severe in both critical and convalescence phases. It is important to mention that multifunctional T cells produced higher levels of those cytokines as measured by intracellular content when compared to single producer T cells. Plasmatic levels of IL27 were not different between groups, but ws+/severe dengue had decrease of IL12 during critical phase when compare to mild dengue and control individuals.

Multifunctional T cells producing IL10 was associated with mild dengue presentation, but not ws+/severe dengue maybe for reduction IL12, related to the emergence and IL10-producing by these cells. Multifunctional T cells seems to be important against DENV because are major producers of IFN γ , TNF and IL10. The proinflammatory response of Th1 associated with a regulatory response provided by the IL10 produced by multifunctional T cells, contributes to balancing effector mechanisms against the virus and preventing pathology.

Footnote: CNPq, CAPES and FAPEMIG

Keywords: Anti-inflammatory cytokines, Dengue, Immunopathology, Multifunctional T cells, Pro-inflammatory cytokines



EPIDEMIOLOGICAL PROFILE OF PATIENTS DIAGNOSED WITH DENGUE, ZIKA AND CHIKUNGUNYA BY RT-PCR BY LACEN- MT IN 2018

Marcelo Adriano Mendes dos Santos ^{2,1}, Nilvanei Aparecido Neves ¹, Janeth Aracely Ramirez Pavon ¹, Elaine Cristina de Oliveira ³, Claudio Luis Campos Souza ³, Ana Elisa Viniski ³, Renata Dezengrini Silhessarenko

¹ UFMT - Universidade Federal de Mato Grosso (Av. Fernando Corrêa da Costa, 2367 - Boa Esperança, Cuiabá - MT, 78060-900), ² UNEMAT - Universidade do Estado de Mato Grosso (Av. Santos Dumont, s/n. Cidade Universitária CEP: 78.200-000), ³ LACEN-MT - Laboratório Central de Estado de Mato Grosso (Travessa Thogo da Silva Pereira, nº 63, Centro, Cuiabá - Mato Grosso CEP: 78.020-500)

Abstract

Introduction: Recently there has been a considerable increase in arboviruses outbreaks in Brazil, especially after the introduction of the zika virus (ZIKV) and chikungunya (CHIKV). The high rates of vector infestation, associated with the favorable climate and susceptible population, make our state a favorable environment for the occurrence of arboviruses outbreaks, justifying the importance of maintaining constant surveillance in epidemics for circulating viruses. **Material and Methods:** Data were obtained from the Laboratory Environment Manager (LAG) of the Central Public Health Laboratory of Mato Grosso-LACEN-MT. The calculations were made with the EPI INFOTM program version 7.2.3.1, with a confidence level of 95%. **Results:** The survey resulted in 531 requests for RT-PCR for CHIKV, with 410 samples tested and 226 positive (overall prevalence of 55.12% - 50.2-59.87) in the period. Most positive samples were from Cuiabá (68.14%; 61.64-74.16), with a prevalence of 59.69% (53.43-65.73). In Cuiabá, the Dom Aquino neighborhood had the highest prevalence (13.16%; 6.49-22.87). CHIKV infection was associated only with race, being more prevalent in the brown population (54%; OR: 1.78; 1.08-2.93; p-value < 0.05). The majority (82.30%) were recorded in patients up to 40 years of age (OR: 1.59; 0.99-2.56; p-value = 0.05). There were 152 requests for RT-PCR for DENV, 112 of which were negative. Of 123 NS1 studies, none were positive. IgM for DENV in 2,507 samples resulted in 1,023 positive results (40.81%; 38.9-42.74). A total of 251 RT-PCR reactions to ZIKV were performed with only one reagent, and the IgG test resulted in a reagent in 193 samples. The most frequent symptoms were fever, 88.71% (78.11-95.34), myalgia 80.65 (68.63-89.58), arthralgia 75.86 (56.46-89.70) and headache 61.29 (48.07-73.40). No patient with CHIKV reported pruritus. **Conclusion:** The high prevalence of CHIKV in 2018 shows the increasing trend of viral circulation in the state. In 2017, the data showed 297 cases (overall prevalence: 41.89% - 38.31-45.56, and in Cuiabá 43.06% - 36.25-50.07). DENV in 2017 was similar to 2018 with 51 requests for RT-PCR, all non-reactive, and 45.33% (42.96-47.73) were positive for IgM. The same trend was verified with ZIKV, without detected viremia and 192 immunity results (IgG). Some confusion with the term pruritus may have prevented its reporting by patients. The LAG system, although robust and practical, lacks epidemiological data, which limited this study.

Keywords: Dengue, Zika Virus infection, Chikungunya Fever, Polymerase Chain Reaction, Health Profile



MODULATION OF HERV EXPRESSION BY DIFFERENT ARBOVIRUSES DURING INFECTION OF HUMAN PRIMARY ASTROCYTES

Fernanda Luz de Castro ¹, Victor Emmanuel Viana Geddes ¹, Liliane Tavares de Faria Cavalcante ¹, Otávio José Fernandes Brustolini ², Joseane Biso de Carvalho ², Guilherme Loss de Moraes ², Ana Tereza Ribeiro de Vasconcelos ², Alexandra Lehmkuhl Gerber ², Iranaia Assunção Miranda ¹, Luciana Ferreira Romão ¹, Amilcar Tanuri ¹, Renato Santana de Aguiar ^{3,1}

¹ UFRJ - Universidade Federal do Rio de Janeiro (PREDIO DO CCS UFRJ, Av. Carlos Chagas Filho, 373, Rio de Janeiro - RJ, 21941-590), ² LNCC - Laboratório Nacional de Computação Científica (Av. Getúlio Vargas, 333 - Quitandinha, Petrópolis - RJ, 25651-075), ³ UFMG - Universidade Federal de Minas Gerais (Av. Pres. Antônio Carlos, 6627 - Pampulha, Belo Horizonte - MG, 31270-901)

Abstract

Viruses are the most common infectious agents associated with encephalitis, including the genera *Bunyavirus*, *Flavivirus* and *Alphavirus*. In viral encephalitis, the nature of astrocyte involvement is still unclear. The human retroelements (HERVs) are sequences of retroviral origin that are fixed in human genome, and their role during inflammatory responses of the CNS is still poor understood. Previous reports showed that HERVs induction in astrocytes stimulates the expression of genes involved in inflammatory regulation. In this work, we attempt to understand the role of HERV expression during the infection of four arboviruses of clinical importance in Brazil and associated to encephalitis sporadic cases. Human primary astrocytes were isolated from the temporal lobe region of patients selected for surgical treatment of temporal lobe epilepsy associated with hippocampus sclerosis. Astrocytes were infected with Zika (ZIKV), Mayaro (MAYV), Oropouche (OROV) and Chikungunya (CHIKV) viruses at a MOI of 1. The infections were confirmed by flow cytometric analysis and only cells with 80% of infectivity levels were considered. Plaque assay was performed to ensure productive infections and cytotoxicity test was carried using CellTiter. RNAseq was performed using infected versus non-infected cells using NextSeq 500/550 Kits High Output v2 (150 cycles) and HERV expression evaluated by TELESCOPE software. Our results showed the first description of MAYV, OROV and CHIKV efficiently infecting astrocytes cells. Considering an adjusted p-value of 0.1, we found, respectively, 14 upregulated HERVs and 13 downregulated by ZIKV, 325 and 135 by CHIKV, 425 and 185 by MAYV and 288 and 204 by OROV. We also found co-modulated HERVs. Among the upregulated HERVs, we identified 18 HERVs co-modulated by CHIKV and OROV, 89 for CHIKV, MAYV and OROV and 45 for MAYV and OROV. A total of 6 HERVs were found to be modulated by all arboviruses, including HERV9_10q23.31, HERV9_11q21a, HERV9_5q13.2, HERVH_14q24.2c, HML3_12q13.12 and HML6_14q24.2. Among the downregulated HERVs, we found 71 HERVs co-modulated by CHIKV, MAYV and OROV, 21 by MAYV and OROV and 11 by CHIKV and OROV. Also 6 HERVs were downregulated for all viruses: HERV1_8p23.1, HERVL_4q26a, HERVL_6p25.2, HML2_3q21.2, HML3_5p15.33b, HML6_Xp11.22. These data and the search for genes whose expression may be affected by these HERVs may help to understand their role during arboviruses infection in the CNS. Financial Support: CAPES/CNPq

Keywords: HERV, Arboviruses, Astrocytes



DROSOPHILA CELLS AS ANTIGEN SUPPORT FOR ZIKA VIRUS DETECTION.

Alane Leite Xalega ¹, Renato Mancini Astray ¹, Soraia Attie Calil Jorge ¹ IB - INSTITUTO BUTANTAN (Avenida Vital Brasil 1500)

Abstract

The Zika virus (ZIKV) is a single-strand positive RNA that belongs to the genus *Flavivirus* in the family Flaviviridae. The ZIKV Envelope (zE) glycoprotein is composed of three ectodomains (DI, DII, and DIII) and is involved in various aspects of the viral cycle, mediating binding and membrane fusion. The availability of tests for the laboratory diagnosis of Zika infection is still very restricted, so there is a need to develop an affordable and more accurate test, reducing the risk of cross-reaction. The Neutralizing antibodies reacting with the EDIII ZIKV are generally specific for each virus and do not cross-neutralize other viruses. In this way, we pretend to express the EDIII domain of ZIKV in *Drosophila* S2 cells to develop a rapid immunofluorescence diagnostic test able to detect the infection of ZIKV in serum of human patients.

Schneider 2 cells (S2 cells), are derived from the late embryonic stage of the *Drosophila melanogaster* (20-24 hours old), which have been used as hosts for the expression of heterologous gene products. They are relatively cheap cells to maintain and produce heterologous proteins translated accurately and correctly processed.

The fragment encoding EDIII-ZIKV was cloned in the pAcV5-HisA-Hygro *Drosophila* vector, and the product was transformed and screened into DH5a. The vector obtained, pAc_ZIKVEDIII-hy, was transfected into S2 cells. After selection period we established the S2Ac_ZIKVEDIII-Hy Tc5 cell line. This recombinant cell line is being used to analyze the expression of zEDIII domain by immunofluorescence and flow cytometry. This cell line showed fluorescence in both techniques, although there is nonspecific wild cell fluorescence.

Financial Support: FAPESP, CAPES, Butantan Foundation.

Keywords: ZIKV, EDIII, S2, Immunofluorescence, diagnosis



BIOKINETIC STUDIES FOR SCALING UP SPODOPTERA FRUGIPERDA (SF9) CELL CULTURE PRODUCING RABIES VIRUS LIKE PARTICLES (VLPs)

Luis Giovanni de Oliveira Guardalini ¹

¹ USP - Universidade de São Paulo (Avenida Prof. Almeida Prado, 1280, Butantã, São Paulo (SP). CEP 05508- 900)

Abstract

Rabies is a globally distributed anthrozoosis that affects mammals, including humans. It is caused by a virus of the genus *Lyssavirus* that causes acute encephalitis, which is fatal in 100% of symptomatic cases. Control of this disease has been a global challenge. While developing countries continue to implement basic measures to prevent human and animal rabies, countries where rabies is a controlled urban infection are looking for effective ways to combat it in the wild. Vaccines available for prevention or treatment after infection are proven effective, but have high production costs and biosecurity risks. An effective vaccine preparation that combines a high neutralizing antibody-mediated immune response with a satisfactory level of cellular immune response may lead to the proposition of a new and more potent rabies vaccine. Several technologies can lead to this preparation, and obtaining a virus-based particle-based vaccine (VLPs) or pseudoparticles containing the rabies immunogenic glycoprotein is a very promising strategy. The use of recombinant baculovirus as a vaccine agent is a strategy that may lead to a protective immune response pattern and has been widely used in other vaccines. For this work, we use the intermediate vector pFastBac1, insert the genes of interest RVM or RVGP, constructing 2 intermediate vectors. We use these plasmids to transpose the bacmid into DH10Bac bacteria. Two recombinant bacmids carrying the RVGP and RVM genes were obtained and transfected into Sf9 cells. Supernatants from transfected cultures were collected yielding the first viral batch (L1). We tested different multiplicities of infection (MOI) for viral lot 2 (L2) and viral lot (L3) infection in order to define the optimal value for better Recombinant Baculovirus production. We also tested different MOI concentrations and collection time to determine what was the best MOI to perform coinfection to produce VLPs. The best MOI and the best harvest time were determined by dot blot, western blot, ELISA and Sf9 ET titration techniques. The assays detected proteins of interest at the expected size in isolation. Thus, we concluded that we obtained a good production of high viral titre batches and infection assays showed us that recombinant baculoviruses are expressing our proteins of interest. We hope to use optimal MOI and harvest data in the next few steps so that we can scale out rabies virus VLP production and perform purification for in vivo testing. asdasd

Keywords: Virus-Like Particles, Rabies virus , Baculovirus, Glycoprotein, Matrix Protein



VIRTUAL SCREENING OF BRAZILIAN NATURAL PRODUCTS TARGETING NSP2 PROTEASE FROM CHIKUNGUNYA VIRUS

Vitor Won-Held Rabelo ¹, Leonardo Santos Corrêa Amorim ¹, Tiago Barbosa Soares ¹, Maria Leonisa Sanchez Nuñez ¹, Caroline De Souza Barros ¹, Cláudio Cesar Cirne Dos Santos ¹, Paula Alvarez Abreu ², Izabel Christina Nunes De Palmer Paixão ¹

¹ UFF - Universidade Federal Fluminense (Rua Outeiro de São João Batista, S/N, Centro, Niterói, RJ, Brasil), ² UFRJ - Universidade Federal Do Rio de Janeiro (Avenida São José Barreto, 764, São José do Barreto, Macaé, RJ, Brasil)

Abstract

Chikungunya virus (CHIKV) is the causative agent of Chikungunya fever, which is responsible for several outbreaks in the last years and have affected over 30 countries. Despite the low mortality rate caused by this disease, it may cause severe manifestations that can persist for even years after acute infection and may compromise patients' quality of life drastically. Currently, there are no vaccines and antivirals for the prevention and treatment of this infection, which makes necessary the search for novel antiviral drugs. In this scenario, the CHIKV nsP2 protease arises as an attractive target since it is crucial for viral replication. The aim of this work is to identify potential inhibitors of CHIKV nsP2 protease from Brazilian biodiversity using molecular modeling tools. To explore the interactions of CHIKV nsP2 with known inhibitors, molecular docking studies were performed using Autodock 4.2.6. Docking results explained the importance of *trans* isomerism to the known inhibitors and revealed important interactions to the enzyme inhibition with residues N1011, C1013, A1046, Y1079, N1082, W1084, L1205, and M1242. Following this, the nsP2 complexed with the most active inhibitors (**C5** and **C19**) and an inactive ligand (**C9**) were submitted to molecular dynamics simulations for 30 ns using AMBER16. Simulations have confirmed the importance of interactions with residues C1013, N1082, H1083, and L1205. Additionally, inhibitors showed lower structural fluctuation and did not affect the cross-dynamic behavior of the enzyme significantly, which allowed discrimination between true and false inhibitors. Afterward, several pharmacophore models were developed using Pharmit and Pharmagist server. The model F was the only one able to identify all inhibitors of nsP2. Therefore, we employed this model to search for compounds within the Brazilian natural products database NUBBE, using the Pharmit server. The compounds were filtered out by their theoretical drug-like, pharmacokinetics and toxicological profiles using the Faf-Drugs4 server, yielding 159 compounds. In conclusion, we identified novel natural products as potential nsP2 inhibitors by computational tools which will be further submitted to molecular docking and simulation studies to prioritize the most promising compounds to the *in vitro* assays, aiding in the search of new anti-CHIKV agents.

Financial Support: CAPES, CNPq, FAPERJ, PROPPI-UFF

Keywords: Chikungunya, nsP2, protease, natural products, molecular modeling



USUV INFECTION DURING PREGNANCY IN MOUSE MODEL AND ITS CONSEQUENCES TO CONCEPTUSES

Marina Alves Fontoura¹, Aline Freitas de Paula Melo¹, Mariana Bortoletto Grizante¹, Rafael Elias Marques², Murilo Carvalho^{1,3}

¹ BM/LNBio/CNPEM - Bioimaging Methods Laboratory, Brazilian Biosciences National Laboratory, Brazilian Center for Research in Energy and Materials (Rua Giuseppe Maximo Scolfaro, 10000, Polo II de Alta Tecnologia, Campinas, São Paulo, Brasil), ² ID/LNBio/CNPEM - Infectious Diseases Laboratory, Brazilian Biosciences National Laboratory, Brazilian Center for Research in Energy and Materials (Rua Giuseppe Maximo Scolfaro, 10000, Polo II de Alta Tecnologia, Campinas, São Paulo, Brasil), ³ LNLS/CNPEM - Brazilian Synchrotron Light Laboratory (LNLS), Brazilian Center for Research in Energy and Materials (CNPEM) (Rua Giuseppe Maximo Scolfaro, 10000, Polo II de Alta Tecnologia, Campinas, São Paulo, Brasil)

Abstract

In humans, fundamental processes to neurogenesis begin very early in pregnancy, they are regulated by very sensitive signaling pathways. Thus, disturbances during neural development may lead to several degrees of damage to the fetus. Some pathogens such as TORCH agents can cross placental barrier and impact embryo development. These agents can cause mild illness in infected mother, vertical transmission to fetus, develop several anomalies in the affected fetus, and in some instances, maternal therapy may not ameliorate fetal prognosis. Such features can also be found in the emerging TORCH agent, Zika virus, which caused an epidemic in Brazil impacting several newborns. Nonetheless, this capability may not be restricted to Zika, but shared among Flaviridae, since other members such as West Nile virus and St Louis encephalitis virus can infect central nervous system, and cross placental barrier leading to fetal demise during pregnancy. Recent reports indicate the emergence of another Flavivirus, Usutu virus (USUV), likely representing risks and impacts to human health. In this scenario, the aim of this study is to develop a *in vivo* mouse model to investigate if it can cross the placental barrier and reach the embryo and what would be the impact to the neurogenesis. We infected wild-type pregnant mouse females (FVB/NJ) at 5.5 and 7.5 days post coitum (dpc) with 10⁶pfu of USUV (Vienna isolate) through intravenous route at retro-orbital sinus. Embryos and placentas were harvested at 10.5dpc, documented and preserved.

Virus detection was performed by PCR amplification. Our preliminary results suggest that adult wild-type mice are resistant to USUV infection. Embryos and placentas from mothers infected in 5.5dpc window do not seem to be severely affected by USUV, although few embryos showed unequivocal evidence of malformation. To investigate the placenta's role in USUV infection, we performed *in vitro* placental explants assays, infecting healthy placentas with the same titer of the virus. Preliminary results of *in vitro* assays indicate that USUV persists but do not replicate in 7.5dpc placenta precursors, while it was undetectable in 10.5dpc placenta. Similar experimental design is also being conducted in Type I IFN receptor knockout mouse (IFN- $\alpha\beta$ R-), which are susceptible to USUV infection. We are now investigating if they can retain embryos during the disease progression and what is the role of placenta to avoid USUV impact on embryo development.

Keywords: USUV, Pregnancy, Embryo, Placenta, Mouse



EVALUATION OF THE KNOWLEDGE ON HIV/AIDS OF AGED PEOPLE LIVING IN SANTA MARIA/RS: AN EPIDEMIOLOGICAL APPROACH.

Huander Andreolla ¹, Adeline Viero ¹, Vitória de Almeida Bassotto ¹

¹ UFN - Universidade Franciscana (Santa Maria/RS - Brasil)

Abstract

Introduction: The changes observed in HIV infection epidemiology profile have been evoked new public health strategies focused on elderly people. Although the latest Brazilian Ministry of Health data have shown a decreasing number of HIV infection and Aids cases since 2012, the population ageing and the growing number of HIV in elderly people, specially in women, has obtained evidence in all levels of care. **Objective:** To verify the level of knowledge of elderly people living in the city of Santa Maria/RS on general characteristics of HIV/Aids infections. **Materials and Methods:** Data were collected through a validated questionnaire on five different domains associated with HIV/Aids. Data collection occurred from April to August 2018 with people aging 60 years or older who presented preserved cognitive abilities and accepted in answering the research instrument. **Results:** Were included 108 subjects, mostly composed by women (94.4%) and aging 72.7 (\pm 6.82) years. On "concept" and "transmission" domains, 40.7% of the participants indicated that people living with the virus always are symptomatic and 27.8% did not know if the mosquito could play a role as infection vector, respectively. About "prevention" and "vulnerability" themes, 23.1% answered they do not know about the existence of female condoms and 16.7% considered Aids a disease still related to men who have sex with men, drug users and prostitutes. When asked questions about "treatment", 31.5% of the participants do not know that AIDS has no cure. **Conclusion:** We observed the need of performing health educational programs that may target people older than 60 years and encourage them to do rapid tests and give them orientation on HIV/Aids treatment, when indicated.

Financial Support: Not applicable

Keywords: HIV/Aids, Ageing, Prevention, Diagnosis, Transmission



INHIBITORY EFFECT OF ISOLATED SUBSTANCES FROM MARINE ALGA LAURENCIA CATARINENSIS AGAINST CHIKUNGUNYA VIRUS

Mariana Oliveira ¹

¹ UFF - Universidade Federal Fluminense (Alameda Barros Terra, s/n - Centro, Niterói - RJ, 24020-150)

Abstract

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that can cause fever and chronic arthritis in humans. Currently, there are no treatments or vaccines available against the virus and, therefore, the search for effective therapeutics is of paramount importance. In this sense, our group's studies with the red alga *Laurencia catarinensis*, originated from the south of Brazil, showed the low cytotoxicity of its isolated substances LCT2 and LCT3, with a CC 50 greater than 400 μM . To evaluate the substance concentration able to inhibit 50% of the production of viral particles (EC 50), vero cells, kept in 24-wells plates with 1×10^5 cells/well density, were infected with CHIKV (1×10^4 PFU) employing 0,1 MOI for one hour at 37°C in 5% CO₂ atmosphere. Afterwards, the cells were treated with the two substances in five concentrations (0.65, 1.25, 2.5, 5 and 10 μM). LCT2 presented an EC 50 of 0.65 μM and LCT3 1.25 μM . Then, the substances in three different concentrations (1.25, 2.5 and 5 μM) were incubated with the virus for an hour and added to vero cells in 24-wells plates in order to analyze its virucidal potential. In the greatest concentration (5 μM) LCT3 demonstrates a 60% percentual of inhibition. Meanwhile, even in the smallest concentration, LCT2 is capable of inhibiting up to 80% of virus entrance in the cells. For the time of addition experiment, CHIKV infected vero cells were treated with the substances in different times (0, 1, 2, 3, 6, 9, 12 e 16h) post-infection. LCT2 was able to inhibit 100% of viral replication for up to three hours. And LCT3 maintained the same inhibition percentage up to six hours post infection, followed by a decreasing in inhibition, but never reaching less than 50% inhibition. These results suggest that LCT2 and LCT3 have a great potential as an antiviral therapeutic drug.

Financial support: CNPq, CAPES, FAPERJ, UFF (PROPI)

Keywords: antiviral, chikungunya, natural products, red algae, laurência



RESPIRATORY SYNCYTIAL VIRUS CIRCULATION IN PEDIATRIC PATIENTS: MOLECULAR EPIDEMIOLOGY AND RESISTENCE

Erick Gustavo Dorlass ¹, Luciano Thomazelli ¹, Celia Pirez ², Daniella Crema, José Maria Lopes, Alfredo Gilio ², Silvia Ibidi ², Ricardo Ambrosio Fock, Edison Luis Durigon ¹, Viviane Botosso, Danielle Bruna Leal Oliveira

¹ ICB - USP - Instituto de Ciências Biomédicas (Avenida Professor Lineu Prestes, 1374), ² HU - USP - Hospital Universitário - Universidade de São Paulo (Avenida Professor Lineu Prestes, 2565), ³ IB - Instituto Butantan (Av. Vital Brasil, 1500)

Abstract

Respiratory Syncytial Virus infection is the main cause of lower respiratory tract illness in pediatric patients. It's estimates up to 3.8 million hospitalizations and 149 thousand deaths every year of children under 5 years old. The virus circulates all year with peaks of infection autumn in subtropical zones. There are two subtypes of RSV, A and B, that characterizes themselves after genomic analyses of the surface glycoprotein G. Both subtypes co-circulate with predominance of one each season. Until this date, the only prophylactic method available is the inhibition of the fusion protein (F) of the viral envelope by Palivizumab, a murinic antibody. Scaping mutants conferring Palivizumab Resistance have been reported around the world. We aim characterize the viral strains circulating in the city of São Paulo and survey for viral mutations conferring Palivizumab resistance. A total of 590 nasopharynges aspirates samples positives for RSV by immunofluorescence assay of children under 5 years old at the University Hospital in the city of São Paulo of January 2017 to December 2018 were used in this study. RNA was extracted with and RT – PCR and PCR reactions were performed for amplification of the G gene, followed by Sanger sequencing analyses of the amplicon for genotyping. Sequences and phylogenetic analyses were performed with DNASTAR Software. The Palivizumab scape mutants surveillance will be performed by pyrosequencing. Our results show that march to June were the months with most samples collection, with 76% of all samples in the study being from this time period, this result is expected in the region of study. Of all samples, 322 were from males patients and 268 from female and 72% were provided from the emergence room. To date, 51% of all samples were amplified, and we were able to obtain 52% of sequences of those. Of all the sequences obtain, 55% were A subtypes and 45% B subtypes. It seems that a substitution of the predominant strain may have occurred, since only 25% of the obtained samples were subtype B in 2017 and 83% in 2018. Phylogenetic analyses showed that 100% of the A subtype cluster with ON1 genotype and 100% of B subtype cluster with BA09. Our results so far indicate that even changing the dominant subtype, the virus seasonality isn't affected. Both genotypes found in the analysed sequences are predominant around the world, this shows evolutionary dominance over the other genotypes of each A and B subtypes.

Financial Support: CAPES

Keywords: RSV, Respiratory, Syncytial, Virus



APPLYING SYNDROMIC SURVEILLANCE IN BRAZIL: EPIDEMIOLOGICAL ANALYSIS OF NEGATIVE CASES OF ARBOVIROSES IN THE STATE OF RIO DE JANEIRO.

Letícia Oliveira Dias¹, Marcelo de Souza Pinto², Guilherme Louzada Silva Meira², Carlos Augusto da Silva Fernandes², Shirlei Ferreira de Aguiar², Elba Regina Sampaio de Lemos¹, Marco Aurelio Pereira Horta¹

¹ IOC/FIOCRUZ - Instituto Oswaldo Cruz (Av. Brasil, 4365, Manguinhos, Rio de Janeiro - RJ, 21040-900.), ² LACEN-RJ - Laboratório Central de Saúde Pública Noel Nutels (Rua México, 128, Centro, Rio de Janeiro-RJ, 20231-092.)

Abstract

Acute febrile illness constitutes a group of common diseases related to infectious agents in tropical countries and, in the Brazilian epidemiological context, the arboviroses are among the main causes of acute febrile illness. With the objective of investigating the etiology of suspected cases of arboviroses in the state of Rio de Janeiro and the epidemiological characteristics related, we analyzed a dataset of patients suspected of arbovirus who had blood sample collected for etiological diagnosis between 2017 and 2018. This is a cross-sectional and retrospective study that uses the database provided by the Brazilian Laboratory Manager System (GAL, in Portuguese). Our results show that in 2017 11,159 tests were performed: 44.2% for Chikungunya, 41.5% for Dengue Fever (DF) and 14.3% for Zika; in 2018 24,913 tests were performed, being: 31.2% for Chikungunya, 25.4% for DF, 9% for Zika and 34.4% with ZDC Test (simultaneous test to detect the three virus agents). For 2017, we found 21.5% positive tests for arboviruses and 78.5% negative and in 2018 40.3% of the tests were positive for arboviruses and 59.7% negative. Despite the increase in suspected cases of arboviroses of 136.8% for the period, we observed a decrease of cases concluded as “*without defined etiology*” of 78.5% in 2017 to 59.7% in 2018. Confirmed and negative cases had the period of higher incidence between March and May. The positive and negative cases had the predominance of women around 60% and 40%, respectively in 2017 and 2018. In relation to age, the higher incidence of confirmed cases to arboviroses were 20-59 years in 2017 and 30-69 years in 2018; the negative cases had a higher incidence among 20-59 years in 2017 and 2018. The pediatric age group had the higher incidence of negative cases for arboviruses. The three most prevalent signs and symptoms are the same in confirmed and negative cases: fever, arthralgia and myalgia. The symptoms that were more prevalent in confirmed cases when comparing to negative cases were arthralgia and articular edema, which can be a direction for the clinical diagnosis. The viral infection detection among suspected cases of these three arboviruses was significantly low (10%) in the state regions of Médio Paraíba and Sul Fluminense, indicating that differential diagnosis of other diseases, as Brazilian spotted fever and hantavirus pulmonary syndrome, among others, is required to reduce the elevated number of cases without an identified pathogen.

Keywords: ARBOVIROSES, ARBOVIRUS, EPIDEMIOLOGICAL SURVEILLANCE , SYNDROMIC SURVEILLANCE



VALIDATION OF TWO TESTS TO RABIES VIRUS NEUTRALIZING ANTIBODY EVALUATION. PRECISION PARAMETER TO SIMPLIFIED FLUORESCENCE INHIBITION MICROTTEST (SFIMT) AND RAPID FLUORESCENT FOCUS INHIBITION TEST (RFFIT) ON MICROPLATES.

Filipe Gabriel M. Pancetti ¹, Rene dos Santos Cunha Neto ¹, Karina Ribeiro da Silva ¹, Adriana Cândido Rodrigues¹, Amanda Siena ¹, Karin Corrêa Scheffer , Iana Suly Santos Katz ¹, Andréa de Cássia Rodrigues da Silva ¹, Luciana Botelho Chaves ¹

¹ IP/SESSP - Instituto Pasteur /Secretaria de Estado da Saúde de São Paulo (Av. Paulista, 393, Cerqueira César, CEP: 01311-000, São Paulo, Brasil)

Abstract

Rabies is a worldwide zoonosis that affects the central nervous system of mammals and is transmitted by the saliva of infected animals by bites, scratches or contact with mucous membranes. Pre-exposure prophylaxis and evaluation of rabies virus neutralizing antibody (RVNA) every six months or once a year is recommended for people at risk of exposure. RVNA titers ≥ 0.50 IU/mL characterize satisfactory immunization. The most commonly used tests for evaluation of RVNA are cell culture neutralization tests such as the Fluorescent Antibody Virus Neutralization Test (FAVN) and the Rapid Fluorescent Focus Inhibition Test (RFFIT). Simplified Fluorescence Inhibition Microtest (SFIMT) is a simpler, faster reading technique that has been successfully used for RVNA evaluation. Test validation evidences the performance of a method within quality specifications, providing valid results. Validation parameters are intra-assay precision (repeatability), intermediate precision, matrix effect, linearity, specificity, accuracy, and stability. The aim of this study was to validate the SFIMT and the RFFIT on microplates following the guidelines DOQ-CGCRE-008 of the General Coordination of Accreditation of Brazil. For the precision parameter, 50 human serum samples from people submitted to pre-exposure prophylaxis for rabies were tested by both tests, RFFIT and SFIMT, repeated three times on alternate days. For the reading was performed by two professionals. The results were analyzed by Geometric Coefficient of Variance (GCV) with a 95% confidence interval (CI). The calculated % GCV of the intra-assay precision (repeatability) for SFIMT was 8.46%, (95% CI: 8.44, 8.48) and the overall % GCV of the intermediate precision was 9.19% (95% CI: 9.15, 9.23), which were within the acceptable limit of $\leq 30\%$. For RFFIT the % GCV of intra-assay precision (repeatability) was 12,93% (95% CI: 12.83, 13.02) and the % GVC intermediate precision was 12,30% (95% CI: 12.24, 12.35). The comparison in samples results for the two readers in SFIMT showed only 8% of differences. In conclusion, the acceptance criteria for precision were met. Financial Support: FEDIP/SESSP

Keywords: Rabies, Serology , Validation, Neutralization, Antibody



SUSCEPTIBILITY OF DIFFERENT CELL TYPES PRESENT IN THE CENTRAL NERVOUS SYSTEM TO ZIKA VIRUS INFECTION

Ricardo Correia da Silva ¹, Sharton Vinicius Antunes Coelho ¹, Yasmim Mucunã Mustafá ¹, Pedro Moreno Pimentel Coelho ¹, Luciana Barros de Arruda ¹

¹ UFRJ - Universidade Federal do Rio de Janeiro (Ilha do Fundão - Cidade Universitária)

Abstract

Zika virus (ZIKV) infection has been associated with neurological abnormalities after congenital infection, and meningoencephalitis in adults. Virus was detected in the brains from microcephaly and meningoencephalitis cases, indicating that ZIKV cross the blood brain barrier and infect CNS cells. Our group demonstrated that ZIKV traversed human cerebral microvascular endothelium (HBMEC) cells that form the blood-brain barrier (BBB), with no alteration of endothelial permeability *in vitro* nor *in vivo*. Interestingly, barrier breakdown was detected at later time points upon systemic mouse infection and this event preceded mice death. The BBB is associated to microglia, astrocytes, and mast cells. Stimulation of those cells induce the production of mediators that may contribute to inflammatory response and to BBB permeability. We hypothesized that, after crossing the BBB, ZIKV could infect and/or activate other cells present in the CNS, what could trigger BBB disruption, amplifying the neuropathology. Here, we aimed to investigate whether ZIKV infects and activates mast cells and glia cells and if this infection affects BBB permeability *in vitro* and *in vivo*. We evaluated the permissivity, viability and type I interferon production by human cell lines derived from mast cells (HMC-1), astrocytes (FHA), and astroglialblastome (U87) upon ZIKV infection. The cells were infected with the ZIKV strains PE243 or MR766 with different MOIs. After different time points, cell lysates and supernatants were harvested, and virus replication was evaluated by qRT-PCR and plaque. IFN- β production was evaluated by qRT-PCR and cell viability was analyzed by XTT assay. We observed that HMC-1 and U87 cells were permissive to ZIKV infection, since the virus RNA levels increased during the time points analyzed and the obtained supernatants were infectious to VERO cells. ZIKV replication induced production of IFN- β and cell death of U87, but not of HMC-1 cells. Importantly, intracerebral inoculation of A129 mice promoted early disruption of the BBB, corroborating with the hypothesis that infection of CNS cells may induce BBB breakdown. We are currently investigating the cell types infected *in vivo* and establishing *in vitro* and *in vivo* models to determine the specific role of each cell type for the alterations in the BBB. We believe that the comprehension of these mechanisms will contribute to the characterization of new targets for anti- ZIKV therapy.

Support: CNPq, CAPES e FAPERJ

Keywords: Brain blood barrier, ZIKV, Mast cells, Astrocytes, central nervous system



MONITORING AND MOLECULAR CHARACTERIZATION OF ARBOVIRUS ISOLATED FROM DIPTERS OF THE BRAZILIAN WEST AMAZON (STATE OF RONDÔNIA)

CAIRO MONTEIRO DE OLIVEIRA ¹, Daniele Bruna Leal de Oliveira ¹, Erney Felício Plessman de Camargo ¹, Marta Maria Geraldês Teixeira ¹, Carla Monadeli Filgueira Rodrigues ¹, Luciana Lima, Edison Luiz Durigon ¹

¹ ICBII-USP - Instituto de Ciências Biomédicas II da Universidade de São Paulo (Av. Prof. Lineu Prestes, 1374 - Butantã, São Paulo - SP, 05508-900)

Abstract

Background: Arboviruses are viruses transmitted by arthropod vectors which predominate mainly in tropical areas of the world. Brazil is a country that holds a large part of its territory taken by tropical forests and other natural ecosystems that allow it ideal conditions the presence of Arbovirus. A number of Arboviruses have already been detected and isolated throughout the territory with many still endemic such as Dengue virus (DENV), Zika virus (ZKV), Chikungunya virus (CHIKV) and many others circulating in nature through reservoir hosts and arthropod vectors such as diptera, ticks, etc. which often cause disease outbreaks in animals and humans. In this context, the Rondônia state which owns ⅓ of its territory covered by the Amazon rainforest has an ecosystem that favors the existence of different arthropod vector species that added to the increasing deforestation rate and the precarious sanitation conditions allow potential transmission and establishment of arboviruses in this region. Objective: Thus, the present work will aim to monitor the presence of Arbovirus of medical importance such as Flavivirus and Alphavirus in the arthropod vectors (dipters) that circulate in the region of the state of Rondônia, Brazil. Methods: In this study, dipters species captured in different regions throughout of the Rondonia state(BRA) between 2017, 2018 and 2019 after a process of maceration and extraction of nucleic acids were submitted to Arbovirus (Flavivirus, Alphavirus) research by Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) assays with SYBR GREEN. Results: To date, of the 9500 dipteros collected were processed and analyzed about 6463 individuals of which 72.85% (4714) belonging to the Culicidae family, 16.5% (1064) Ceratopogonidae, 8.6% (555) psychodidae, 2% (127) Tabanidae and 0.05% (three) Simuliidae divided into more than 35 distinct species. From the total dipters analyzed no Flavivirus or Alphavirus was detected. Conclusion: Despite the no detection of Flavivirus or Alphavirus in the samples analyzed so far the possibility of circulation of these viruses in the study region should not be ruled out since it provides all the necessary environment for the establishment of arboviruses.

Keywords: Arbovirus, Dipters, Brazil, Rondonia, Monitoring



POS-MORTEN DIAGNOSIS OF HANTAVIROSI S AT THE MATO GROSSO STATE DEATH VERIFICATION SERVICE

Lucas Silva Dias ¹, Letícia Lerner Lopes , Emanuelle Soares Camolesi , Adriana Yuki Mello Prado , Tiago Ferreira Portela

¹ UFMT - Universidade Federal do Mato Grosso (Cuiabá, MT)

Abstract

Hantavirus Cardiopulmonary Syndrome (SCPH) is a highly lethal disease whose etiological agent is the positive single- stranded RNA virus of the family Bunyaviridae of the genus Hantavirus. Viruses representing this genus are subdivided into three groups: associated with Renal Syndrome Hemorrhagic Fever (FHSR), causing Hantavirus Cardiopulmonary Syndrome (SCPH) and those detected in wild rodents. Viral particles are generally transmitted to humans by wild rodent vectors by inhalation of excreta aerosols or by saliva. The most common symptoms related to hantavirus infection are fever, dyspnea, headache, myalgia and cough. In addition, SCPH-related viral types use B3 integrins as a receptor for platelet infection, thereby also generating thrombopenia and increased likelihood of bleeding episodes. Confirmatory diagnosis of infection is given by Hantavirus-specific IgM positivity, positive tissue immunohistochemistry or positive RT-PCR. In this context, a quantitative analysis of cases with suspected Hantavirus infection between 2016 and 2018, of necropsies of the Mato Grosso State Death Verification Service, based in Cuiabá, Brazil, was performed. Confirmation of the diagnostic suspicion of hantavirus infection was made by enzyme immunoassay (IgM and IgG) for Hantavirus, corroborating the clinical, epidemiological and histopathological findings. There were eight suspected cases of hantavirus, two of which were confirmed by enzyme immunoassays: a twenty-two-year-old man and a ten-year-old adolescent. The main symptoms reported were dyspnea, fever, cough, headache, myalgia and asthenia. There was a strong correspondence between infection and circulation in rural areas in the week before the appearance of symptoms. The disease evolved rapidly, leading to death within 72 hours. Therefore, the notification of hantavirus cases is of utmost importance for public health due to its rapid evolution.

Keywords: hantavirus, cardiopulmonary syndrome, pos-mortem, zoonosis, rodent virus RNA



INTERACTION CHARACTERIZATION OF RABIES DIAGNOSIS IN THE LABORATORIES NETWORK OF THE STATE OF SÃO PAULO, IN THE CONTEXT OF RABIES CONTROL AND SURVEILLANCE

Micheli Cocchi¹, Nayara Ugeda¹, Daniela Barroso Brogliatto¹, Valéria Gentil de Tommaso¹, Keila Iamamoto¹, Adriana Maria Lopes Vieira¹, Luciana Hardt¹, Andréa de Cássia Rodrigues¹

¹ IP - Instituto Pasteur (Av. Paulista, 393 - Cerqueira César, São Paulo - SP, 01311-000)

Abstract

Rabies is an anthroozoonosis transmitted by the inoculation of the virus contained in the saliva of infected animals and is characterized by a highly lethal acute viral encephalitis. It represents a serious public health problem, in addition to producing major economic losses to livestock. The Rabies Surveillance and Control Program aims to promote systematic actions to combat rabies as one of the health policy priorities. Due to the importance of a structured, organized and collaborative laboratory network to support epidemiological surveillance, the aim of the study was to characterize the performance of the network of laboratories in the state of São Paulo to perform virological and serological rabies diagnostic tests and to evaluate structural, operational capacity and resources. A quantitative descriptive observational design based on primary and secondary data obtained from questionnaires, interviews and documents from the laboratories was performed to perform examinations related to the surveillance and control program of the State of São Paulo, from 2014 to 2018. During the study period, it was observed that the Pasteur Institute (IP) received approximately 56% of all samples and the Zoonoses Control Center (CCZ) 30%, while the remainder was distributed among the other laboratories of the network. Overall, 2.85% were positive. The techniques used by all laboratories were Direct Fluorescent Antibody test (DAFt) and Mouse Viral Isolation. For CCZ we add Cell Culture Viral Isolation, RT-PCR and RT-qPCR and for Pasteur Institute RT-PCR and genetic sequencing. Serological diagnosis is performed only by IP (SFIMT and RFFIT) and CCZ (SFIMT and FAVN). It was observed that all laboratories are able to perform virological diagnosis and ability to work cooperatively, but the inputs and the number of employees were interfering in operational capacity. The results of this study demonstrate that there is no homogeneous distribution of samples between laboratories, although they have equipment and processing capacity. The formalization of a collaborative laboratory network for the diagnosis of rabies aims to balance the distribution of samples, improving the capillarity of epidemiological surveillance and promoting agility in health care actions involved in the prophylaxis and treatment of the disease.

Financial Support: Instituto Pasteur - Secretaria de Estado da Saúde de São Paulo.

Keywords: rabies, diagnosis, virological, serological, surveillance



EVALUATION OF ANTIHERPES ACTIVITY OF TOTAL ALKALOIDS FRACTION OF *Fusaea longifolia* (Aubl.) Saff.

Suzy Hellen Alves Dourado ¹, Cristiângela Silva Santos ¹, Catarina Fumagali Caetano ¹, Dênia Mendes De Sousa Valladão ¹, Carla Regina Andrighetti ¹

¹ UFMT/CUS - Universidade Federal de Mato Grosso/Campus Universitário de Sinop (Av. Alexandre Ferronato, 1200, Sinop-MT)

Abstract

Infectious diseases are of great concern worldwide, particularly viral infections, among which those caused by the herpes viruses occur with high incidence. Herpes simplex is caused by two DNA enveloped viruses, Herpes Simplex Virus 1 (HSV-1) and Herpes Simplex Virus 2 (HSV-2). In some cases, treatment with Acyclovir (ACV) is ineffective because of the emergence of ACV-resistant mutant strains, particularly in immunocompromised patients. The rapid emergence of such ACV-resistant strains calls for to the need to discover and develop new antiherpes compounds with mechanisms of action that are different from that of ACV. In this context, natural products provide an important source of biologically active substances, playing a key role in the research and development of novel antiherpes products. Chemical and pharmacological studies of Annonaceae have shown the accumulation of secondary metabolites with important biological activities such as antiprotozoal, cytotoxic and antiviral agents. The objective of this research was to evaluate the antiviral activity of Total Alkaloids Fraction from the branches of *Fusaea longifolia* (Aubl.) Saff. against Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). Cytotoxicity was evaluated on Vero E6 cells by using MTT assay, and the potential antiherpes activity was evaluated by viral plaque assay. The tested viruses were Herpes Simplex Virus types 1 (HSV-1, KOS strain) and 2 (HSV-2, strain 333), and an acyclovir resistant strain (HSV-1 29R). Results were expressed as 50% cytotoxic concentrations (CC50) and 50% viral replication inhibitory concentrations (IC50), respectively, in order to calculate the selectivity index (SI=CC50/IC50). The evaluation of cytotoxicity of Total Alkaloids Fraction of *F. longifolia* resulted in values of CC50 of $72,9 \pm 0,6 \mu\text{g/mL}$. Concerning antiviral activity, the SI values were 4.31 for HSV-1 KOS strain, 3.48 for HSV-1 29-R strain and 4.76 for HSV-2 333 strain. Total Alkaloids Fraction presented virucidal activity against HSV-2 with a IC50 value of

Keywords: HSV, natural products, antiviral activity, *Fusaea longifolia*



CYTOKINE ANALYSIS ON THE DEVELOPMENT OF FACTOR VIII INHIBITORS IN HAEMOPHILIA PATIENTS INFECTED BY HEPATITIS C VIRUS (HCV)

Eduarda Bolina-Santos ¹, Daniel Gonçalves Chaves ², Ricardo Andrade Carmo ², Maria Clara Fernandes da Silva Malta ², Edel Figueiredo Barbosa-Stancioli ¹, Marina Lobato Martins ²

¹UFMG - Universidade Federal de Minas Gerais (Av. Pres. Antônio Carlos, 6627 - Pampulha, Belo Horizonte - MG, 31270-901), ² Hemominas - Fundação Hemominas (Alameda Ezequiel Dias, 321 - Santa Efigênia, Belo Horizonte - MG, 30130-110)

Abstract

The development of inhibitors against factor VIII (INB) is the most significant treatment complication seen in patients with haemophilia A. This study aims to examine whether hepatitis C virus (HCV) infection could be associated with the development of INB in haemophilia A patients by modulation immune response. The medical records and serum samples were collected from 197 males accompanied at the hemocenter of Belo Horizonte of the Fundação Hemominas between January 1985 and January 2015. These patients were classified into six groups were evaluated: uninfected without and with history of INB (NI^{INB-}, n = 62 and NI^{INB+}, n = 34); with HCV clearance without and with a history of INB (cHCV^{INB-}, n = 19 and cHCV^{INB+}, n = 24); with chronic HCV (HCV RNA +) without and with history of INB (HCV^{+ INB-}, n = 31 and HCV^{+ INB+}, n = 27). Cytokines (IL2, IL4, IL6, IL10, TNF, IFN γ e IL17A) were measured with BD CBA Th1/Th2/Th17 Cytokine Kit by flow cytometry in a sample. All cytokines were observed in higher concentrations in patients with INB compared to those without INB, both in the NI group and in those with past or chronic HCV infection. Patients without INB compare to the NI group showed significantly higher concentrations of IL4 compared to cHCV and HCV⁺ groups, and TNF compared to HCV⁺ group. IL4 concentrations were also significantly elevated in patients with active HCV infection and no history of INB development compared to viral clearance and no history of INB development (HCV^{+ INB-} vs. cHCV^{INB-}). In the groups with INB⁺ we observed lower IL2 concentration in the NI group compared to cHCV and HCV⁺; higher IL4 and IFN γ concentrations in the NI group compared to cHCV and HCV⁺ and elevated TNF in the NI group compared to cHCV. Among INB⁺ patients with a history of HCV infection, there was a difference only in IFN γ concentration, being higher in HCV⁺. There was no significant difference in IL6, IL10 and IL17A concentrations between patients without INB, regardless of history of HCV infection (NI^{INB-}, cHCV^{INB-} e HCV^{+ INB-}), as well as, INB⁺ (NI^{INB+}, cHCV^{INB+} e HCV^{+ INB+}). In conclusion, increased serum concentrations of proinflammatory cytokines (IL2, TNF and IFN γ), regulated by IL4 and IL10 are characteristic of patients with a history of INB development, however, this profile appears to be modulated by the HCV immune response. It is complex to use these cytokines as biomarkers of risk for inhibitor development without discussing HCV infection.

Keywords: Cytokines, Factor VIII inhibitors, Haemophilia A, HCV



DETECTION OF HUMAN RESPIRATORY SYNCYTIAL VIRUS IN PEDIATRIC PATIENTS USING A HIGH SPECIFIC RAPID ANTIGEN DETECTION

CAMILA SOARES¹, Nathalia Utecht Soares¹, Erick Gustavo Dorlass¹, Daniella Crema³, Célia Pirez³, Viviane Botosso², Danielle Bruna Oliveira¹, Edison Luiz Durigon¹

¹ USP - Universidade de São Paulo (Cidade Universitária, CEP: 05508-900), ² IB - Instituto Butantan (Av. Vital Brasil, 1500 - Butantã, São Paulo - SP, 05503-900), ³ HU - Hospital Universitário (Av. Prof. Lineu Prestes, 2565 - Butantã, São Paulo - SP, 05508-000)

Abstract

Human Respiratory Syncytial Virus (hRSV) is a world widely distributed virus and is the main virus involved in lower respiratory tract infections. It is a common virus that cause respiratory infections in children under 5 years old, immunocompromised patients and the elderly. The hRSV is a member of the family *Pneumoviridae* and genus *Pneumovirus*, its genome is composed of a single-stranded RNA molecule and encodes 11 proteins.. The similarity between the symptoms caused by hRSV infection with others respiratory viruses impairs the accurate clinical diagnosis, in this context, laboratory diagnostic methods are extremely important. At first, because they prevent the common practice of antibiotic prescribing and also because patient isolation measures can be applied, decreasing the viral spread. In this study a rapid test is evaluated (Eco Diagnóstica® ECO F RSV Ag), this test promises a result in 15 minutes, compared to almost 90 minutes for a conventional IFA test and 2-3 h for enzyme (ELISA). The ECO F RSV Ag® test is a dipstick immunoassay, which allows a rapid, qualitative detection of hRSV antigen (viral fusion protein). We analyzed nasopharyngeal aspirate (NPA) samples from 29 children under five years old with symptoms of acute respiratory infection (ARI), including upper and lower respiratory tract disease who had ARI and were cared for an emergency room, as outpatients, or hospitalized in the Pediatrics Department, University Hospital, University of São Paulo (HU-USP). A preliminary result showed 10 positive samples by immunofluorescence assays, 9 (90%) were also positive by the rapid antigen detection test.

However in comparison with a RT- qPCR test, the rapid antigen detection test is not very sensible. The RT- qPCR test showed that 9 positive samples for hRSV were also positive for others respiratory viruses, but only 5 (55,5%) were positive in the ECO F RSV Ag® test. These 4 samples, negative in the rapid test but positive in the RT- qPCR, presented a higher presence of the other viruses than hRSV. The ECO F RSV Ag® Test showed sensitivity of 73.6%, specificity of 100%, predictive positive value of 100%, and negative predictive value of 66.6% and agreement κ index of 0.65 in comparison to immunofluorescence assay and RT- qPCR. Financial Support by Fundação de Apoio à Pesquisa e à Extensão, Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo para Pesquisa do Estado de São Paulo

Keywords: hRSV, PEDIATRIC, nasopharyngeal, IFA, Qpcr



MOLECULAR DETECTION OF YELLOW FEVER VIRUS IN HUMAN SAMPLES, CENTRAL WEST, 2018, BRAZIL

Rachel Cruz Alves¹, José Henrique Francisco Roma^{2,1}, Ludiele Souza Castro¹, Marcio José Ferreira¹, Claudinéia de Araújo¹, Bruno Moreira Carneiro¹, Renata Dezengrini Silhessarenko³, Juliana Helena Chavez Pavoni¹

¹ UFMT - Curso de Graduação em Medicina. Câmpus Universitário de Rondonópolis. Universidade Federal de Mato Grosso. (Rondonópolis, MT), ² UNICAMP - Programa de Pós-Graduação em Clínica Médica. Faculdade de Ciências Médicas. Universidade Estadual de Campinas (Campinas, SP), ³ UFMT - Departamento de Ciências Básicas em Saúdes. Faculdade de Medicina. Univesidade Federal de Mato Grosso (Cuiabá/MT)

Abstract

Yellow fever (YF) is an arboviral disease which remains a public health issue in endemic areas despite the availability of a safe and effective vaccine. At the end of 2016, Brazil experienced an unprecedented YF outbreak. Human cases are still diagnosed although public health authorities attempt to reach higher vaccination coverage in risk areas. From December 2018 through January 2019, 36 confirmed human cases, including eight deaths, have been reported in São Paulo and Paraná. Furthermore, surveillance of non-human primate (NHP) has confirmed 25 epizootics reported in five federal entities: São Paulo (13), Rio de Janeiro (8), Minas Gerais (1), Mato Grosso (2) and Parana (1) from July 2018 through January 2019. Here, we report the molecular detection of YF in two serum samples from patients with acute febrile illness. Patients were referred to Laboratório Central in Rondonópolis, Mato Grosso State, to test for dengue, zika and chikungunya serology at the end of 2018. The age of these female patients were 31 and 61 years old and their occupation were secretary and housewife, respectively. The main symptoms were headache, myalgia and fever. Sample of both patients were collected up to five days after onset of symptoms and were also tested by RT-PCR for flavivirus. Results showed a 250 pb amplicon in nested-multiplex reaction for eleven flavivirus. A single RT-PCR reaction confirmed the amplification of YF genome. Amplicons were purified and sequenced by Sanger method. Blast results showed that both sequences had high homology to YF strain. Isolation in C6/36 cells and amplification of different genome regions for further sequencing is being performed. Both patients have informed no travel history to endemic areas in the past months. One patient declared immunization against YF in 2012, while other patient was not able to inform his YF vaccination status. Both patients have not declared disease complications. These results corroborate to epizootics reports for Mato Grosso State and may reinforce surveillance and vaccination coverage for YF.

Keywords: Yellow Fever, Acute febrile illness, molecular detection, Mato Grosso



ANTIVIRAL ACTIVITY OF SAPONIN FRACTIONS FROM QUILLAJA SPP. AGAINST CHIKUNGUNYA VIRUS

Karoline Schallenberger¹, Francini Pereira da Silva¹, Simone Gasparin Verza¹, Fernando Ferreira², Spilki Fernando Rosado¹, Fleck Juliane Deise¹

¹ Feevale - Universidade Feevale (Novo Hamburgo, RS - Brazil), ² Udelar - Universidad de La República (Montevideo - Uruguay)

Abstract

Viral diseases can cause many problems to living beings and the currently available treatment for most of these infections is restricted, making the search for new molecules with antiviral activity an important task. Amongst them, the Chikungunya virus (CHIKV) (*Togaviridae* family, *Alphavirus* genus) is a disease transmitted by mosquitoes of the genus *Aedes*, that can cause fever, muscle and joint pain. The saponins are plant metabolites with reported pharmacological activities, such as antiviral action. These metabolites are found in purified fractions from *Quillaja saponaria* (Quil-A[®]) barks and *Q. brasiliensis* (Fraction B) leaves. Thus, this work aims to evaluate the antiviral activity of Quil-A[®] and Fraction B against CHIKV virus. Firstly, the maximum non-toxic concentration was determined by the MTT cell viability assay, employing Vero cells (*Cercopithecus aethiops* kidney). The evaluation of fractions of saponins with anti-CHIKV activity and their mechanisms (virucidal, pretreatment and simultaneous addition) were performed by the plaque reduction assay. It was conducted according to previous standardization and the results were obtained from at least three independent experiments. Serial dilutions of both saponin fractions were used in the assays. The maximum non-toxic concentration of Quil-A[®] and Fraction B on Vero cells was 6 µg / mL. Both fractions demonstrated dose-dependent antiviral activity. The reduction of the CHIKV plaques number in the treatment with Quil-A[®] was 0% (1 µg/mL) at 99% (6 µg/mL). In treatment with Fraction B, the reduction in the same concentrations was 23% at 96%, respectively. Quil-A[®] demonstrated a high percentage reduction at higher concentrations for the three mechanisms evaluated, while the Fraction B showed the highest percentage reduction in the concentration of 6 µg/mL only in their cells protective mechanism (pretreatment), about 69%. These results demonstrated that both saponin fractions, at highest tested concentrations, were able to inhibit the replication of the CHIKV. Thus, *Quillaja* saponins can be promising candidates for the development of new drugs to treat CHIKV infections.

Keywords: Arbovirus, Fraction B, Quil-A[®], Plaque reduction assay, Vero cell



MODULATION HUMAN CHEMOKINE ASSOCIATED WITH THE DEVELOPMENT OF FACTOR VIII INHIBITORS IN HEMOPHILIA PATIENTS INFECTED BY HEPATITIS C VIRUS (HCV)

Eduarda Bolina-Santos ¹, Daniel Gonçalves Chaves ², Ricardo Andrade Carmo ², Maria Clara Fernandes da Silva Malta ², Edel Figueiredo Barbosa-Stancioli ¹, Marina Lobato Martins ²

¹UFMG - Universidade Federal de Minas Gerais (Av. Pres. Antônio Carlos, 6627 - Pampulha, Belo Horizonte - MG, 31270-901), ² Hemominas - Fundação Hemominas (Alameda Ezequiel Dias, 321 - Santa Efigênia, Belo Horizonte - MG, 30130-110)

Abstract

The development of inhibitors against factor VIII (INB) is the most significant treatment complication seen in patients with haemophilia A. This study aims to examine whether hepatitis C virus (HCV) infection could be associated with the development of INB in haemophilia A patients by modulation immune response. The medical records and serum samples were collected from 197 males accompanied at the hemocenter of Belo Horizonte of the Fundação Hemominas between January 1985 and January 2015. These patients were classified into six groups were evaluated: uninfected without and with history of INB (NI^{INB-}, n = 62 and NI^{INB+}, n = 34); with HCV clearance without and with a history of INB (cHCV^{INB-}, n = 19 and cHCV^{INB+}, n = 24); with chronic HCV (HCV RNA +) without and with history of INB (HCV^{+ INB-}, n = 31 and HCV^{+ INB+}, n = 27). Chemokines (IL8, CCL2 (MCP-1), CCL5 (RANTES), CXCL9 (MIG) e CXCL10 (IP-10) were measured with BD CBA Human Chemokines Kit by flow cytometry in a sample. Our results present higher serum concentrations of IL8 observed in the cHCV^{INB-} and HCV^{+ INB-} as compared to the NI^{INB-}. On the other hand, INB⁺ groups have IL8 concentrations are higher than in the NI compared to cHCV and HCV⁺. The groups with a history of HCV infection had higher serum CCL2 concentrations compared to the NI group only when patients had no history of INB development. Besides this, CXCL10 presented higher serum concentrations in cHCV and HCV⁺ groups when compared to NI with and without a history of the development of INB. Moreover, CXCL9 concentrations were higher in cHCV^{INB+} patients compared to the NI^{INB+} group, with a trend for significant difference between HCV^{+ INB+} vs. NI^{INB+}. To finish, CCL5 was not associated with a history of the development of INB or HCV infection. In conclusion, HCV infection appears to modulate the serum concentrations of some chemokines that appear to act as protective factors in the development of INB, such as IL8 and CCL2. However, CXCL9 and CXCL10 behave as risk factors for the generation of these antibodies. Thus, the use of chemokines as biomarkers of INB development should take into the presence of HCV infection.

Financial Support: Fundação HEMOMINAS, Ministry of Health, FAPEMIG and CNPq.

Keywords: Chemokines, Factor VIII inhibitors, Haemophilia A, HCV



ANTIVIRAL ACTIVITY OF *CHIOCOCCA ALBA* (L.) HITCHC. AGAINST MAYARO VIRUS

Ellen Caroline Feitoza Pires², Karoline Schallenger¹, Francini Pereira da Silva¹, Fabrício Souza Campos², Raimundo Wagner de Souza Aguiar², Juliane Deise Fleck¹

¹ Feevale - Universidade Feevale (Novo Hamburgo, RS - Brazil), ² UFT - Universidade Federal do Tocantins (Gurupi, TO - Brazil)

Abstract

Mayaro virus (MAYV), belonging to the Togaviridae family and genus *Alphavirus*, is an infectious disease whose symptoms are headaches, acute fever, rash and, in the most severe condition, persistent arthralgias. These conditions are similar to those manifested in Zika and Dengue virus infections, which are arboviruses transmitted by the *Aedes* mosquito. Although it presents itself as an emerging virus, there are no licensed vaccines to prevent infection, or effective antiviral therapy to treat it. For this reason, an alternative would be to use plants that have secondary metabolites with possible antiviral effect. The bush *Chiococca alba* (L.) Hitchc. of the Rubiaceae family, abundant in all regions of Brazil, besides being employed in folk medicines antiophidic, anti-inflammatory, antirheumatic and laxative, there are studies related to the presence of metabolites with antiviral capacity in the roots, such as saponins, tannins, flavonoids, alkaloids and anthracenic derivatives. The aim of this study was to analyze two methanolic extracts, one obtained by maceration (MM) and the other by Soxhlet (MS), from the roots of *C. alba*. against the MAYV. Before antiviral assay, cytotoxicity assays were performed to determine the cytotoxic concentration for 50% of cells (CC₅₀) through lysosomal viability by Neutral Red. For this purpose, VERO cells were treated with serial dilutions of the plant extract (from 9.8 to 1000 µg/mL) for 48h. Antiviral activity was assessed by plaque reduction assay, in which the cells were infected with MAYV (100 PFU/well) and after viral adsorption were treated with non-toxic concentrations of the extracts (40–100 µg/mL). Viral suspension and culture medium were used as viral and cellular control, respectively. After 48h of incubation (5% CO₂, 37°C), the cells were fixed with 4% formaldehyde, stained with violet crystal and then the lysis plates were counted. The CC₅₀ of 100.20 and 101.16 µg/mL was obtained for MM and MS, respectively. Viral inhibition for extract MM was 79, 90, 93 and 98% at concentrations 40, 60, 80 and 100 µg/mL, respectively. While for extract MS, was 84, 93 and 100% at the same concentrations. The results showed that regardless of the extraction method used extracts MM and MS had similar behavior in both cytotoxic and antiviral assay. Therefore, these data indicate potential antiviral actions against MAYV in the compounds present in roots from *C. alba*.

Financial support: CAPES, CNPq, Universidade Feevale, UFT.

Keywords: Maceration, Methanolic extracts, Plaque reduction assay, Soxhlet, Vero cell



THREE POST-MORTEM DIAGNOSED CASES OF SEVERE ATYPICAL CHIKUNGUNYA FEVER WITH PNEUMOLOGICAL MANIFESTATIONS IN A DEATH VERIFICATION SERVICE

Tiago Ferreira Portela¹, Pedro Henrique Souza Dos Santos Menezes¹, Lucas Silva Dias¹, Amanda Amorim Souza Rondon¹, Rafaela Andrade Donalsonso¹, Adriana Yuki Mello Prado¹

¹ UFMT - Universidade Federal de Mato Grosso (Av. Fernando Corrêa da Costa, 2367 - Boa Esperança, Cuiabá - MT, 78060-900)

Abstract

Although Chikungunya has gained relevance as another epidemiologically important arbovirus infection in Brazil and is associated with high chronic morbidity, manifested mainly in the form of severe chronic arthralgia, it is not recognized for severe manifestations in the acute phase, unlike other infections such as yellow fever and dengue fever. In fact, 99% of Chikungunya fever cases are typical, manifesting with fever, arthralgia, and rash and have a lethality of only 0.1%. However, it can be atypical in 0.3-1% of cases, this atypical form is characterized by also including pneumological, neurological, cardiovascular, hepatic, renal or ocular manifestations, and in these cases, lethality reaches 10%. In addition, 0.1% of symptomatic cases and about 1/3 of atypical cases may manifest in the severe atypical form defined as requiring the support of at least one vital function such as mechanical ventilation. Of the severe atypical form, 30% of cases are fatal. A quantitative analysis of suspected cases of arbovirus infection, from 2016 to 2018, of necropsies performed in a death verification service confirmed by real-time RT-PCR for Chikungunya, comparing clinical condition, histopathological and presence of comorbidities. Of 11 suspected cases, three were confirmed, and one case was ruled out because it was confirmed only by IgM ELISA, plus positive results for yellow fever immunohistochemistry and dengue virus IgM ELISA, which prevented the attribution of death to Chikungunya fever only. Of the confirmed cases of chikungunya 100% female; average of 41 years, 1 case > 45 years; 100% with dyspnea, 66.7% arthralgia and 33.4% with neurological symptoms; < 4 days of evolution to death; 100% with diffuse alveolar damage, hyaline membrane and foci of pulmonary hemorrhage; one case had comorbidity (hypertension, heart disease and diabetes mellitus). Postmortem diagnosis of Chikungunya fever is of great epidemiological importance, considering the low lethality of the disease and because the clinical diagnosis can be confused with other febrile hemorrhagic syndromes with a higher lethality, which underestimates the real lethality of this infection. In addition, it leads clinicians to consider monitoring for patients who are usually discharged after diagnosis of these syndromes. This hypothesis, for Chikungunya fever, should be considered mainly in the presence of non-typical symptoms such as the respiratory symptoms in the three cases described.

Keywords: Chikungunya fever, Chikungunya virus, Fatal cases, Pneumological manifestations, Severe atypical form



CASE REPORT OF COINFECTION ARBOVIRUS DENGUE, CHICKUNGUNA, AND YELLOW FEVER, LEADING TO DEATH

Tiago Ferreira Portela ¹, Pedro Henrique Souza Dos Santos Menezes ¹, Rafaela Andrade Donalsonso ¹, Amanda Amorim Souza Rondon ¹, Paulo Kentaro Fugiyama ¹, Lucas Silva Dias ¹, Adriana Yuki Mello Prado ¹

¹ UFMT - Universidade Federal de Mato Grosso (Av. Fernando Corrêa da Costa, 2367 - Boa Esperança, Cuiabá - MT, 78060-900)

Abstract

The arboviruses are frequently endemics in tropical and subtropical countries, mainly in America and Africa. Yellow fever, dengue, and Chikungunya fever follow a sylvatic cycle with nonhuman primates and an urban cycle involving humans and primarily mosquitoes from *Aedes* genus. The co-infection cases by the dengue virus (DENV) and chikungunya virus (CHIKV) are not uncommon, but just a few cases include yellow fever virus owing to the prevalence of the urban cycles for DENV and CHIKV, while it was just noticed sylvatic ways of infection to yellow fever virus since 1942 in Brazil. An 82-year-old woman, from Campo Grande, visiting Cuiabá, started symptoms with body ache, diarrhea, and low fever, evolving with hyporexia, ulcerated wounds in her oral cavity, dyspnea and asthenia. This clinical condition made her bedridden. The day before her death, she presented hematemesis and was hospitalized, followed with septic shock, which lead her to death after 8 days from the beginning of the clinical condition. The patient was a carrier of hypothyroidism and hypertension. She was sent to a death verification service to clarify the cause of death. The necropsy data revealed diffuse gastrointestinal bleeding and diffuse pulmonary congestion, and the diagnosis of the death was hemorrhagic fever syndrome and hypovolemic shock. Serum and lung and liver tissue samples were collected and sent for analysis, which showed positive IgG ELISA for Chikungunya and Dengue and positive immunohistochemistry for Yellow Fever in liver biopsy, with histological findings with councilman corpuscle presence, confirming the occurrence of chick, dengue and yellow fever co-infection in the same individual. Arbovirus co-infections make the condition more severe and increase the chances of atypical clinical manifestations. In addition, co-infections reduce the time of disease progression, so patients die rapidly.

Keywords: Arbovirus, Chikungunya fever, Coinfection, Dengue fever, Yellow fever



ANALYSIS OF GENOTROPISM IN HIV-1 SUBTYPES B, BBR AND F1 INFECTED INDIVIDUALS FAILING TO COMBINED ANTIRETROVIRAL THERAPY

Júlia Guimarães Barcellos de Abreu ¹, Carlos Silva de Jesus ¹, José Carlos Couto-Fernandez ¹, Monick Lindenmeyer Guimarães ¹

¹ IOC - Fiocruz - Instituto Oswaldo Cruz (Av. Brasil, 4365 - Manguinhos, Rio de Janeiro)

Abstract

Many studies have shown that various aspects of HIV-1 infection, such as disease progression and pathogenesis, can be affected by its highly diversity. At early stages of infection, the virus preferably uses coreceptor CCR5 to enter the host cell, and through the AIDS progression, around 40% of HIV-1 subtype B viruses switch to using coreceptor CXCR4. However, it's been demonstrated that correceptor use varies according to the viral subtype. Chemokine receptor antagonist Maraviroc, to which only individuals presenting CCR5 tropic viruses are eligible, has been used in Brasil since 2013. Yet, there are still few studies evaluating the relationship between HIV-1 prevalent subtypes/variants in Brazil and the coreceptor use. Thus, we aim to analyze a set of HIV-1 subtype B (pandemic and BBR) and F1 chronically infected individuals to evaluate the influence of the subtype/variant on the CCR5 or CXCR4 tropism, as well as to indicate the percentage of individuals who would be eligible to Maraviroc therapy. We selected samples from 90 HIV-1 individuals failing to combined antiretroviral treatment previously genotyped in protease region as F1 (n=30) or B (n=60). The DNA was extracted from whole blood samples, then amplified by nested PCR for the C2V3 region of the viral envelope and sequenced. The phylogenetic analysis was performed using the Neighbor-Joining method and Tamura-Nei model, to determine the HIV-1 subtypes. Finally, the coreceptor tropism prediction was obtained using the program Geno2Pheno, FPR=10%. So far, 96 samples were successfully sequenced. Based on the phylogenetic envelope analysis, we confirmed that 46 of those were Bpol/ Bpanenv, 15 were Bpol/BBRenv, 3 were Bpol/F1env, 29 were F1pol/F1env, 3 were F1pol/Benv. We verified that 26% of BBR (n=4) and 10% of F1 (n=3) samples showed CXCR4 tropism, while a higher number of 37% (n=18) of Bpan showed tropism for that coreceptor, thus, evidencing the difference of the coreceptor usage according to the subtype. Financial support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Instituto Oswaldo Cruz (IOC)

Keywords: correceptor, genotropism, HIV, maraviroc, resistance



EVIDENCE OF LYMPHOCYTIC CORIOMENINGITIS VIRUS INFECTION IN EMPLOYEES WHO HANDLE ANIMALS AND RODENTS IN RIO DE JANEIRO STATE, BRAZIL

Gabriel Cavalcanti Rosa Gabriel Cavalcanti Rosa ¹, Jorlan Fernandes de Jesus Jorlan Fernandes de Jesus ¹, Fernando de Oliveira Santos Fernando de Oliveira Santos ¹, Vanessa Borges Morgado Vanessa Borges Morgado ², Maria Inês Doria Maria Inês Doria ², Bernardo Teixeira Bernardo Teixeira ¹, Paulo Sérgio D`Andrea Paulo Sérgio D`Andrea ¹, ELBA REGINA SAMPAIO DE LEMOS ¹, Renata Carvalho de Oliveira Renata Carvalho de Oliveira ¹

¹ IOC/FIOCRUZ - Instituto Oswaldo Cruz/FIOCRUZ (Av Brasil 4365 Manguinhos Rio de Janeiro, RJ CEP 21045-360), ² ICTB/FIOCRUZ - Instituto de Ciência e Tecnologia em Biomodelos/Fiocruz (Av Brasil 4365 Manguinhos Rio de Janeiro, RJ CEP 21045-360)

Abstract

The arenavirus, genus *Mammarenavirus*, can cause highly lethal diseases such as hemorrhagic fevers and infections of the central nervous system caused mainly by lymphocytic choriomeningitis virus (LCMV). Humans are infected by inhalation of aerosolized excreta, saliva and nasal secretions from infected rodents. These virus are classified into two large groups, the Lassa-LCM complex and the Tacaribe complex, which comprise respectively Old World viruses and New World viruses.

Exception to this rule, the LCMV, included in the Lassa-LCM complex, whose reservoir is the rodent of the *Mus musculus* species, can be found in the American continent. The LCMV is named due to the zoonosis that it causes, lymphocytic choriomeningitis, which in severe cases can induce aseptic meningitis and encephalitis and, during pregnancy, can cause abortion or malformation of the fetus. In this context, the professionals who handle animals, especially rodents are a potential risk group of infection by these viral agents. The arenavirus infections are considered underestimated in Brazil, where rare studies are performed in human population and rodent reservoirs. Thus, sera of 212 professionals who work in an institute of animal breeding were analyzed and three workers, submitted to ELISA for anti-LCMV IgG antibodies, were seroreactive. All three seroreactive workers reported who they did not wear frequently any protective personal equipment (PPE). Additionally, 300 serum samples of the synanthropic rodents from different regions of Brazil were tested and 49 samples (49/300 - 16%) presented anti-LCMV IgG antibodies: 2 *Rattus rattus* (2/50), 2 *Rattus norvegicus* (2/95) and 45 *Mus musculus* (45/155). The data obtained indicate the presence of the LCMV in the country and the need of measures in the institute for monitoring these zoonotic infections as occupational risk.

Keywords: Arenavirus, Employees who Handle Animals, Lymphocytic Coriomeningitis Virus, Rodents, Zoonosis



HANTAVIRUS SEROPREVALENCE IN A RURAL POPULATION OF SUGARCANE CUTTERS IN THE STATE OF GOIÁS: PRELIMINARY RESULTS

Renata Malachia Maia ¹, Luciana Helena Bassan Vicente ¹, Jorlan Fernandes de Jesus ¹, Karlla Antonieta Amorim Caetano ², Megmar Aparecida dos Santos Carneiro ², Regina Maria Bringel Martins ², Sheila Araujo Teles ², ELBA REGINA SAMPAIO DE LEMOS ¹, Renata Carvalho de Oliveira ¹
¹ IOC - Instituto Oswaldo Cruz (Av. Brasil 4365, Manguinhos Rio de Janeiro, RJ CEP 21045-360), ² UFG - Universidade Federal de Goiás (Av. Esperança, s/n - Chácara de Recreio Samambaia, Goiânia - GO, 74690-900)

Abstract

Hantavirus is a cosmopolitan zoonosis mainly associated with wild and synanthropic rodents of different species. Transmission usually occurs from inhalation of viral particles from secretions or excreta from infected rodents. The disease has two clinical forms, Asia-Europe-restricted renal syndrome (HFRS), and hantavirus pulmonary syndrome (HPS), described in the Americas, including Brazil with an average lethality of 46%. Laboratory diagnosis can be performed by screening for specific IgM and IgG antibodies from serological tests against viral nucleoprotein. The most commonly used test is the Enzyme-Linked Immunosorbent Assay (ELISA). Few studies analyze the seroprevalence of hantavirus despite high case-fatality rate of the HPS. Agricultural activities lead to greater exposure to wild rodents, especially sugar cane cutters that perform manual management. The state of Goiás has a predominance of the Cerrado biome, which is considered a habitat of the *Necromys lasiurus*, rodent reservoir of the pathogenic viral genotype known as Araraquara virus. To better understand the risk of hantavirus infection in the state of Goiás, a serological survey in a rural population of manual sugar cane cutters is under development by detecting IgG anti-Andes antibodies. A total of 654 serum samples from hand-cane cutters were analyzed, 38 of which were anti-Andes IgG seroreactive at 1: 400 titration, with a seroprevalence of 5,8%. The epidemiological survey will be conducted from online consultation of the database made publicly available on the DATASUS/SINAN website and in scientific articles. The results will be correlated with sociodemographic data of the population. The relationship between the variables will be performed by the chi-square test (χ^2) and data analysis by the R program (version 3.1.1). With the development of the present study is expected to gain more knowledge about this viral zoonosis, and to collaborate with HPS surveillance measures in the state, especially in populations considered vulnerable.

Keywords: Hantavirus, seroprevalence, sugarcane cutters, preliminary results



CARBON PASTE ELECTRODE MODIFIED WITH GOLD NANOPARTICLES AND/OR GRAPHITE POWDER WITH ELECTRODEPOSITED GOLD AND ITS FUNCTIONALIZATION: APLICATION IN ELETROCHEMICAL BIOSENSOR FOR HANTAVIRUSES

MARIELENA VOGEL SAIVISH¹, Maria Luiza Lopes Sierra e Silva¹, Vivaldo Gomes da Costa^{1,3}, Roger Luiz Rodrigues^{1,4}, Gildiberto Mendonça de Oliveira², Tatiane Moraes Arantes^{1,2}, Marcos Lázaro Moreli¹

¹ UFG - Universidade Federal de Goiás (Programa de Pós-Graduação em Ciências Aplicadas à Saúde, Universidade Federal de Goiás (Cidade Universitária, BR 364, km 195, nº3800, CEP 75801-615, Jataí-GO), ² UFG - Universidade Federal de Goiás (Programa de Pós-Graduação em Química, Universidade Federal de Goiás (Cidade Universitária, BR 364, km 195, nº3800, CEP 75801-615, Jataí-GO), ³ UnB - Universidade de Brasília (Programa de Pós-Graduação em Biotecnologia e Biodiversidade (Brasília, DF, 70910-900), ⁴ USP - Universidade de São Paulo (Programa de Pós-Graduação em Biologia Celular e Molecular (Av. Bandeirantes, 3900, Monte Alegre, Ribeirão Preto - SP)

Abstract

In this work, the use of a Hantavirus protein as a possible bioreceptor to obtain an electrochemical biosensor was investigated by cyclic voltammetry and factorial design. A modified carbon paste electrode (MCPE) and the ferro/ferricyanide couple were used as a working electrode and redox indicator, respectively. The investigated variables in the factorial planning 2^4 were: 1, gold nanoparticles (NpAu); 2, graphite powder coated with electrodeposited gold (AuPg); 3, functionalization with Hantavirus protein and 4, UV or Acetone drying process. This factorial planning was used to investigate the effects of the MCPE composition and preparation over the voltammetric behave of the redox indicator. By complete factorial planning 2^5 , with the same variables of the 2^4 , were investigated the effect of MCPE composition and preparation in two serological samples, including as factor 5: IgG negative (IgG-) or IgG positive (IgG+). Using factorial planning 2^4 , it was verified that the functionalization decreases the anodic peak current (I_{pa}) and increases the difference between anodic- cathodic peak potential (ΔE). It is also verified that NpAu leads to a lower ΔE . The complete factorial planning 2^5 showed that the functionalization also was the main variable. It was verified that the lowest ΔE and highest I_{pa} occur when the functionalization is performed in the serological sample IgG+. In these voltammetric studies no cathodic or anodic current peaks were observed that were directly related to the protein or to IgG + or IgG-. The results obtained until the moment, indicate that the functionalization of NpAu or AuPg with the Hantavirus protein is promising for the differentiation between IgG + and IgG-

Keywords: biosensor, nanomaterials, diagnosis, hantavirus.

Keywords: biosensor, nanomaterials, diagnosis, hantavirus



DETERMINATION OF POTENTIAL ANTIGENIC TARGETS OF MAYARO VIRUS GLYCOPROTEIN E2

MARIELENA VOGEL SAIVISH¹, Roger Luiz Rodrigues^{1,2}, Gabriela de Lima Menezes¹, Maristela Pereira³, Roosevelt Alves da Silva¹, Vivaldo Gomes da Costa^{1,4}, Marcos Lázaro Moreli¹

¹ UFG - Universidade Federal de Goiás (Programa de Pós-Graduação em Ciências Aplicadas à Saúde, Universidade Federal de Goiás (Cidade Universitária, BR 364, km 195, nº3800, CEP 75801-615, Jataí-GO), ² USP - Universidade de São Paulo (Programa de Pós-Graduação em Biologia Celular e Molecular (Av. Bandeirantes, 3900, Monte Alegre, Ribeirão Preto - SP), ³ UFG - Universidade Federal de Goiás (Laboratório de Biologia Molecular, Instituto de Ciências Biológicas (Avenida Esperança, s/n, Campus Samambaia (Campus II)), ⁴ UnB - Universidade de Brasília (Programa de Pós-Graduação em Biotecnologia e Biodiversidade (Brasília, DF, 70910-900)

Abstract

Mayaro Virus is endemic in South and Central America and the involvement of mosquitoes of the genus *Aedes* sp. in its transmission is a risk for outbreaks of greater proportions. It causes a potentially disabling illness known as Mayaro fever, similar to the Chikungunya Virus. The co-circulation of both, their clinical and structural similarities and the absence of prophylactic and therapeutic measures highlight the need for studies that seek to understand the Mayaro Virus. Using *in silico* approach we identified an antigenic and specific epitope (p_MAYV4) in domain A of the E2 glycoprotein of Mayaro Virus. This epitope is stable and exposed on the surface of the protein with key properties to enable it to interact with neutralizing antibodies. These characteristics make it an interesting target in the development of immunodiagnostic platforms. Structural analyzes from molecular dynamics simulations showed that the PHE95 residue in the E1 fusion loop region is conserved in the Alphavirus family and interact with hydrophobic residues of E2 glycoprotein to form a structure of cage34 shaped, and it is critical to assembly and stability of the E1/E2 heterodimer structure. These results provide important insights for the advancement of diagnostic platforms and the study of therapeutic alternatives.

Keywords: MAYV, E1/E2, Peptides, antigenicity, immunodiagnosis, Molecular Dynamics.

Keywords: MAYV, E1/E2, Peptides, antigenicity, immunodiagnosis



INFECTION OF LYMPH NODES BY RESPIRATORY SYNCYTIAL VIRUS

Bruna Lais Santos de Jesus¹, Ricardo de Souza Cardoso¹, Juliano Paula Souza¹, Ítalo Araújo Castro¹, Ronaldo Bragança Martins¹, Miriã Ferreira Criado¹, Fernando Chahud¹, Eurico Arruda¹
¹USP - Universidade de São Paulo (Avenida bandeirantes, 3900, Monte Alegre, Ribeirão Preto - São Paulo)

Abstract

Human respiratory syncytial virus (HRSV) is the single most important viral cause of severe respiratory infections in children worldwide. HRSV infects the respiratory epithelium and is also frequently detected by RT-PCR in tonsillar tissues from asymptomatic patients. Prompted by that, this study was done to investigate whether HRSV infects lymph nodes. Paraffin-embedded tissues from 16 cervical and 17 infra-diaphragmatic lymph nodes from 33 patients (2014-2017) were tested for HRSV by real-time RT-PCR and immunohistochemistry. Results revealed that 7 of 16 (44%) cervical and 5 of 17 (29%) infra-diaphragmatic lymph nodes were positive for HRSV A. HRSV B was not detected. HRSV antigen was detected in follicular and inter-follicular regions, in B and CD4+ T lymphocytes, macrophages and dendritic cells. The susceptibility of B and CD4+ lymphocytes was confirmed by in vitro infection of primary cultures obtained from dissociated human palatine tonsils. We also inoculated C57bl/6 intranasally with HRSV and sacrificed the animals at 7, 15, 40, 80, 100, 120 and 150 days post infection. RT-PCR and immunohistochemistry analysis showed that HRSV genome and structural proteins were present in lymphoid and tissues for up to 150 days post infection. It has been known that during persisting viral infections there is production of IL-10 by dendritic cells, B and CD4+ T cells, macrophages and innate and regulatory T cells. Since these cell types can harbor HRSV acute infection, we investigated whether they also support persisting HRSV in IL-10 knockout C57b/6 mice. IL-10 is important in the modulation of HRSV persistence in mice. To the best of our knowledge, this is the first report of HRSV detection in human lymph nodes from adult patients without ARI symptoms. In addition, persisting HRSV infection can be established in lymphoid tissues from experimentally infected mice, with as yet unknown immunological consequences.

Financial support: FAPESP, CNPq and CAPES.

Keywords: Human respiratory syncytial virus , lymphoid tissues , persistence



NON-HUMAN PRIMATES CARRY FLAVIVIRUS IN SÃO PAULO STATE- BRAZIL

Leonardo La Serra¹, Larissa Mayumi Bueno¹, Gilberto Sabino-Santos Jr¹, Luiz Tadeu Moraes Figueiredo¹

¹Center for Virology Research, Ribeirão Preto Medical School of University of São Paulo, Ribeirão Preto, São Paulo, Brazil

Abstract

Non-human primates have an essential role in the maintenance and diffusion of many arboviruses in a zoonotic and epizootic scenario where the mosquitoes, reservoir-vectors, are present. In the past decades, arboviruses reappeared as major public health concern since outbreaks of viruses such as Zika, yellow fever and chikungunya spread throughout the world and hit Brazilian population. However, our lack of knowledge on the dynamics of those arboviruses sylvatic cycle and host interaction hinder public efforts to understand, better prevent and control arbovirus outbreaks before it happens. In our study, from January 2017 to June 2019, we collected 265 biological samples (whole blood, saliva, and excreta from living animals, and brain, liver and salivary glands from deceased animals) of captive and wild non-human primates from the northeastern and northwestern region of São Paulo State, Brazil. Samples were collected from an overall of 227 *Callithrix penicillata*, 1 *Callithrix jacchus*, 1 *Callithrix geoffroyi*, 11 *Allouatta caraya*, 1 *Allouatta guariba*, 4 *Saguinus midas*, 10 *Sapajus apella*, 7 *Sapajus libidinosus* and 2 *Sapajus* spp.. and 1 *Saimiri sciureus*. From the 265 primates' samples 42 were tested by a semi-nested multiplex RT-PCR for viruses from the Flaviviridae family, including Dengue, Zika, Yellow fever, West Nile virus and Saint Louis encephalitis virus, and the Alphaviridae family, Mayaro and Chikungunya virus. Ten of the 42 non-human primates' samples (5 *Sapajus apella*, 3 *Callithrix penicillata*, 1 *Saguinus midas* and 1 *Allouatta caraya*.) presented positive results for a Flavivirus currently on investigation for species. These results suggested Flavivirus circulation among non-human primates from the northeastern and northwestern regions of São Paulo State, Brazil. This is an ongoing study and the remaining samples will be analyzed and with the results we intend to generate a risk map for arbovirus outbreak in São Paulo State, Brazil.

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BMC INFECTIOUS DISEASES

MULTIPLEX qPCR FACILITATES IDENTIFICATION OF BETAHERPESVIRUSES IN PATIENTS WITH ACUTE LIVER FAILURE OF UNKNOWN ETIOLOGY

Jéssica Vasques Raposo¹, Arthur Daniel Rocha Alves², Alexandre dos Santos da Silva², Damião Carlos dos Santos⁴, Juliana Gil Melgaço, Otacílio C. Moreira³, Marcelo Alves Pinto², Vanessa Salete de Paula^{1*}.

¹Laboratory of Molecular Virology; Oswaldo Cruz Institute / Fiocruz; Rio de Janeiro, Brazil;

²Laboratory of Technological Development in Virology; Oswaldo Cruz Institute / Fiocruz; Rio de Janeiro, Brazil; ³Laboratory of Molecular Biology and Endemic Diseases; Oswaldo Cruz Institute / Fiocruz; Rio de Janeiro, Brazil; ⁴Estácio de Sá University, Rio de Janeiro, Brazil.

*Address for correspondence: PhD. Vanessa Salete de Paula, Oswaldo Cruz Foundation, IOC – Av. Brasil 4365-Manguinhos, Pav. Helio e Peggy Pereira B10, 21040-360. Rio de Janeiro, Brazil. Tel (+55 21) 2562- 1823

E-mails: jessicavasquesr@gmail.com(JVR), arthur.alves@ioc.fiocruz.br(ARA), alesantosbio@yahoo.com.br (ASS), damiaio.santos@hotmail.com (DCS), otaciliomoreira@gmail.com (OCM), julana.melgaco@gmail.com (JGM), marcelop@ioc.fiocruz.br (MAP); vdepaula@ioc.fiocruz.br - corresponding author (VSP)

Abstract

Background: The etiology of acute liver failure (ALF) is often unknown and reported to be associated with herpesviruses in a number of cases. In this study, we examined for betaherpesviruses infections in patients with ALF of unknown etiology using a multiplex qPCR to Betaherpesviruses subfamily.

Methods: Liver explant and serum samples from 27 patients with ALF of unknown etiology were analyzed with the aid of multiplex qPCR to identify betaherpesviruses. All positive samples were sequenced to confirm herpes infection and liver enzyme levels evaluated.

Results: Betaherpesviruses infection was effectively detected using multiplex qPCR. Six (22%) HHV-6, one (3%) HCMV and two (7%) dual infections (one with HHV-7/HHV-6, and the other with HHV-7/ HCMV). Interestingly, HHV-7 was only detected in the presence of other betaherpesviruses. Sequencing information confirmed betaherpesviruses infection. High hepatic enzyme levels and INR values >1.5 were determined in all betaherpesvirus-positive patients.

Conclusions: Multiplex qPCR facilitated efficient quantification, indicating that differentiation between betaherpesviruses is possible with the sole use of real-time PCR. Liver explant and serum samples were positive for some betaherpesviruses, and coinfection of HHV-7 with HHV-6 and HCMV was additionally detected. Based on these results, we propose that ALF patients should be screened for the presence of betaherpesviruses.

Keywords: Betaherpesviruses, Acute liver failure, qPCR, multiplex, Liver



PRODUCTION AND PROTOTYPING OF AN ENZYME-LINKED IMMUNOASSAY FOR DIAGNOSIS AND SURVEILLANCE OF CHIKUNGUNYA

J Bagno, F. F.^{1,2}; Godoi, L. C.^{1,3}; Figueiredo, M.M. ¹; Sérgio, S.A.R.¹; Carvalho, G. P.⁴, Fonseca, M.S.P.^{1,3}; Da, Fonseca, F. G.^{1,2}.

¹Centro de Tecnologia de Vacinas (CT Vacinas), BH-Tec, UFMG. Belo Horizonte, MG, Brasil.

²Laboratório de Virologia Molecular e Aplicada, Depto de Microbiologia, ICB/UFMG, Belo Horizonte, MG, Brasil. ³Colégio Técnico da UFMG (COLTEC), Belo Horizonte, MG, Brasil.

⁴Fundação Ezequiel Dias (FUNED), Belo Horizonte, MG, Brasil.

Abstract

Chikungunya virus is a re-emerging alphavirus that causes a disease characterized by febrile illness associated with arthralgia and may result in long term sequelae, including prolonged joint pain and chronic arthritis. In order to provide an accessible diagnostic tool to detect CHIKV infections, we successfully developed an indirect ELISA assay which could detect IgG antibodies against CHIKV from human sera. A total of 104 clinical samples were tested in this study in which we evaluate sensitivity, specificity, repeatability, reproducibility and stability of the developed method based on current recommendations for the development of bioanalytical products. The results demonstrated that the ELISA kit was able to detect IgG antibodies against CHIKV with high sensitivity (100%) and specificity (96%) compared with a commercially available reference kit. No cross-reactivity was found against positive sera for Dengue, Zika and rheumatoid factor. The repeatability and reproducibility assessments indicated a coefficient of variation (CV) <10%, and there was no distinct difference among three recombinant protein batches tested during the intra-assay study. Furthermore, the kit showed shelf stability of approximately 2.3 years. These data suggested that our ELISA assay has good repeatability, stability and shelf life, reaching required standards of commercially acceptable ELISA tests. The developed ELISA assay provides a convenient and specific method for the large-scale determination of CHIKV infections in human sera samples with high accuracy

Keywords: Chikungunya virus, Chikungunya Fever, arboviruses, diagnosis, ELISA, prototyping.



RECONSTRUCTION OF THE SPATIAL AND TEMPORAL DYNAMICS OF HEPATITIS B VIRUS GENOTYPE D IN THE AMERICAS

Natália Spitz¹, Francisco C. A. Mello², Aline S. Moreira³, Carolina S. Gusatti⁴, Regina M. B. Martins⁵, Selma A. Gomes¹, Gonzalo Bello⁶, Natalia M. Araujo¹

¹ Laboratory of Molecular Virology, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil, ² Laboratory of Viral Hepatitis, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil, ³ Laboratory of Functional Genomics and Bioinformatics, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil, ⁴ Centre for Scientific and Technological Development, State Foundation on Medical Production and Research, Porto Alegre, Brazil, ⁵ Institute of Tropical Pathology and Public Health, Federal University of Goiás, Goiânia, Brazil, ⁶ Laboratory of AIDS and Molecular Immunology, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil

Abstract

Hepatitis B virus (HBV) genotype D (HBV/D) is globally widespread, and ten subgenotypes (D1 to D10) showing distinct geographic distributions have been described to date. The evolutionary history of HBV/D and its subgenotypes, for which few complete genome sequences are available, in the Americas is not well understood. The main objective of the current study was to determine the full-length genomic sequences of HBV/D isolates from Brazil and frequency, origin and spread of HBV/D subgenotypes in the Americas. Complete HBV/D genomes isolated from 39 Brazilian patients infected with subgenotypes D1 (n = 1), D2 (n = 10), D3 (n = 27), and D4 (n = 1) were sequenced and analyzed together with reference sequences using the Bayesian coalescent and phylogeographic framework. A search for HBV/D sequences available in GenBank revealed 209 complete and 926 partial genomes from American countries (Argentina, Brazil, Canada, Chile, Colombia, Cuba, Haiti, Martinique, Mexico, USA and Venezuela), with the major circulating subgenotypes identified as D1 (26%), D2 (17%), D3 (36%), D4 (21%), and D7 (1%) within the continent. The detailed evolutionary history of HBV/D in the Americas was investigated by using different evolutionary time scales. Spatiotemporal reconstruction analyses using short-term substitution rates suggested times of the most recent common ancestor for the American HBV/D subgenotypes coincident with mass migratory movements to Americas during the 19th and 20th centuries. In particular, significant linkages between Argentina and Syria (D1), Brazil and Central/Eastern Europe (D2), USA and India (D2), and Brazil and Southern Europe (D3) were estimated, consistent with historical and epidemiological data

IMMUNOBIOLOGICALS





EVALUATION OF CYTOKINE PRODUCTION AND IMMUNODOMINANCE PROFILE OF T CELL RESPONSE TO ZIKA VIRUS

Tertuliano Alves Pereira Neto ¹, Camila Pereira de Queiroz ¹, Marcela Helena Gonçalves Pereira de Oliveira ¹, Andréa Teixeira de Carvalho ², Olindo Assis Martins Filho ², Helton da Costa Santiago ¹¹ UFMG - Universidade Federal Minas Gerais (Belo Horizonte, MG), ² Fiocruz Minas - Instituto René Rachou (Belo Horizonte, MG)

Abstract

The ZIKV genome encodes a polyprotein with three structural proteins: capsid, pre-membrane, envelope, and seven nonstructural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. The detailed understanding of cellular immunity to ZIKV and its association to antigenic regions of the virus remains unclear. Thus, this study aimed to evaluate the profile of the immune response and the role of multifunctional T cells in individuals exposed to ZIKV. We used a library of 671 synthetic peptides expanding the whole polyprotein of ZIKV, in which pools corresponding to each viral proteins were used to stimulate PBMCs from individuals previously exposed or not exposed to ZIKV. Investigation of cytokine production by CD4⁺ and CD8⁺ T cells was performed by intracellular cytokine staining combined with flow cytometry. We observed production of IFN- γ and TNF- α by CD8⁺ T cells stimulated with prM (IFN- γ , p=0.0267; TNF- α , p=0.0033), capsid (IFN- γ , p=0.0011; TNF- α , p⁺ T cells. In addition, there was an increase in the frequency of CD8⁺IL-10⁺ T cells after stimulation with prM (p=0.0116), capsid (p=0.0017), NS1 (p⁺ T cells with capsid (p=0.0017) and NS1 (p=0.0448). Multifunctional cytokine production was observed in ZIKV infected individuals, with triple production of: IFN- γ ⁺ TNF- α ⁺ IL-10⁺ in cells stimulated with prM (CD8⁺, p=0.0078), capsid (CD8⁺, p=0.0020; CD4⁺ p=0.0117), NS1 (CD8⁺, p=0.0234) and NS3 (CD8⁺, p=0.0156), and double production of IFN- γ ⁺TNF- α ⁺ in cells stimulated with envelope (CD8⁺, p=0.0156, CD4⁺, p=0.0391), prM (CD8⁺, p=0.0195; CD4⁺ p=0.0195), capsid (CD8⁺, p=0.0078, CD4⁺ p=0.0078), NS1 (CD8⁺, p=0.0156; CD4⁺, p=0.0039) and NS3 (CD8⁺,p=0.0313; CD4⁺, p=0.0078). In addition, a double production of IFN- γ ⁺IL-10⁺ cells stimulated with prM (CD8⁺, p=0.0195), capsid (CD8⁺, p=0.0020; CD4⁺, p=0.0020) and NS1 (CD4⁺, p=0.0078) was detected. These data indicate a prominent and multifunctional T CD8⁺ response targeting mainly structural proteins, while the CD4⁺ T cell response was distributed to structural and non-structural proteins similarly.

Keywords: Zika Virus, T cells, cytokine, immunodominance, immunity



STUDY OF THE INTERACTIONS BETWEEN PEPTIDES DERIVED FROM THE YELLOW FEVER VIRUS AND THE YF-17D VACCINE WITH HLA CLASSES I AND II: AN IN SILICO APPROACH

Francisco Javier Romero Mercado ¹, Stephanie Almeida ¹, Helton Santiago ¹

¹ UFMG - Universidade Federal De Minas Gerais (Avenida Antonio Carlos 6627)

Abstract

The attenuated virus YF-17D is used as a vaccine against yellow fever virus (YFV) and confers protective immunity in 90 to 98% of vaccinated populations. The mechanisms of 2 to 10% in vaccination failures are still not well understood. A possible reason of vaccination failures might be defective antigenic presentation by HLA repertoire of the vaccine. We believe that knowing the amplitude and strength of the interactions between HLA class I and II with the YF-17 and YFV peptides can help us understand cases of vaccine failure. The objective of this research was to predict the amplitude and strength of the interactions between the proteins of the vaccine virus YF-17D and the HLAs of class I and II.

We used bioinformatic tools at EIDB to predict the IC50 values of the interactions between the most common HLA class I and II alleles and polyprotein peptides and viral proteins such as capsid (C), pre-membrane (PrM), envelope (E), NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 of the YFV and YF -17D. Predicted IC50 values 50 nM and 500 nM were considered low affinity and non-significant interaction.

The proteins with the highest number of significant interactions for both, class I and II HLAs were NS5, which showed 977 and 4462 interactions respectively, NS3 (568, 3118), E (517, 5250) and NS4a (480, 1774), probably associated to their bigger sizes when compared to other virus proteins.

In addition, we found that alleles HLA-A*02:03, HLA-A*68:02, HLA-A*02:01, HLA-A*02:06 of class I HLAs and the alleles HLA-DRB1*01:01, HLA-DQA1*05:01/DQB1*03:01, HLA-DRB5*01:01, HLA-DRB1*07:01 and HLA-DRB1*11:01 HLA class II display higher number of interaction with different peptides, which can be classified as good presenters of antigens of the YF-17D. On the other hand, HLAs such as HLA-A*01:01, HLA-B*53:01 and HLA-A*26:01 of class I, and HLA-DQA1*05:01/DQB1*02:01, HLA-DQA1*03:01/DQB1*03:02 and HLA-DPA1*02:01/DPB1*05:01 of class II showed a limited number of interactions with the YF-17D peptides, which makes them poor antigen presenting.

There are differences in the capacity of different HLA alleles to present peptide antigens of Yellow Fever vaccine, which may underlie vaccination failure.

Finacial support: CAPES

Keywords: Human leukocyte antigen, yellow fever vaccine, viral proteins



REFOLDING OF RECOMBINANT EDIII ZIKV PROTEIN GENERATED UNDER HIGH PRESSURE CONDITIONS PRESERVES ANTIGENICITY AND IMMUNOGENICITY IN MICE.

Lennon Ramos Pereira ¹, Aléxia Adrienne Venceslau Brito Carvalho ¹, Samuel Santos Pereira ¹, Rosa Maria Chura-Chambi ², Maria Fernanda Castro-Amarante ¹, Rúbens Prince dos Santos Alves ¹, Robert Andreato-Santos ¹, Lígia Ely Morganti Ferreira Dias ², Luís Carlos de Souza Ferreira ¹

¹ ICB/USP - Instituto de Ciências Biomédicas/Universidade de São Paulo (Avenida Professor Lineu Prestes nº 1374 CEVAT Gene 2 - Sala 118 - Anexo Didático - Cidade Universitária - SP - CEP: 05.508- 000), ² IPEN - Instituto de Pesquisas Energéticas e Nucleares (Avenida Lineu Prestes, 2242 - Cidade Universitária - São Paulo - SP CEP 05508-000)

Abstract

Zika virus (ZIKV) belongs to the *Flavivirus* genus (family Flaviviridae) has a wide geographical distribution and is transmitted to humans by mosquito vectors. Although most infected individuals do not develop symptoms, symptomatic cases cause dramatic consequences to neonates, such as microcephaly and other neurological disorders. Despite efforts, there are no specific and effective preventive or therapeutic methods available against this infection. In this sense, the induction of neutralizing antibodies has been correlated with virus protection. In particular, antibodies targeting the domain III of E ZIKV protein (EDIII ZIKV), responsible for attachment of the virus to the cell, represents an important target for antibodies capable to block virus infection. Thus, the present study aimed to produce and characterize a recombinant form of EDIII ZIKV protein with antigenic and immunogenic features compatible with vaccine applications. The expression of EDIII ZIKV protein was carried out with *E. coli* BL21 (DE3) strain. As strategy to obtain the EDIII protein from insoluble fraction of the bacterial lysate, we optimized an *in vitro* refolding method using High hydrostatic pressure (HHP) associated with alkaline pH. This technique allowed the recovery of EDIII ZIKV at yields up to 27 fold higher (~111 mg/L) than conventional methodologies and the protein was obtained with high purity. In addition, the EDIII ZIKV antigenicity (reactivity against antibodies generated following infection by ZIKV) and its biological function (binding to cellular receptors) were preserved, as demonstrated by ELISA and flow cytometry. To evaluate the EDIII immunogenicity, we carried out immunizations on C57BL/6 mice using the target protein associated with different adjuvants (Alum and a recombinant derivative of the heat-labile toxin (LT) originally produced by enterotoxigenic *E. coli*). After three doses, high of EDIII-specific titers were detected in sera of immunized mice, particularly after immunization with the LT derivative. Thus, this study confirms the antigenic and immunogenic properties of the recombinant EDIII ZIKV protein produced under HHP conditions. Importantly, these results open the possibility of the use of this antigen in the development of new vaccine strategies against ZIKV.

Keywords: Zika virus, Domain III from E protein, EDIII, Vaccine, Flavivirus



RECOMBINANT EXPRESSION OF ZIKA VIRUS-LIKE PARTICLES (VLPs)

Renata Gois de Mello ¹, Thaissa Consoni Bernardino ¹, Renato Mancini Astray ¹, Soraia Attie Callil Jorge ¹

¹ IB - Instituto Butantan (São Paulo-SP, Brasil)

Abstract

In recent years, arboviruses have become a major public health problem in the tropical and subtropical regions of the world. The Zika virus (ZIKV) is an arboviral disease prevalent in the Americas, Africa and Asia and has increased its area of endemicity and it is considered a major public health problem in our country. The diagnosis of the infection is made through molecular techniques and serological tests, but these may be non-specific. Due to this and to the advances of the infections caused by the ZIKV it is of extreme importance the development of tools that allow the adequate combat to the ZIKV. Virus-like particles (VLPs) vaccines appear as an enormous potential for use as extremely effective antiviral vaccines, since they mimic the viral particle, inducing immune response and, as they don't have the genetic material of the virus they won't replicate making them safe as viral particles. In this work, we established a methodology for production and characterization of VLPs containing the structural proteins C, prM and E of ZIKV produced in insect cells and using gene expression system derived from baculovirus. In order to obtain the recombinant baculovirus (BV-ZIKV), vectors containing the sequences of the proteins of interest were constructed. The bacmid was transfected into *Spodoptera frugiperda* (Sf-9) insect cells and stocks of BV-ZIKV were obtained for infection. Infection kinetics were performed to determine the multiplicity of viral infection (MOI) of the inoculum to be used to infect the cells and the best time for BV-ZIKV and VLPs production. Sf-9 cells were infected with different MOIs of BV-ZIKV and were collected at the times 24h to 144h. The best MOI for infection and the best BV-ZIKV collection time was determined by dot blot, western blot and indirect immunofluorescence techniques. The purification step to separate the VLPs from the BV-ZIKV were done by sucrose and iodixanol gradients. The work evidenced the correct expression of ZIKV proteins in this system and were observed by SDS-PAGE and western blot. The VLPs were analyzed by transmission electron microscopy. The results of this project can generate important tools in the development of a vaccine method against the ZIKV.

Financial Support: FAPESP, CAPES and Butantan Foundation

Keywords: Zika virus, Virus-like particles, Recombinant baculovirus



GENERATION OF A LIBRARY OF HYBRIDOMAS WITH ANTI-MAYARO VIRUS REACTIVITY

Lais Durço Coimbra ^{1,2}, Rebeca Froes Rocha ^{1,2}, Luís Antonio Peroni ^{1,2}, Rafael Elias Marques ^{1,2}

¹ CNPEM - Centro Nacional de Pesquisa em Energia e Materiais (Rua Giuseppe Máximo Scolfaro, 10000 - Bosque das Palmeiras, Campinas), ² UNICAMP - Universidade Estadual de Campinas (Cidade Universitária Zeferino Vaz - Barão Geraldo, Campinas)

Abstract

Mayaro virus (MAYV) is an *Alphavirus* present in Latin America. MAYV is transmitted by mosquitos and causes an acute febrile disease characterized by rash, retro-orbital pain and polyarthralgia. Chronic clinical manifestations are associated with incapacitating articular pain which may persist for months. There is no treatment or vaccine available against MAYV and also no specific diagnostic test for MAYV, as serological cross-reactivity to more prevalent alphaviruses takes place. By developing a hybridoma-based anti-MAYV library we expect to select a wide range of specific antibodies able to detect MAYV and potentially neutralize it. Female Balb/c mice were immunized with a MAYV strain isolated from a patient at Sao Jose do Rio Preto. Serum samples were collected 30 days p.i. and antibody titer was accessed by Indirect ELISA. All sera seroconverted after immunization. In order to obtain neutralizing or highly specific anti-MAYV monoclonal antibody (Mabs), the splenocytes from the previously immunized mice were fused with myeloma cells (SP2/O). Next, the supernatant was collected and the reactivity against MAYV was determined by indirect ELISA followed by ROC curve analysis ensuring a 100% of sensitivity and 92% of specificity. We identified 42 MAYV antibody-secreting hybridomas (Absorbance (Abs)/ml of serum $\geq 0,100$). Of those, 76% were considered low positive (0,100 - 0,399 Abs) and 24% were considered high positive anti-MAYV ($\geq 0,400$ Abs). To characterize the Mabs isotype, the supernatant was collected purified by protein G chromatography affinity. We observed IgG1 subclass was predominantly isotype. IgG2b subclass was also detected, but in lower concentrations. In summary, the fusion of splenocytes from immunized mice with SP2 cells allowed us to create a hybridoma library in which 24% of the cells produce highly reactivity antibodies against MAYV. Further experiments are needed to characterize potential neutralizing Mabs and also, specificity against MAYV. Financial support: FAPESP/Capes

Keywords: MAYV, hybridomas, immunization, monoclonal antibodies, IgG



AN IMMUNOENZYMATIC ASSAY FOR ZIKA VIRUS INFECTION DIAGNOSIS UTILIZING IMMUNOGLOBULIN Y

Wagner Luis da Costa Nunes Pimentel Coelho ¹, Alexandre dos Santos da Silva ¹, Fernanda de Oliveira Bottino ¹, Arthur Daniel Rocha Alves ¹, Marcelo Alves Pinto ¹

¹ LADTV/IOC/Fiocruz - Laboratory of Technological Development in Virology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (Rio de Janeiro, RJ, Brazil)

Abstract

The Zika virus (ZIKV) is an arbovirus from family Flaviviridae. Its main form of transmission is through the bite of an infected *Aedes aegypti* mosquito. ZIKV clinical manifestations are similar to those manifestations caused by other arboviruses, such as fever, headache, arthralgia, myalgia, and rash. Besides that, ZIKV infection is related to microcephaly in newborns and Guillain-Barré syndrome in adults. The similarities between arboviruses lead to cross-reactivity in immunoassays, hindering the differential diagnosis. Therefore, this work aimed to standardize a sandwich ELISA utilizing immunoglobulin Y (IgY), an antibody that may be found in birds serum and eggs. IgY presents several advantages when compared to IgG, such as high concentration in egg yolks, no cross-reactivity with IgG, reduction of background in immunoenzymatic assays and it can be obtained by the egg yolks harvest. Eight Isa Brown hens were selected and divided in two groups. The first group was inoculated with two doses of ZIKV by intramuscular via to produce IgY anti-ZIKV, while the second group was inoculated with PBS. Then, the eggs were collected and submitted to the purification method described by Polson. After that, the purified yolks were submitted to polyacrylamide gel electrophoresis to demonstrate the presence of IgY, and then to Western Blotting, to demonstrate the specificity of these antibodies. IgY was conjugated to peroxidase, and then several tests were done to standardize the ELISA utilizing IgY as conjugated and capture antibody. The electrophoresis showed the presence of IgY in the purified and, through the Western Blotting, was observed that these antibodies are specific to the E protein of the ZIKV. Besides that, there was specific binding between ZIKV and IgY anti-ZIKV on ELISA assay. Results showed be possible the immunization of laying hens against ZIKV and the obtaining of IgY anti-ZIKV. Furthermore, the IgY was able to bind to the ZIKV on ELISA test, demonstrating that IgY anti-ZIKV can be used to diagnose Zika infection.

Financial Support: Oswaldo Cruz Institute

Keywords: ELISA, IgY, Zika



DEVELOPMENT OF A ELISA BASED ON PEPTIDE ASSAY (ELISA-PEPTIDE) FOR SPECIFIC DETECTION OF ZIKA VIRUS: PRELIMINARY RESULTS

MarcosL Lazaro Moreli ¹, Marielena Saivish ¹, Roger Luis Rodrigues ¹, Flavio Henrique Lima Fernandes ¹, Vivaldo Gomes da Costa ¹

¹ UFJ - Universidade Federal de Jatai (Rod BR 364 km 192 Pq Industrial 3800)

Abstract

Introduction: ZIKV transmitted by *Aedes* is included in the genus *Flavivirus*, family *Flaviviridae*, which include other important viruses such as Dengue and Yellow Fever. In acute phase different of Dengue IgM antibodies profile antibodies against ZIKV could detect after 3 days of symptoms. Cross-reactivity in diagnostic tests is common but a major problem to address due to epidemiological importance of this viruses as well as their severity associated with ZIKV as microcephaly in newborns, Guillain Barré syndrome and subclinical infections that may go unnoticed, and even. In this work we have started the standardizing a simple, inexpensive, sensitive ZIKV-specific serological test to perform within 5 days of disease. Alternatives to NS1 protein used commonly in the literature, here we have used a peptide from domain III region selected after silico analysis. Aim: The aim was to develop a diagnostic differential ELISA-peptide assay to detect IgM antibodies against ZIKV in acute phase of infection. Methodology: The work was approved by ethical commit COEP at Federal University. For this study retrospective samples of 2017-2018 were collected and the infection by ZIKV was confirmed by RT-PCR using specific primers. To standardizing the reaction different block titration assays were used starting a range of different concentration of peptide vary from 1 to 15 µg/mL in at least two buffers (carbonate, pH 9,6 and saline buffer, pH 7,4 following the addiction of primary antibodies (MIAF – Mouse Immune ascitic fluid and human positive sera for ZIKV) and conjugate peroxidase antibodies. The analysis for this step was made in triplicate with other sera using mean plus 3 standard deviation (SD). Results. On the preliminary test the best results for these assays indicate buffer carbonate was more sensible for coat and the concentration of peptide that indicate the presence of peptide on the plate against antibodies was 15 µg/mL and the dilution of sera and MIAF was 1:100. Conclusion: The results presented here shows the initial steps for standardization of ELISA-Peptide using part of Domain III of E protein of ZIKV is promising, however additional tests using this peptide is necessary for next steps to discrimination of response to others acute phase samples for others arboviruses.

Finantial support: FAPEG, 04/2017

Keywords: vírus Zika, ELISA-Peptide, Diagnostic, Flavivirus



ANTIGENIC AND PHYSICOCHEMICAL CHARACTERIZATION OF HBSAG VLPS

José Luiz Lopes ², Denise Cristina Andre Oliveira ¹, Carla Lilian De Agostini Utescher ¹, Wagner Quintilio ¹, Elisabeth Christina Nunes Tenório ¹, Cristiano Luis Pinto De Oliveira ², Marcia Carvalho De Abreu Fantini ², Martin Kjærulf Rasmussen ³, Heloisa Nunes Bordallo ³, Osvaldo Augusto Sant'Anna ¹, Viviane Fongaro Botosso ¹

¹ IBut. - INSTITUTO BUTANTAN (Av. VITAL BRASIL, 1500), ² IF/USP - Instituto de Física da Universidade de São Paulo (Rua do Matão Nr.1371), ³ University of Copenhagen - University Of Copenhagen (Universitetsparken 5 2100 Copenhagen Denmark)

Abstract

The main component of the recombinant hepatitis B vaccine is the surface antigen (HBsAg), a protein that self-assembles into 22 nm spherical virus-like particles (VLPs) which contain lipids, derived from the host cell, and around 80-100 monomers of HBsAg in a particle. The effectiveness of this vaccine is cleared related to the maintaining of the native conformation of the main epitopes within the VLP antigen in all stages of its manufacturing process and storage. The aim of the present work was characterized the HBsAg during each step of the production and storage in order to established the critical quality attribute of the vaccine (CQA). HBsAg diluted in different solutions used routinely during the manufacturing process were analyzed by biophysical methods [small angle X-ray scattering (SAXS), synchrotron radiation circular dichroism (SRCD), fluorescence spectroscopies and surface plasmon resonance (SPR)] as well as *in vivo* and *in vitro* potency assays. The antigen submitted to different storage temperature (4°C, room temperature and – 20°C) was also studied. Data obtained from SRCD and fluorescence spectroscopies indicate that the HBsAg is a well-ordered globular protein, with a mixed α/β conformation (24% helix, 21% β -strands) in solution, preserving its Trp residues from exposure to water molecules. The native secondary and tertiary structure of the protein is high stable in a large range of pH (from 5.5 to 8.5) and at different temperatures studied. But it can change after treatment at high temperatures, especially after its melting point (56°C), where the protein could unfold. When frozen the protein tend to form large aggregates (>200 nm) and precipitates causing a loss of antigen content measure by ELISA. Some level of aggregates and precipitation were also observed in extremes of acid pH. However, these alterations didn't interfere in the interaction with antibodies, analyzed by SPR instrumentation, and in the *in vivo* assay. These results showed that the conformational structure of the protein was preserved and it is crucial for the immune response. The high stability of the protein, even at room temperature, could improve the distribution of the vaccine and consequently the efficiency of global vaccination programs.

FINANCIAL SUPPORT: FUNDAÇÃO BUTANTAN; FAPESP; CNPq

Keywords: Hepatitis B vaccine, HBsAg, pH stability, Thermal stability



NANOMULTILAMELLAR LIPID VESICLES POTENTIALIZE THE IGG ANTIBODY RESPONSES AGAINST ZIKA VIRUS NS1 PROTEIN.

Mônica Josiane Rodrigues De Jesus ¹, Marianna Teixeira de Pinho Favaro ¹, Wesley Luzetti Fotoran Wesley Luzetti Fotoran ¹, Roberta Mansini Cardoso ², Gerhard Wunderlich ¹, Koiti Araki ², Luís Carlos de Souza Ferreira ¹

¹ ICB/USP - Institute of Biomedical Sciences / University of Sao Paulo (Av. Professor Lineu Prestes, 1374, Cidade Universitária, São Paulo, SP), ² IQ/USP - Chemistry Institute / University of Sao Paulo (Av. Professor Lineu Prestes, 748, Cidade Universitária, São Paulo, SP)

Abstract

The search for safe vaccines against Zika virus (ZIKV) infection is a worldwide priority due to its association with severe manifestations, such as Guillain-Barré Syndrome, microcephaly and congenital malformations. The lack of effective treatments and vaccines support the development of new approaches that can circumvent the occurrence of cases in endemic regions, such as Brazil. In this sense, the present work aimed to develop a vaccine formulation composed of lipid nanostructured particles (NMVs) combined with a recombinant non-structural protein 1 (Δ NS1) of ZIKV. To this end, the Δ NS1 protein was expressed in a prokaryote system with subsequent standardization of expression and purification conditions. The antigenicity of Δ NS1-ZIKV was confirmed against murine anti-ZIKV sera. The recombinant vesicles (NMV Δ NS1-ZIKV) were characterized by charge, size, polydispersion, percentage of incorporated protein and release rates. The cytotoxic effects of the NMV Δ NS1-ZIKV also determined under *in vitro* conditions. The immunological effects of the NMV Δ NS1-ZIKV combined with monophosphoryl lipid A adjuvant (MPL-A) were evaluated after intramuscular (i.m.) immunization of female C57/BL6 wild-type mice. The induction of antigen-specific IgG and subclass responses was measured by ELISA. Under the tested conditions, the NMV Δ NS1-ZIKV were capable to induce higher specific IgG Δ NS1-ZIKV titers when compared to protein-only administration, as well as high levels of IgG1 and IgG2c subclasses, with predominance of IgG1. Antibodies generated after the third dose were able to recognize native NS1 protein present in virus-infected VERO cells. Thus, the results obtained so far offer a basis for further evaluation of antibody functionality and induction of protective immunity to ZIKV infection under pre-clinical conditions.

FINANCIAL SUPPORT:

CAPES – Processo: 88887.185337/2018-00 / **FAPESP** - Processo: 2016/20045-7

Keywords: Nanoparticles, Vaccine, NS1, ZIKV, Protein



CROSS-REACTIVE NEUTRALIZING HUMAN SURVIVOR MONOCLONAL ANTIBODY BDBV223 TARGETS THE EBOLAVIRUS STALK

Liam King ^{1,2}

¹ TSRI - The Scripps Research Institute (10550 N. Torrey Pines Rd, San Diego, CA, United States),

²UNICAMP - Universidade de Campinas (Cidade Universitária Zeferino Vaz - Barão Geraldo, Campinas - SP, 13083-970)

Abstract

Introduction: Three viruses in the Ebolavirus genus cause lethal disease and lack targeted therapeutics: Ebola virus, Sudan virus and Bundibugyo virus. Cocktails of monoclonal antibodies (mAbs) present a potential therapeutic strategy. BDBV223 IgG, which was identified in a human survivor of Bundibugyo virus disease, neutralizes both Bundibugyo virus and Ebola virus. Here we report two crystal structures of the antibody BDBV223, alone and complexed with its epitope on the glycoprotein (GP) stalk. This site is particularly attractive for therapeutic/vaccine design due to its high sequence conservation among ebolaviruses. **Materials and Methods:** BDBV223 IgG was expressed in hybridoma cells generated from a survivor of Bundibugyo virus disease. Purified BDBV223 Fab was co-crystallized with a synthetic peptide representing residues 620–635 of the BDBV GP stalk epitope. Crystals of the BDBV223-GP2 stalk complex were diffracted at SSRL Beamline 12–2 to 3.7 Å and 2 Å resolution, respectively; phases were solved using molecular replacement. Based on structural findings, mutant BDBV223 antibodies were generated to broaden cross-reactivity. Mutants were tested for binding via ELISA and via viral neutralization assays against live filovirus in a biosafety level 4 facility. **Results and Conclusions:** The structures of the BDBV223-GP stalk complex as well as the apo-BDBV223 Fab reveal an unexpected binding orientation of the antibody. The structure suggests that BDBV223 binding may interfere with both the trimeric bundle assembly and the viral membrane. This indicates that BDBV223 likely stabilizes a conformation in which the monomers are separated by lifting or bending of the glycoprotein. Interestingly, mutagenesis of BDBV223 against SUDV indicates that additional determinants of antibody binding likely lie outside the visualized interactions, perhaps involving quaternary assembly or membrane-interacting regions. These quaternary movements are akin to dynamics seen in stalk binding antibodies of HIV and are important to consider in the context of immunogen design. These results reveal new information about the filovirus stalk and can be used to better design or elicit antibodies against this conserved epitope. **Financial Support:** The Scripps Research Institute Graduate Program, NIH F30AI136410, and NIH R01AI089498

Keywords: Antibody, Crystallography, Ebola, Filovirus, Structure



GUANOSINE IS NOT PROTECTIVE IN THE CONTEXT OF USUTU VIRUS INFECTION

Rebeca de Paiva Froes Rocha ^{1,2}, Lais Coimbra ^{1,2}, Alexandre Borin ^{1,2}, Giulliana Eboli Sotorilli ^{1,2}, Julio Cesar Santos ^{1,3}, Bruno Henrique Araujo Torres ¹, Rafael Elias Marques ^{1,2}

¹ CNPEM - Centro Nacional de Pesquisa em Energia e Materias (Rua Giuseppe Maximo Scolfaro 10000, Campinas, SP), ² UNICAMP - Universidade Estadual de Campinas (idade Universitária Zeferino Vaz - Barão Geraldo, Campinas - SP, 13083-970), ³ UNIFESP - Universidade Federal de São Paulo (Rua Silva Jardim, 136 - Vila Mathias - Santos/SP)

Abstract

Usutu virus (USUV) is an arbovirus capable of causing encephalitis in humans and other vertebrates. This pathogen demands greater attention due to increased detection in mosquitos and birds throughout Africa and Central Europe. The lack of specific treatments or vaccines only heightens this need. Guanosine is a neuroprotective compound which has been used in the treatment of various neuropathologies. We hypothesized that the neuroprotective properties of guanosine may be beneficial in the context of USUV infection. C57BL/6 mice (8-12 weeks old) intracranially infected with 10^1 (LD80) and 10^3 PFU (LD100) of USUV presented signs of neurological disease including hunched back, paralysis, and conjunctivitis. Mice succumbed to these doses of virus after 10 and 7 days, respectively. Increased viral loads and Inflammatory cytokines such as CXCL1, CCL5, IFN- γ , TNF- α were only observed in the brain, indicating USUV is neurovirulent but not viscerotropic. Guanosine treatment to C57 WT mice infected with 10^1 PFU of USUV is not protective. We therefore evaluated whether guanosine treatment would be protective *in vitro*. USUV infection at a multiplicity of infection (MOI) of 0,1 was cytotoxic to human neuroblastoma cells (SH-SY5Y), leading to 10^7 viral PFU, 48h post infection (p.i.). Treatment with guanosine in SH-SY5Y cells did not improve cell viability or interfered in the viral load. Diversely, we were not able to recover virus from induced pluripotent stem cell-derived *microglia* infected with USUV. When we treated USUV-infected *microglia* with guanosine, we recovered 10^4 viral units, which indicates that guanosine treatment potentiates USUV replication in these cells. This finding correlates to our previous result, that guanosine is not protective *in vivo*. Overall summary, our data indicates the intracranial infection with USUV causes encephalitis in adult wild type mice. Further, we observed that guanosine does not prevent mortality *in vivo*. In accordance, guanosine does not improve cell viability or interferes in USUV viral load in neuroblastoma cells. However, the treatment led to increased susceptibility to infection in *iPSC-MGLCs*.

Keywords: Encephalitis, Guanosine, Neuroprotection, Usutu vírus



THE MARBURGVIRUS-NEUTRALIZING HUMAN MONOCLONAL ANTIBODY MR191 TARGETS A CONSERVED SITE TO BLOCK VIRUS RECEPTOR BINDING

Liam King ^{2,1}

² UNICAMP - Universidade de Campinas (Cidade Universitária Zeferino Vaz - Barão Geraldo, Campinas - SP, 13083-970), ³ TSRI - The Scripps Research Institute (10550 N. Torrey Pines Rd, San Diego, CA, United States)

Abstract

Introduction: Since its first identification 50 years ago, marburgviruses have emerged several times, with 83-90% lethality in the largest outbreaks. Although no vaccines or therapeutics are available for human use, the human IgG antibody MR191 provides complete protection in nonhuman primates when delivered several days after inoculation of a lethal marburgvirus dose. The detailed neutralization mechanism of MR191 remains poorly understood. This study analyzes the structural biology, biochemistry, and immunology behind MR191 mediated viral neutralization and protection. **Materials and Methods:** Marburgvirus glycoprotein (GP) was expressed in *Drosophila* S2 cells. MR191 IgG was expressed in hybridoma cells generated using B-cells collected from a Marburg virus disease survivor; IgG was subsequently cleaved to yield Fab fragments. Purified GP and MR191 Fab were complexed and subsequently crystallized by hanging drop crystallization. Crystals were diffracted at the Advanced Photon Source to a resolution of 3.2 Å, with phases solved using molecular replacement. To generate escape mutants to MR191, a chimeric vesicular stomatitis virus displaying GP was incubated with serial dilutions of MR191, and any antibody-resistant viruses were subsequently sequenced. **Results and Conclusions:** Here we present a 3.2 Å crystal structure of MR191 complexed with a trimeric marburgvirus surface glycoprotein (GP). MR191 neutralizes by occupying the conserved receptor-binding site and competing with the host receptor Niemann-Pick C1. The structure illuminates several previously disordered regions of GP including part of the critical marburgvirus-specific “wing” antibody epitope. Virus escape mutations mapped far outside the MR191 receptor-binding site footprint suggest a role for these other regions in the GP quaternary structure and antibody recognition. Fc-immune effector function assays revealed a role for monocytes in MR191 mediated phagocytosis. Finally, MR191 escape mutants were generated and provided insight into the role of unresolved regions of the protein in the protection of the receptor binding site. The combined biochemical and structural data suggest that MR191 is an appropriate first immunotherapeutic for development against marburgvirus disease. **Financial Support:** The Scripps Research Institute Graduate Program, NIH F30AI136410, and NIH R01AI089498

Keywords: Antibody, Crystallography, Ebola, Filovirus, Structure



USE OF ECO DIAGNOSTICA® RAPID TESTS FOR THE DETECTION OF RESPIRATORY SYNCYTIAL VIRUS ECO F ADENOVIRUS AG (ADV) AS A DIAGNOSTIC TOOL FOR USE IN PEDIATRICS.

Fabyano Leal ¹, Flavio Mesquita , Celia Pirez ¹, Jose Lopes ¹, Alfredo Gilio ¹, Ricardo Fock ¹, Silvia Ibdia ¹, Vanessa Silveira ¹, Luciano Thomazelli , Viviane Botosso ¹, Danielle Oliveira ¹, Erick Dorlas , Edison Durigon ¹

¹ USP - UNIVERSIDADE DE SÃO PAULO (AV.PROF. LINEU PRESTES 1374 -BUTANTÃ)

Abstract

INTRODUCTION: Human Adenovirus (AdV) is an important pathogen responsible for many types of diseases, such as respiratory, gastrointestinal, ophthalmological, genitourinary and neurological diseases. Present, there are 79 known types of HAdVs divided into seven species (HVV-A to HAdV-G) and / or after subspecies B1, we find serotypes 3,7, 16, 21 and 50 in many cases associated with respiratory respiration. ECO Diagnostics has the ECO F Adenovirus Fluorescence Detection (FIA) kit for detecting Adenovirus infection using EUROPIO core technology that enables detection of most target analytes. **OBJECTIVES:** To determine the sensitivity and specificity of the ECO F Adenovirus Ag rapid test, compared with Immunofluorescence (IF), to be used as a diagnosis of AdV in hospitals and pediatric clinics. **MATERIAL AND METHOD:** Forty-five samples from the University Hospital of USP (HU) and Santa Casa were used in this study from children who had symptoms of respiratory diseases and were hospitalized in these places. The samples were divided into 4 groups: AdV positive (+) (20 samples); Negative (-) for AdV / + for other viruses (10); - for respiratory virus (9 samples); + for Adv / + for other viruses (6 samples). The tests were performed strictly following the instructions for use of the product, with a maximum time of 15 min and the reading was performed on the ECO F200 equipment. **CONCLUSION:** Of the forty-five samples tested, 4 were divergent between the ECO rapid test and the IFI, the qPCR was performed for the tiebreaker and all samples were positive for AdV, in agreement with the IFI. Thus, the ECO F Adenovirus Kit analyzed, promotes the detection of the viral target in a very specific way, presenting sensitivity of 80% and specificity of 100% when compared to IFI. **KEYWORD:** Adenovirus, diseases, immunofluorescence, test, virus, diagnosis.

Keywords: Adenovirus , Doenças, Imunofluorescencia, teste, virus

PLANT AND INVERTEBRATE VIROLOGY





MOLECULAR DETECTION OF HONEY BEE VIRUSES IN APIARIES OF SOUTHERN BRAZIL

Domitila Brzuskowski Chagas¹, Francielle Liz Monteiro¹, Lariane da Silva Barcelos¹, Alice Silveira Becker¹, Matheus Iuri Frühauf¹, Mara Helena Saafeld², Luis Fernando Wolff³, Marcelo de Lima¹, Silvia de Oliveira Hübner¹, Geferson Fischer¹

¹ UFPel - Universidade Federal de Pelotas (Campus Universitário Capão do Leão, Prédio 1, Faculdade de Veterinária, Laboratório de Virologia e Imunologia.), ² EMATER/RS - EMATER/RS (Rua General Osório, 305 - Ganguçu/RS), ³ EMBRAPA - EMBRAPA Clima Temperado (Rodovia BR-392, Km 78 - Pelotas/RS)

Abstract

Bees are very important insects for agriculture, fulfilling an important role in pollination and renewal of the ecosystem. However, in several countries significant losses of colonies and population decline of honeybees and native bees have been reported, which are influenced by biotic and abiotic factors, including the effects of multiple pathogen infection and/or pesticide exposure. The majority of the viruses that have already been isolated and characterized in bees are classified as positive-sense single-stranded RNA viruses within the order *Picornavirales*, comprising the families *Dicistroviridae* and *Iflaviridae*. Prominent viruses include acute bee paralysis virus (ABPV), deformed wing virus (DWV), black queen cell virus (BQCV), sacbrood bee virus (SBV) and israeli acute bee paralysis (IAPV). Thus, the objective of this study was to detect the main bee viruses in apiaries in southern Brazil. Samples of honeycomb (larva and pupa) and adult bees were collected in the apiaries and kept under refrigeration until transportation to the Laboratory. Immediately the larvae and pupae were homogenized to TRIzol Reagent and frozen at -70 °C. Adult bees collected and stored in closed bottles were directly frozen. Six pupae, larvae and adult bee abdomen were submitted to RNA extraction and cDNA synthesis, followed by two multiplex polymerase chain reaction (PCR) (1: ABPV, CBPV and SBV; 2: BQCV, DWV and IAPV). All cDNA samples were tested with the endogenous control (GAPDH) to verify the efficiency of the whole process. RNA extracted from the bees' pool was used as negative control and gBlock® Gene Fragments were used as positive control of honey bee viruses. To date, 75 samples were obtained, mainly from southern Rio Grande do Sul, two of which were positive for IAPV (3,5%), three for ABPV (5,4%) and twenty-five for BQCV (33,3%), totaling 40% (30/75) of positive samples. All detected viruses were obtained from adult bees, and the identity of these viruses was confirmed by nucleotide sequencing. No viruses were detected in samples of larvae and pupae. These results demonstrate that ABPV, BQCV and IAPV viruses are present in apiaries in the South region of Brazil, with a high percentage of positivity for BQCV, and may, together with other factors, contribute for the bee population decline. This study is underway in order to increase the number of samples collected and phylogenetic characterization of the viruses detected.

Financial Support: CAPES.

Keywords: ABPV, *Apis mellifera*, BQCV, IAPV, RT-PCR multiplex



BACULOVIRUS INFECTION TRIGGERS DIFFERENT CYTOSOLIC DNA SENSING PATHWAYS IN MAMMALIAN CELLS

Sabrina Amalfi¹, Guido Nicolás Molina¹, Oscar Taboga¹, Victoria Alfonso¹

¹ IABIMO-INTA - Agricultural Biotechnology and Molecular Biology Institute. Nacional Institute of Agriculture Technology (De los Reseros y Nicolás Repetto, Hurlingham s/n, Buenos Aires, Argentina)

Abstract

The baculovirus AcMNPV is an enveloped virus with a dsDNA genome and is a pathogen of insects. The budded vir capable of transducing genes under the control of an adequate promoter in mammalian cells, although it cannot its genome in this host. Among the applications of baculoviruses as a biotechnological tool in mammals, it mentioning their use for vaccine development, gene delivery and as immunomodulators. BVs induce a strong innate response in mammals that is capable of generating an unspecific antiviral state, independent of TLR pathways. Our reports using a reporter BV showed that the cytoplasm is the main destination reached by their genome in diff lines. Thus, this work aims to study the role of baculoviral cytoplasmic nucleic acids in the production of an antivir non-immune mammalian cells. We studied the involvement of different cytosolic DNA sensors in murine and hu infected with BV. We evaluated the production of cytokines by qPCR and antiviral activity by protection against vesicular virus infection. In first place, we demonstrate that RNA Pol III does not participate in the establishme antiviral state. We then studied the cGAS-STING pathway by CRISPR-Cas9 gene editing in murine cells and complementation of cGAS or cGAS and STING in HEK293 and HEK293 T (human epithelial cells), respectively. Th showed that STING was required for the establishment of an antiviral state in mammalian cells. Moreover, at different signaling pathways had an impact on STING and contributed to the baculovirus induced antiviral s detection of the viral genome by cGAS sensing induced the strongest cellular response and it was necessar production of IFN β . Additionally, the cGAS-independent STING activation produced an antiviral state in human cells where the production of IFN λ 1 was involved. In conclusion, the results of this work show that the geno baculovirus AcMNPV has a relevant role in the establishment of an antiviral state and in the production of IFN through its impact on the nucleic acids sensing pathway cGAS-STING.

Financial Support: PICT 2015 1334, ANPCyT; PIP N° 11220130100258, CONICET; PNBIO-1131034, INTA.

Keywords: BACULOVIRUS, cGAS, INTERFERON, MAMMALS, STING



THE INHIBITORS OF APOPTOSIS GENES LIMITS THE IN VITRO HOST RANGE OF CHRYSODEIXES INCLUDENS NUCLEOPOLYHEDROVIRUS

Fabricio da Silva Morgado¹, Bergmann Morais Ribeiro¹

¹ UnB - Universidade de Brasília (Laboratório de Baculovírus, Instituto de Biologia, Campus Darcy Ribeiro, Brasília, DF)

Abstract

The larvae of *Chrysodeixes includens* and *Anticarsia gemmatalis* are two important defoliators in soy fields of Brazil. The baculovirus biocontrol agents applied in the fields to control each have low efficacy on the other species and little is known regarding the exact cause of host restriction of each baculovirus. There are many potential barriers to infection *in vivo*, ranging from physical and chemical barriers within the midgut to innate cellular defenses that inhibits viral proliferation and systemic spread in the host. Apoptosis, or programmed cell death, is an efficient method to limit systemic infection by a baculovirus. The viral genes that function as inhibitors of apoptosis (*iap*) evolved to inactivate specific enzymes in the biochemical cascade that leads to apoptosis within the host cell. This makes the *iap* functions crucial to viral proliferation and fitness. In this work we isolated a new *Chin*NPV strain (*Chin*NPV-UNB1) and generated a recombinant virus containing the fluorescence reporter *gfp* gene controlled by the constitutive *hsp70* promoter (*Chin*NPV-GFP). We used these viruses to assess the infectivity in UFLAg-286 cell lines, derived from *A. gemmatalis*. Light and fluorescence microscopy revealed that the infection leads to apoptosis with subsequent increase of effector caspase activity as measured by chemiluminescent assays. To further our understanding of this phenomenon, we cloned *Chin*NPV-UNB antiapoptotic *iap2* and *iap3* genes in *p35* defective *AcMNPV* bacmid vectors to assess their specific activities. While the *iap2* containing bacmid induced some apoptosis, both *iap2* and *iap3* were capable of infecting *Trichoplusia ni* derived Tn5B leading to the production of occlusion bodies, a hallmark of successful baculovirus infection. In contrast, both bacmids failed to inhibit apoptosis in UFLAg-286. These results demonstrate that *Chin*NPV inhibition of apoptosis genes limits its host range *in vitro* to Plusiinae derived cell lines. This suggests that *Chin*NPV *iaps* are strong determinants of host range *in vivo*.

Financial support: CAPES, CNPq, FINATEC and FAPDF.

Keywords: baculovirus, IAP, biocontrol, *Anticarsia gemmatalis*, *Chrysodeixes includens*



ULTRASTRUCTURAL STUDIES OF THE *COTESIA FLAVIPES* OVARIES AND ITS ENDOSYMBIOTIC POLYDNA VIRUS

Fabricio da Silva Morgado¹, Daniela Carrilho de Jesus¹, Bergmann Morais Ribeiro¹

¹ UnB - Universidade de Brasília (Laboratório de Baculovirus, Instituto de Biologia, Campus Darcy Ribeiro, Brasília, DF)

Abstract

The parasitoid wasp *Cotesia flavipes* has been introduced in Brazil to serve as a biocontrol agent of lepidopteran pests of corn and sugarcane. *C. flavipes* is an efficient hunter of *Diatreia saccharalis* larvae that lives within the sugarcane stems, effectively protected from chemical insecticides applied over the fields. Once a female *C. flavipes* finds the larval host, it injects its matured eggs and calyx fluid contained in the ovaries into the hemolymph of the host. The eggs then develop into larvae within the lepidopteran larvae, feeding on the nutrients of the hemolymph until they breach out of their hosts, to pupate outside and morph into an adult wasp. Lepidopteran larvae have innate immune defenses against parasitoids such as melanization that can encapsulate and asphyxiate the wasp's eggs. Throughout the evolutionary history of this parasite-host interaction, the Braconidae group of wasps has incorporated an ancestral Nudivirus into its genome and further evolved it into an endosymbiotic Polydnavirus. A specific group of calyx cells within the ovaries of *C. flavipes* produces abundant viral particles that contains circular segments of the Bracovirus genome (CfBV). These viral particles are secreted into the ovarian lumen and are also injected with the eggs into the larval host, acting as gene delivery agents into cells of the hemolymph, expressing viral genes that disrupts the metabolism and their defense functions for the organism. The study of the biology of CfBV and its symbiotic relationship to the wasp is crucial to understand the evolution of its parasitoid life. It should also yield new information on how to improve biological control methods. Here we present the first images of the *C. flavipes* CfBV particles within the wasp's ovaries by Transmission Electron Microscopy (TEM), demonstrating that the ovarian fluid possesses abundant viral particles composed of multiple nucleocapsids with a common envelope. We also purified viral particles by ultracentrifugation and visualized them by Negative Staining TEM. A single nucleocapsid has clear physical traits such as the end-cap, ring-like and helix-tail structures with variable lengths, possibly due to the different sizes of the DNA segments contained in each virion.

Keywords: Parasitoid, Biocontrol, *Cotesia flavipes*, *Diatreia saccharalis*, Poly DNA vírus



AN IN-SILICO APPROACH TO VALIDATE THE CAPSID ARCHITECTURE OF NEW PUTATIVE ICOSAHEDRAL VIRUSES: GEMINIVIRIDAE AS CASE STUDY.

Luca Cestari ¹, Gabriel Bonfim ¹, Tatiana Domitrovic ¹, Simone da Graça Ribeiro ²

¹ UFRJ - Universidade Federal do Rio de Janeiro (Bloco I, sala 14 - PREDIO DO CCS UFRJ, Av. Carlos Chagas Filho, 373, Rio de Janeiro - RJ, 21941-590), ² EMBRAPA - Embrapa Recursos Genéticos e Biotecnologia (Parque Estação Biológica - PqEB s/nº. Brasília, DF - Brasil - CEP 70770-901)

Abstract

With the advent of metagenomics approaches, a diversity of known and unknown viruses has been identified in various types of samples. The sequence-based only taxonomic classification of these viruses is a challenge, and more tools are needed to understand and predict biological aspects of this viral-dark matter. Many capsid proteins from icosahedral viruses have a positively charged domain (R-arm) that is important for genome packaging and particle stability but that have poor sequence conservation. Recently, we developed a computational approach based on the net charge calculation of discrete protein segments that can automatically identify R-arms and calculate its electrostatic net-charge. With this analysis, we identified various virus families with ssDNA, ssRNA, and dsDNA genomes, for each the genome packaging capacity is highly correlated with the total number of capsid subunits (capsid architecture). Therefore, we propose that by knowing the capsid protein sequence and the total genome size, we could apply this analysis to check if a putative new member of a given viral family complies with the particle morphology expected for that group. In this work, we tested this hypothesis with geminiviruses (*Geminiviridae*), that were among the viruses that use capsid R-arms to stabilize their capsids. These ssDNA plant viruses are divided into two main genera, *Begomovirus* and *Mastrevirus*, which contain the viruses with known tridimensional capsid structure: a geminated capsid, formed by two T=1 icosahedral capsids joined by a missing pentameric vertex, totalizing 110 repeated subunits. The family also comprises other 7 minor genera, 2 unsigned species and several new species. We applied our program to calculate the R-arm net charge for all *Geminiviridae* sequences included in the 10th ICTV report (n=466) and other putative isolates. We observed a linear correlation between the R-arm net charge and the genome size for most of the data-set. All minor genera had similar genome/capsid charge ratio, corroborating the assumption that they share the same geminated capsid architecture. Importantly, our plot was able to predict that a virus closely related *Genomoviridae* family (T=1, 60 subunits) did not comply with the geminated architecture. Moreover, our analysis indicated that mulberry mosaic dwarf associated virus that is yet unassigned to a genus in the family *Geminiviridae*, could have an alternative T=1 virus architecture based on the R-arm charge and genome size.

Keywords: Bioinformatics, Capsid, Geminiviridae



EMERGENCE AND ADAPTATION OF TOMATO BEGOMOVIRUSES IN BRAZIL: ASSESSING REPLICATIVE AND TRANSMISSION FITNESS

César Xavier ¹, Angélica Nogueira ¹, Anelise Orílio ¹, Vinícius Bello ², Renate Krause-Sakate ², Francisco Murilo Zerbini ¹

¹ UFV - Dep. de Fitopatologia, Universidade Federal de Viçosa (Viçosa, MG, 36570-900, Brazil), ² Unesp - Departamento de Proteção Vegetal, Universidade Estadual Paulista (Av. Universitária, 3780 - Altos do Paraíso, Botucatu - SP, 18610-034)

Abstract

The prevalence of only a few begomoviruses infecting tomatoes in Brazil is an intriguing fact, on light of the great begomovirus diversity that has been reported in this crop. Most studies that have been performed in an attempt to understand the begomovirus emergence process and consequent epidemics in Brazil have focused on the genetic structure and dispersion patterns of viral populations. Studies addressing the underlying mechanisms leading to emergence and the current patterns of prevalence have not been conducted. Here, we quantified the replicative fitness of two begomoviruses infecting tomato in Brazil, *Tomato severe rugose virus* (ToSRV) and *Tomato yellow spot virus* (ToYSV), in tomato plants and in non-cultivated hosts to which each virus has often been associated, and quantified the transmission efficiency by *B. tabaci* Middle East-Asia Minor 1 (MEAM1) and *B. tabaci* Mediterranean (MED). Interestingly, ToSRV and ToYSV presented similar adaptation levels in tomato. No fitness trade-off across hosts was observed for ToYSV when viral accumulation was evaluated in tomato and in the wild host *L. sibiricus*. In contrast, ToSRV performed better in tomato than in *N. physaloides*. These results reinforce that ToSRV is well adapted to tomato and occasionally spills back to wild hosts, while ToYSV is well adapted to both tomato and *L. sibiricus*. We also compared the replicative fitness of ToSRV and ToYSV during single or mixed infection. Interestingly, while there were no differences in fitness between the two viruses at 14 days after inoculation (dpi), ToYSV had a gain in fitness from 21 to 28 dpi. Furthermore, a negative interference of ToSRV over ToYSV was observed, as ToYSV reached a higher accumulation in single infection than in mixed infection with ToSRV. Together, these results suggest that adaptation to the host does not explain the prevalence of ToSRV over ToYSV in the field. However, while ToSRV was transmitted by *B. tabaci* MEAM1 and MED, ToYSV was not, which could be the reason why this virus is not widespread in the field.

Financial Support: CAPES, CNPq, FAPEMIG

Keywords: begomovirus, *Bemisia tabaci*, geminivirus, mixed infection, transmission



ASPECTS OF THE ASSOCIATION BETWEEN LEONURUS YELLOW SPOT ALPHASATELLITE AND BIPARTITE BEGOMOVIRUSES: EFFECTS ON INFECTION AND TRANSMISSION BY BEMISIA TABACI MIDDLE EAST-ASIA MINOR 1

Francisco Murilo Zerbini ¹, Monique Nascimento ^{2,1}, Angélica Nogueira ¹, Tarsiane Barbosa ¹, Ayane Quadros ¹, Danielle Barros ²

¹ UFV - Dep. de Fitopatologia, Universidade Federal de Viçosa (Viçosa, MG, 36570-900, Brazil), ² UFPel - Dep. de Fitossanidade, Universidade Federal de Pelotas (Pelotas, RS 96010-000, Brazil)

Abstract

The genus *Begomovirus* (family *Geminiviridae*) includes plant viruses with circular, single-stranded DNA (ssDNA) genomes which are transmitted by the whitefly *Bemisia tabaci*. Begomoviruses in the New World can be found in association with alphasatellites, which are circular, ssDNA molecules capable of autonomous replication, but dependent on the helper begomovirus for encapsidation, systemic infection and insect transmission. The impact of the interaction between alphasatellites and begomoviruses is unknown. The objective of this work was to verify the effect of *Leonurus yellow spot alphasatellite* (LeYSA) in the infection of *Tomato yellow spot virus* (ToYSV), *Tomato severe rugose virus* (ToSRV) and *Euphorbia yellow mosaic virus* (EuYMV) in three hosts, *Leonurus sibiricus*, *Nicotiana benthamiana* and tomato. The plants were inoculated with each virus in the presence or absence of the alphasatellite. Infectivity and symptom development for each begomovirus alone or in the presence of LeYSA were evaluated, and viral DNA accumulation was quantified for each virus and virus-satellite combination. The association of LeYSA with ToYSV was less efficient in tomato than in *L. sibiricus* and *N. benthamiana*, as measured by a lower percentage of plants in which the presence of the alphasatellite was detected. The association between ToSRV and LeYSA was similar in both tomato and *N. benthamiana*. The association between EuYMV and LeYSA in tomato was the least efficient, and in *N. benthamiana* the presence of the alphasatellite was not detected in any of the plants infected with EuYMV. Together, these results indicate distinct levels of interaction between the alphasatellite and different begomoviruses. Quantification of ToYSV and ToSRV DNA-A accumulation indicated that LeYSA does not interfere in the accumulation of these begomoviruses. However, symptoms were more severe in the presence of LeYSA for both viruses and in all hosts. There was a variation in the accumulation of LeYSA relative to the host and the associated begomovirus. Together with previous studies, these results highlight the potential risk of the association between begomoviruses and alphasatellites in both cultivated and non-cultivated plants.

Financial Support: CAPES, CNPq, FAPEMIG

Keywords: alphasatellite, begomovirus, geminivirus, *Leonurus*, tomato



MALVAVISCUS YELLOW MOSAIC VIRUS, A BEGOMOVIRUS CARRYING A NANOVIRUS-LIKE NONANUCLEOTIDE AND A MODIFIED STEM-LOOP STRUCTURE

Anelise Franco Orilio¹, Alison Talis Martins Lima^{1,5}, Mariana Martins Severo Almeida², Carolina da Silva Rocha^{1,6}, Danielle Ribeiro Barros^{1,7}, Gloria Patricia Castillo Urquiza^{1,8}, Fabio Nascimento Silva^{1,9}, Poliane Alfenas Zerbini³, Júlio C. Barbosa⁴, Leonardo Cunha Albuquerque², Alice Kazuko Inoue Nagata², Elliot Watanabe Kitajima⁴, Francisco Murilo Zerbini¹

¹ UFV - Universidade Federal de Viçosa (Dep. de Fitopatologia/BIOAGRO, UFV, Viçosa, MG, 36570-900, Brazil), ² CNPH - Embrapa Hortaliça (Brasília, DF), ³ UFV - Universidade Federal de Viçosa (Dep. de Microbiologia/BIOAGRO, UFV, Viçosa, MG, 36570-000, Brazil), ⁴ ESALQ-USP - Escola Superior de Agricultura Luiz de Queiroz (NAP/MEPA, ESALQ-USP, Piracicaba, SP, 13418-900, Brazil), ⁵ UFU - Universidade Federal de Uberlândia (Instituto de Ciências Agrárias, UFU, Uberlândia, MG 38400-902, Brazil), ⁶ FuturaGene - FuturaGene (FuturaGene Brasil, Avenida José Lembo 1010, Itapetinga, SP, 18210-780, Brazil), ⁷ UFPel - Universidade Federal de Pelotas (Dep. de Fitossanidade, UFPel, Pelotas, RS, 96010-000, Brazil), ⁸ CORPOICA - Corporación Colombiana de Investigación Agropecuaria (Corporación Colombiana de Investigación Agropecuaria, Magdalena, Colombia), ⁹ UDESC - Universidade do Estado de Santa Catarina (Centro de Ciências Agroveterinárias, UDESC, Lages, SC 88520-000, Brazil)

Abstract

Begomoviruses (family *Geminiviridae*) are whitefly-transmitted viruses with a circular, ssDNA genome encapsidated in twinned icosahedral particles. In Brazil, a high number of begomoviruses infecting non-cultivated plants have been described. These plants may act as natural begomovirus reservoirs and as sources of genetic variability. Here we describe a novel bipartite begomovirus infecting *Malvaviscus arboreus* (Malvaceae) plants showing a bright yellow mosaic, collected at Campinas, São Paulo state in May 2005 and Rio de Janeiro, Rio de Janeiro state in August 2009 and February 2011. Total DNA was extracted and the viral genome was amplified by RCA, cloned and sequenced. Sequence analysis indicated that the virus corresponds to novel species, for which the name Malvaviscus yellow mosaic virus (MaLYMV) is proposed. Successful infection by biolistic of *Nicotiana benthamiana* and *Malvaviscus arboreus* was confirmed by PCR, RCA and Southern blot hybridization. Symptoms observed in infected *Malvaviscus arboreus* plants consisted in bright yellow mosaic while in *N. benthamiana* showed slight mosaic and leaf deformation. The progeny virus population present in biolistic infected plants was isolated and identity to the original isolate was confirmed by sequencing. Therefore, Koch's postulates were fulfilled. Strikingly, MaLYMV has a nanovirus- and alphasatellite-like nonanucleotide (5'-TAGTATTAC-3'). Moreover, a short sequence located 5' of the nonanucleotide potentially forms a minor hairpin structure embedded in the major hairpin. Intramolecular interactions involving the sequence of the atypical nonanucleotide were predicted. To biologically characterize the replication origin of this distinct begomovirus, three different mutants were obtained. The mutant that rescues the begomovirus nonanucleotide (TAATATTAC) was able to infect *N. benthamiana* plants, showing that the point mutation at the nonanucleotide does not disrupt MaLYMV replication. On the other hand, the short sequence located 5' of the nonanucleotide seems to be essential for infection. Although MaLYMV has been collected in Brazil, it is phylogenetically closer to viruses from Central and North America. The *M. arboreus* plant at Campinas has been displaying the observed yellow mosaic symptoms since



at least the 1960's, which suggests that MalYMV may be poorly transmitted (or not transmitted at all) by local whitefly populations.

Financial Support: CAPES, CNPq, FAPEMIG

Keywords: Geminiviridae, Malvaceae, replication origin , hairpin structure



COMPOSITION OF BEGOMOVIRUS POPULATIONS IN CULTIVATED AND NON-CULTIVATED HOSTS DETERMINED BY HIGH-THROUGHPUT SEQUENCING

Ayane F. F. Quadros ¹, Camila G. Ferro ¹, Márcio T. Godinho ¹, César A. D. Xavier ¹, Rafael R. Rezende

², Angélica M. Nogueira ¹, Poliane Alfenas-Zerbini ², F. Murilo Zerbini ¹

¹ UFV - Universidade Federal de Viçosa (Dep. de Fitopatologia, Campus Universitário), ² UFV - Universidade Federal de Viçosa (Dep. de Microbiologia, Campus Universitário)

Abstract

The genus *Begomovirus* (family *Geminiviridae*) includes single-stranded DNA plant viruses transmitted by whiteflies. Begomoviruses are among the most damaging plant pathogens, causing epidemics in economically important crops worldwide. Tomato-infecting begomoviruses emerged in Brazil in the early 1990's following the introduction of *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1, previously known as *B. tabaci* biotype B). Several lines of evidence suggest that these viruses evolved from indigenous viruses infecting non-cultivated hosts. However, tomato-infecting viruses are only rarely found in non-cultivated hosts, and vice-versa. It is possible that viral populations in a given host are composed primarily of viruses which are better adapted to this host, but also include a very small proportion of viruses which are poorly adapted. Then, after transfer to a different host by the whitefly vector, the composition of the viral population shifts rapidly, with the viruses which are better adapted to the new host becoming predominant. To test this hypothesis, we collected tomato and *Sida* sp. plants, growing next to each other, at two locations (Coimbra and Florestal, both in Minas Gerais state, Brazil), in 2014 and 2018. Viral infection was confirmed by polymerase chain reaction (PCR) using specific primers. Total DNA from one tomato and one *Sida* sp. sample from each location and year were subjected to high-throughput sequencing (HTS). Following a highly stringent set of criteria, reads were mapped to a data set including all New World begomoviruses. The reads were classified as (i) *Tomato severe rugose virus* (ToSRV), (ii) *Sida micrantha mosaic virus* (SiMMV) and (iii) *Sida common mosaic virus* (SiCmMV), when the first three hits were isolates of these species, or (iv) begomovirus, when the first three hits included isolates of different species. For the 2014 samples, >98% of the reads from *Sida* sp. mapped to SiMMV, but 0.01% of the reads mapped to ToSRV. Conversely, >99% of the reads from tomato mapped to ToSRV, with 0.001% mapping to SiMMV. For the 2018 samples, >99% of the *Sida* reads mapped to three *Sida*-infecting viruses, and 0.1% of the reads mapped to ToSRV. These results are consistent with the hypothesis that viral populations in a given host are composed primarily of the virus that is most adapted to this host but also includes a very small proportion of viruses that are less adapted.

Keywords: Geminivirus, host adaptation, viral population



THE OVEREXPRESSION OF SIDJ1 PROTEIN IN NICOTIANA BENTHAMIANA LEADS TO DECREASED INFECTION BY TURNIP MOSAIC VIRUS

Pamela Magalhães ¹, Fernanda Prieto Bruckner ¹, Poliane Alfenas Zerbini ¹

¹ UFV - Universidade Federal de Viçosa (Av. P. H. Rolfs, s/n. Viçosa, MG)

Abstract

Potyviridae is one of the largest and most important families of viruses that infect plants, being *Potyvirus* the largest genus in the family. Potyvirus genome consists of one single strand positive-sense RNA component, which encodes about eleven proteins that can interact with the host, manipulating the plant cell for the benefit of the virus. A previous analysis of genes differentially expressed in tomato plants infected by the potyvirus *Pepper yellow mosaic virus* (PepYMV) led to the identification of the gene *SIDj1* as induced by the infection. *SIDj1* is a member of the DnaJ protein family, also known as Hsp40. These proteins may act as co-chaperones of Hsp70 proteins, regulating their activity, or they may also act as chaperones. The DnaJ-Hsp70 complex is involved in cellular processes such as protein folding, regulation of protein degradation and protein complexes assemble, among others. The downregulation of *SIDj1* homologs in *Nicotiana benthamiana* plants leads to decreased infection by both the potyviruses PepYMV and *Turnip mosaic virus* (TuMV). Silenced plants also present a phenotype similar to the symptoms of viral infection. Confocal microscopy analyses demonstrated the co-localization of *SIDj1* with vesicles associated with TuMV replication in infected plants. In order to understand the role of *SIDj1* in potyvirus infection, *SIDj1* was fused to the GFP and overexpressed transiently in leaves of *N. benthamiana* by agroinfiltration. Twenty-four hours later, the same leaves were agroinoculated with TuMV and the virus infection was analyzed. The overexpression of *SIDj1*-GFP was confirmed by Western Blot, using antiserum anti-GFP, and the viral accumulation was quantified by qRT-PCR, using specific primers. The plants overexpressing *SIDj1* accumulated fewer viruses than control plants. These results indicate that *SIDj1* is involved in the process of infection by potyviruses. Further studies are necessary to unravel the role of *SIDj1* in potyvirus infection. Financial support: CAPES, CNPq, FAPEMIG.

Keywords: Plant-virus interaction, Chaperones, Potyvirus



SPECIFIC NUCLEOTIDES IN THE COMMON REGION OF THE BEGOMOVIRUS TOMATO RUGOSE MOSAIC VIRUS (TORMV) ARE RESPONSIBLE FOR THE NEGATIVE INTERFERENCE OVER TOMATO SEVERE RUGOSE VIRUS (TOSRV) IN MIXED INFECTION

Angélica M. Nogueira ¹, Anelise Franco Orilio ¹, Marcela C.J. Bigão ¹, César A.D. Xavier ¹, Camila G. Ferro ¹, Francisco Murilo Zerbini ¹

¹ UFV - Universidade Federal de Viçosa (Dep. de Fitopatologia, UFV, Viçosa, MG, 36570-900, Brazil)

Abstract

Natural mixed infections with two or more begomoviruses are common and may alter infectivity, symptom severity and viral accumulation in comparison to single infections. The begomoviruses Tomato rugose mosaic virus (ToRMV) and Tomato severe rugose virus (ToSRV) have genomes with a high degree of sequence identity, including the cis-elements (iterons) in the common region (CR) and their specific recognition sites within the Rep gene (iteron-related domain, IRD), which are essential for initiating viral replication. Previous work has shown that the interaction between these begomoviruses in mixed infection is complex, with ToRMV negatively interfering in infectivity and in the accumulation of ToSRV. In this work we investigated if divergent sites in the CR and IRD of these begomoviruses are involved in this interference. ToSRV DNA-A mutants containing the same nucleotides of ToRMV DNA-A at the divergent positions of the CR (ToSRV-A(CR)), of the Rep gene IRD (ToSRV-A(IRD)) and in both regions (ToSRV-A(CR+IRD)) were constructed. Infectivity and viral accumulation of ToSRV in single infection were not affected by mutations in either of the two regions. However, in mixed inoculation of ToRMV with ToSRV-A(CR), high infectivity of both viruses and high accumulation of ToSRV-A(CR) DNA in relation to wild-type ToSRV was observed. This was not observed when plants were inoculated with ToRMV and ToSRV-A(IRD). These results suggest that the mutated CR sites serve as specific recognition sites for Rep binding, increasing the viral replication rate and viral DNA accumulation. On the other hand, decreased viral accumulation in plants inoculated with ToSRV-A(CR+IRD) suggests that the divergent amino acids in the IRD do not offer an advantage for ToSRV replication efficiency.

Financial Support: CAPES, CNPq, FAPEMIG

Keywords: Geminiviridae, tomato, replication, interaction



RSIBR1, AN INOVIRUS THAT CAN MODULATE MOTILITY AND BIOFILM PRODUCTION OF THE PHYTOPATHOGEN RALSTONIA PSEUDOSOLANACEARUM

Maria Eduarda Vieira de Arruda de Souza ¹, Rafael Reis de Rezende ¹, Poliane Alfenas Zerbine ¹
¹ UFV - Universidade Federal de Viçosa (Avenida Peter Henry Rolfs, s/n - Campus Universitário, Viçosa - MG, 36570-900)

Abstract

Filamentous bacteriophages contain a single-stranded DNA genome and have a peculiar lifestyle compared to other bacteriophages once they do not cause host cell lysis, but actually establish a persistent association with host, often causing behavioral changes, with unpredictable effects on bacterial ecology. In a previous work, we reported the RSIBR1, an Inovirus capable to remarkably attenuate the virulence of the phytopathogenic *R. pseudosolanacearum* GMI1000 besides provoking innumerable alterations in phenotypic and physiological parameters of the host bacterium. The presence of GMI1000 phage-infected (GMI1000 PI) in xylem vessels of plants without symptoms after 3 months confirms that the infected isolates are able to colonize the plant without causing disease, showing that the phage infection changed the behavior of these pathogens. However, it is not clear how the virus modulates the bacteria pathogenesis. Motility and biofilm production are important components for establishing bacterial wilt in plants caused by *R. solanacearum*. In this work, we evaluate the effects of RSIBR1 in *R. solanacearum* motility (swimming and Slipping) and the production as well as composition of biofilm. In order to better understand this phenotypic change, the isolate of *R. pseudosolanacearum* GMI1000 was infected with the inovirus RSIBR1 and its motility and biofilm production were evaluated. Motility was evaluated by motility assay in culture medium CP 0,3% agar, 0,7% agar and Minimal Medium. The biofilm production was evaluated on 96 well plates and biofilm composition was determined using (DNASE 1) to quantify eDNA, Proteinase K was used to quantify proteins while sodium periodate was used to quantify polysaccharide. The *R. pseudosolanacearum* GMI1000 infected with RSIBR1 showed an increase of motility (swimming and slipping) and a reduction in total biofilm production. We also observed a reduction of polysaccharides on biofilm produced by *R. pseudosolanacearum* GMI1000 infected with RSIBR1. These results can help us to explain the phenotypes of reduction or loss of virulence that have been reported for *Ralstonia* spp infected by inoviruses.

Keywords: Inovirus , *Ralstonia pseudosolanacearum*, Biocontrol, Biofilm, Phytopathogen



SYSTEMIC INFECTION OF PLANTS BY A GEMYCIRCULARVIRUS (FAMILY GENOMOVIRIDAE)

Tales Mendes¹, Anelise Olírio¹, Rafael Rezende², Poliane Alfenas Zerbini², F. Murilo Zerbini¹

¹ UFV - Dep. de Fitopatologia, Universidade Federal de Viçosa (Viçosa, MG, 36570-900, Brazil), ² UFV - Dep. de Microbiologia, Universidade Federal de Viçosa (Viçosa, MG, 36570-900, Brazil)

Abstract

The *Genomoviridae* family is one of the most recently established ssDNA virus families. Genomovirids are non-enveloped, ssDNA viruses with circular genomes ranging from 2 to 2.4 kb, containing two ORFs separated by an intergenic non-coding region (IR). One of the ORFs, located in the virion-sense strand, encodes the putative coat protein (CP), and the other, in the complementary-sense strand, encodes a putative replication-associated protein (Rep) similar to the Rep found in members of the family *Geminiviridae*. Currently, the *Genomoviridae* family is comprised of 73 viral species classified into nine genera, with *Gemycircularvirus* as the largest genus. Members of the family have been identified in mammals, birds, invertebrates, in a variety of environmental samples, and also in plants. However, despite this large number of reports and their pervasive presence in the environment, the infectivity of genomovirids to specific hosts remains largely unknown. The gemycircularvirus SsHADV-1 remains the only genomovirid with a known (fungal) host. Interestingly, the genus *Gemycircularvirus* includes all plant-associated genomovirids found so far. It is possible that gemycircularviruses play relevant ecological roles in association with plants. Here, we report the first case of systemic infection of plants by a genomovirid, *Euphorbia heterophylla* associated gemycircularvirus (EuaGmV). A dimeric clone (~4.4 kb) of EuaGmV was used for the biolistic inoculation of eight plants of *Euphorbia heterophylla* and 10 plants of *Nicotiana benthamiana*. Control plants (three of each species) were inoculated with the geminivirus *Euphorbia* yellow mosaic virus (EuYMV) or with water. At 21 and 28 days after inoculation (dai), non-inoculated upper leaves of the EuaGmV-inoculated plants (which did not show any symptoms) were collected for PCR-based analysis of the presence of the virus. Six plants of *E. heterophylla* and all 10 plants of *N. benthamiana* were PCR-positive for the presence of EuaGmV. Amplicons (612 bp) obtained from both plant species were sequenced, and a 99.6% identity with the sequence of the EuaGmV clone was obtained. These results demonstrate, for the first time, that a gemycircularvirus (family *Genomoviridae*) is capable of systemically infecting plants.

Financial support: CAPES, CNPq, Fapemig

Keywords: metagenomics, genomoviridae, gemycircularvirus, *Euphorbia heterophylla*, CRESS DNA virus



INTERCEPTION OF BARLEY STRIPE MOSAIC VIRUS-BSMV: A QUARANTINE VIRUS ABSENT IN BRAZIL DETECTED IN IMPORTED BARLEY GERmplasm

Tallyrand Moreira Jorcelino ^{1,3}, Giovana Curcio Guimarães ^{1,2}, Thaina Berbert Gelelete ^{1,2}, Priscila Alves Noronha ³, Norton Polo Benito ¹, Barbara Eckstein ¹, Marcio Martinello Sanches ¹, Marília Santos Silva ¹

¹ Cenargen - Embrapa Recursos Genéticos e Biotecnologia (Brasília - DF, Brasil), ² UniCEUB - Centro Universitário de Brasília (Brasília - DF, Brasil), ³ UnB - Universidade de Brasília (Brasília - DF, Brasil)

Abstract

Barley stripe mosaic virus (BSMV), a quarantine virus absent in Brazil, was intercepted in 2019 in imported barley (*Hordeum vulgare*) seeds. Expressive and typical symptoms of virus infection were observed in young leaves in seedlings from the abovementioned barley material (confidential process). The presence of BSMV in these symptomatic leaflets was confirmed by Enzyme-Linked Immunosorbent Assay (ELISA), using antibodies against BSMV (Agdia SRA 19500/0096) in biological and technical independent duplicates, resulting in clear detection of BSMV, once the spectrophotometry value was 1,063 times higher in barley samples than the experimental control samples. Visual inspection of symptoms and Polymerase Chain Reaction (PCR) for phytoplasma detection were negative. As BSMV is a regulated quarantine pest absent in Brazil (according to the Normative Instruction n. 39, 01/10/2018- Brazilian Ministry of Agriculture, Livestock and Food Supply), destruction of the respective imported seeds was recommended (incineration) and a report with technical information justifying such recommendation was issued. Samples of BSMV infected barley leaves were stored and will be used as positive control for future detection procedures. The presently reported BSMV quarantine interception of barley to be imported into Brazil reinforces the need for research on quarantine intelligence, development and validation of more sensitive molecular diagnostic methods, and preventive genetic improvement to combat the vulnerability of introduction of quarantine pests absent in the country, which can seriously jeopardize the primary sector of the national economy. This work is aligned with the Brazilian policy, which promote prevention and surveillance of quarantine absent pests, according to the "Portaria nº 131, 27/jun/2019 - Programa Nacional de Prevenção e Vigilância de Pragas Quarentenárias Ausentes (PNPV-PQA)".

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Keywords: Grass-plants, Phytovirus, Prevention, Serological-molecular diagnosis, Surveillance



AN ASYMPTOMATIC IFLAVIRUSES COVERTLY INFECTING BRAZILIAN STINK BUGS: MOLECULAR AND ULTRASTRUCTURAL CHARACTERIZATION

Ethiane Rozo dos Santos ¹, Luana Beló Trentin ¹, Assis Ecker ¹, Leonardo Assis Silva ², Miguel Borges ³, Bergmann Morais Ribeiro ², Joseph D. Mowery ⁴, Robert L. Harrison ⁴, Daniel Mendes Pereira Ardisson-Araújo ¹

¹ UFSM - Federal University of Santa Maria (Santa Maria, RS - Brazil.), ² UnB - University of Brasilia (Brasília, DF - Brazil.), ³ Embrapa - Embrapa Recursos Genéticos e Biotecnologia (Brasília, DF - Brazil.), ⁴ USDA - Agricultural Research Service (Beltsville, MD - USA)

Abstract

Iflaviruses belong to the picorna-like virus family *Iflaviridae* (order *Picornavirales*) and are classified within a single genus called *Iflavirus*. The members of this genus have a broad host spectrum and are characterized by a positive ssRNA genome organized into one single ORF that codes for one polyprotein organized into structural and non- structural peptides. *Iflavirus* infection is still unclear and seems to be different depending on the host type, but the majority of iflavirus infections do not result in visible signs or any apparent disease. An analysis of transcriptomes derived from the antennae of the South American stink bugs *Euschistus heros* (Fabricius, 1794), *Chinavia ubica* (Rolston, 1983), and *Dichelops melacanthus* (Dallas, 1851) revealed the presence of picorna-like virus genome-length RNAs with high sequence identity to the genome of Halyomorpha halys virus (HhV), originally discovered in the transcriptome of the brown marmorated stink bug, *Halyomorpha halys* (Stål). Features of the genome sequences, their phylogenetic relationships to other insect picorna-like viruses, and the appearances of virus-like particles isolated from host stink bugs all confirm that these viruses are iflaviruses. Comparison of the predicted capsid amino acid sequences of these viruses indicate that all four viruses are isolates of an undescribed species of genus *Iflavirus*. Iflavirus RNAs were present at high levels, with iflavirus reads significantly outnumbering actin mRNA reads in all stink bug transcriptomes that were examined. Approximately 40% to 90% of transcriptome reads in the stink bug antennal transcriptomes mapped to the virus genome sequences found in these transcriptomes. In whole-insect transcriptomes of *H. halys*, HhV reads were >500-fold more abundant in adults than in nymphs. No iflavirus sequences were detected in the genomic DNA datasets for *E. heros*, *C. ubica*, *D. melacanthus* or *H. halys*, suggesting that the iflavirus RNAs detected in the stink bug transcriptomes are not derived from copies of iflavirus sequences integrated into host genomes. The results of the analysis suggest that these iflaviruses are able to produce large quantities of their RNAs without causing any obvious pathology to their hosts.

Financial Support: CAPES

Keywords: covert infection, Halyomorpha halys, Iflaviridae, iflavirus, Pentatomidae



NOVEL VIRUSES IN SALIVARY GLANDS OF ANOPHELES MOSQUITOES FROM MATO GROSSO, BRAZIL

Nilvanei Aparecido da Silva Neves ¹, Fernando Lucas de Melo ², Andressa Zelenski Lara Pinto ¹, Raquel da Silva Ferreira ¹, Laura Maia Siqueira Maia ¹, Fábio Assis de Campos Junior ¹, Michellen Santos de Carvalho ¹, Bergman Moraes Ribeiro ², Renata Dezengrini Silhessarenko ¹

¹ UFMT - Universidade Federal de Mato Grosso (Av. Fernando Corrêa da Costa, 2367 - Boa Esperança, Cuiabá), ² UnB - Universidade de Brasília (Brasília, DF, 70910-900)

Abstract

Anopheles species are the main vectors of Malaria. However these mosquitoes have been associated to the transmission of few arboviruses such as the alphaviruses Mayaro virus and O'nyong nyong virus to date. Metagenomic studies busted the discovery of novel insect viruses and their evolutionary relationships. Here we report the identification of three viruses in salivary glands of 353 female anopheles specimens allocated into 12 pools according to genus, capture location and climatic period (dry, transitional, rainy). In total, 6 pools (197 specimens of 13 species; 55.8%) from High Pantanal, 5 pools (126 specimens of 8 species; 35.7%) from Chapada dos Guimarães National Park and 1 pool (30 specimens of 3 species; 8.5%) from Cuiabá were subjected to RNA extraction, dscDNA synthesis and randomic PCR; the purified DNA was sequenced on an Illumina HiSeq 2500 platform. Anopheles nimbus / thomasi (70; 19.8%) was the most frequent along with 18 sampled species. Three viruses were found: segments VP1 and VP3 of a putative novel reovirus named Purunga orbivirus, with 74% and 65% of identity with Changuinola virus and Urongo virus, was identified in an Anopheles pool (30 Anopheles benarrochi and 3 Anoptheles spp.) from Pantanal. This virus was isolated in Vero cells. A novel rhabdovirus named Coxipó dielmovirus was identified in an Anopheles spp. pool from Cuiabá and presents 54% identity with Merida virus. Also, Anopheles triannulatus orthophasmavirus (92% identity) was detected in this study in an Anopheles lutzi pool from Chapada dos Guimarães. Anopheles mosquitoes can be infected with insect-specific viruses and arboviruses. Evolutionary studies have shown that insect-specific viruses are more ancient than arboviruses. Although these agents comprise different phylogenetic groups, they share the same viral families and a common ancestral.

Financial Support: Capes, CNPq rede pró-centro oeste

Keywords: arbovirus, High throughput sequencing, ISV, novel viruses, phylogeny



COMPARISON OF PARAMETERS FOR *CHRYSODEIXIS INCLUDENS* NUCLEOPOLYHEDROVIRUS IN VIVO PRODUCTION

Marcio Martinello Sanches¹, Claudia Efigenia Pereira Silva^{1,2}, Ana Lis Rangel Santos^{1,3}, Norton Polo Benito¹, William Sihler¹, Marlinda Lobo Souza¹

¹ Cenargen - Embrapa Recursos Genéticos e Biotecnologia (Pqeb W5 Norte, Asa Norte, Brasília-DF), ²UNIP - Universidade Paulista (Brasília-DF), ³ UNICEUB - Centro Universitário de Brasília (Brasília-DF)

Abstract

The *Chrysodeixis includens nucleopolyhedrovirus* (ChinNPV), genus *Alphabaculovirus*, family *Baculoviridae* is pathogenic to *C. includens* larvae, which is an important soybean pest. The ChinNPV-Buritis isolated from Brazilian savanna demonstrated potential for biocontrol of this pest. However, there are limiting factors for the large-scale *in vivo* production of ChinNPV, such as disruption of larvae integument and restraints on soybean looper mass rearing due to endogamy and colony depletion. In order to improve *in vivo* production of ChinNPV some parameters were tested. The first assay was conducted comparing larvae of 3rd instar and 4th instar, three different temperatures (23°C, 26°C and 29°C) for larvae incubation and two different viral concentrations (5×10^6 and 5×10^7 Occlusion bodies-OBs/ml). The experiment was performed with 30 larvae/treatment and 30 larvae for control without virus. The larvae were kept individually in 50ml plastic cups with artificial diet. OBs concentration in the larvae was determined using a Neubauer chamber under an optical microscope. The evaluations were performed at 3 to 7 d.p.i. The mass of infected and control larvae was measured in a precision balance. The second assay was performed comparing the incubation of 3rd instar larvae individually and groups of 30 larvae in 300ml plastic recipients and two temperatures for larvae incubation (23°C and 26°C). Each treatment was performed with 30 larvae inoculated with 5×10^6 OBs/ml and 30 larvae for control without virus. OBs and mass evaluation were performed at 6 d.p.i. Statistical analysis were performed in R program using ANOVA for mass and GLM-quasipoisson for OBs analysis. In the first experiment the best OBs production occurred at 6 d.p.i. No significant differences in OBs production (*pin vivo* production to avoid the disruption of larvae integument. The rearing of larvae in groups and lower concentration of virus as inoculum are indicated to reduce the costs of production. Financial Support: FAP-DF

Keywords: ChinNPV, baculovirus, biological control, soybean looper



VIRAL METAGENOMICS OF HEMATOPHAGOUS INSECTS COLLECTED IN THE COMPLEXO MINERADOR DE CARAJÁS AREA, STATE OF PARÁ

Camila margalho braga ¹, Sandro Patroca da Silva ², Hamilton Antonio de Oliveira Monteiro ², jedson ferreira Cardoso ², Joaquim Pinto Nunes Neto ², Lívia Carício Martins ²

¹ UEPA - Universidade do Estado do Para (Tv. Perebebuí, 2623 - Marco, Belém - PA, 66087-662),

² IEC - Instituto Evandro Chagas (Rodovia BR-316 km 7 s/n - Levilândia - 67030-000 - Ananindeua / Pará / Brasil)

Abstract

The Amazon region presents favorable environmental factors for the maintenance of viruses and some anthropic actions contribute mainly to the dispersion of vectors. Metagenomic studies are essential to elucidate the arthropod virosphere and provide relevant data on viral diversity and evolution. A total of 40 hematophagous insects of 2 mosquito species (*Haemagogus janthinomys* and *Sabethes glaucodaemon*) and a genus of *ceratopogonidae* (biting midges) were collected in 3 municipalities: Curionópolis, Marabá e Canaã dos Carajás, state of Pará (2014-2016). It was performed the extraction of viral RNA, double strand cDNA and sequencing illumina Miniseq. In the Bioinformatic step, the programs SortMeRNA, CD-HIT, assembly with IDBA-UD and SPADES, alignment with Diamond and curation with Geneious were used. The JModelTest and RaXML programs were used for phylogeny. Viral sequences belonging to more than 15 different RNA virus families were detected and annotated. The most abundant families were *Flaviviridae*, *Chuviridae*, *Rhabdoviridae*, *Phasmaviridae* and *Phenuiviridae*. The *Sabethes glaucodaemon* pool had the highest viral diversity and the highest number of contigs for the *Xincheng Mosquito Virus*, presenting a low identity (35-56%) in BLAST, which may indicate a new virus. Among the members of the *Flaviviridae* family were identified the *Mercadeo virus* and *Culiseta flavivirus* which are insect-specific flaviviruses also detected in North American countries. The viruses *Hubei tombus-like virus 28*, *Hubei diptera virus 17*, *Wuhan insect virus 8*, *Hubei dimarhabdovirus virus 2*, *Wuhan insect virus 8*, *Hubei virga-like virus 11*, *Hubei chuvirus-like virus 3* and *Sanxia water strider virus 9* found in this work were recently discovered in a chinese study made in 2016. A large number of non-classified contigs were observed in the database, and a detailed curation was necessary to identify the existing viruses. The majority of the viruses found in this work do not have a well defined biological importance because they are newly discovered viruses, thus other molecular biology studies are necessary to elucidate their role in the arthropod virome and possible pathogenic effects on vertebrates.

Financial Support: Fundação Instituto para o desenvolvimento da Amazônia- FIDESa, Capes-CNPQ, Instituto Evandro Chagas, Universidade do Estado do Pará.

Keywords: Amazon, Carajas, insects, metagenomic, viroses



NEW VIRUS IN MOSQUITOES FROM CANAA DOS CARAJAS, NORTH BRAZIL

Camila margalho braga ¹, Sandro Patroca da Silva ², Hamilton Antonio de Oliveira Monteiro ², jedson ferreira Cardoso ², Joaquim Pinto Nunes Neto ², Livia Caricio Martins ²

¹ UEPA - Universidade do Estado do Pará (Tv. Perebebuí, 2623 - Marco, Belém - PA, 66087-662),
² IEC

- Instituto Evandro Chagas (Rodovia BR-316 km 7 s/n - Levilândia - 67030-000 - Ananindeua / Pará / Brasil)

Abstract

New viruses and its variations keep on emerging and studies are needed to elucidate the virosphere profile and to locate new viruses in mosquito samples, as mosquitoes are vectors of many viruses that infect animals and humans. A total of 5 mosquitoes of *Sabethes glaucodaemon* specie, collected in Canaã dos Carajás (Pará) in 2016, was subjected to the extraction of viral RNA, double strand of cDNA and sequency with Illumina Miniseq technology. The softwares used in this work were: SortMeRNA and CD-HIT (preprocessing of the readings); Spades and IDBA-UD (assembly); Diamond (BLAST of the contigs); besides Geneious versão 9.1.4 program and BLAST in the NCBI site (curating of the contigs). The new virus found in this work was named *Canaa Virus* (VCAN). The assembling of the contigs revealed a low identity (35-56%) with the *Xincheng mosquito virus* when individually subjected to the BLASTx of the NCBI. The structure of this virus is similar to the structure of the *Xincheng mosquito virus*, which presents 3 non-structural genes (VP1, VP2 and VP3) and 2 structures, glycoprotein (G) and RNA polymerase (L). The molecular weight of which structure is given in kilodaltons (KDa): VP1 (46.1 KDa), VP2 (18,4 KDa), VP3(48,1 KDa), G (72 KDa) e L (231,1 KDa). To make the phylogeny of *Canaa Virus*, the gene corresponding to L, which is generally more conserved among the species, was used. The database was assembled with reference genomes of the viral families grouped in the order of Mononegavirales. The *Canaa Virus* showed a greater similarity with the *Xincheng Mosquito Virus*, an *Anphevirus* of the *Xinmoviridae* family, grouping in the same clade in the phylogenetic tree. The phylogenetic analysis has demonstrated that *Canaa Virus* is closely related to the *Xincheng Mosquito Virus*, being their genomic structure the same, presenting the regions VP1, VP2, VP3, G e L, which have similar sizes. This work emphasised the utility of a metagenomic sequencing approach to the discovery of a new virus, besides contributing, in the future, to the deposition of a cured sequence in Genbank.

Financial Support: Fundação Instituto para o desenvolvimento da amazônia- FIDESA, Capes-CNPQ, Instituto Evandro Chagas, Universidade do Estado do Pará.

Keywords: Amazon, metagenomic, vírus



NOVEL VIRUSES IN MOSQUITOES FROM BRAZILIAN PANTANAL

Laura Marina Siqueira Maia ¹, Fernando Lucas de Melo ², Andressa Zelenski de Lara Pinto ¹, Michellen Santos de Carvalho ¹, Douglas Oliveira Morais ¹, Bergmann Morais Ribeiro ², Renata Dezengrini Shessarenko ¹

¹ UFMT - Graduate Program in Health Sciences. Virology Laboratory. Federal University of Mato Grosso. (2367, Avenue Fernando Correa da Costa, Cuiabá, MT. 78060900, Brazil.), ² UnB - Department of Cellular Biology, Institute of Biological Sciences, University of Brasilia. (No number, Avenue L3 norte, Brasília, Federal District. 70910900, Brazil.)

Abstract

Viruses are ubiquitous and diverse microorganisms. Emerging viruses arise as a result of novel interactions between populations of hosts and pathogens, and can threaten the health and wellbeing of the entire spectrum of biodiversity. Here we herein report the presence of different viruses in salivary glands of 1,657 mosquitoes, major infectious disease vectors worldwide, classified over 28 culicinae species captured in the Brazilian Pantanal wetland. After identification in a dormant state, mosquitoes were allocated in 29 pools, which were subjected to RNA extraction, to randomic RT-PCR and to IlluminaHiSeq 2500 sequencing platform. In total, 12 viruses were found after sequence analysis, eight putative novel viruses with relatively low similarity with the pre-existing species of viruses according to classification criteria within their respective families, named with traditional terms from Mato Grosso: Pirizal iflavivirus, Furrundu phlebovirus and Pixé phlebovirus, Guampa vesiculovirus, Chacororé flavivirus, Rasqueado orbivirus, Uru chuvirus and Bororo circovirus. Viruses were additionally confirmed with iflavivirus-like, rhabdovirus-like and circovirus-like and Phlebovirus-like RT-PCR protocols and through viral isolation in C6/36 cells. We also found the already described Lobeira dielmorhabdovirus, Sabethes flavivirus, Araticum partitivirus and Murici totivirus. Phylogeny revealed these viruses clustered with insect-specific viruses within their respective genus/family members and, surprisingly, ssRNA- viruses, which are evolutionarily more recent than dsRNA and ssRNA+, were more frequently detected in this study. Therefore, these findings underscore the vast diversity of culicinae and novel viruses yet to be explored in Pantanal, the largest wetland in the planet.

Financial support: Capes, CNPq rede pró centro-oeste

Keywords: arboviruses, brazillian pantanal, culicinae mosquitoes, insect-specific viruses, novel viroses



CHARACTERIZATION OF SPODOPTERA ERIDANIA NUCLEPOLYHEDROVIRUS ISOLATE VPN165 AND THE EVOLUTION OF A BACTERIAL CHONDROITIN LYASE HOMOLOG ACQUIRED BY BACULOVIRUSES

Daniela Teixeira Rodrigues ¹, Daniel Sosa-Gómez ², Bergmann Morais Ribeiro ³, Daniel Mendes Pereira Ardisson de Araújo ¹

¹ UFSM - Universidade Federal de Santa Maria (Santa Maria, RS, 97105-900, Brasil), ² Embrapa Soja - Empresa Brasileira de Pesquisa Agropecuária (Londrina, PR, 86001-970, Brasil), ³ UnB - Universidade de Brasília (Brasília, DF, 70910-900, Brasil)

Abstract

The Southern armyworm *Spodoptera eridania* (Cramer, 1782) (Lepidoptera: Noctuidae) is native to the American tropics and considered a pest with intensive polyphagous habit. In Brazil, *S. eridania* went from a secondary plague status to an expanding pest of great importance in crops of soybean, cotton, fruits, and weeds. In this work, we characterized a second baculovirus isolated from *S. eridania* at structural, biological, and molecular level. The virus was found in extracts of caterpillars died with symptoms of baculovirus infection and called *Spodoptera eridania* nucleopolyhedrovirus isolate VPN165 (SperNPV-VPN165). Analysis by Scanning electron microscopy and transmission electron microscopy showed that SperNPV-VPN165 OBs are polyhedral with a mean diameter of $2.7 \mu\text{m} \pm 0.4$ and contained several virions with multiple rod-shaped nucleocapsids per envelope. Bioassays confirmed that the virus was lethal to the caterpillars of *S. eridania* at third instar with an LC₅₀ of 1.04×10^2 OB/ml. SperNPV-VPN165 is member of a new tentative species inside *Alphabaculovirus*, with a genome of 137.373 bp in size with a G+C content of 42.8%. We annotated 152 ORFs with 16 ORFs unique in baculoviruses. The genome has no typical homologous region. SperNPV-VPN165 isolate is closely related to the *Spodoptera*-infecting viruses, which include SeMNPV, SINPV-II, and SpeNPV-251 viruses. Surprisingly, SperNPV-VPN165 has only one copy of *odv-e66*, a bacterial acquired *chondroitin lyase* gene, whereas sister viruses present two copies. Therefore, we took advantage of this feature, and analyzed the evolution of *odv-e66* in *Baculoviridae*. We found 13 deletions, 16 acquisitions, and 1 duplication of *odv-e66* among baculoviruses. The analyses suggest that the SperNPV-VPN165 genome seems to have independently lost this gene. The study of baculovirus allows a better understanding of the virus evolution, providing important information for the development and improvement of tools for biological control.

Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES.

Keywords: Baculovirus genome, Alphabaculovirus, *Spodoptera eridania* nucleopolyhedrovirus, chondroitin lyase, *odv-e66*



A CPD-PHOTOLYASE-CONTAINING ALPHABACULOVIRUS INFECTIOUS TO THE PLUSIINAEAN SOYBEAN LOOPER RACHIPLUSIA NU PRODUCES TETRAHEDRAL OCCLUSION BODIES AND CLARIFIES THE EVOLUTION OF DNA REPAIR GENES IN BACULOVIRUS

Luana Beló Trentin ¹, Ethiane Rozo dos Santos ¹, Admilton Gonçalves de Oliveira Junior ³, Daniel Ricardo Sosa-Gomez ³, Bergmann Morais Ribeiro ⁵, Daniel Mendes Pereira Ardisson de Araújo ¹
¹ UFSM - Universidade Federal de Santa Maria (Santa Maria, RS, 97105-900, Brasil), ² UFSM - Universidade Federal de Santa Maria (Santa Maria, RS, 97105-900, Brasil), ³ Embrapa - Empresa Brasileira de Pesquisa Agropecuária (Londrina, PR, 86057-970, Brasil), ⁴ Embrapa - Empresa Brasileira de Pesquisa Agropecuária (Londrina, PR, 86057-970, Brasil), ⁵ UnB - Universidade de Brasília (Brasília, DF, 70910-900, Brazil), ⁶ UFSM - Universidade Federal de Santa Maria (Santa Maria, RS, 97105-900, Brasil)

Abstract

We described a novel alphabaculovirus isolated from the polyphagous insect pest *Rachiplusia nu*. After analysis by scanning electron microscopy and transmission electron microscopy, we found a peculiar feature, never described in baculovirus before: the virus presented pyramidal-shaped occlusion bodies (OBs) with singly-embed nucleocapsids. The usual OB morphology of alphabaculovirus is polyhedral. The major protein responsible for OB formation is polyhedrin and RanuNPV polyhedrin presents one punctual mutation (A197N) in comparison to other closely related polyhedral OB-forming viruses, *i.e.* ChchNPV and ChinNPV. The tetrahedral OBs allowed for a dose mortality response of 6.9×10^3 OBs/ml to third-instar larvae of *R. nu*. Moreover, it is not clear the molecular basis of OB shape in baculoviruses. As remark for this viruses, we also found in its genome several auxiliary genes with homologs in other baculoviruses, such as a *CPD-photolyase* (*phr*), responsible for removing UV-caused pyrimidine dimers in the virus genome. The lack of *phr* by other plusiinaean-infecting baculovirus genomes is rather intriguing. Since the acquisition happened by HGT from lepidopteran to baculovirus and that solely some species retained the gene, this fact prompted us to investigate what could happen with the expression of a plusiinaean host *phr* in a context of infection by a plusiinaean-isolated baculovirus that lacks *phr*. Therefore, we used as infection model the AcMNPV infecting *T. ni* and checked the expression level of the host *phr* along the infection course in midgut cells. The majority of host transcripts is downregulated during baculovirus infection since early stages (6 h p.i.). Surprisingly, we found no difference in host *phr* transcription during baculovirus infection when compared to the mock-infection control. Indeed, the host *phr* transcription in the midgut is not changed by the virus infection and seems to be advantageous maintaining the transcripts level throughout the infection. Importantly, baculovirus infection triggers a positive phototactic response in caterpillars to induce 'tree-top' disease, a host behavior manipulation that enhances virus transmission and survival. Therefore, the UV exposure intensification might be counteracted by the virus to avoid DNA damage by pyrimidine dimer formation.

Financial Support: CAPES.

Keywords: Tetrahedral occlusion bodies, Alphabaculovirus, *Rachiplusia nu* nucleopolyhedrovirus, Photolyase, Plusiinae



THE VIROME OF WILD ACCESSIONS OF CAPSICUM SPP.: LOW DIVERSITY OF VIRUS SPECIES MAY SUGGEST NEW SOURCES OF RESISTANCE TO PLANT VIRUSES.

Jefferson Bertin Vélez-Olmedo ^{1,2}, Fernando Lucas Melo ², Liliana Corozo Quiñonez ^{1,4}, Renato de Oliveira Resende ³

¹ UTM - Universidad Técnica de Manabí (Av, Ché Guevara, Portoviejo, 130105 Ecuador.), ² UnB - Universidade de Brasília (Departamento de Fitopatologia), ³ UnB - Universidade de Brasília (Departamento de Biologia Celular), ⁴ UNAL - Universidad Nacional de Colombia (Sede-Palmira)

Abstract

The Andean region in South America is the primary center of origin and diversity of the *Capsicum* genus. To characterize the viral diversity in wild accessions of *Capsicum* spp., samples of *C. baccatum*, *C. chinense* and *C. frutescens* from several localities were cultivated under high inoculum pressure. Frozen leaves samples were used for total RNA extraction using the commercial kit RNeasy Plant Mini kit. The samples were pooled together according to the *Capsicum* species, and the libraries were prepared using TruSeq Library Prep Kit and sequenced by Illumina HiSeq 2500 platform at Macrogen Inc, South Korea. The resulting reads: 24.527.146 (MS3=*C. frutescens*), 31.495.892 (MS4=*C. chinense*) and 29.188.784 (MS5=*C. baccatum*) were quality trimmed, and *de novo* assembled using CLC Genomics Workbench version 6.3. All contigs were compared to a viral protein RefSeq database using Blastx implemented in Geneious R11. All sequences with hits matching the viral database were then subjected to a Blastx search against the nr database. To confirm the assembly results and further extend incomplete genomes, trimmed reads were mapped back to the viral contigs and reassembled, until genome completion or no further extension. The final sequences of the virus genomes were obtained from the majority consensus of the mapping assembly and annotated using Geneious R11. In MS3 pool, only one contig related to probably new *Solendovirus* was found with 170.651 reads mapped. In MS4 pool, only one contig related to Pepper mild mottle virus-PMMoV was found with 25.838.128 reads mapped. In MS5 pool, only one contig related to PMMoV was found with 19.393 reads mapped. Overall, we sequenced 36 accessions, and only three viruses were found. Even *Capsicum frutescens* endornavirus 1-CFEV 1, previously reported from wild accessions from South America was not found. Indeed, disease and infection risk increases with the level of human management and appears to be associated with low species diversity, low genetic diversity, and high host plant density. However, the reduced number of viruses found infecting the wild accessions may suggest they are resistant to common viruses described in *C. annum* from Ecuador, since all accession, including *C. annum*, were cultivated in the same area. Therefore, an initial virome characterization may assist the identification of accessions without viral infection and/or with a lower virus accumulation, helping in the search for sources of resistance to plant viruses.

Keywords: CAPSICUM, NGS, RESISTANCE, WILD SPECIES



HIGH-RESOLUTION METATRANSCRIPTOMIC REVEALS SEVERAL NEW VIRUSES IN *CAPSICUM ANNUUM* SAMPLES COLLECTED IN ECUADOR

Jefferson Bertin Vélez-Olmedo ^{1,2}, Fernando Lucas Melo ², Sergio Miguel Vélez-Zambrano ^{3,2}, Renato de Oliveira Resende ⁴

¹ UTM - Universidad Técnica de Manabí (Departamento de Ciencias Agronómicas, Av. Ché Guevara, Portoviejo, 130105 Ecuador.), ² UnB - Universidade de Brasília (Departamento de Fitopatologia, Brasília DF, Brasil), ³ ESPAM - Escuela Superior Politécnica Agropecuaria de Manabí Manuel Félix López (Carrera de Ingeniería Agrícola, Campus Politécnico El Limón, Km 2.7 Vía Calceta-El Limón.), ⁴ UnB - Universidade de Brasília (Departamento de Biología Celular, Brasília, DF, Brasil)

Abstract

The Andes is considered the primary center and diversity of *Capsicum* spp., therefore, it is likely that this region harbors great viral diversity. Aiming to reveal the viral diversity in *C. annuum* from Ecuador, several samples with typical viral symptoms were collected in Guayas, Manabí and Santa Elena provinces and stored at -80 °C. Frozen leaves samples were used for total RNA extraction using the commercial kit RNeasy Plant Mini kit. The samples were pooled according to the sampling location, and the libraries were prepared using TruSeq Library Prep Kit and sequenced by Illumina HiSeq 2500 platform. The raw reads (37.696.482=GP1, 29.129.762=MC1, 31.686.936=MC7 and 27.772.706=SE6) were quality trimmed, and de novo assembled using CLC Genomics Workbench version 6.3. The resulting contigs were compared to a viral protein RefSeq database using Blastx implemented in Geneious R11. All sequences with hits matching the viral database were then subjected to a Blastx search against the nt database. To confirm the assembly results and further extend incomplete genomes, trimmed reads were mapped back to the viral contigs and reassembled, until genome completion or no further extension. The final sequences of the virus genomes were obtained from the majority consensus of the mapping assembly and annotated using Geneious R11. Blastx comparisons revealed great plant viruses diversity. In SE6 pool, we were able to identify Bell pepper endornavirus-BPEV, Cucumber mosaic virus-CMV, Melon yellow spot virus-MYSV, Papaya ring spot virus- PRSV, Pepper mild mottle virus-PMMoV, Peru tomato mosaic virus-PTV, Tomato yellow vein streak-ToYVSV, two contigs were to news *Enamovirus* and *Solendovirus*. In MC7 pool, we found BPEV, *Capsicum frutescens* endornavirus 1-CFEV, Maize yellow dwarf virus-MYDV, PMMoV, and PTV. In MS1 pool, we found contigs related to BPEV, Pepper cryptic virus 1-PCV1, Pepper cryptic virus 2-PCV2, PMMoV and one contig related to a new *Solendovirus*. In GP1 pool, contigs related to BPEV, CMV, PMMoV, PTV and five contigs were related to probably novel viruses belonging to *Enamoravirus*, *Luteovirus*, *Potexvirus*, *Potyvirus*, and *Umbravirus*. Sequencing only 29 plants we identified 11 known viruses and six previously unknown viral species in *C. annuum*. Overall, our results suggest that many plant viruses remain to be discovered, and that sampling at the primary center of genetic diversity may increase the discovery rate of novel viruses of the cultivated plants.

Keywords: DISCOVERY, DIVERSITY, NGS, PEPPERS



CONSTRUCTION OF INFECTIOUS CLONE OF CUCURBIT APHID-BORNE YELLOWS VIRUS BRAZILIAN MELON ISOLATE

Thiago Marques Costa ¹, Ayoub Maachi ², Lizandra Costa Pereira Brandt ¹, Tatsuya Nagata ¹
¹ UnB - Universidade de Brasília (UnB - Brasília, DF, 70910-900), ² INRA - Institut National de la Recherche Agronomique INRA - Centre Régional de Kénitra (Km 9, route de Sidi Yahia El Gharb - BP. 257, Kénitra 14000 - Morocco)

Abstract

The Northeast region is the largest melon producer in Brazil, contributing about 90% of the national production. Brazil occupies the 11th place in the world ranking of melon production, being this fruit one of the most exported in recent years in the country. The main disease of melon crop in Brazil is caused by virus called “Amarelão do meloeiro”. This disease is associated with the carlavirus (*Betaflexiviridae*) *Melon yellow-associated virus* (MYaV) and the polerovirus (*Luteoviridae*) *Cucurbit aphid-borne yellows virus* (CABYV). CABYV was detected by the Next Generation Sequencing in Brazil in 2018. CABYV genomic RNA is about 5.7kb and encapsidated in icosahedral particle. The symptoms caused by solely CABYV in melon are not well-elucidated due to the mixed infection with MYaV. Therefore, the objective of this work was to construct an infectious CABYV clone. Total RNA was extracted from M3 isolate of CABYV using silica-based nucleic acid extraction protocol. The complete CABYV genome was amplified in two fragments by RT-PCR. For this, cDNA was synthesized with random or specific reverse primer using SuperScript IV reverse transcriptase. The 5' and 3' region fragments were amplified and, then, cloned in pCR4 Topo cloning kit using *Escherichia coli* (DH10B). The two fragments were reamplified and, then, joined by Gibson Assembly using pJL89 as background vector. The construct was cloned into *Agrobacterium tumefaciens* (GV3101). The sequence of clones obtained from *E. coli* and *A. tumefaciens* was confirmed by Sanger sequencing. To evaluate the infectious clone, CABYV clones were agroinoculated in cucumber (redneck and Japanese), melon and *Nicotiana benthamiana* plants and the infection was evaluated by RT-PCR 10 days post-agroinoculation (dpa). Strong chlorosis symptoms were observed in all *N. benthamiana* leaves. In cucumber and melon plants, symptoms of internodal chlorosis were observed 10 dpa. CABYV was detected by RT-PCR with specific primers in all plants evaluated in the experiment. In conclusion, the complete CABYV M3 isolate genome clone obtained from the Gibson Assembly methodology is infectious.

Financial Support: CNPq, UnB.

Keywords: CABYV, Infectious clone, Polerovirus



TWO NEW VIRUSES NATURALLY FOUND CO-INFECTING LEGUMINOUS FORAGE PLANTS IN BRAZIL, BELONG TO A NEW PUTATIVE GENUS OF THE POTYVIRIDAE FAMILY TRANSMITTED BY WHITEFLY

Jamile Mendes De Souza ¹, Karina Nascimento da Silva ¹, Anelise Franco Orílio ¹, Fernando Lucas Mello ¹, Tatsuya Nagata ¹, Celso Dornelas Fernandes ², José Raul Valério ², Fabrícia Torres ², Renato Oliveira Resende ¹

¹ UnB - University of Brasilia (Department of Cell Biology), ² CNPq - Embrapa beef Cattle (Campo Grande-MS)

Abstract

In Brazil, forage crops represent large areas of tropical pasture for cattle feeding. These pastures can be natural or planted and are composed of grasses and forage legumes. The use of forage legumes of the genus *Stylosanthes* has increased in Brazil due to its nutritional value and great potential of nitrogen fixation. Recently, virus-like mosaic symptoms were observed in *Stylosanthes guianensis* cv. Mineirão in the experimental fields of the Embrapa Gado de Corte. These samples were collected and submitted to high performance sequencing (HTS) in the Plataforma Illumina HiSeq 2000. The sequences obtained were assembled using the CLC Genomics Workbench 7.0 program and after that, were analyzed in the BLASTX program against the database (GenBank). The results obtained in these symptomatic samples revealed the presence of two new viruses belonging to the *Potyviridae* family and have been tentatively named as *Stylosanthes Mosaic-associated virus 1* (StyMaV-1) and *Stylosanthes Mosaic-associated Virus 2* (StyMaV-2). Simultaneously, the contigs were assembled and then RT-PCR detection tools were developed with specific primers from the consensus. The fragments were amplified, sequenced by the Sanger method and comparative phylogenetic analyzes of nucleotides and amino acids of the viral proteins based on the polyprotein were performed. These analyzes demonstrated that these viruses are new genus within the family and have been tentatively named *Stylomovirus*. Based on the taxonomic criterion of demarcation of species of the *Potyviridae* family, which is related with the sequences identity of the nucleotide and amino acid of the protein coat (CP), the StyMaV-1 and StyMaV-2 viruses have 46% and 44% of the nucleotide sequence identity (respectively) with *Blackberry Virus Y* (*Brambyvirus*). In the biological assay these viruses were transmitted mechanically to *N. benthamiana* and different varieties of *Glycine max*. A whitefly transmission assay was performed and the results revealed transmission of the "Stylomovirus" by this vector.

The present work aims to study the virus/host/vector interaction to understand the existing epidemiological relationship and propose effective control measures.

Financial Support: CAPES; CNPq; UnB.

Keywords: Beef Cattle, forage, legume, HTS, *Stylosanthes*



PEPPER MILD MOTTLE VIRUS (PMMOV) DETECTED IN IMPORTED QUARANTINE CHILI PEPPER GERMPLASM

Tallyrand Moreira Jorcelino ^{1,3}, Giovana Curcio Guimarães ^{1,4}, Thaina Berbert Gelelete ^{1,4}, Priscila Alves Noronha ³, Norton Polo Benito ¹, Barbara Eckstein ¹, Alice Kazuko Inoue Nagata ², Marcio Martinello Sanches ¹, Marília Santos Silva ¹

¹ Cenargen - Embrapa Recursos Genéticos e Biotecnologia (Brasília - DF, Brasil), ² CNPH - Embrapa Hortaliças (Gama - DF, Brasil), ³ UnB - Universidade de Brasília (Brasília - DF, Brasil), ⁴ UniCEUB - Centro Universitário de Brasília (Brasília - DF, Brasil)

Abstract

Recently (2019) the presence of *Pepper mild mottle virus* (PMMoV) was detected in imported chili pepper (*Capsicum* sp.) seeds through visual analysis of symptoms in chili pepper plants (germinated seeds), mechanical inoculation of indicator plants with symptomatic leaf extract of chili pepper and Enzyme-Linked Immunosorbent Assay (ELISA). A symptomatic chili pepper plant was identified, whose extract mechanically inoculated in indicator plants generated typical symptoms of virus in sweet pepper (*Capsicum annuum*). ELISA confirmed the presence of PMMoV as well as absence of *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Tomato bushy stunt virus* (TBSV) and *Potyvirus*. Polymerase Chain Reaction (PCR) molecular tests confirmed absence of *Potyvirus*, *Tobamovirus* and *phytoplasma*. As PMMoV is a non-quarantine pest and is already present in Brazil, the corresponding chili pepper seed samples were released for importation, with the information of PMMoV presence. Consultation of scientific publications and discussion with virologist colleagues corroborated decision making. Therefore, the detection of PMMoV in this quarantine importation process of chili pepper seeds exemplifies the relevance of the plant quarantine service of import / export of materials for research. In addition, this service is effective preventive control of plant viruses and contributes to impacting scientific research on plant genetic improvement and phytosanity as well as for agriculture and national food security within Brazil. This work is aligned with the Brazilian policy, which promote prevention and surveillance of quarantine absent pests, according to the “Portaria nº 131, 27/jun/2019 - Programa Nacional de Prevenção e Vigilância de Pragas Quarentenárias Ausentes (PNPV-PQA)”.

Financial support: Empresa Brasileira de Pesquisa Agropecuária - Embrapa

Keywords: Prevention, Quarantine, Serological-molecular diagnosis, Surveillance, Vegetables



P0 AND P4 FROM CLRDV SHOW SYNERGIST EFFECT WITH VIRUS FROM DISTINCT FAMILIES

Dania Pereira Lobaina¹, Andreia Santino¹, Alex Moura¹, Rhuana Oliveira Santos¹, José Leonardo Santos Jimenez, Maite Vaslin De Freitas Silva¹

¹ UFRJ - Universidade Federal do Rio de Janeiro (Ilha do Fundão, Rio de Janeiro, RJ, Brasil)

Abstract

Polerovirus are ssRNA+ virus largely distributed around the world imposing severe lost in agriculture. Virus of this genus impact potato, pepper and other solanaceas plants as well beet and even cotton crops. In South America, especially in Argentine and Brazil, cotton diseases associate to CLRDV are largely crops. The suppressor of gene silencing protein of CLRDV, the P0 protein, was already reported as a possible effector of disease and is associated to virulence. It was also already shown for PLRV and CABYV that the movement protein P4 may induce local silencing suppression (Fusaro et al., 2017). In order to understand the paper of CLRDV P0 and P4 during virus infection, infectious clones of PVX and TRV viruses presenting a GFP insertion were agroinfiltrated in *Nicotiana benthamiana* plants in the presence of CLRDV P0 and/or P4. We observed the induction of necrotic areas in the agroinfiltrated leaves when P0 was co-infiltrated with TRV. P0 also improved the systemic spread of TRV. CLRDV P4 however didn't affect TRV systemic movement but induced a faster local cell-cell movement. Necrosis wasn't induced by CLRDV P4. When TRV was co-infiltrated with both P0 and P4, a stronger local and systemic spread of the virus was observed, showing synergistic effects. Looking for PVX, we could observe the induction of strong necrosis in the infiltrated area and in systemic leaves in all proteins combinations. The induction of necrosis for PVX was associated with the presence of the SSP P19 from TSBG in all infections. However, it could be observe that the virus was able to leave necrotic tissues and spread to healthy parts of the leaves. The presence of P4 increased the number PVX spots both locally and systemically. P0 however seems to reduce PVX systemic spread. We can conclude that P0 and P4 could act in a synergistic way with virus from these two other families, probably helping them to evade the anti-viral silencing defense.

Keywords: P0, P4, CLRDV, synergy



VETERINARY VIROLOGY





MULTIPLICATION OF BOVINE HERPESVIRUS TYPE 1 (BOHV-1) AND 5 (BOHV-5) IN DIFFERENT CELL LINES

Jaqueline Peruzzo ¹, Anne Caroline Ramos dos Santos ¹, Fernando Finoketti ¹, André Ferreira Hennigen ¹, Ricardo Rohweder ¹, Paulo Michel Roehe ¹

¹ UFRGS - Universidade Federal do Rio Grande do Sul (Av. Sarmento Leite 500, Farroupilha - Porto Alegre - RS)

Abstract

Bovine herpesviruses types 1 (BoHV-1) and 5 (BoHV-5) share genomic homology equal to 85% or higher, depending on the viral strain. In the present study, a comparison was performed between the permissivity of two cell lines, "Cell Resistant to Infection with BVDV" (CRIB) and Baby Hamster Kidney (BHK-21), to six BoHV strains of different subtypes: BoHV-1.1 (EVI-123), BoHV-1.1 (SV63/06), BoHV-1.1 (56/06), BoHV-1 (SV1613), BoHV-1.2b (PG1779) e BoHV-5 (N569). All viruses were multiplied on both cell lines and titrated in 96-well microplates. Infectious titers were calculated and expressed in 50% cell culture infectious doses per 50 μ L (CCID₅₀). In BHK-21, the BoHV-1 strains achieved the following titers: $10^{4,25}$ (EVI-123/BoHV-1.1), 10^3 (SV63/06/BoHV-1.1), 10^2 (56/06/BoHV-1.1), 10^1 (SV1613/BoHV-1) and 10^2 (PG1779/BoHV-1.2 b), while BoHV-5 strain achieved an infectious titer of 10^7 (N569/BoHV5). With CRIB the titers obtained after viruses' multiplication were: $10^{5,75}$ (EVI- 123/BoHV-1.1), $10^{7,25}$ (SV63/06/BoHV-1.1), $10^{7,5}$ (56/06/BoHV-1.1), $10^{7,5}$ (SV1613/BoHV-1), $10^{7,5}$ (PG1779/BoHV-1.2 b) and $10^{5,5}$ (N569/BoHV5). The results of this study show that BoHV-1 strains significantly produced higher titers ($\geq 1 \log_{10}$) in CRIB, while BoHV-5 strains produced higher titers in BHK-21 ($\geq 1 \log_{10}$). We conclude that both cells are permissive to viral replication when in contact with these herpesviruses. There is an apparent relation between virus type and cell line that seems to affect the productive performance of the infections. Subsequent studies will be executed to uncover the reasons for these *in vitro* behavior differences. Financial support: CAPES, CNPq, FINEP.

Keywords: Herpesvirus, Titration, Cell Culture



CHARACTERIZATION OF THE VIROME IN NASAL CAVITY OF NURSERY PIGLETS

Anne Caroline Ramos dos Santos ¹, Márcia Regina Loiko ³, Deisi Maria Pereira ¹, Ana Paula Mutterle Varela ¹, Raíssa Nunes dos Santos ¹, André Ferreira Hennigen ¹, Fabiana Quos Mayer ², Paulo Michel Roehle ¹

¹ UFRGS - Universidade Federal do Rio Grande do Sul (Rua Sarmento Leite, 500 - 90050-170 Bairro Farroupilha Porto Alegre/RS Brasil), ² IPVDF - Instituto de Pesquisas Veterinárias Desidério Finamor (Estrada Do Conde, 6000 - Sans Souci, Eldorado do Sul - RS, 92990-000), ³ Feevale - Universidade Feevale (Av. Edgar Hoffmeister, 500, Zona Industrial Norte, Campo Bom, RS - CEP 93700-000)

Abstract

The microbiota of piglets is of particular interest, as it may affect the susceptibility to infections and interfere with growth performance. To date, the characterization of the respiratory virome of piglets in the initial periods of life has been scarcely investigated. In this study, the nasal virome of 4-5 weeks old nursery pigs with or without signs of respiratory disease was examined by high throughput sequencing (HTS). Nasal swabs were collected from 60 piglets (30 healthy and 30 diseased). The samples were pooled and submitted to viral DNA and RNA extractions and sequenced in the MiSeq (Illumina) platform. The reads obtained were filtered with Trimmomatic 0.39, reassembled with Spades meta 3.10.1 and analyzed with Geneious software. The total number of reads sequenced pool varied between 84.580 to 183.976. These were compared to the database of viral sequences at the protein level using K-mer to lowest common ancestor mapping. On average, 11,77% of the reads showed similarity to viral sequences deposited at GenBank database. Among the viral contigs, 64,4% presented homology with sequences of eukaryotic virus genomes and 35,6% with prokaryotic viral genomes. Higher viral diversity was detected in diseased piglets. Most of the genomes in the diseased group displayed similarity with members of the families *Astroviridae*, *Caliciviridae*, *Circoviridae*, *Coronaviridae*, *Herpesviridae*, *Parvoviridae*, *Picobirnaviridae*, and *Reoviridae*. The most abundantly identified viral genomes in diseased group were porcine cytomegalovirus (PCMV, 63.6%), porcine astrovirus (PAsV, 9.6%) and porcine sapovirus (PSaV, 7.2%). Whereas in healthy group, most genomes identified were members of families *Circoviridae*, *Herpesviridae*, *Parvoviridae*, and *Picobirnaviridae*. The most abundant viral genomes in healthy group were PCMV (95.6%) and circular single-strand DNA virus (ssDNA, 3.2%). The results showed higher viral variability in respiratory disease-affected piglets, where PCMV was the predominant viral genome in both groups.

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Keywords: metagenomic analyses, piglet, sequencing, vírus



CORONAVIRUS DETECTED IN BATS FROM PARK OF INSTITUTO BUTANTAN, SÃO PAULO, BRAZIL.

Amanda De Oliveira Viana ¹, Carla Menequim Barbosa ¹, Erick Gustavo Dorlass ¹, Elizabete Captivo Lourenço ³, Erika Hingst-Zaher ², Edison Luiz Durigon ¹

¹ USP - Universidade de São Paulo (Av. Prof. Lineu Prestes, 1374 - Butanta, São Paulo - SP, 05508-900.), ² IBu - Instituto Butantan (Av. Vital Brasil, nº 1500. Butantã, São Paulo.), ³ UERJ - Universidade do Estado do Rio de Janeiro (Rua São Francisco Xavier, 524, Maracanã, Rio de Janeiro .)

Abstract

Bats are very important to one health, having a particular interest as a host for some viruses. Usually the RNA viruses are more associated with these mammals. Among the RNA viruses found in bats the Coronavirus, cause of Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). Coronavirus are divided in four genus: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus*. *Alpha* and *Betacoronavirus* emerged in bats. We analyzed the presence of Coronavirus in a population of bats in Park of Instituto Butantan, in urban area of São Paulo City. Oral and rectal swab samples were collected from 47 bats, captured between 2017 and 2018, with mist nets in the Butantan Park, in São Paulo city, Brazil. Swabs were placed in cryotubes containing 500µL of VTM and kept in ultra-freezer (-80°C). DNA extraction was *automated* performed with the “MagMAX™ Express” (Applied Biosystems®) using the “MagMAX™-96 Total Nucleic Acid Isolation Kit” according to the *manufacturer’s instructions*. For an enhanced sensibility, detection was made using a protocol to avian Coronavirus developed by Poon et al. (2009) and vaccine was used as positive control. Sequencing was performed with Sanger’s technique using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and ABI PRISM 3130XL DNA Sequencer (Applied Biosystems). An oral and rectal swab from six individuals of bats showed to be positive for *Alphacoronavirus*. One male and one female of *Artibeus fimbriatus* and a male of *Glossophaga soricina* were detected with an *Alphacoronavirus* not yet identified and found in Costa Rica. Only this species of bats, among seven species of bats was found with this virus. The virus found this work was found to others researches in Costa Rica and São Paulo. Considering the importance of bats, Coronavirus and the location of this study, is very important to screen this population of bats.

Keywords: Coronavirus, Alphaviridae, Bats, São Paulo



DETECTION OF TESCHOVIRUS A IN DIFFERENT BREEDING STAGES OF A SWINE HERD

Meriane Demoliner ^{1,2}, Ana Karolina Antunes ^{1,2}, Paula Rodrigues de Almeida ^{1,2}, Fernando Rosado Spilki ^{1,2}

¹ Feevale - Câmpus II - Universidade Feevale (ERS 239, 2755 - Vila Nova, Novo Hamburgo - RS, 93525-075), ² Feevale Techpark - Universidade Feevale (Av. Edgar Hoffmeister, 600 - Zona Industrial Norte, Campo Bom - RS, 93700-000)

Abstract

Teschovirus A (TVA) is a non-enveloped RNA virus in the genus *Teschovirus*, family *Picornaviridae*. Some species of TVA frequently circulate in swine populations without causing clinical signs. However, the clinical manifestations may include polioencephalomyelitis, mainly by TVA-1 that is responsible for causing severe encephalomyelitis outbreaks. *Senecavirus A* (SVA) belongs to the same family but in the *Senecavirus* genus. It is an emerging virus, responsible to cause idiopathic vesicular disease in swine. In the beginning of 2019, a swine herd from Santa Catarina, Brazil started to present ulcerative lesions on limbs and nostrils, affecting animals from nursery over 130 days until the termination stage. In this case, the foot-and-mouth disease possibility had been discarded by the responsible veterinarian. The aim of this research was to investigate the circulation of TVA and SVA in this herd. To this, swine were necropsied and samples of skin lesion, liver, lung, kidney, lymph nodes, tonsil, paw, bladder, central nervous system (CNS), serum, cerebrospinal fluid, urine and, feces were collected. Additionally, skin swab, serum saliva and feces were collected from several breeding stages of the herd. Lastly, ration samples from the affected farms were collected too. The samples were previously macerated and added in 1mL Eagle's Minimum Essential Medium, after 1h incubation at 4°C, the viral RNA extraction was performed and immediately the cDNA was synthesized. To molecular detection, a PCR was performed for SVA genome and, a nested PCR was performed for TVA genome. Both molecular techniques used primers that targeting the 5'-UTR region. Of a total of 94 samples, 31 were positive to TVA. Among the positive samples, 14 were from faecal sample, 14 from skin swab, 3 from saliva, and ration samples were all negative. High prevalence of TVA circulation was observed in swines from nursery to termination of the herd. The cause of the lesions could not be addressed, since skin is often contaminated with feces, and in these cases the animals were excreting TVA in feces. *Picornaviridae* family members are known to cause CNS, upper respiratory, myocardial and skin infections, such as SVA in swine and enterovirus 71 and coxsackie virus A infections in humans. Further sequencing and isolation of the TVA detected here must be performed in order to understand its phylogenetic relationships and role as a contaminant or a causative agent of these lesions.

CAPES, CNPq, FEEVALE

Keywords: TVA, Picornaviridae, Swine



EVALUATION OF RABIES LYSSAVIRUS REPLICATION AND CELL GROWTH IN DIFFERENT CONCENTRATIONS OF N2A CELL LINE

Tamires Santos de Arruda ¹, William de Oliveira FAHL ¹, Iana Suly Santos Katz ¹, Graciane Maria Medeiros Caporale ¹, Francielle Cristina de Freitas Montibeller ¹, Keila Iamamoto Nogi ¹

¹ IP - Instituto Pasteur (Avenida Paulista, 393 - Cerqueira César - São Paulo/SP - CEP: 01311000)

Abstract

The laboratory diagnosis of rabies has the direct fluorescent antibody test (DFAT) as the gold standard test, but the WHO recommends performing the viral isolation test in mice or cell culture (VICC). This project aimed to evaluate the viral replication of the VICC test when initial concentrations of 5×10^5 and 2.5×10^5 cells/ml were used and to compare cell growth curves when final concentrations of 1.6×10^4 cells/mL and 1×10^4 cells/ml were used. A total of 14 samples with minimal viral titer of 10^3 TCID₅₀/ml were selected, and two positive controls. For the evaluation of viral replication, samples were inoculated into 96-well plates and observed at time intervals (24, 48, 72 and 96 hours) by adding cells at a concentration of 5×10^5 cells/ml and 2.5×10^5 cells/ml. After each interval, DFAT was performed for each plate. For the cell growth curve analysis, cells were added in quadruplicate in 96-well plates with final concentrations of 1.1×10^4 cells/ml and 1.6×10^4 cells/ml at the same time intervals (24, 48, 72 and 96 hours), and the procedure was repeated three times. The cells were suspended with culture medium and Trypan blue, and they were counted in a Neubauer chamber. Viral replication was observed in all samples from 24h until 96h, for both initial cell concentrations. However, it was possible to observe the presence of larger foci at the initial concentration of 2.5×10^5 cells/ml when compared to the concentration 5×10^5 cells/ml. In the cell growth curve evaluation, at a final concentration of 1.1×10^4 cells/ml, the following cell growth rate was observed in relation to the time intervals: 24-48h - 219%, 48-72h - 14% and 72-96h - 17%. Already at the final concentration of 1.6×10^4 cells/ml: 24-48h - 63%, 28-72h - 4%, 72-96h - 38%. Student's t-test showed a difference in cell growth at 24 and 72 (p=0.0031), but no difference was observed at 48 and 96 hours. The results suggest that when there are lower concentrations of cells, there is less nutrient consumption and longer preservation of environmental conditions, increasing the phase of cell proliferation, which may favor the formation of larger foci in viral isolation.

Financial Support: Instituto Pasteur, São Paulo, Brazil (IP10/2018)

Keywords: N2A CELL LINE, VIRAL ISOLATION, RABIES LYSSAVIRUS, DIAGNOSIS, CELL CONCENTRATION



FIRST DETECTION OF ANTI-OROPOUCHE NEUTRALIZING ANTIBODIES IN SERA SAMPLES FROM SLOTHS (*BRADYPUS VARIEGATUS*) FROM ALAGOAS STATE

Thiago Pina Goes De Araujo ¹, Danilo Machado De Melo ¹, Flávio Martins Dos Santos ¹, Thayna Maria Da Silva Torres ¹, Ana Carla Eugênio Lima ¹, Maria Luisa Beserra De Faria Ferreira Leite ¹, Ana Rachel Vasconcelos De Lima ¹, Alessandra Abel Borges ¹

¹ ICBS - UFAL - Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas (av. Lourival de melo mota s/n, cep: 57072-900, Maceió – AL, Brazil)

Abstract

Oropouche virus (OROV) belongs to the *Orthobunyavirus* genus, *Peribunyaviridae* family (order *Bunyavirales*), and is a causative agent of arboviral febrile illness in Brazil. OROV presents two transmission cycles: 1) urban cycle (*Culicoides paraensis* is the primary vector); and 2) sylvatic cycle, in which there is evidence that sloths (*Bradypus tridactylus*), nonhuman primates and wild birds play a role as vertebrate hosts. The potential of OROV to spread to other geographical areas has been recognized in recent years, increasing the possibility of the disease to emerge to non-endemic areas of Brazil. Laboratory diagnosis of Oropouche fever is achieved by viral isolation, as well as by detection of NT, HI, and IgM antibodies. Our aim was to standardize a 50% plaque reduction neutralization test (PRNT50) to use as screening test of sera samples collected from quarantined wild animals, at Instituto Brasileiro do Meio Ambiente (IBAMA) from Maceió city. For this, Vero E6 cell monolayers at 70% confluency were infected with a mix of OROV (50-100 PFU of strain BeAn19991 P/3) plus 2-fold dilutions of the heat-inactivated test sera, incubated at 37°C for 1 hour covered with carboxymethylcellulose overlay, followed by incubation at 37°C for 3 days. In this study, we screened 29 serum samples from *Bradypus variegatus* sloths by PRNT50, which 9 (34.61%) presented neutralizing antibodies with titers ranging from 1: 4 to 1: 8. The samples were further tested for viral genome detection by one-step RT-PCR, but all of them were negative. This was the first study that investigated the presence of antibodies against OROV in sloths from Alagoas. Our data evidence the epidemiological importance of the study, since it demonstrates the existence of the contact of these sentinel animals with this virus. Therefore, our finding generates a warning signal for the health surveillance organs for the possibility of epidemic outbreaks caused by OROV.

Financial support: Ministério da Saúde/CNPq/SESAU-AL/ FAPEAL

Keywords: *Bradypus variegatus*, Neutralizing Antibodies, Oropouche virus



SURVEILLANCE OF INFLUENZA A VIRUS IN DOMESTIC PIGS IN NORTHEAST BRAZIL

Adalúcia Silva ¹, Edmilson Oliveira-Filho ², Gustavo Lima ¹, Antônio Filho ³, Rafael Rosa ¹, Christian Reis ¹, Abelardo Júnior ⁴, Laura Gil ¹, Alexandre Machado ¹, Daniel Perez ⁶, Lindomar José Pena ¹
¹ IAM - Fundação Oswaldo Cruz-Fiocruz, Instituto Aggeu Magalhães (Recife), ² Institute of Virology, Berlin, Germany - Institute of Virology, Berlin, Germany (Germany), ³ UFRPE - Universidade Federal Rural de Pernambuco (Recife), ⁴ UFV - Universidade Federal de Viçosa (Viçosa), ⁵ CPqRR - Fundação Oswaldo Cruz-Fiocruz, Centro de Pesquisa René Rachou/I (Belo Horizonte), ⁶ University of Georgia - University of Georgia (USA)

Abstract

Swine production in Brazil has grown over the last 15 years due to several improvements in the production chain, such as farm technification, genetic improvement and control of the main diseases that hinder production. Influenza A virus (IAV) is a major cause of respiratory disease in pigs, but the epidemiology of swine influenza in Brazil is still poorly understood. The main objective of this work was survey IAV in domestic pigs in the state of Pernambuco, Brazil. To this, we collected nasal swabs and/or lungs samples from 500 pigs in farms and slaughterhouses in different regions of Pernambuco State between 2017 and 2018 and tested them using the OIE qRT-PCR protocol. We found 48 (9.6%) samples positive for IAV. We have also collected blood from 340 animals who and tested them by hemagglutination inhibition test (HI) using A/California/04/2009 (H1N1) and A/Pernambuco/01/2019 (H3N2) viruses. We found 86% positive for H3N2 and 71% positive for H1N1, suggesting that IAV is widely spread in Pernambuco. Thus, we have identified, for the first time, IAV in pigs in this region of the country. These studies provide valuable information for swine producers, health authorities and the scientific community regarding the epidemiology of IAV in pigs in Brazil.

Keywords: Influenza A, Swine, Epidemiology



MULTIPLEX REAL-TIME PCR VALIDATION FOR DETECTION OF PCV2A AND PCV2B

Victor Hugo da Silva¹, Camila Alves Ferreira¹, Paula Martins Uchoa de Sousa¹, Alessandra Marnie Martins Gomes de Castro¹

¹ FMU - Centro Universitário das Faculdades Metropolitanas Unidas (Rua Ministro Néelson Hungria, 541 - Vila Tramontano, São Paulo - SP, 05690-050)

Abstract

Porcine circovirus type 2 (PCV2), discovered initially in 1998, has been associated with several disease manifestations in pigs denominated PCV2 associated disease. Today it is well established that several main PCV2 genotypes circulate in pigs also known as PCV2a, PCV2b and PCV2d worldwide. Real-time or quantitative PCR (qPCR) assays have been used for specific identification of the target sequence by fluorescent probes and so improves the specificity of assays. Another feature of qPCR is the ability to perform multiplex assays by using differences of fluorescent probes in the same reactions. In this report, a multiplex real-time PCR (multiplex qPCR) was validated for simultaneous detection of PCV2a and PCV2b. Briefly, fragments containing specific primer and probe annealing sequences for PCV2a and PCV2b were synthesized, inserted into a plasmid vector and cloned into an E. coli K12 DH10B™ T1R. The plasmid DNA from the transformed bacteria was purified, the concentrations determined by spectrophotometer and sequenced to verify sequence identity. Serial dilutions were performed and used as stand curve. The reactions were performed in a final volume of 25 μ L containing 5.0 μ L DNA; 13 μ L of TaqMan™ Universal Master Mix II (Thermo Fischer Scientific, USA), 0.5 μ L of each probe at 10 μ M (PCV2a VIC- GGG GAC CAA CAA AAT CTC TAT ACC CTT T-MGBNF and PCV2b FAM- CTC AAA CCC CCG CTC TGT GCC C-QSY); 1 μ L of 10 each primer at 10 μ M (PCV2abF: 5 'GGCGGTGGACATGATGAGA 3' and PCV2abR: 5 'GCAGGGCCAGAATTCAACC 3') and sterile MilliQ water qsp. The amplifications conditions were 50 °C for 2 minutes, 95 °C for 10 minutes followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute and run on a QuantiStudio3 (Applied Biosystems, USA). The detection limit of the multiplex qPCR was 25.4 and 25.2 copies of DNA/ μ L for PCV2a and PCV2b, respectively. The standard curves of multiplex qPCR showed that all parameters analyzed were within the acceptable range, i.e. the efficiency (ϵ) from 98.9 to 101.9; correlation coefficients (R²) from 0.998 to 0.989 and slope from -3.32 to 3.24. Similar detection limit and standard curves parameters results were obtained from the singular qPCR assays with both viruses separately. The specificity of the multiplex qPCR was performed using 10 clinical samples that were previously sequenced and the assay was able to classify correctly the PCV2a and PCV2b samples.

Financial support: FAPESP 2015/07994-7 and 2017/21592-4.

Keywords: Diagnosis, Molecular biology, Swine, Virus



EVALUATION OF THE EFFECTS OF AN HVT-INFECTIOUS BURSAL DISEASE VECTOR AND AN IMMUNOCOMPLEXED VACCINE ON THE IMMUNE SYSTEM AND PRODUCTION PARAMETERS OF COMMERCIAL BROILERS

Tobias Fernandes Filho ¹, Melquiades Dvojatzi Junior ¹, Lourenço Sausen ¹, Leandro Selau ¹, Filipe Santos Fernando ¹

¹ BI - Boehringer Ingelheim (Rochaverá Corporate Towers. Av. Nações Unidas, 14.171 - Torre Marble – 18º andar - Santo Amaro, São Paulo - SP, 04794-000, Brazil)

Abstract

The infectious bursal disease (IBD) is a widespread infection of broilers caused by an avian *Birnavirus* with higher mortality, condemnations and feed conversion rates leading to production losses in poultry industry. Most of the impact is due to the viral replication in B lymphocytes progenitors in the bursa, resulting in severe immunosuppression not just in humoral but also in cellular immune response. An effective manner to prevent from IBD is through the use of IBDV vaccines at hatchery with replicating attenuated intermediate plus strains of IBDV viruses (Winterfield 2512) complexed with antibodies or with vectored HVT (Fc-126) vaccines expressing IBDV VP2 protein. Although both vaccine technologies are effective in Gumboro disease control the use of a replicating intermediate plus strain of the IBDV virus would mimic the same impact of wild IBDV on bursa infection and consequent impairment of humoral and cellular immune response, in more attenuated way but still demanding energy from the birds to deal with viral replication and its apoptosis and inflammation effects.

To understand if immunocomplexed Winterfield 2512 IBDV vaccines could lead broiler to an immunosuppression condition in the field, broilers vaccinated *in ovo* with W2512 IBDV-antibody complex or HVT-IBD vector vaccines were grown in a large Brazilian broiler company in the state of Paraná. To access the impact of both IBD vaccines in bursa damage and cellular immune response birds in the field had bursa collected to measure histological lymphoid depletion and quantification of total leukocytes, T CD8 and B lymphocytes and heterophil phagocytosis activity through flow cytometry at 21 and 28 days of age. The results brought to evidence the damage in the bursa due to a virus complexed with higher scores for lymphoid depletion and a leucopenia especially for B and T CD8 lymphocytes that increased with age. Heterophil phagocytosis was also affected within the group that have received W2512 immunocomplexed vaccine, with higher phagocytosis indexes. Nevertheless, the feed conversion rate was lower for the group that had been vaccinated with vectored HVT-IBD. In conclusion, adopting a vaccination with a live intermediate plus strain of IBDV in the hatchery for broilers causes damage to the bursa and also to both innate and adaptive immune response which result in a certain degree of immunosuppression, resulting in higher cost of production and higher feed conversion rate.

Keywords: Infectious bursal disease, Immunossuppression, Gumboro disease, Broilers, B lymphocytes



EPIDEMIOLOGIC SURVEY OF EQUINE INFECTIOUS ANEMIA VIRUS IN EQUIDAE FROM NORTHEASTERN BRAZIL

Camila de Sousa Bezerra ¹, Denise Batista Nogueira ¹, Davidianne de Andrade Morais ¹, Clebert José Alves ¹, Maria Luana Cristiny Rodrigues Silva ¹, Sérgio Santos de Azevedo ¹

¹ UFCG/CSTR - Universidade Federal de Campina Grande Centro de Saúde e Tecnologia Rural (Avenida Universitária, SN, Santa Cecília, Patos, PB CEP: 58700-970 Brazil)

Abstract

Equine infectious anemia (EIA) is caused by EIAV (equine infectious anemia virus), a member of the Retroviridae family, genus Lentivirus. This disease causes economic impact, because in Brazil positive animals must be sacrificed, as established in the PNSE of the Ministry of Agriculture, Livestock and Supply. In Brazil, the frequency of infection has already been observed in the states of Rondônia (9.6%), Acre (7.5%), Pará (17%), Minas Gerais (3.1%) and Mato Grosso do Sul (24, 8%). The objective of this work was to determine the epidemiological profile of EIAV in equidae from the states of Paraíba, Pernambuco, Rio Grande do Norte and Ceará, during the rainy (May and June) and dry (October and November) periods of 2017 and 2018. For the serological diagnosis of EIA the agar gel immunodiffusion test (IDGA) was used. During the rainy season of 2017, 22 of the 1,842 (1.2%) animals tested were positive for EIAV (12 from Ceará, eight from Paraíba and two from Rio Grande do Norte states). In the dry season, of the 1,564 animals sampled, 29 (1.9%) were positive in the serology, of which 20 belonged to Ceará, seven to Paraíba and two to Rio Grande do Norte. There was no positive result in the state of Pernambuco in the months corresponding to the study in 2017. In the analysis of data from 2018, it was observed that during the rainy season, 26 of the 1,636 horses were seroreactive (1.6%), with 19 cases resulting from Ceará, four from Paraíba and three from Pernambuco, with no positive animals occurring in the state of Rio Grande do Norte during this period. While in the dry season, 32 out of 1,526 animals were seroreactive to the EIAV, of which 26 were from Ceará, three from Paraíba, one from Rio Grande do Norte and two from Pernambuco. Among the four states represented in the present study, Ceará presented the largest number of positive animals, this being the holder of large herd of equidae, as well as the occurrence of horse events, with the largest contact of infected and susceptible animals. The increase in EIA cases in the dry period compared to the rainy season may be associated with increased vector proliferation, which usually occurs between the end of the rainy season and the beginning of the dry period. The results observed in this study demonstrate the circulation of EIAV in four states of Northeastern Brazil, suggesting a correlation between the dry period and the increased frequency of infection.

Keywords: Equine infectious anemia , Infection, Horse, Agar gel immunodiffusion test , Caatinga



BAT INFLUENZA A VIRUS H18N11 IN BRAZILIAN BATS ARTIBEUS LITURATUS

Angélica Cristine de Almeida Campos ^{1,2}, Luiz Gustavo Bentim Góes ^{1,2}, Andrés Moreira-Soto ², Cristiano Carvalho ³, Guilherme Ambar ⁴, Anna-Lena Sander ², Carlo Fischer ², Adriana Ruckert Rosa ⁵, Débora Cardoso Oliveira ⁵, Ana Paula Gerales Kataoka ⁵, Wagner André Pedro ³, Luzia Fátima Alves Martorelli ¹, Luzia Helena Queiroz ³, Ariovaldo Pereira Cruz-Neto ⁴, Edison Luiz Durigon ¹, Jan Felix Drexler ²

¹ USP - Universidade de São Paulo (Av. Prof. Lineu Prestes, 1374 - sala 225 - Depto Microbiologia - ICB II), ² CHARITÉ - Charité - Universitätsmedizin Berlin (Helmut-Ruska-Haus Charitéplatz 1 10117 Berlin, Germany), ³ UNESP - Universidade Estadual Paulista "Júlio de Mesquita Filho" - Faculdade de Medicina Veterinária - Câmpus de Araçatuba (Rua Clóvis Pestana, 793 - Dona Amélia - Araçatuba, SP/SP - CEP 16050-680), ⁴ UNESP - Universidade Estadual Paulista "Júlio de Mesquita Filho" - Instituto de Biociências - Câmpus de Rio Claro (Avenida 24 A, 1515 - Bela Vista - Rio Claro/SP - CEP 13506-900), ⁵ CCZ-São Paulo - Centro de Controle de Zoonoses, São Paulo, SP, Brazil (R. Santa Eulália, 86 - Santana, São Paulo - SP, 02031-020)

Abstract

Influenza A viruses (IAV) are major causes of human disease. Until recently, IAV were deemed to be maintained in avian reservoirs. In first studies from 2012 and 2013, the first bat IAV termed H17N10 and H18N11 were discovered in *Sturnira lilium* (little yellow-shouldered bat) and *Artibeus planirostris* (flat-faced fruit-eating bat). So far, only four individual bat specimens yielded IAV genomic sequences during the pivotal investigations. To investigate bat IAV epidemiology, we sampled 533 individual bats representing 27 species and 3 families across 28 sampling sites located in southern/south-western Brazil during 2010-2014. Intestine specimens from all bats were tested using two highly sensitive, broadly reactive nested RT-PCR assays targeting different regions of the IAV PB1 gene. In two samples of *Artibeus lituratus* bat species was found H18N11 Influenza A virus. High concentrations, tested by real-time-PCR, suggested fecal shedding. Genomic characterizations revealed conservation of viral genes across different host species, countries and sampling years, suggesting a conserved cellular receptor and wide-ranging occurrence of bat influenza A viruses.

Keywords: Influenza A virus, Bats, H18N11, Atlantic Rain Forest



NEWCASTLE DISEASE VIRUS REPLICATION IN DIFFERENT CELL SYSTEMS

Maria Angela Orsi ¹, Ana Paula de Moraes ¹, Matheus C. Martini ¹, Laís S. Rizotto ², Hikary Moriyama

¹, Ana Cristina Alves de Almeida ¹, Tânia Rosária Pereira Freitas ³, Clarice Weis Arns ¹

¹ UNICAMP - Department of Genetics, Evolution, Microbiology and Immunology, Institute of Biology, University of Campinas (Rua Monteiro Lobato, 255, Campinas, São Paulo), ² USP - Faculdade de Medicina Veterinária and Animal Science, São Paulo University (Piracicaba, São Paulo), ³ LFDA/MG, - Federal Agricultural Defense Laboratory in Minas Gerais (Pedro Leopoldo, Minas Gerais, Brazil.)

Abstract

Repeated studies have been shown that isolates of vaccine-derived Newcastle disease virus (NDV) from different species of wild birds and the number of recovered vaccine-derived virus coincident with the most widely vaccine used in the world (B1 and Lasota). Usually, the NDV has been isolated in chicken embryos (CE) as standard method before cell line adaptation. This present study reports the trials to B1 strain infection establishment in five different cell lines: Chicken embryos related (CER); Baby hamster kidney (BHK-21); Mouse macrophages (J774 A.1); *Aedes Albopictus* larvae (C6/36) and *Eidolon Helvum* kidney (EidNi/41). For each cell line flasks (25cm²) were seeded with 3X10⁴ cells/mL in culture medium for each cell line and supplemented 10% fetal bovine serum. At 85% cell confluence, were exposed 900 µL of B1 strain (10^{8.5} DIE₅₀/mL) inoculated directly on each monolayer's different cells. Four mammalian cells: CER, BHK-21, EidNi/41 and J774 were incubated by 45 min/37°C and for insect cell C6/36 the incubation was for 75 min/28°C. The cell medium was replaced and the flasks incubated at 37°C and for C3/36 at 28°C until the cytopathic effect (CPE) was observed over 70% or 5 days post infection (PI). The cells passages were frozen, thawed, centrifuged and virus supernatant aliquots keep at -80°C. It was repeated for three passages. The virus CPE has been observed at least 24 hours (h) until 120h PI. For J774 cell lines until 72 h PI, BHK-21 and CER until 96 h PI, C6/36 and EidNi/41 until 120 h PI. The comparative kinetics of the NDV proliferation among the cells assays and compare with the standard CE inoculation was performed by quantitate qPCR. Briefly, the RNA was extracted using the QIAamp viral RNA mini kit, Taq Man RNA to CT1step kit (Applied) was used to perform qRT-PCR assays. The fragments of matrix gene (121pb) were amplified and detected using the primer; F (M+4100), R (M-4220) and P (M+4169). The infection at the all cell subcultures have varied. The CT in the first cell passage ranged from 14.00 to 18.38, in the 2nd 15.80 to 25.50, in the 3rd 20.40 to 30.73. From the 2nd to the first passage, the difference ranged from -0.2 to 8.8 Ct. in the 3rd to 2nd passage 0.05-7.82 Ct. The best performance was from the CER, but until 2nd passage was CER, J774 and EidN1/41 cells lines. However, preliminary results showed susceptibility to all five cell lines evaluated against NDV.

Acknowledgment and financial support: CNPq, FAPESP, FUNCAMP AND IB-UNICAMP

Keywords: Newcastle disease virus, Replication , different cells systems



IN VITRO CHARACTERIZATION OF DIFFERENT GENETIC LINES OF RABIES VIRUS ISOLATED FROM NON- HEMATOPHAGOUS BATS

Jaíne Gonçalves Garcia ^{1,2}, Camila Mosca Barboza ^{1,2}, Marcélia Emanuele Sad Fernandes ^{1,2}, Raphaela Zamudio ², Ana Lee Francisco ^{1,2}, Adriana Candido Rodrigues ², Karin Correa Scheffer Ferreira ², Helena Beatriz de Carvalho Ruthner Batista ^{2,1}

¹ UFABC - Universidade Federal do ABC (Av. dos Estados, 5001 – Bairro Santa Terezinha, Santo André – SP), ² IP - Instituto Pasteur de São Paulo (Avenida Paulista 393 - Cerqueira César SP)

Abstract

Rabies is one of the oldest known human diseases. Caused by the rabies virus (RABV), this zoonosis has worldwide distribution and still causes the death of approximately 60,000 people per year. Despite advances in research, there are still gaps in knowledge about the maintenance of RABV in nature, especially aspects related to its biology and its mechanisms of adaptation to hosts. In recent years, reports of isolation of RABV in different species of bats have raised Public Health concern. The possibility of these animals developing synanthropic habits, considering that they are reservoirs for RABV, may cause a higher risk of human infections. This study aimed to elucidate adaptive aspects of the maintenance of RABV in different species of non-hematophagous bats. Therefore, 9 samples of RABV isolated from different species of bats were selected. These samples were subjected to genetic characterization of RABV and genetic identification of the species. After selection and characterization of the samples, they were submitted to passage in *Swiss albino* mice and later adapted to *in vitro* growth in Human Embryonic Kidney (HEK 293T) cells. Each serial passage in cells is subjected to Direct Immunofluorescence Test. The following genetic lineages of RABV were identified: genetic lineage from frugivorous bat *Artibeus lituratus* (3 samples), genetic lineages from insectivorous bats from the specie *Eptesicus furinalis* (3 samples) and from the specie *Tadarida brasiliensis* (3 samples). With the genetic identification of species, it was possible to identify that each genetic lineage of RABV was maintained by the respective bat specie, and no spillover cases were verified. So far, RABV samples have been subjected to 6 passages in HEK293T cells, and one of the isolated samples of *A. lituratus* (1233) and one of the isolated samples of *E. furinalis* (964) are already adapted *in vitro*. These are preliminary results that show that the RABV is more adapted to such species, considering the samples used in this study. At the end of the *in vitro* adaptation of the samples, they will be submitted to viral replication kinetics assays. With this work, it will be possible to propose diagnostic and surveillance tools to control rabies specific in non-hematophagous bats.

Keywords: In vitro characterization, Non-hematophagous bat, Rabies



VIROME OF THE NASAL CAVITY OF SWINE PRIOR TO SLAUGHTER

André Ferreira Hennigen¹, Anne Caroline Ramos dos Santos¹, Márcia Regina Loiko¹, Deisi Maria Pereira¹, Ana Paula Muterle Varela¹, Fabiana Quoos Mayer², Paulo Michel Roehe¹

¹ UFRGS - Universidade Federal do Rio Grande do Sul (Av. Sarmiento Leite, 500 - Farroupilha - Porto Alegre - RS), ² IPVDF - Instituto de Pesquisas Veterinárias Desidério Finamor (Estrada Do Conde, 6000 - Sans Souci, Eldorado do Sul - RS)

Abstract

High-Throughput Sequencing (HTS) allows the assessment of genetic diversity of microorganisms present in biological samples. Knowledge on the microbiome of swine is important as it may affect the susceptibility to pathogens and interfere in productivity. In this study, we analyzed swine nasal virome using HTS. At 173rd day of age, 15 days before slaughtering, sixty pigs had their nasal secretions collected with sterile swabs at a swine farm with respiratory disease history. The viral DNA was extracted by a standard phenol method and the RNA was extracted with TRIzol-chloroform. The DNA and cDNA libraries were prepared with the Nextera XT kit and HTS performed on an Illumina Miseq sequencer. The reads obtained from sequencing were filtered with Trimmomatic 0.39, assembled with metaSPAdes 3.10.1 and analyzed with Geneious 9.1.8 software. The total number of reads after the trimming process varied between 131,180 to 141,089. These were compared to the viral sequences database at the protein level using K-mer to lowest common ancestor mapping. On average, 18.58% of the reads showed identity to viral sequences deposited at the GenBank database. Among the viral contigs, 93.25% presented similarities with sequences of eukaryotic virus genomes and 6.75% with prokaryotic viral genomes. The virome results show a low diversity of viruses. Sequences with significant identity to porcine picobirnaviruses (PPBV) and circular viruses were detected. Among these, picobirnavirus related sequences were the most abundant. PBV (picobirnaviruses) are commonly found in the gastroenteric tract of different species. However, the lack of data regarding the presence of PBV in the respiratory tract of pigs hinders the identification of a possible pathogenic role in this species. The circular virus related sequences found here display identity to porcine stool-associated circular virus and porcine associated porprismacovirus, both groups of viruses to date not yet associated to diseases. The low diversity of viral families found on the animals' nasal cavity may have been influenced by a number of environmental factors that will be further investigated to understand the relations of this host with its viruses and other microorganisms. Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Pesquisa (CNPq), Financiadora de Estudos e Projetos (FINEP).

Keywords: Virome, Swine, High-Throughput Sequencing



DETECTION OF NEUROPATHOGENIC GENOTYPIC VARIANT OF EQUINE HERPESVIRUS TYPE 1 (EHV-1) ASSOCIATED WITH ABORTION AND REPRODUCTIVE PROBLEMS IN BRAZIL: DIAGNOSIS AND CASE DESCRIPTION

Luisa Feliciano de Souza Franklin ¹, Marcelo Ferreira Catizane ¹, Aila Solimar Gonçalves Silva ¹, Ana Carolina Diniz Matos ¹, Anna Gabriella Guimarães ¹, Grazielle Cossenzo Florentino Gallinari ¹, Izabela de Assis Rocha ¹, Maria Isabel Maldonado Coelho Guedes ¹, Raffaella Bertoni Cavalcanti Teixeira ¹, Zélia Inês Portela Lobato ¹, Erica Azevedo Costa ¹

¹ UFMG - Universidade Federal de Minas Gerais (Av. Pres. Antônio Carlos, 6627 - São Luiz, Belo Horizonte - MG, 31270-901)

Abstract

Equine herpesvirus type 1 (EHV-1) is a highly prevalent virus, affecting up to 80% of the world's equine population, causing extensive economic losses in the equine industry. The virus causes respiratory and neurological diseases, neonatal mortality, and outbreak of abortion, usually in the final third of pregnancy. Over the past decade, the incidence of abortion caused by EHV-1 has decreased, possibly due to vaccination practices. In contrast, cases of neurological disease increased significantly over the past 15 years. Different strains vary in their abortogenic potential, as well as in neuropathogenicity. Currently, one of the major markers for neuropathogenic potential of EHV-1 strains is related to a single nucleotide polymorphism of viral DNA polymerase gene (ORF 30). Infections with this marker are associated with a high and prolonged viremia, which may indicate a selective advantage of the neuropathogenic strain, which is refractory to vaccination and is related to abortion cases in some countries. In this study, 35 animals with reproductive problems were analyzed, 30 cases of abortion, 3 cases of neonatal mortality and 2 cases of stillbirths. The animals belonged to 15 properties of four different states of Brazil (Minas Gerais, Rio de Janeiro, São Paulo and Mato Grosso do Sul). A PCR reaction assay was performed to detect partial gene that encodes viral ORF 30, which was posteriorly confirmed by sequencing. The DNA of EHV-1 was detected in 7 of 30 abortion cases and the neuropathogenic marker was found in 6 of 7 EHV-1 positive cases by sequencing positive samples. Neuropathogenic EHV-1 was detected in mares vaccinated against EHV-1 and EHV-4 and the vaccination protocol was performed as recommended by the manufacturer. DNA of EHV-4 was detected in only one of the abortion cases analyzed. The results demonstrates the relevance of neuropathogenic strain as the cause of abortion in pregnant mares, even with vaccine control in the Brazilian properties. Financial support: CNPq, FAPEMIG, CAPES

Keywords: herpesviridae, reproductive failure, stillbirth, neonatology



DETECTION OF EQUINE GAMMAHERPESVIRUS 2 IN HORSES

Sofia Cicolo ², Angélica Cristine Gois de Almeida Campos ¹, Cairo Monteiro de Oliveira ¹, Daniele Bruna Leal de Oliveira ¹, Edison Luiz Durigon ¹, Vanessa Barbosa da Silveira ¹, Luiz Gustavo Bentim Góes ¹, THAÍS POLTRONIERI DOS SANTOS ², Carla Bargi Belli ², Raquel Yvone Arantes Baccarin ²
¹ ICBII-USP - Instituto de Ciências Biomédicas II da Universidade de São Paulo (Av. Prof. Lineu Prestes, 1374 - Butantã, São Paulo - SP, 05508-900), ² FMVZ-USP - Faculdade de Medicina Veterinária e Zootécnica da Universidade de São Paulo (Av. Prof. Orlando Marques de Paiva, 87 - Butantã, São Paulo - SP, 05508-010)

Abstract

Introduction: Equine herpesvirus (EHV) comprises a group of viruses that are divided into three subfamilies: Alpha-, Beta- and Gammaherpesvirinae, responsible for affecting different species of horses. Among the EHV species, EHV-2 is an important gammaherpesvirus known to cause infection in equine populations worldwide. This virus is often transmitted horizontally from its mother to newborn foals via the nasopharyngeal pathway or through contact with other foals, which may or may not show clinical signs of disease. EHV-2 is most commonly associated with upper respiratory tract infections of the animal, causing pharyngitis that can progress to more severe forms of disease, occasionally causing death of the animal. Few studies report the circulation of this virus in horses in the Brazil. **Objective:** The aim of this study is to evaluate the presence of herpesvirus in different equine samples. **Methods:** Blood, urine, cerebrospinal fluid, brain and swab samples were collected from seventeen symptomatic and asymptomatic horses from the University Hospital of the Faculty of Veterinary Medicine and Zootechnics of the University of São Paulo (HOVET-USP). The analysis of the samples was performed from the automated extraction of total nucleic acids, followed by amplification by PCR and nested assays targeting the Herpesviridae viral polymerase gene. **Results:** Herpesvirus was detected in two samples (serum and nasal swab) from two horses (11.7% - 2/17). The analyzed fragments were segregated with samples from the Equine Gammaherpesvirus 2 (EHV-2) group. **Conclusion:** This study shows that despite the few reports and information about this virus in Brazil, it has circulated, causing infections and subsequent death of horses in the country.

Keywords: EQUINE, HERPESVIRUS, GAMMAHERPESVIRUS 2, DETECTION



AMINO ACID VARIATIONS IN PARTIAL HA SEQUENCES OF H1 SWINE PANDEMIC FLU, FROM 2009 TO 2015

Ana Luiza Soares Fraiha ¹, Ana Carolina Diniz Matos ¹, Beatriz Senra Álvares da Silva Santos ², Maria Isabel Maldonado Guedes ¹, Erica Azevedo Costa ¹, Alexandre de Magalhães Vieira Machado ², Zélia Inês Portela Lobato ¹

¹ UFMG - Universidade Federal de Minas Gerais (Av. Pres. Antônio Carlos, 6627 - São Luiz, Belo Horizonte - MG, 31270-901), ² Fiocruz René Rachou - Fundação Oswaldo Cruz René Rachou (Av. Augusto de Lima, 1715 - Barro Preto, Belo Horizonte - MG, 30190-002)

Abstract

Since the 2009 pandemic, Influenza A virus (IAV) has threatened Brazilian swine herds. Although there are different subtypes circulating in the country, 2009 pandemic H1N1 virus (H1N1pdm09) has higher prevalence reports. In 2014, a vaccine against H1N1pdm09 was licensed and is the only one available for swine influenza prevention in Brazil. IAV has a segmented negative-sense genomic RNA contributing for rearrangements and RNA- polymerase lack of proofreading during replication may results in mutations, specially at the glycoproteins haemagglutinin (HA) and neuraminidase (NA) which are the main targets of host immune response. Five antigenic sites were identified at HA: Sa, Sb, Ca1, Ca2, Cb and substitutions of amino acids at these sites, during infection, are associated with antigenic changes. Monitoring the changes is important to understand IAV evolution and selection of vaccine strains. The objective of the study was to compare partial sequences of H1N1pdm09 virus detected in swine during the years 2012-2015 to other Brazilian sequences already deposited at GenBank. Forty- five samples of RNA extracted from clinical samples of swine, positive for H1N1pdm09 by a Nested RT-PCR, were used in the study. Eighteen samples were from 2012/2013 and twenty-seven from 2014/2015. Samples were subjected to a RT-PCR to amplify a 616bp conserved region of HA. The amplicons were sequenced and nucleotide sequence data analyzed. Consensus sequences were aligned with Brazilian H1N1pdm09 sequences detected in swine from 2009-2010 and HA sequences of H1N1pdm09 used in human and Brazilian swine vaccines, previously deposited at GenBank. Amino acid translation was performed and variations among the sequences were analyzed. Twenty-three amplicons were sequenced, 6 from 2012/2013 and 17 from 2014/2015. Amino acid analysis allowed the identification of Sa, Ca2 and Sb antigenic sites. Four partial sequences from 2012/2013 presented amino acid variations at Sa and six from 2014/2015 at Ca2. Punctual variations in other residues could also be observed in samples from 2012-2015 compared to those from 2009/2010 and vaccine strains. The data suggests that H1N1pdm09 virus may have evolved over the years and changes in immunologically important epitopes may have occurred. Surveillance of swine IAV is important to monitoring variation which could help establish updates for vaccine production and also to compare genetic characterization data with human IAV. **Financial support: CNPq, FAPEMIG.**

Keywords: Brazil, hemagglutinin, sequencing, swine Influenza Virus



FELINE IMMUNODEFICIENCY VIRUS (FIV): OCCURRENCE IN NORTHERN REGION OF CEARÁ, BRAZIL

Bruno Marques Teixeira ¹, Sueli Akemi Taniwaki ², Ana Kétylla Ponte Prado Rodrigues ¹, Meylling Mayara Linhares Magalhães ¹, Thiago Luiz Mendes Arcebispo ³, Gissandra Farias Braz ¹, Paulo Eduardo Brandão ², Marcos Bryan Heinemann ², Marcos Xavier Silva ³, Margaret J. Hosie ⁴

¹ UNINTA - Centro Universitário INTA - UNINTA (R. Antônio Rodrigues Magalhães, 359 - Dom Expedito, Sobral - CE, 62050-100), ² VPS - FMVZ - USP - Departamentos of Preventive Veterinary Medicine and Animal Health, School of Veterinary Medicine and Animal Science, University of São Paulo (Av. Prof. Dr. Orlando Marques de Paiva, 87 Cidade Universitária "Armando Salles de Oliveira" São Paulo – SP CEP 05508-270), ³ DMVP - EV - UFMG - Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais (Av. Antônio Carlos 6627 Caixa Postal 567, Campus Pampulha da UFMG CEP: 31270-901. Belo Horizonte, MG), ⁴ MRC - CVR - University of Glasgow - Medical Research Council; Centre for Virus Research, University of Glasgow, Glasgow, UK. (464 Bearsden Rd, Bearsden, Glasgow G61 1QH, United Kingdom)

Abstract

The prevalence of feline immunodeficiency virus (FIV) was investigated in domestic cats in northern Ceará, Brazil. Samples from 296 cats were collected and tested using anti-FIV antibody screening, with confirmation of positive results by polymerase chain reaction (PCR). Seventeen cats (5.74%) tested positive for FIV, two female (0.67%) and fifteen males (5.06%). Phylogenetic analysis of *gag* and *pol* gene sequences indicated that the FIV isolates circulating in the study area belonged to subtype B.

Financial Support: Centro Universitário INTA – UNINTA; CNPq; FUNCAP

Keywords: Feline immunodeficiency virus, Domestic cat, Brazilian Northeast, Ceará



FIRST COMPLETE GENOME CHARACTERIZATION OF A BRAZILIAN BEAK AND FEATHER DISEASE VIRUS ISOLATE

Matheus A. Duarte ¹, João M. F. Silva ¹, Tatsuya Nagata ¹, Clara R. Brito ¹, Danilo S. Teixeira ¹, Fernando L. Melo ¹, Bergmann M. Ribeiro ¹, Fabrício Souza Campos ²

¹ UnB - Universidade de Brasília (Campus Universitário Darcy Ribeiro, Bloco E s/n 1º andar - Asa Norte, DF, 70910-900), ² UFT - Universidade Federal do Tocantins (Rua Badejós, Chácara 69/72 s/n Zona Rural, Gurupi - TO)

Abstract

Circoviruses are non-enveloped, single-stranded, circular DNA viruses. They belong to the family *Circoviridae* and have genome size ranging between 1.7-2.3 kb. The group was taxonomically reviewed in 2016. Currently, the family is composed by two genera, *Circovirus* and *Cyclovirus*. *Circoviridae* comprises important animal pathogens, like beak and feather disease virus (BFDV) and porcine circovirus 2 (PCV-2), causes important environmental and economic losses in Psittaciformes species and pig industry, respectively. Genome sequences of these two circoviruses are the most reported in the family. However, no complete genome sequence of a Brazilian BFDV isolate was described yet. Based on this scenario, a high-throughput sequence method was employed to perform this genomic characterization. Feces samples of *Amazona aestiva* were collected from the Veterinary Hospital, University of Brasilia. The sequencing was performed using Illumina HiSeq 2500, 100 paired-end. Reads were trimmed and the contigs *de novo* assembled. Genome-wide pairwise identity is used as species demarcation criteria in *Circoviridae* with 80% identity as threshold and was applied for BFDV contig analysis. The present isolate is phylogenetically closer to the Polish isolate BFDV-U_PL-543_2008 (JX221029.1). Nucleotide identity was 94.9 %. The complete genome sequence found has 1,991 nt in size and is in accordance to other BFDVs. A 270- fold coverage was obtained, and 5339 reads were assembled. The two major ORFs (Rep and CP) were identified. However, differently from other BFDVs isolates, a point mutation was detected. A change of a cytosine (C) to a thymine (T) drives a premature stop codon producing a truncated CP, that is supported by a 345-fold coverage. Another ORF that codes for a hypothetical protein has high identity to C-terminus of CP. The circovirus' conserved nonanucleotide motif (TAGTATTAC) present in *ori* region was also observed. Similar to other circoviruses, the rolling circle replication (RCR) motifs at N-terminus of Rep were identified, motif I (FTLNN), motif II (PHLQG) and motif III (YCSK). Moreover, the superfamily 3 (SF3) helicases motifs, Walker-A (GPPGCGKS), Walker- B (VLDDF) and motif C (IITSN), were detected in Rep. These different features may help to explain the epidemiology of this viral disease in the country, that has great important in the veterinary medicine.

Financial Support: CNPq, FAPDF

Keywords: circovirus, high-throughput sequence, CP, Rep, ssDNA virus



THE PROPOSED AVIAN CHAPPARVOVIRUS: A NOVEL PARVOVIRUS FOUND IN BRAZILIAN WILD BIRDS' FECES BY A METAGENOMIC APPROACH

Matheus A. Duarte ¹, João M. F. Silva ¹, Tatsuya Nagata ¹, Clara R. Brito ¹, Danilo S. Teixeira ¹, Fernando L. Melo ¹, Bergmann M. Ribeiro ¹, Fabrício Souza Campos ⁴

¹ UnB - Universidade de Brasília (Campus Universitário Darcy Ribeiro, Bloco E s/n 1º andar - Asa Norte, DF, 70910-900), ⁴ UFT - Universidade Federal do Tocantins (Rua Badejós, Chácaras 69/72 s/n Zona Rural, Gurupi - TO)

Abstract

Chapparvovirus is a new virus genus recently proposed that belongs to *Parvoviridae*. This family comprises small non-enveloped ssDNA viruses with non-segmented and linear genome of 4 - 6.3 kb in size, involved in many clinical and subclinical animal infections. Chapparvoviruses exhibit a wide host range, infecting birds, dogs, rodents, bats, Tasmanian devils and swines, and have been identified by metagenomics analyses in feces as well as integrated to mammalian and avian genomes. The Brazilian Cerrado fauna shows very wide diversity and can be a potential viral reservoir. However, the wild animal virome of this biome is unknown. Based on this scenario, a high-throughput sequencing (HTS) constitutes a robust tool for the identification of new virus species in this environment and was applied in the present study. Feces samples of Cerrado birds (*Amazona aestiva* and *Sicalis flaveola*) were collected from the Veterinary Hospital, University of Brasilia. The sequencing was performed using Illumina HiSeq 2500, 100 paired-end. The reads were trimmed and the contigs *de novo* assembled. This new virus showed closer sequence identity to turkey parvovirus TP1-2012/HUN, a Chapparvovirus genus member, with 45.3% NS1 amino acid sequence identity. This protein is used as demarcation criteria for genus and species in *Parvoviridae* family, with 30% identity as threshold to novel genus determination. The genomic sequence found has 4425 nt in size and is in accordance to other chapparvoviruses. The 5'- and 3'-ends showed palindromic sequences that are responsible for the folding of the parvoviruses' terminal hairpins, essential to DNA virus replication. Two main ORFs were identified (NS1 and VP1), occurring a 62 nt overlap between them, that is the biggest one observed in this group or in any parvovirus genus of vertebrate hosts. The parvoviruses' conserved motifs (GPXNTGKS) and (HVH) were found coded in the genome. Similar to other chapparvoviruses, this new virus lacks the phospholipase A2 motif, recognized for playing a role in release of virus particle from endosomes in *Parvoviridae*. The phylogenetic trees of NS1 protein and virus genome sustain with high bootstrap values that this genus is monophyletic and closer to *Muscovy duck parvovirus*. These different features attach importance to study Chapparvovirus and understand your biology.

Financial Support: CNPq, FAPDF

Keywords: high-throughput sequencing, Cerrado, NS1, VP1, ssDNA virus



WILD ANIMALS AND THEIR IMPORTANCE FOR THE MAINTENANCE OF RABIES IN THE STATE OF SÃO PAULO – DATA COLLECTION

Ana Lee Aparecida Francisco ^{1,2}, Gilmara de Sousa Silva ², Marlon Benedito N. Santos ², Helena Beatriz de C. R. Batista ^{2,1}, Keila Iamamoto ², Karen M. Asano ², Enio Mori ², Willian de O. Fahl ², Karin C. Scheffer ²

¹ UFABC - Universidade Federal do ABC (Avenida dos Estados, 5001 Santo André), ² IPSP - Instituto Pasteur de São Paulo (Avenida Paulista, 393)

Abstract

Rabies is an acute and progressive encephalitis caused by a virus that belongs to *Rhabdoviridae* family, *Lyssavirus* genus and *Rabies lyssavirus* (RABV) species. Considering the decrease in rabies cases in dogs and cats and the maintenance of control measures against rabies in herbivores, wild animals have been highlighted as important reservoirs of this disease. In addition, it is worth noting the increased risk to humans due to the synanthropic habits of some wildlife species. The aim of this study was to conduct a retrospective study on rabies positivity in wild animals, referred for diagnosis in the reference laboratory of the State of São Paulo. For this, we used the results obtained by direct immunofluorescence and viral isolation s from different groups of wild animals received from 1996 to 2016, from the State of São Paulo. During this period, the total number of wild animal samples received was 49,310, of which 881 (1.79%) were diagnosed as positive for rabies virus. Evaluating the results by animal group the positivity in the group of chiroptera was 1.80% (865 / 47,937), while the group of wild canids obtained a positivity of 4,8% (7/145) and the group of non-human primates with 1.11% (6/541). The group of cervids, marsupials and procionids obtained a positive sample during the study period. The data obtained in the present study reiterate the importance of wild animals in strategies to limit the spread of rabies, such as coordinated epidemiological surveillance, laboratory diagnostic procedures that allow the integrated study of genetics and ecology, thus providing knowledge of the dynamics of rabies in rabies. wildlife beyond the relevance of epidemiological surveillance, where the strategies adopted for adequate surveillance and control of rabies according to the epidemiological cycle in circulation and the region are evaluated.

Keywords: Rabies, wild animals, diagnosis, direct immunofluorescence, São Paulo



EQUINE INFECTIOUS ANEMIA (EIA) IN DONKEYS, NORTHEAST, BRAZIL

Bruno Marques Teixeira¹, Viviane Maria Dias Costa^{2,1}, Andreia Elisa Cursino², Ana Paula Moreira Franco Luiz³, Gissandra Farias Braz¹, Betânia Paiva Drumond², Erna Geessien Kroon²

¹ UNINTA - CENTRO UNIVERSITÁRIO INTA (R. Antônio Rodrigues Magalhães, 359 - Dom Expedito, Sobral - CE, 62050-100), ² ICB/UFMG - Laboratório de Vírus- Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (Av. Pres. Antônio Carlos, 6627), ³ Viriontech do Brasil - Viriontech do Brasil (Avenida José Cândido da Silveira)

Abstract

The population of donkeys (*Equus asinus*) is concentrated in Northeastern Brazil for around 90%. Due to the agricultural modernization, these animals are being abandoned by their owners. As such, it is common to come across many of these animals wandering through the roads with ignored sanitary status, being potential transmitters of infectious agents. Among the infectious agents that can be transmitted by these animals, equine infectious anemia virus (EIAV) can be included. EIAV belongs to the family *Retroviridae* and genus *Lentivirus*. AGID is the official test designated by OIE for the worldwide diagnostic of EIA and is a highly specific test to identify infected animals, however, it has a low diagnostic sensitivity. Therefore, this test has a large number of false negative results, especially in donkeys, a species which is resistant to viral replication and shows a low viral load and late humoral response to the virus when compared to *Equus caballus*. This study aims to examine the serological status of EIAV in the wandering donkeys, using different techniques and also identifies the EIAV molecularly. A total of 124 donkeys were randomly selected in the state of Ceará. For each animal a data sheet with identification, clinical examination and body scoring were recorded. Blood samples were collected for accomplishment of the three diagnostic tests for EIA (AGID, ELISA recombinant protein gp90 and p26) and the detection of the proviral DNA. Immunological tests confirm AIEV in donkeys, however in AGID only one animal was positive (0.81%), compared with 21,8% (27/124) in the rgp90 ELISA and 10,5% (13/124) in the rp26 ELISA. In 8,1% (10/124) of the samples proviral DNA was detected. Thus, in light of the results it can be concluded that donkeys can also be carriers of the EIAV and may be possible sources of infection for horses, mainly in the Brazilian northeast.

Financial Support: Centro Universitário INTA – UNINTA; CNPq; FUNCAP; FAPEMIG.

Keywords: Equine infectious anemia virus - EIAV, Donkey, Diagnostic tests



SUPPRESSION OF STAPHYLOCOCCUS AUREUS BIOFILM FORMATION BY BACTERIOPHAGE VB_SAUM_UFV4 IN A DYNAMIC AND STATIC SYSTEM

Adrielle do Carmo ¹, Larissa Araújo ¹, Clara Laguardia ¹, Isabela Paes ¹, Roberto Dias ¹, Marcella Vieira¹, Jéssica Silva ¹, Vinícius Duarte ¹, Sérgio Paula ¹

¹ UFV - Universidade Federal de Viçosa (Avenida Peter Henry Rolfs, s/n - Campus Universitário, Viçosa - MG, 36570-977)

Abstract

Staphylococcus aureus is an opportunistic pathogen that affects humans and animals, being considered the main causative agent of bovine mastitis, one of the main diseases that affect dairy herds, reducing milk production and quality. Because they cause infections in the mammary glands, it is often associated with milk contamination and is responsible for outbreaks of diseases transmitted by the consumption of contaminated milk and dairy products. This microorganism causes great economic losses for the sector, as it is difficult to control, due to the expression of multiple resistance genes and the formation of biofilm. Biofilms are aggregates of microbial cells surrounded by a exopolymers matrix, highly organized and is an important virulence factor for species, because cells present in the biofilm are more resistant to antimicrobial agents, sanitizers and the action of the host immune system. Thus, an alternative method for biofilm biocontrol formed by *S. aureus* is phage therapy. Phages have been used since the early 20th century to treat bacterial infections, and have been shown to decrease biofilm formation due to enzymatic degradation of the layer surrounding and protecting microorganisms. In this work, the potential of bacteriophage vB_SauM_UFV4 was assessed in the preventive action of biofilm formation developed by multidrug-resistant *S. aureus* isolate obtained from milk samples collected from a mastitis casuistic production system. Biofilm formation in the presence of virus (MOI 1.0) was investigated under dynamic conditions through flow cells with polycarbonate coupons (FC BST Biosurface Technologies Corporation© 71) and under static conditions in 96 well flat bottom polystyrene plates, in both experiments occurred at 37 ° C for 72 hours. Bacterial growth was monitored (D.O600) and biomass of biofilm was determined by the microtiter plate-based crystal violet assay, measuring the absorbance of the supernatant obtained after the biofilm discoloration step. In addition, the action of phage on biofilm formation in both systems was assessed by fluorescence microscopy using the dyes 4,6-diamino-2-phenylidol (DAPI), propidium iodide and fluorescein isothiocyanate (FITC). From the data obtained in this work it was possible to observe that vB_SauM_UFV4 interfered negatively in biofilm formation in both analyzed systems, showing to be a promising strategy in biofilm biocontrol formed by *S. aureus* such as pipes, milk cans and milk cooling tanks.

Keywords: Bacteriophage, Biocontrol, Biofilm



METAGENOMIC DETECTION OF HEPACIVIRUS IN ORAL AND RECTAL MICROBIOME OF OPOSSUMS FROM CAMPINAS METROPOLITAN REGION, STATE OF SÃO PAULO, BRAZIL

Paulo Vitor Marques Simas¹, Leonardo Cardia Caserta^{1,2,3}, Gabriela Mansano do Nascimento^{2,3}, Lok Raj Joshi^{2,3}, Raphael Mausbach Simão⁴, Michael Edward Miller¹, Diego G. Diel^{2,3}, Clarice Weis Arns¹

¹ UNICAMP - University of Campinas (Laboratory of Animal Virology, Institute of Biology, Campinas/SP, 13083-970, Brazil), ² SDSU - South Dakota State University (Animal Disease Research and Diagnostic Laboratory, Department of Veterinary and Biomedical Sciences, Brookings, SD 57007), ³ CORNELL - Cornell University (Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Ithaca, NY 14853, USA), ⁴ USP - University of São Paulo (FZEA, Pirassununga/SP, 13635-900, Brazil)

Abstract

The significant biodiversity found in Brazil is a potential for the emergence of zoonosis. Identifying natural reservoir species and characterizing which traits are associated with pathogens occurrence can be key to control emerging infectious diseases. Marsupials *Didelphis spp.* are widely distributed in the Americas and the species *Didelphis albiventris* and *D. aurita* are common in the most populated areas of Brazil. They adapt to a broad variety of habitats, including great urban centers and secondary forests modified by human action. However, their potential as reservoir species for zoonotic pathogens has not been deeply studied yet. Here we describe the oral and rectal/fecal microbiome (including viruses and bacteria) of 16 healthy opossums (*D. albiventris* and *D. aurita*) captured in three forest fragments in Campinas Metropolitan Region, São Paulo State, Brazil, using high throughput sequencing. Differences between composition and origin of microbiomes were observed. Oral microbiome presented higher bacterial diversity than anal. At species level, we detected sequences from *Campylobacter*, *Salmonella*, *Burkholderia*, *Chlamydia* and *Hepacivirus*, which are microorganisms of high zoonotic potential. Some pathogens species were detected only to one opossum specie or only one sampling location. Extrinsic factors like habitat fragmentation as well intrinsic factors like diet and phylogeny could potentially play a role on the patterns observed. *Hepacivirus* was detected in all samples, suggesting that opossums may be reservoir for this zoonotic virus and should be investigated as possible hosts to other viruses from the *Flaviviridae* family as well. The detection of these pathogens in such broadly distributed animal species warns to the possibility of disease emergence in other species including humans, especially when their habitats overlap.

Keywords: *Didelphis albiventris* and *D. aurita*, Microbiome, Opossum, Reservoir, Zoonotic diseases



CHARACTERIZATION OF ROTAVIRUS POSSESSING A DS-1-LIKE VP3 GENE FROM PIGS IN BRAZIL: EVIDENCE FOR ZOOANTHROPONOTIC TRANSMISSION.

Mayara Neves ¹, Alcines Sousa Junior ¹, Patrícia Lobo ¹, Renato Bandeira ¹, Luana da Silva Soares Farias ¹, Joana Mascarenhas ¹

¹ IEC - Instituto Evandro Chagas (Br 316, km 07, s/n)

Abstract

Porcine group A rotavirus (RVA) strains SUI15A and SUI24A were suggested to have genes of human origin and VP3 gene possessing DS-1-like backbone. The aim of the present study was to analyse the genome of two strains (SUI15A and SUI24A) and understand the evolution of a rare human-like M2 genotype in pigs. On partial genomic analysis, strains SUI24A (G3-P[13]-I5-R1-C1-M2-A8-N1-T7-E1-H1) and SUI15A (G3-P[x]-Ix-R1-C1-M2-Ax-Nx-T7-E1-H1) were found to have VP3 gene RVA different from those of typical porcine RVA strains described in Brazil and worldwide. This genotypic constellation was a novel constellation that has not been reported previously in both humans and pigs. Furthermore, on phylogenetic analysis, of VP3 gene of strains appeared to be of human origin. Therefore, suggested to have evidence for human-to-porcine zoonanthropotic transmission.

Financial support: CNPq, FAPESPA.

Keywords: Group A rotavirus, Zoonanthropotic transmission, Swine



GENETIC DIVERSITY OF CANINE MORBILLIVIRUS GENOTYPE CIRCULATING IN THE WEST-CENTRAL REGION, BRAZIL

Vivaldo Gomes da Costa ¹, Marielena Vogel Saivish ², Rebeca Francielle de Lima Silva ², Roger Luis Rodrigues ², Marcos Lázaro Moreli ², Ricardo Henrique Kruger ¹

¹ UnB - Universidade de Brasília (Asa Norte, Brasília-Distrito Federal, 70910-900), ² UFG - Universidade Federal de Goiás (Br364, Km 195, Setor Industrial, Jataí-Goiás, 75801-615)

Abstract

Canine morbillivirus (previously known as canine distemper virus (CDV)) is an important viral agent that causes severe and highly contagious diseases in domestic dogs (*Canis familiaris*). CDV is enveloped with single-stranded, negative sense and nonsegmented RNA genetic material, belonging to the genus *Morbillivirus* (family *Paramyxoviridae*). The virus has a large genetic diversity that divides it into several genotypes. Consequently, the hemagglutinin (H) gene has become the most suitable target to investigate the CDV variability. In view of there is little data that analyze the genetic variability, the objective of this study was to perform a molecular characterization of the H gene from CDV in the clinical samples from dogs, which had biological samples collected between the years 2017 and 2018 in the municipality of Jataí-Goiás (Ethics Committee on the Use of animals-UFG: 054/17). The molecular characterization was performed for all CDV RNA positive samples by nested RT-PCR (detection of the CDV nucleoprotein gene) sequence, for the obtainment of an amplicon from the H gene, it was done an RT-PCR test with specific primers generating a product with 1189 bp. PCR product was confirmed by DNA sequencing and Neighbor Joining phylogenetic inferences was performed by Mega 7.0 software. The results showed that only three (3/30) whole blood samples from dogs with distemper had amplicons for the H gene. The H gene phylogeny showed characterization of the lineage in the genotype of South America-I/Europe, with greater similarity to the isolated strain of Uruguay (KM280689.1). Thus, a phylogenetic proximity between our isolates and other isolates from Latin America and Europe was observed. To our knowledge, this is the first study in the region about CDV molecular research and will contribute to molecular surveillance and trace the epidemiological profile of CDV in the study region.

Financial Support: FAPDF, CNPq and CAPES.

Keywords: Canine distemper virus, Canine morbillivirus, Paramyxoviridae, Domestic dogs, Morbillivirus



CHARACTERIZATION OF THE HEMAGGLUTININ PROTEIN GENE OF CANINE MORBILLIVIRUS FROM NATURALLY INFECTED DOGS IN THE STATE OF MATO GROSSO

¹Mayara Lima Kawasaki ¹, Daniel Moura de Aguiar ¹, Michele Lunardi ², Luiz Donizete Campeiro Junior

¹ UFMT - Universidade Federal de Mato Grosso (Av. Fernando Corrêa da Costa, nº 2367 - Bairro Boa Esperança. Cuiabá - MT - 78060-900), ² UNIC - Universidade de Cuiabá (Av. Manoel José de Arruda, 3100 - Jardim Europa, Cuiabá - MT, 78065-700)

Abstract

Canine morbillivirus (CDV) causes one of the major infectious diseases of high morbidity and mortality in dogs and wildlife, called canine distemper (CD), belonging to the family *Paramyxoviridae* and genus *Morbilivirus*. The viral genome contains transcriptional units that encodes eight proteins that include nucleoprotein (N) and Hemagglutinin (H). The hemagglutinin (H) is expressed in the viral envelope and has the highest genetic variability among CDV and has been used to characterize phylogenetically into nine strains named America I, America II, Asia I, Asia II, Europe Wildlife, Arctic, South Africa, South America I / Europe and South America II. In the present study, hemagglutinin (H) protein gene was characterized in CDV from naturally infected dogs in the state of Mato Grosso. Central nervous tissue from 6 dogs were collected post mortem between August 2018 and April 2019 and submitted to RT-PCR to detect the nucleocapsid gene (N) of CDV. From the positives, the H gene were amplified, and the nucleotide sequence was aligned by the MUSCLE program and a phylogenetic tree was inferred by the Neighbor-Joining method. Sequenced samples were classified into MT1, MT2, MT3, MT4, MT5 and MT6. Samples MT1 to MT4 were from the municipality of Poconé-MT and MT5, MT6 from Cuiabá-MT. Phylogenetic analysis revealed that the samples positioned in two distinct groups: samples MT1, MT3, MT5 and MT6 were genetically related to isolates from Brazil, Italy and Spain, and the samples MT2 and MT4 were grouped in clade composed by strains from South America, Europe, South Africa and classic vaccine strains (Convac, CDV3, Snyder Hill, and Onderstepoort). All sequences were classified within the South America I / Europe genotype. Financial support: Ministry of Education of Brazil (MEC), Federal Agency for the Support and Improvement of Higher Education (CAPES) and National Council for Scientific and Technological Development (CNPQ).

Keywords: Canine distemper, Nucleoprotein, Molecular Detection, RT-PCR, Phylogenetic analysis



CHARACTERIZATION OF BRAZILIAN GENETIC LINEAGES OF THE RABIES VIRUS COMPATIBLE WITH SAMPLES ISOLATED IN DOMESTIC AND WILD CANIDS AND VAMPIRE BATS IN RT-QPCR ASSAY

Maria Eduarda Rodrigues Chierato ¹, Débora Fernanda Pedrozo Pavani ², Alex Domingos Reis ², Yasmin Machado de Freitas ², Karin Corrêa Scheffer ², Helena Beatriz de Carvalho Ruthner Batista², Karen Miyuki Asano ², Willian de Oliveira Fahl ², Keila Iamamoto ², Enio Mori ^{1,2}

¹ FMVZ-USP - Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (Av. Prof. Dr. Orlando Marques de Paiva, 87 - Butantã, São Paulo - SP, 05508-900), ² IP-SP - Instituto Pasteur de São Paulo (Av. Paulista, 393 - Cerqueira César, São Paulo - SP, 01311-000)

Abstract

Rabies is a progressive acute viral encephalitis, caused by Rabies virus (RABV), which belongs to the family *Rhabdoviridae*, genus *Lyssavirus*. The disease is characterized by a central nervous system (CNS) disorder, that presented a high lethality (almost 100%). Because of the RABV adaptation to the determinate animal hosts (reservoirs) over the time, different genomes were formed and are classified as variants (AgV) and/or distinct genetic lineages. In Brazil, the main reservoirs are domestic dogs (AgV1 and AgV2), vampire bats *Desmodus rotundus* (AgV3) and non-hematophagous frugivorous bats *Artibeus lituratus* (AgV3). The aim of this study was to develop a RT-qPCR assay for the detection and genetic lineages characterization of the different variants of the RABV for diagnosis in the Pasteur Institute of Sao Paulo. Samples from cattle CNS (n=40) and domestic and wild canids CNS (n=16), compatible to AgV3 and to AgV2, respectively, were used in order to analyze the specific character of the probes designed to AgV3 and to AgV2 for the RT-qPCR assay. The cattle samples were obtained between the years 2015 and 2016 from the Southeast Brazil and the domestic and wild canids samples were obtained between the years 2007 and 2018 from the North and Northeastern Brazil. Total RNA was extracted from all 56 CNS samples by the guanidinium thiocyanate (TRIzol reagent) method and the RT-qPCR assay was performed using primers and probes to amplify the N gene. The results showed that the probes are specific and sensitive for the respective genetic lineages. Furthermore, the probe for AgV3 did not amplify the wild canids samples, and the probe for AgV2 did not amplify the cattle samples. The results also suggest that the different genetic lineages may be analyzed in a real time RT-PCR assay without the need of sequencing. Besides, this assay may discriminate the main variants of RABV in Brazil and once introduced into the laboratory routine can help implementing surveillance measures and rabies control.

Financial Support: CNPq (404065/2016-3); FAPESP (15/17807-0); CAPES (Finance Code 001).

Keywords: Genetic lineages, Rabies, RNA, RT-qPCR



A CONTEMPORARY BRAZILIAN SENECAVIRUS A ISOLATE: IN VITRO CHARACTERIZATION - PARTIAL RESULTS -

Manuela Muller ¹, Sergio Abreu Machado ², Diego Gustavo Diel ³, Mathias Martins ¹

¹ UNOESC - Universidade do Oeste de Santa Catarina (Laboratório de Virologia, Rodovia Rovilho Bortoluzzi, SC480, Km 3.5, Bloco C, Xanxere, SC, Brazil. 89820-000), ² UNOESC - Universidade do Oeste de Santa Catarina (Laboratório de Biologia Molecular, Bloco C, Xanxere, SC, Brazil. 89820-000), ³ SDSU - South Dakota State University (Animal Disease Research and Diagnostic Laboratory, Department of Veterinary and Biomedical Sciences, Brookings, SD, United States. 57007)

Abstract

Senecavirus A (SVA) belongs to the *Picornaviridae* family and was firstly described in 2002 in United States as a cell culture contaminant and non-pathogenic for animals. However, SVA became a problem between the end of 2014 and early 2015 when it was detected in piglets presenting vesicular disease, diarrhea and death in Brazil. Although the virus remained endemic in the country since then, field veterinarians have reported an increase in the number of cases and a change in the clinical form of the disease in 2019. The virus is apparently more virulent than the one first detected in 2014, especially in finishing pigs. The disease caused by SVA has generated disturbance to the swine production chain in Brazil since it is on the list of diseases that can be confused with foot-and-mouth disease. In this study, a contemporary Brazilian Senecavirus A was isolated. The sample was collected using swab from vesicle in finishing swine with 160 to 170 days old, kept in a farm with 6000 pigs in Minas Gerais State, Brazil. The morbidity was about 20 percent and mortality rate less than 3 percent. The diagnosis was confirmed by PCR using primers that determine amplification of an internal region of the 3D gene of the SVA genome. Simultaneously, the sample was inoculated in cell culture for viral detection. The virus isolation was performed in baby hamster kidney cells (BHK-21). In the first passage of the material under cultivation, a cytopathogenic effect compatible with SVA replication was observed (cell rounding and detachment). The identity of virus was confirmed using two additional techniques: nucleotide sequencing of PCR amplicons and indirect immunofluorescence assay (IFA) using monoclonal antibody (mAb). Both tests confirmed that it was an SVA. The next step of the research is to obtain the complete genome of the isolated virus and compare with the SVA that circulated in Brazil in 2014.

Keywords: emerging infectious disease, idiopathic vesicular disease, picornavirus, Seneca Valley virus, swine



PHYLOGENY OF CIRCULATING STRAINS OF CAPRINE ARTHRITIS ENCEPHALITIS VIRUS FROM GOATS OF THE SÃO PAULO STATE, BRAZIL

Ingrid Bortolin Affonso Lux Hoppe ¹, Igor Renan Honorato Gatto ¹, Andressa de Souza-Pollo ¹, Andréa Souza Ramos de Medeiros ¹, Kayo José Garcia de Almeida Castilho Neto ¹, Samir Issa Samara ¹, Adolorata Aparecida Bianco Carvalho ¹

¹ FCAV/Unesp - Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (Via de Acesso Prof. Paulo Donato Castellane, s/nº, CEP:14.884-900, Jaboticabal, São Paulo, Brazil)

Abstract

Caprine arthritis encephalitis virus (CAEV) and Maedi-Visna virus (MVV), also known as Small Ruminant Lentiviruses (SRLVs), cause slow and persistent inflammatory diseases in goat and sheep, respectively, leading to important productive and economic losses worldwide. For a long time, these infections have been considered species-specific, although, several reports showed that natural cross-species infections may occur. Therefore, monitoring the genetic diversity of SRLVs in sheep and goat herds is useful mainly to improve the diagnostic tools used in the control or eradication programs. The present study aimed to characterize genetically SRLVs strains obtained from naturally infected dairy goat herds of the municipality of Jaboticabal, São Paulo State, Brazil. Thus, blood samples of three CAEV-positive goats (121, 171 and 191) detected by agar gel immunodiffusion test were submitted to nested PCR to amplify part of the *gag* gene of the SRLVs and perform the sequencing. In addition, a nested PCR-positive goat sample (V23.2), obtained from another herd of the same municipality, was included in the study. None of the animals have presented clinical signs of SRLVs infection. In the phylogenetic tree, the sequences of this study were grouped in CAEV group type B, subtype B1. Sequences of the goats 121, 171 and 191 were grouped in the same cluster, separately from the sequence V23.2, that showed to be genetically distant from the other sequences obtained in this study. These results indicate that the occurrence of SRLVs strains belonging to subtype B1 remains predominant in Brazil, as reported in previous studies. CAEV is a major disease of goat production and the continued study of its molecular epidemiology is especially important owing to its constant change, related to animal movement, cross-species transmission and the rapid evolutionary rate.

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Keywords: *gag* gene, phylogeny, Small Ruminant Lentiviruses, SRLVs



GENETIC CHARACTERIZATION OF AVIAN POXVIRUS IN SOUTHERN BRAZIL

¹Leonardo Clasen Ribeiro ¹, Francielle Liz Monteiro ¹, Matheus Iuri Fruhauf ¹, Renata Nobre da Fonseca ¹, Gilberto D'Avila Vargas ¹, Marcelo de Lima ¹, Geferson Fischer ¹, Sílvia de Oliveira Hübner

¹ UFPel - Universidade Federal de Pelotas (Campus Universitário Capão do Leão, Prédio 1, Faculdade de Veterinária, Laboratório de Virologia e Imunologia)

Abstract

Avian poxvirus (APV) is an enveloped double-stranded DNA virus which affects a wide variety of domestic and wild birds worldwide. APVs belong to the subfamily *Chordopoxvirinae* and to the family *Poxviridae*. In the genus *Avipoxvirus* there are ten recognized species, being the nomenclature given in accordance with the species in which they were first described. However, new molecular characterization studies corroborate the idea that the same strain may affect different bird species. Thus, the objective of this study was to characterize genetically APVs detected in different bird species in southern Brazil. The samples were received by the pathology department and sent for confirmatory diagnosis at the Laboratory. DNA was extracted from frozen tissue of the lesions and submitted to the PCR, targeting the polymerase and P4b gene. PCR product was purified, and amplicons were sequenced bidirectionally. Results were analyzed to obtain a consensus sequence from each duplicate. Phylogenetic analysis was conducted by the MEGA 6.0, using the *neighbor-joining method*, and the evolutionary distances were computed using the *Kimura 2-parameter model*. Ten samples were analyzed: a sample of domestic fowl (*Gallus gallus domesticus*) grouped in clade A1, and five samples, one of hawk (*Mivalgo chimango*), one of canary (*Serinus canaria*) and three of dove (*Columba livia*), grouped in clade A2. Two canary samples were grouped in clade B1 and one in clade B2. Curiously, the samples of canary and hawk grouped in clade A, which represented by *Fowlpox virus*. These samples demonstrated 90.7 to 100% identity between them, demonstrating that different bird species can be infected by very similar viruses. Studies conducted in Brazil are generally based on clinical diagnosis and histopathology. Thus, the constant monitoring of the evolution of APVs is important an eventual outbreak of the disease and the evolutionary biology of these viruses.

Financial Support: CAPES.

Keywords: APV, phylogenetic analysis, P4b gene, polymerase gene.



TEMPORAL AND SPACE CHARACTERIZATION OF RABIES IN LIVESTOCK IN THE STATE OF MATO GROSSO BETWEEN 2009 AND 2018

Emmanuelle Rosa Mutzenberg ^{1,2}, Áquila José Gonçalves Delfino ¹, Ernani Machado de Lima ², Selma Maria Nassarden ², Rísia Lopes Negreiros ², Anderson Castro Soares de Oliveira ¹, Daniel Moura de Aguiar ¹

¹ UFMT - Universidade Federal de Mato Grosso (Avenida Fernando Corrêa da Costa, 2367, Cuiabá- MT), ² INDEA - Instituto de Defesa Agropecuária do Estado de Mato Grosso (Avenida Jornalista Arquimedes Pereira Lima, 1000, Cuiabá-MT)

Abstract

Rabies is a zoonosis caused by *Lyssavirus*, causing acute fatal encephalomyelitis in farm animals and causing serious damage to livestock. The disease is endemic in the state of Mato Grosso (MT). The present work carried out an epidemiological analysis of rabies outbreaks in MT registered by the state animal health defense between 2009 and 2018. The following data were extracted from the Disease Investigation Forms: number of outbreaks, cases, animal species, positivity rate, and number of notified municipalities. The data of focus, mean, standard deviation and coefficient of variation were evaluated by the *Rcmdr* package of the R software. Maps with focus concentration / km² were constructed using the *QGIS* software. The ARIMA (1,1,1) statistical model to determine the historical series of outbreaks was built by the *TSA* package of the R software. Of the total, 589 cases were diagnosed in 538 outbreaks with an average of 53.8 outbreaks per year and a coefficient of variation of 0.39. It was found a higher occurrence in cattle (89%) and horses (10%), with the highest positivity rate, 35.8% and 33.7%, respectively. Direct immunofluorescence testing diagnosed 89.3% of cases. Ninety-five of 141 municipalities (67.3%) recorded outbreaks that shifted over the years, concentrating mainly on the western (Amazon biome), central and eastern (Cerrado biome) regions of MT. Outbreaks increased from May to July, but without seasonality patterns ($p > 0.01$), with 1% significance level. There was a decreasing trend of outbreaks (p

Financial Support: National Council for Scientific and Technological Development (CNPQ)

Keywords: Lyssavirus, epidemiological survey, rural cycle, Brazil, animal health defense



IN VITRO SUSCEPTIBILITY OF BOVINE CELLS TO SMALL RUMINANTS LENTIVIRUS

Maria Aurea Azevedo Nogueira ¹, Marcelo Tigre Moura ¹, Giselle Ramos da Silva ¹, Davi Santos Rodrigues ¹, José Wilton Pinheiro Junior ¹, Rita de Cássia Carvalho Maia ¹, Sergio Alves do Nascimento ¹

¹ UFRPE - Universidade Federal Rural de Pernambuco (Rua Dom Manoel de Medeiros, Dois Irmãos, Recife. PE.)

Abstract

The susceptibility of a primary bovine cell line to viral agents of small ruminants, which do not cause natural infection in this species, was tested. Thus, ear biopsy samples from male bovine with no defined breed, and approximately two years old, were obtained from slaughterhouses. After tissue processing and fragmentation, 1- 2mm explants were plated on 60mm petri dishes containing 3mL Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% Fetal Bovine Serum (FBS). Afterwards, weekly passages were performed, where the cultures were trypsinized and subcultivated in 25cm² flasks, containing DMEM with Amphotericin B, Streptomycin and Penicillin, and incubated at 37°C. During cell culture 10% FBS was used and for viral inoculation 2% FBS was preferred. The cells obtained (CFBov) were inoculated with Caprine Arthritis-Encephalitis virus (CAEV-Co) and Maedi Visna virus (MVV) (K1514), and karyotyping was performed for chromosome analysis. The CFBov cells were cultivated continually for 18 months and 48 passages. The monolayers did not show any morphological modifications or reduction in multiplication rate. The CFBov primary cells from bovine ear samples showed susceptibility during *in vitro* infection with both CAEV and Maedi-Visna viruses. The chromosomic analysis of twelve intact cells, showed 2n=60 with all acrocentric autosomes and a submetacentric X, thus confirming that the cells have shape, size and number characteristic of bovine species. In addition to the position of the centromere, the size and bands were evaluated and confirmed, which are the light and dark regions along the chromosome. Each band is distributed differently and specific for each species, leading to an effective analysis. This study corroborates the importance of cell culture techniques as a key player in a veterinary virology laboratory. Due to their growth characteristics, the cells have behaved as a continuous lineage, which will be confirmed with the continuation of the passages. Finally, it is possible to state that the CFBov cells have demonstrated *in vitro* susceptibility to small ruminant virus, and further studies on the pathogenesis of the etiological agent in this species are necessary.

Keywords: Cell culture, Virus susceptibility, Chromosomic analysis



DETECTION OF HOBI-LIKE PESTIVIRUS IN AN OUTBREAK OF RESPIRATORY DISEASE IN CALVES OF SÃO PAULO STATE, BRAZIL

Ingrid Bortolin Affonso Lux Hoppe ¹, Andressa de Souza-Pollo ¹, Adolorata Aparecida Bianco Carvalho¹

¹ FCAV/Unesp - Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (Via de Acesso Prof. Paulo Donato Castellane, s/nº, CEP:14.884-900, Jaboticabal, São Paulo, Brasil)

Abstract

HoBi-like is an emerging pestivirus of the family *Flaviviridae* detected in cattle herds and biological products, such as fetal bovine serum, in many parts of the world. The virus is associated with a variety of clinical manifestations resembling the infections by bovine viral diarrhoea virus (BVDV), such as reproductive and respiratory disorders, persistent infection and mucosal disease. Clinical signs caused by HoBi-like pestivirus can also be confused with those caused by other viruses, making the diagnosis difficult. This study reports the detection of HoBi-like pestivirus in an outbreak of respiratory disease in calves of a dairy cattle herd in São Paulo State, Southeastern Brazil. For that, serum samples and nasal swabs were collected from 44 calves up to one year old, presenting or not clinical signs of respiratory disease. RT-PCR was performed to detect pestiviruses (BVDV-1, BVDV-2 and HoBi-like), bovine respiratory syncytial virus (BRSV) and bovine parainfluenza-3 (BPIV-3); and, PCR was utilized to detect the bovine herpesvirus-1 (BoHV-1) DNA. Both serum samples and nasal swabs of two animals aging 0-3 months and two older calves (6-12 months) were positive for pestiviruses. Sequencing results of the amplified 5'UTR and E2 regions of the nasal swabs identified the HoBi-like pestivirus. The phylogenetic tree of the concatenated sequences of the 5'UTR and E2 regions showed a close genetic similarity among the sequences obtained in this study which were grouped in a same cluster, nonetheless, the sequences were separated in different subgroups according to the age of the calves, evidencing the genetic particularities between the sequences obtained from younger and older calves. Only one Brazilian sequence of HoBi-like, from the State of Mato Grosso do Sul, showed genetic relation with the sequences obtained in this study, but presenting high genetic distance from them (0.033), and the other Brazilian sequences showed to be even more genetically distant (≥ 0.047). This is the first detection of HoBi-like pestivirus in nasal secretions of calves in an outbreak of respiratory disease in Brazil, and the increasing detection of this virus at field indicates the necessity of a differential diagnosis to enable the implementation of appropriate measures of control and prophylaxis.

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Keywords: calves, nasal swab, pestiviruses, phylogeny



OCCURRENCE OF GENOGROUP I PICOBIRNAVIRUS IN SHEEP FLOCKS FROM PARANA.

Caroline Do Nascimento Ferreira¹, Daniela Lorencena¹, Patrick Kaiser Knabben¹, Joice Aparecida De Andrade¹, Petronio Pinheiro Porto², Francielle Gibson Da Silva Zacarias², Liza Ogawa², Elisabete Takiuchi¹

¹ UFPR - UNIVERSIDADE FEDERAL DO PARANÁ (SETOR PALOTINA - Rua Pioneiro, 2153. Jardim Dallas.

CEP 85950-000. Palotina - PR.), ² UENP - UNIVERSIDADE ESTADUAL DO NORTE DO PARANA (RODOVIA BR-369 KM 54, VILA MARIA, CEP 86360-000, BANDEIRANTES, PR, BRAZIL)

Abstract

Picobirnaviruses (PBV) are non-enveloped virus, with a bisegmented double stranded RNA genome. PBV are classified into genogroups I and II due to the high genetic variability of the segment 2 that encodes its RdRp. PBV is considered an opportunistic pathogen and its role as a causative agent of diarrhea is uncertain. Although PBV have already been detected in a wide range of susceptible hosts, data pertaining to its presence in small ruminants are still limited. In order to assess the occurrence of PBV in ovine host, stool samples from 203 animals were collected between 2017 and 2018 in four sheep flocks located in the municipality of Bandeirantes, Parana State. Sampling was performed from animals with (n=68) or without (n=135) signs of diarrhea. The PBV diagnosis was carried out by silver-stained 7.5% polyacrylamide gel electrophoresis (SS-PAGE) and RT-PCR using the primers PicoB25 (5'TGGTGTGGATGTTTC 3') and PicoB43 (5'A(GA)TG(CT)TGGTCTGA ACT T 3') that amplify a 201 bp fragment of the RdRp gene of GI PBV. The PBV was detected (positive results by SS-PAGE and/or RT-PCR) in all four herds surveyed, with frequencies of 24.3% (18/74); 30% (3/10); 30.6% (11/36) and 50.6% (42/83). PBV was found in 29.4% (20/68) of the diarrheic and 40% (54/135) of the non-diarrheic samples. Out of the total samples, PBV was detected in 4.93% (10/203) and 34% (69/203) by ss-PAGE and RT-PCR, respectively. From the 10 SS-PAGE positive fecal samples, five were not successfully amplified using GI specific primers, suggesting the presence of PCR inhibitors in the samples or these samples may still belong to the GII PBV. However, since we have not tested the GII specific primer set it is not possible to assign to these PBV this classification. The low sensitivity of the SS-PAGE technique could be considered as a limiting factor for PBV diagnosis. However, the positive results obtained in this assay suggest intense viral replication with faecal excretion of PBV in high titers by these animals. Besides, all positive samples by SS-PAGE were non-diarrheic that reinforces the hypothesis that PBV may not be the primary etiological agent in diarrhea episodes. Although much remains to be understood about the epidemiology of PBV, this study confirms the GI PBV is widely distributed in sheep flocks from Parana State.

Keywords: Picobirnavirus, sheep, genogroup I, PAGE, RT-PCR



DETECTION OF PICOBIRNAVIRUS IN FECAL SAMPLES FROM PIGS AND PIG FARM WORKERS IN THE WESTERN REGION OF PARANÁ.

Daniela Lorencena ¹, Caroline Do Nascimento Ferreira ¹, Thiago Henrique Belle ¹, Leonardo Ereno Tadielo ¹, Janaina Lustosa De Mello ¹, Elisabete Takiuchi ¹

¹ UFPR - UNIVERSIDADE FEDERAL DO PARANÁ (SETOR PALOTINA - Rua Pioneiro, 2153. Jardim Dallas. CEP 85950-000. Palotina - PR.)

Abstract

Picobirnavirus (PBV) constitute a group of emerging non-enveloped virus with a bisegmented double-stranded RNA (dsRNA) genome. PBV has been detected in a wide range of host species, including terrestrial and marine mammals, reptiles, and birds. Some studies of PBV has been suggested the possibility of inter-species transmission, highlighting the zoonotic potential of PBV in pigs and humans. However, most of the studies have compared PBV sequences obtained from the swine host and from humans without any epidemiological relation between them. This work aimed to investigate the PBV excretion in fecal samples from pigs and their contacts pig farm workers. In order to investigate the PBV infection in swine and human in close contact with these animals, stool samples from 133 pigs and nine pig farm workers were collected in 2018 in three commercial farms located in the western region of Parana State. Fecal samples were submitted to nucleic acid extraction by the combination of phenol chloroform-isoamyl alcohol and silica/guanidine isothiocyanate. The PBV diagnosis was carried by silver- stained polyacrylamide gel electrophoresis (SS-PAGE) for visualization of the dsRNA genome. PBV was detected in all three pig farms surveyed, with frequencies of 24.4% (11/45); 41.3% (19/46); 45.2% (19/42) in pigs and of 0% (0/2); 100% (3/3); 50% (2/4) in their respective pig farm workers. Considering the low sensitivity of the SS-PAGE, the detection of PBV dsRNA using this technique demonstrates the elimination of high titers of viruses in both host species. This report constitutes a preliminary investigation; future studies will include analysis of all samples by RT-PCR and sequencing of the amplified products to assess the identity of the sequences obtained from swine and humans in the same space/time locations. Due to the close relationship between pig farms workers and pigs, new epidemiological studies should be conducted to evaluate the pathogenesis of PBV and to elucidate the role of these viruses in populations exposed to infection.

Keywords: picobirnavirus, pigs, humans, PAGE



CRITICAL ANALYSIS OF FACTORS THAT MAY INFLUENCE THE DOMESTIC HERBIVORE RABIES DIAGNOSIS

Vitória Bueno Vilela Silveira ¹, Maria Eduarda Rodrigues Chierato ², Debóra Fernanda Pedrozo Pavani ¹, Samira Maria Achkar ¹, Helena Beatriz de Carvalho Ruthner Batista ¹, Karen Miyuki Asano ¹, Keila Iamamoto ¹, Willian de Oliveira Fahl ¹, Enio Mori ^{1,2}

¹ IP-SP - Instituto Pasteur de São Paulo (Av. Paulista, 393 - Cerqueira César, São Paulo - SP, 01311-000), ² FMVZ-USP - Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (Av. Prof. Dr. Orlando Marques de Paiva, 87 - Butantã, São Paulo - SP)

Abstract

Direct Fluorescent Antibody (DFA) test is the golden standard for rabies diagnosis because it is fast and has high sensitivity and specificity. However, since rabies is almost 100% fatal and the DFA may present low sensitivity in some specific cases, a confirmatory test is recommended. It has been postulated that the bovine species has near 100% of sensitivity in the DFA test, while equine sensitivity may be lower. For this reason, the Pasteur Institute of Sao Paulo uses the RT-PCR as a second test for equines. This study aimed to make a survey of the DFA results from February 2017 to July 2019, and compare the different techniques used for the domestic herbivore rabies diagnosis such as DFA, RT-PCR, and virus isolation (VI) in N2A cells. During this period, the Pasteur Institute tested 457 bovine central nervous system (CNS) samples and 382 equine CNS samples for rabies. Spite of 211 bovines were DFA negative, 8 of them were positive in the RT-PCR. At the same, 248 equines were DFA negative; however, 25 were positive in the RT-PCR. Therefore, 3.8% of bovines and 10% of equines were false negative in the DFA test. Moreover, 3 bovines and 3 equines were positive in the VI test. These results highlight that bovines and equines are indicating that some factors may influence DFA sensitivity. 100% of the false negative bovines (n=8) showed a fast disease progression (2 days), and 50% of them were euthanized. Therefore, the samples could have a low viral load, which may hamper the visualization of the inclusion corpuscle. Due to the lack of data, the same information cannot be applied to equines. Also, the sampling used for the DFA may not have been infected since the virus does not infect the CNS uniformly, which is why a transversal section of the brain stem could be required as recommended in these cases by WHO. On the other hand, especially for bovines, the VI test proved to be a satisfactory technique as a backup of the DFA test. A false negative diagnosis may carry tragic effects such as the death of humans due to the lack of post exposure prophylaxis. Nonetheless, additional studies are required in order to have a better understanding of the causes that may influence the rabies diagnosis and also, how the processing of CNS samples must be performed.

Financial Support: Pasteur Institute of Sao Paulo (IP-07/2019)

Keywords: Cattle, DFA, Equine, Rabies, RT-PCR



MOLECULAR MODELING AND STRUCTURAL ANALYSIS OF THE NS5B POLYMERASE OF NOVEL HEPACIVIRUS AND PEGIVIRUSES INFECTING HORSES

Pedro Pereira Lira Furtado de Albuquerque ^{2,1}, Lucianna Helene Silva dos Santos ^{5,6,7,8}, Deborah Antunes ^{3,4,1}, Ernesto Raul Caffarena ^{3,4,1}, Andreza Soriano Figueiredo ^{2,1}

¹ LADTV, IOC - Laboratório de Desenvolvimento Tecnológico em Virologia, Instituto Oswaldo Cruz (Avenida Brasil, 4.365, Pavilhão Hélio e Peggy Pereira - Manguinhos, RJ, Brasil), ² PROCC, IOC - Grupo de Biofísica Computacional e Modelagem Molecular, Programa de Computação Científica, Instituto Oswaldo Cruz (Avenida Brasil, 4.365, Manguinhos, RJ, Brasil), ³ LMMPF, UFMG - Laboratório de Modelagem Molecular e Planejamento de Fármacos, Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (Av. Antônio Carlos, 6627, Pampulha - Belo Horizonte - MG)

Abstract

Defining the three-dimensional (3D) structure of a viral protein has many biological, evolutionary, therapeutic and prophylactic perspectives. The Non-structural 5B (NS5B) protein of the *Flaviviridae* family is known for its conservation, and as an important drug target. Containing the RNA-dependent-RNA-polymerase (RdRp) domain, the NS5B is responsible for viral replication, though its conformation for the equine hepacivirus (EHCV), equine pegivirus (EPgV) and Theiler's disease associated virus (TDAV), novel equine viral infections from the *Hepacivirus* and *Pegivirus* genus, have not yet been elucidated. Thus, this work aimed to build the first *in silico* 3D model of the EHCV, EPgV and TDAV NS5B protein. EHCV subtypes 1 and 2, obtained in previous epidemiological investigations in Rio de Janeiro, and reference sequences from the EPgV and TDAV were submitted to comparative modeling using hepatitis C virus (HCV) crystallographic structures as templates: PDB 3HHK and 4WTG in open (elongation phase) and closed (chemically active phase) conformation. The following amino acid (aa) numbers are based on HCV reference isolate H77. Primary structure analysis revealed conservation of the catalytic motif A (DxxxD 220-225) and motif C (GDD 318-320). Essential aa for the RdRp activity were present such as G283, T287, N291 in motif B and K153, R158 and L/I160 in motif F, responsible for sugar selection and interaction with the incoming nucleotide, respectively. Some aa, which studies with mutation-induced analysis showed decreased or abolished HCV polymerase activity, were partially conserved in motif B, D and E. Secondary and tertiary analysis revealed conserved folding structures in both open and closed conformation among these flavivirus family members (high TM-score of 0.99 and low RMSD values, thumb domain, characteristic of the HCV enzyme. In conclusion, primary, secondary and tertiary structure analysis argue toward a similar RdRp mechanism of action for the EHCV, EPgV and TDAV, while the variability may suggest different polymerization rate or efficiency, comparatively to HCV polymerase, although further research is needed.

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Plano de Objetivos e Metas – POM, Instituto Oswaldo Cruz, Fiocruz.

Keywords: Hepacivirus equino, Pegivirus equino, Theilers Disease Associated Virus, RNA polimerase dependente de RNA, Análise estrutural



DIAGNOSTIC ACCURACY OF LENTZ BODY INCLUSIONS TEST FOR CANINE MORBILLIVIRUS DETECTION

Vivaldo Gomes da Costa ¹, Priscila Gomes de Oliveira ², Marielena Vogel Saivish ², Andréia Vitor Couto do Amaral ², Marcos Lázaro Moreli ², Ricardo Henrique Kruger ¹

¹ UnB - Universidade de Brasília (Asa Norte, Brasília-Distrito Federal, 70910-900), ² UFG - Universidade Federal de Goiás (Br364, Km 195, Setor Industrial, Jataí-Goiás, 75801-615)

Abstract

Rapid detection tests are widely used in veterinary clinical laboratories. In this context, the observation of Lentz bodies in erythrocytes and leukocytes is considered definitive diagnosis of distemper; however, its absence does not rule out the possible existence of the distemper. This disease is caused by canine distemper virus (CDV, currently termed canine morbillivirus, family *Paramyxoviridae*); it has worldwide distribution, affects mainly puppies dogs and is responsible for respiratory, gastrointestinal and neurological complications. Therefore, in view of the significant impact of CDV infections on the health of domestic dogs, the aim of this study was to evaluate the accuracy of CDV diagnosis by Lentz bodies in blood samples from dogs clinically suspected of distemper in comparison to gold standard (nested RT-PCR). The present study was approved by the ethics committee on the use of animals of the UFG (N° 054/17). Samples from dogs (n=40) were collected between 2017 and 2018 in the Veterinary Hospital of the UFG, municipality of Jataí, located in the Goiás state, Brazil. CDV RNA was extracted with QIAamp viral RNA[®] and subsequently nested-RT-PCR were performed for the purpose of detection of the CDV nucleoprotein gene. The Lentz corpuscle survey consisted of blood smear on a glass slide for microscopy, with subsequent staining by Panotic[®] kit. Analysis of the slides was performed under a 100X objective optical microscope in immersion oil. Sensitivity of the Lentz bodies test was 77.7% (90% CI: 54-100), while the specificity was 74.2% (90% CI: 61-87). The proportion of agreement between the tests by Kappa index had a value of $k = 42\%$. In the Kappa index scale this value is considered of regular agreement. Among the possible causes of false-negative number of Lentz bodies test are the high sensitivity/specificity of nested RT-PCR. While, false- positive number probably occurred due to the formation of artifacts in the staining of slides. These preliminary results demonstrated the highest possibility of occurrence of false positives in Lentz body inclusions research; however future studies must be conducted to better evaluate these hypotheses.

Financial Support: FAPDF, CNPq and CAPES.

Keywords: Canine distemper virus, Canine morbillivirus, Paramyxoviridae, Domestic dogs, Morbillivirus



PCV2a AND PCV2b DETECTION IN DOGS FROM A NON-VACCINATED PCV2 POSITIVE PIGS' HERD

Camila Alves Ferreira¹, Victor Hugo da Silva¹, Paula Martins Uchoa de Sousa¹, Alessandra Marnie Martins Gomes de Castro¹

¹ FMU - Faculdades Metropolitanas Unidas (Rua Ministro Nelson Hungria, 541 - São Paulo)

Abstract

Porcine circovirus type 2 (PCV2), discovered initially in 1998, has been associated with several disease manifestations in pigs denominated PCV2 associated disease. Recently, many researchers have revealed PCV2 could infect many other mammals like mice, calves, minks, dogs and goats. PCV2 has been currently classified (PCV2a - PCV2f), of which PCV2a, PCV2b and PCV2d are predominant. The current study aims to investigate PCV2a and PCV2b in feces samples of dogs from non-vaccinated PCV2 positive pigs' herd. The study was conducted in a PCV2-positive pig herd that has never used a PCV2-vaccine protocol. The herd had four dogs that always had access to installation and had contact with food, feces, oral fluids and mummified from the pigs' herd. A total of 15 fecal swabs were collected from four (C1 to C4) dogs that have access to the pig installations in four different moments (M1 to M4). The swabs were stored in 2 mL microtubes containing 1 mL sterile saline solution. All the samples were storage at -20 °C into DNA extraction that was performed using QIAamp DNA mini kit to quantify the genomic DNA copy numbers of PCV2 by SYBR green quantitative real-time PCR (qPCR). The reactions were performed in a final volume of 25 µL containing 5.0 µL DNA; 13 µL of TaqMan™ Universal Master Mix II (Thermo Fischer Scientific, USA), 0.5 µL of each probe at 10 µM (PCV2a VIC-GGG GAC CAA CAA AAT CTC TAT ACC CTT T-MGBNF and PCV2b FAM- CTC AAA CCC CCG CTC TGT GCC C-QSY); 1 µL of 10 each primer at 10 µM (PCV2abF: 5 'GGCGGTGGACATGATGAGA 3' and PCV2abR: 5 'GCAGGGCCAGAATCAACC 3') and sterile MilliQ water qsp. The amplifications conditions were 50°C for 2 minutes, 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute and run on a QuantiStudio3 (Applied Biosystems, USA). PCV2 cycle threshold values were converted into copy number per swabs using standard curve data. Samples with no signal by a cycle-threshold (CT) of 40 were considered negative (number of DNA copies < of the analytical sensitivity). A total of 3/12 samples were PCV2 positive, being two for PCV2a from the same dog (C1) in two different (M1 and M4) and one for PCV2b from dog C3 in one moment (M4). The viral load for PCV2 was of 30 copies of DNA/swabs and for PCV2b ranged from 41 to 51 copies of DNA/swabs. These findings suggested the possibility of PCV2a and PCV2b cross-species transmission in herds with high virus circulation.

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Keywords: Dogs, feces, Pig's herd, Porcine Circovirus, type 2



IMPROVING INFECTIOUS BRONCHITIS VIRUS ANTIGEN PROCEDURES FOR SEROLOGICAL ASSAYS

Maria Angela Orsi¹, Ana Cristina Alves De Almeida¹, Hikary Moriyama¹, Ana Paula Moraes¹, Laís Santos Rizotto, Paulo Vitor Marques Simas², Tânia Rosária Pereira Freitas³, Clarice Weis Arns¹
¹ UNICAMP - DEPARTAMENTO DE GENÉTICA, EVOLUÇÃO, MICROBIOLOGIA E IMUNOLOGIA, INSTITUTO DE BIOLOGIA, UNIVERSIDADE ESTADUAL DE CAMPINAS (RUA MONTEIRO LOBATO, INSTITUTO DE BIOLOGIA, BLOCO E, CIDADE UNIVERSITÁRIA "ZEFERINO VAZ", BARÃO GERALDO, CAMPINAS-SP, Rua rur), ² EMBRAPA - EMBRAPA- SÃO CARLOS (SÃO CARLOS), ³ LFDA/MG - FEDERAL AGRICULTURAL DEFENSE LABORATORY (PEDRO LEOPOLDO- MINAS GERAIS)

Abstract

Infectious bronchitis virus (IBV) causes a highly contagious acute avian disease caused by a coronavirus of the family *coronaviridae*. Several serological methods are applied to determine the level of protection of vaccines and infections. The Hemagglutination Inhibition (HI) test should be applied to IBV differentiation (or subtype).

This study describes an improvement in the procedure for obtaining IBV antigens for the HI test. The Massachusetts-41 (M41) IBV strain was inoculated at 10^4 DIE₅₀ into 11-day-old SPF chicken eggs incubated at 37°C. After 72h observing a typical lesion of IBV, allantoic-amniotic fluid (AAF) was collected, clarified at low-speed centrifugation at 1,100 X g for 30 minutes, titrated, filtered (0.22- μ m) and kept at -80 ° C. The AAF was then performed to ultracentrifugation at 77,000X g for 2 hours. The pellet was submitted to two distinct treatments. Primarily, pellets with a 60% concentration of antigen was treated with 10 mL phosphate buffer [0.15 M pH 7.2], after than treated with 3 mL of two different solutions, Solution-A (0.01 M Tris HCL buffer - pH 6,4) and solution- B (NaCl, KCl, CaCl₂, MgSO₄). The second treatment (40% of concentrated virus) was performed to Solution-A, then added to Solution-A and B. Phospholipase C type 1 was added to the both treatments and reached the final concentration of 2.3 and 2.6 units/mL for each treatment. Then, those treatments were incubated for 37°C for 45 minutes, fractionated and kept at -28°C. The antigens prepared above were tested against homologous IBV-Mass- 41, avian metapneumovirus, bursal Infectious virus, Newcastle diseases virus sera and human Ig. Our preliminary results showed that the Hemagglutination (HA) titer for each treatment reached 1: 2,048 and 1: 512, respectively. That means, the second treatment showed no advantage over the first treatment. The sensitivity of both antigens was 72.72%. In these assays, the Hemagglutination Inhibition (HI) test was performed without cross reactivity. The next step will be to evaluate the antigen produced in this study with variant IB-virus sera.

Financial support: CNPq, FAPESP, FUNCAMP AND IB-UNICAMP

Keywords: INFECTIOUS BRONCHITIS VIRUS, ANTIGEN , SEROLOGICAL ASSAYS



BIOCHEMICAL AND HEMATOLOGICAL PROFILE OF PREGNANT RHESUS MONKEYS (*MACACA MULATTA*) EXPERIMENTALLY INFECTED WITH ZIKA VIRUS AND TREATED WITH SOFOSBUVIR – A DESCRIPTIVE ANALYSIS

Fernanda de Oliveira Bottino ^{1,2}, Noemi Rovaris Gardinali ², Juliana Gil Melgaço ², Tatiana Kugelmeier³, Gisela Trindade Freitas ⁴, Jaqueline Mendes de Oliveira ², Marcelo Alves Pinto ²

¹ EPSJV/ Fiocruz - Escola Politécnica de Saúde Joaquim Venâncio (Rio de Janeiro, Brasil), ² IOC/ Fiocruz - Instituto Oswaldo Cruz (Rio de Janeiro, Brasil), ³ ICTB/ Fiocruz - Instituto de Ciência e Tecnologia em Biomodelos (Rio de Janeiro, Brasil), ⁴ Fiocruz - Bio-manguinhos (Rio de Janeiro, Brasil)

Abstract

Zika virus (ZIKV) infection is highly relevant for public health since a strong association between the occurrence of infection in pregnant women and congenital malformation. However, little is known about hematological and biochemical changes during ZIKV infection in pregnancy. Non-human primates may mimic both ZIKV infection and fetal neuropathogenesis. Our study aimed to perform a descriptive analysis of the biochemical and hematological profile of *Macaca mulatta* experimentally infected with ZIKV and treated with antiviral sofosbuvir (SOF). Four pregnant rhesus monkeys were inoculated subcutaneously with a ZIKV suspension containing 10^7 plaque-forming unit /mL and treated subcutaneously with SOF at 5 mg/kg/day for 15 days after 2nd day post-infection (dpi). Samples were collected from baseline (day 0) and 2, 4, 8 and 12 dpi, followed by biweekly samples until the end of pregnancy. Viral RNA isolation was confirmed by RT-qPCR. Biochemical and hematological analyses were performed by the commercial laboratory Laborlife Clinical Analyzes (Rio de Janeiro), including: aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), c-reactive protein (PCR), total cholesterol (TC), glucose (GLU), leukogram, erythrogram and platelet count. Elevation in CK values in the acute phase of infection (2 dpi) were observed in two of four animals (AB28 e AA14). AST, ALT, TC and GLU values remained within the expected normal range for the species, with animals AB18 and AB28 showing, respectively, isolated increases of TC (2 and 8 dpi) and GLU (84 dpi). Anemia, characterized by hematocrit, red blood cell count and hemoglobin dosage decreased, was observed in all animals since the beginning of the experiment. Leukopenia and lymphopenia were also observed in all four animals, both of which were more pronounced during the acute phase. After this phase ends, eosinophilia was observed in three of four animals (AD18, AB28 and AB18). All animals had mild neutrophilia and monocytosis at baseline (day 0), showing no major changes during the experiment. These results suggest a cause-effect relationship between ZIKV infection during pregnancy and changes in biochemical and hematological parameters, such as CK, leukocytes, lymphocytes and eosinophils counts.

Keywords: Zika virus, *Macaca mulatta*, Sofosbuvir, Hematology, Biochemistry



IMMUNOGENICITY OF AN INACTIVATED VACCINE AGAINST BOVINE ALPHAHERPESVIRUS TYPE 5, ASSOCIATED WITH THE THERMOLABILE ENTEROTOXIN OF ESCHERICHIA COLI

Cristina Mendes Peter ¹, Matheus Iuri Fruhauf ¹, Nadálin Yandra Botton ¹, Lariane da Silva Barcelos ¹, Rodrigo Bozembecker de Almeida ¹, Marcelo de Lima ¹, Silvia de Oliveira Hübner ¹, Geferson Fischer¹

¹ UFPel - Universidade Federal de Pelotas (Campus Universitário Capão do Leão, Prédio 1, Faculdade de Veterinária, Laboratório de Virologia e Imunologia)

Abstract

The mucosal immune system represents the initial barrier against several pathogens that use these surfaces as a gateway to the body, such as bovine alphaherpesviruses (BoHV), which use the mucous membranes, mainly nasal and genital, as the starting point of replication. The secretory immunoglobulin A (IgA) plays a fundamental role in the humoral immunity of mucosal surfaces through viral neutralization, an essential mechanism in the defense of the genital mucosa against genitally transmitted pathogens. Due to the great importance of mucosal pathways in BoHV transmission, interest in the development of vaccines that provide mucosal immunity against BoHV becomes evident. In the present study, experimental vaccines containing inactivated BoHV-5 associated with the subunit B recombinant of *Escherichia coli* thermolabile enterotoxin (rLTB) were made for intravaginal application. Thirty bovine females were divided into five groups: Placebo (PL: ova consisting of gelatin + E-MEM), Antigen (AG: gelatin + BoHV-5 + E-MEM), Adjuvant rLTB (rLTB100: gelatin + 100 µg / dose of rLTB + E-MEM), rLTB50 (rLTB50: gelatin + BoHV-5 + 50 µg rLTB + E-MEM) and rLTB100 (rLTB100: gelatin + BoHV-5 + 100 µg rLTB). Two doses of each vaccine were applied at 21-day intervals. The humoral (IgA and IgG) local (vaginal mucus and nasal swab) and systemic (serum) induced response in the inoculated animals was measured by indirect ELISA. Analysis of variance (ANOVA) was used to compare antibody levels in the ELISA test. As a result, the rLTB-containing vaccine at both concentrations was shown to increase IgA and IgG levels in the vaginal and nasal mucosa, and in animal serum (p

Financial Support: CAPES.

Keywords: Mucosal Immunity, BoHV-5, Cattle, IgA, IgG.



MOLECULAR INVESTIGATION OF THE PRESENCE OF FELINE PARAMYXOVIRUS RNA IN KIDNEYS OF DOMESTIC CATS FROM CUIABÁ, MATO GROSSO

Káryta Maria de Lima Bezerra Bertti ¹, Gabriela Molinari Darold ¹, Glaycenyra Cecília Pinheiro da Silva ¹, Jordan Gregorio Lucca Cardoso ¹, Michele Lunardi ¹

¹ UNIC - Universidade de Cuiabá (Avenida Manoel José de Arruda, 3100 - Bairro Jardim Europa - Cuiabá, Mato Grosso)

Abstract

Paramyxoviruses are enveloped, single-stranded, negative RNA viruses, which have been previously identified in a wide variety of vertebrate hosts. Feline morbillivirus (FeMV) is an emerging member of *Morbillivirus* that was first discovered in Hong Kong in 2012. Recently, its circulation was also demonstrated within cat populations from Japan, Italy, Germany, USA, Turkey, and Brazil. Upon discovery, the presence of FeMV[ML1] was associated with histopathologically confirmed tubulointerstitial nephritis (TIN) and chronic kidney disease (CKD) in cats, however the possible role of the virus as a triggering event of a disease with major systemic changes such as CKD is still uncertain. Moreover, data on the correlation between CKD and FeMV infection are insufficient and have not yet been investigated in Brazil. Thus, the aim of this study was to investigate through RT-PCR the presence of feline paramyxovirus infection in renal tissues of domestic cats, which died due to several causes, in Cuiabá, Mato Grosso state. Kidney samples of 62 domestic cats that died in veterinary clinics and at the Veterinary Hospital of the University of Cuiabá, were evaluated for presence of FeMV RNA by reverse transcription followed by semi-nested PCR assay, in order to amplify a partial fragment of the paramyxoviral L gene. Only five out of the 62 evaluated cats (8,0%) had the presence of the viral RNA in renal tissue. Data of this study showed an overall prevalence of FeMV similar to previously reported frequencies observed in other geographic regions, which ranged from 7 to 44%. This result may be due to sampling from cats that died from various causes, unlike other studies using samples from cats with some nephropathy or lower urinary tract diseases. Nevertheless, the pathogenicity of FeMV is not clear yet due to paucity of isolation of viral strains from diverse geographical regions and the chronic nature of involved diseases, thus requiring further studies to better evaluate the pathogeny of FeMV and establish if this virus is associated with other diseases. Our results confirm the circulation of the FeMV within domestic cats and this is the first study to show the presence of FeMV infection in kidneys of cats in Brazil.

Financial Support: University of Cuiabá (UNIC)

Keywords: Brazil, Chronic kidney disease, Feline morbillivirus, RT-PCR



THE FIRST REPORT OF CANINE MORBILLIVIRUS INFECTION IN GIANT ANTEATER (*MYRMECOPHAGA TRIDACTYLA*) IN BRAZIL

Melissa Belizário¹, Mayara Lima Kawasaki¹, Adriane Jorge Mendonça¹, Marisol Alves de Barros¹, Thais Oliveira Morgado¹, Carolina Fontana¹, Daniel Moura de Aguiar¹, Mateus de Assis Bianchini¹, Aneliza de Oliveira Souza¹, Amanda Raiza Gonçalves Lima Oliveira Santos¹, Edson Moleta Colodel¹, Lucas Avelino Dandolini Pavelegini¹

¹ UFMT - Universidade Federal de Mato Grosso (Av. Fernando Corrêa da Costa, nº 2367 - Bairro Boa Esperança. Cuiabá - MT - 78060-900), ² UNIC - Universidade de Cuiabá (Av. Manoel José de Arruda, 3100 - Jardim Europa, Cuiabá - MT, 78065-700)

Abstract

Canine Distemper (CD) is a multisystemic and contagious disease caused by Canine Morbillivirus (CDV), an enveloped RNA virus that replicates in epithelial, nervous and lymphoid tissues; it is released in urine, feces, saliva, oral and nasal secretions and its major infection route is from respiratory system. Beside dogs, the disease and natural hosts of CDV include species of wild terrestrial carnivores. The present study reports the natural infection of *Myrmecophaga tridactyla*, a threatened species in Brazil named the Giant anteater, that was maintained hospitalized due to health problems in consequence of mistreatment. During a hematological exam, the presence of Lentz corpuscles were visualized in leukocytes. Posterior analysis of CDV rapid test combined with RT-PCR of N and H genes sequencing and pathological findings confirmed infection by CDV. The consensus sequence generated from the N gene amplicon was deposited in the GenBank under the accession number MK552116. Phylogenetic tree of H gene was inferred by the Neighbor-Joining method and our sequence fell into a clade composed of other genotypes classified as South America isolates. The sequence of the H gene generated in this study was deposited in GenBank under accession number MN208239. Macroscopy alterations were skin hyperkeratosis and evidence of lobular septa in lung. Histopathologic findings were eosinophilic intracytoplasmic and intranuclear inclusion corpuscles in urinary bladder, kidney, lung, stomach, duodenum and jejunum tissues' cells. Both macroscopy and microscopy alterations observed during necropsy were related to CD disease. Lentz corpuscles usually appears in the early stage of CD with the firsts evidence of infection which is associated with prostration, anorexia, diarrhea, nasal and ocular secretion that started after hospitalization. The results confirmed the first report of CM in a threatened species occurring in the American continent, *Myrmecophaga tridactyla*. This is the second report of CDV infection in the order Pilosa and family *Myrmecophagidae* in the Midwestern region of Brazil. Financial support: Ministry of Education of Brazil (MEC), Federal Agency for the Support and Improvement of Higher Education (CAPES) and National Council for Scientific and Technological Development (CNPQ).

Keywords: Canine distemper, Infection, wild life, sequencing, RT-PCR



PCR SURVEY OF BOVINE ALPHAHERPESVIRUS 1 DNA IN SEMEN FROM BULLS FROM MATO GROSSO STATE

Raphael Campos Quinteiro ¹, Sandro Ribeiro da Costa ¹, Gabriela Molinari Darold ¹, Glaucenyra Cecília Pinheiro da Silva ¹, Jordan Gregorio Lucca Cardoso ¹, Michele Lunardi ¹

¹ UNIC - Universidade de Cuiabá (Rua Manoel José de Arruda, 3100, Jardim Europa, Cuiabá - MT. CEP: 78065-900)

Abstract

Bovine alphaherpesvirus 1 is regarded as one of main viral pathogens associated with negative impacts on reproduction of both beef and dairy cattle. Clinical consequences of this viral infection can occur after acute infection as well as when viral recrudescence takes place after a period of viral latency. Infected bulls usually excrete virus in the semen specially when experiencing the genital disease known as infectious pustular balanoposthitis. In infected cows, endometritis, infertility, abortions, and occasional birth of stillborn or weak calves may be seen. In order to study the frequency of excretion of bovine alphaherpesvirus 1 in semen from bulls from beef and dairy herds of Mato Grosso state, 99 animals aging ³ 24 months from eight different cattle herds, without history of reproduction failure and specific vaccination, belonging to eight municipalities, located in six out of seven macroregions of the Mato Grosso state, had an aliquot of fresh semen evaluated for the presence of viral DNA by using PCR. Total DNA was extracted with QIAamp DNA mini kit (Qiagen), following manufacturer's instructions. To amplify a partial fragment of glycoprotein C gene of bovine alphaherpesvirus 1, a PCR assay employing Platinum *Taq* DNA polymerase (Invitrogen) and the primer pair B1/Bcon was carried out. Despite the amplification of a PCR product with the expected molecular size in some semen samples, through direct sequencing of obtained amplicons the purified DNA was shown to be a result of inespecific amplification. In this investigation, excretion of bovine alphaherpesvirus 1 through semen was not observed in the bulls evaluated. Since we collected only one aliquot of semen for each bull included in this study, it is important to highlight that it is not possible to exclude presence of infection in its latent form. Once bovine alphaherpesvirus 1 can be spread by breeding infected bulls through semen, knowing the frequency of viral excretion in this body fluid of bulls from Mato Grosso is of importance so measures of prevention and control for this specific viral pathogen can be implemented to avoid or limit reproductive losses in breeding female cows.

Financial Support: University of Cuiabá (UNIC)

Keywords: brazil, cattle, glycoprotein C, PCR, reproduction



CLINICAL AND LIVER HISTOLOGICAL FINDINGS OF A CHRONIC EQUINE HEPACIVIRUS (HEPACIVIRUS A, EQHV) INFECTED HORSE

Caroline Roberta Soares Salgado ¹, Fernanda Nascimento de Godoi ⁶, Tatianne Leme Oliveira Santos Godoi ⁷, Bruno Gonçalves de Souza ⁴, Cassia Maria Molinaro Coelho ⁴, Cristiane Divan Baldani ⁵, Anna Paula Balesdent Barreira ³, Leonardo Rodrigues de Lima ³, Renato Sergio Marchevsky ², Fernando Queiroz de Almeida ³, Marcelo Alves Pinto ¹, Andreza Soriano Figueiredo ¹
¹ LADTV - Laboratório de Desenvolvimento Tecnológico em Virologia, Instituto Oswaldo Cruz (Av. Brasil, 4365- Manguinhos, Rio de Janeiro-RJ), ² LANEU - Laboratório de Neurovirulência, Biomanguinhos/Fiocruz (Av. Brasil, 4365- Manguinhos, Rio de Janeiro-RJ), ³ UFRRJ - Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro (Rodovia BR465, Km 07, s/n Zona Rual-Seropédica, RJ), ⁴ UFRRJ - Hospital Veterinário, Universidade Federal Rural do Rio de Janeiro (Rodovia BR465, Km 07, s/n Zona Rual-Seropédica, RJ), ⁵ UFRRJ - Laboratório de Patologia Clínica, Universidade Federal Rural do Rio de Janeiro (Rodovia BR465, Km 07, s/n Zona Rual-Seropédica, RJ), ⁶ UFRRJ - Instituto de Zootecnia, Universidade Federal Rural do Rio de Janeiro (Rodovia BR465, Km 07, s/n Zona Rual, Seropédica), ⁷ UFRRJ - Sede Administrativa da Fazenda Universitária, Universidade Federal Rural do Rio de Janeiro (Rodovia BR465, Km 07, s/n Zona Rual, Seropédica)

Abstract

The equine hepacivirus (EqHV, *hepacivirus A*) is the closest genetic relative to hepatitis C virus (HCV) and also shares pathogenic similarities. Infection with both hepatotropic viruses may evolve to clearance or chronicity, but unlike HCV, a few equines become chronic infected. The clinical and pathological consequences of the chronic EqHV infection are not well established with limited data available from experimental infection studies. The elucidation of the equine infection characteristics is important to help veterinary clinicians facing this new pathogen and is an interesting comparative model of hepatitis C. The aim of this study was to evaluate the clinical and histological aspects of an EqHV chronically infected horse by analyzing viral load, serum biochemical alterations and the presence of liver damage. A male 10-years-old gelding horse from Rio de Janeiro state, positive for EqHV RNA since 2014 was subjected to blood collection and ultrasound-guided liver biopsy. Blood count, serum levels of liver associated proteins, serum viral load, Hematoxylin and eosin stain (H&E) performen on ultrasound-guided liver biopsies and liver ultrasound images were obtained in June 2018. Liver associated proteins were slightly above reference values in serum: AST 370 IU/L (<366 IU/L), GGT14 IU/L (<13.4 IU/L), total bilirubin 2.8 mg/dL (<2.0 mg/dL), conjugated bilirubin=0.46 mg/dL (<0.4 mg/dL) and unconjugated bilirubin2.34 mg/dL (<2.0 mg/dL). Serum viral load was $\leq 8.0E+3$ copies/mL. Ultrasound examination revealed the liver to be isoechoic to the spleen, which may suggest a diffuse hyperechogenicity of the liver. This can be associated to chronic hepatic changes, but ultrasound-guided biopsy gives more sensitive and specific information in diffuse lesions. H&E staining of the liver tissues showed the presence of focal discrete lymphocyte infiltrates, areas of anucleated hepatocytes, ballooning and fibrosis. There were no alterations in hematological parameters., The results demonstrate a chronic course of EqHV infection of at least 4 years presenting low viral load with mild clinical and histological alterations.

Financial support: Instituto Oswaldo Cruz/Fiocruz and CNPq/Pibic

Keywords: Equine Hepacivirus, Chronic infection, Histopathology, Clinical aspects



FIRST DESCRIPTION OF THEILER'S DISEASE ASSOCIATED VIRUS (TDAV) IN BRAZIL

Maria Vitória dos Santos de Moraes¹, Caroline Cordeiro Soares², Flávia Lowen Levy Chalhoub³, Ana Maria Bispo de Filippis³, Debora Regina Lopes dos Santos⁴, Fernando Queiroz de Almeida⁴, Tatianne Leme Oliveira Santos Godoi⁵, Aline Moreira de Souza⁶, Tatiana Rozental Burdman⁷, Elba Regina Sampaio de Lemos⁷, Jenner Karlisson Pimenta dos Reis⁸, Oswaldo Gonçalves Cruz⁹, Marcelo Alves Pinto¹, Andreza Soriano Figueiredo¹

¹ LADTV - Laboratório de Desenvolvimento Tecnológico em Virologia, Instituto Oswaldo Cruz, Fiocruz (Avenida Brasil, 4365 - Manguinhos - Rio de Janeiro - Brasil), ² LVM - Laboratório de Virologia Molecular, Instituto Oswaldo Cruz, Fiocruz (Avenida Brasil, 4365 - Manguinhos - Rio de Janeiro - Brasil), ³ LF - Laboratório de Flavivírus, Instituto Oswaldo Cruz, Fiocruz (Avenida Brasil, 4365 - Manguinhos - Rio de Janeiro - Brasil), ⁴ UFRRJ - Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro (Rodovia BR 465, Km 07, s/n Zona Rural, Seropédica - Rio de Janeiro - Brasil), ⁵ UFRRJ - Coordenação de Produção Integrada ao Ensino, Pesquisa e Extensão, Reitoria, Universidade Federal Rural do Rio de Janeiro (Rodovia BR 465, Km 07, s/n Zona Rural, Seropédica - Rio de Janeiro - Brasil), ⁶ UFF - Laboratório de Pesquisa Clínica e Diagnóstico Molecular Professor Marcílio Dias do Nascimento, Departamento de Patologia e Clínica Veterinária, Faculdade de Veterinária, Universidade Federal Fluminens (Av. Alm. Ary Parreiras, 507 - Icaraí, Niterói - Rio de Janeiro - Brasil), ⁷ LHR - Laboratório de Hantavírus e Rickettsioses, Instituto Oswaldo Cruz, Fiocruz (Avenida Brasil, 4365 - Manguinhos - Rio de Janeiro - Brasil), ⁸ UFMG - Laboratório de Retrovírus, Escola de Veterinária, Universidade Federal de Minas Gerais (Av. Pres. Antônio Carlos, 6627 - Pampulha, Belo Horizonte - Minas Gerais - Brasil), ⁹ PROCC - Programa de Computação Científica, Fiocruz (Avenida Brasil, 4365 - Manguinhos - Rio de Janeiro - Brasil)

Abstract

Theiler's disease associated virus (Pegivirus D, TDAV) is a newly described member of the Pegivirus genus (Flaviviridae family) infecting horses. TDAV was described as the causative agent of an acute hepatitis outbreak (also known as Theiler's disease) in the USA, when 4 horses presented elevated hepatic enzymes with icterus, lethargy, hyporexia and photodermatitis, while another 4 horses presented elevated hepatic enzymes without clinical manifestations. Since then, TDAV has been detected as a contaminant of equine-derived serum, but not in the horse population. The aims of this study were to investigate the presence of TDAV in Brazil, to evaluate possible risk factors, presence of liver damage and genetic variability of virus. Study population comprised 500 horses from Rio de Janeiro, Mato Grosso do Sul, Minas Gerais and Espírito Santo states. Information such as age, sex, breed, activity, geographical location and management system were recorded for epidemiological analysis. TDAV was detected by real time RT-PCR using DNA intercalating SYBR Green with primers directed to 5'NC region. RT nested-PCR and semi-nested PCR directed to NS3 region were performed for phylogenetic analysis. For clinical biochemistry analyses, serum was tested in automated analyzer to determine AST, GGT and GLDH levels in either RNA positive or negative horses. To find possible risk factors associated with TDAV infection, univariate and multivariate logistic regression analyses were performed. Prevalence was 1.6% (8/500), detected only in Rio de Janeiro state, in 5 of 6 mesoregions (Metropolitan, Central, North, Northwest and Coastal



Lowlands, South being the exception). The age ranged from 3 months to 12 years, but the majority were Sequencing and phylogenetic analysis were performed together with all TDAV nucleotide sequences available in the GenBank. The nucleotide genetic distance within the Brazilian isolates was 7.9%, while within Brazilian isolates and the commercial serum isolates was 11.1%. Tree topology demonstrated the formation of two strongly supported clades. None of the horses had serum biochemical alterations. In this study we demonstrated for the first time the detection of TDAV RNA-positive horses outside the USA without any clinical signs. Infection was not statistically associated with any of the analyzed variables. TDAV has similar circulating isolates worldwide.

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Keywords: Theiler's disease associated virus, Prevalence, Risk factors, Phylogenetic analysis



IDENTIFICATION OF CLADE E AVIPOXVIRUS IN BRAZIL

Leonardo Clasen Ribeiro¹, Francielle Liz Monteiro¹, Domitila Brzoskowski Chagas¹, Tamires Ellen Tomio¹, Marcelo de Lima¹, Geferson Fischer¹, Gilberto D'Ávila Vargas¹, Sílvia de Oliveira Hübner¹

¹ UFPel - Universidade Federal de Pelotas (Campus Universitário Capão do Leão, Prédio 1, Faculdade de Veterinária, Laboratório de Virologia e Imunologia.)

Abstract

Avipoxviruses (APVs) cause fowlpox (FP) disease which results in significant economic losses in domestic poultry due decreased egg production, reduced growth, and increased mortality. APVs are enveloped viruses with a double-stranded DNA genome, belonging to the family *Poxviridae*. Three main clades (A to C) are differentiated into APVs, represented by Fowlpox virus (Clade A), Canarypox virus (Clade B) and Psitacinepox virus (Clade C). Two additional clades (D and E) were also proposed. The constant monitoring of the evolution of APVs with the genetic characterization of new isolates is an important approach to study an eventual outbreak of the disease and the evolutionary biology of these viruses. Thus, the objective of this study is to report the identification of clade E *Avipoxvirus* in Brazil. The sample was obtained from a periocular tumor-like skin lesion found in a young domestic fowl (*Gallus gallus domesticus*). The lesion was nodular, round shaped, crusty and blackened. The bird was kept in a backyard system with thirty animals of the same species, without any vaccination protocol or zootechnical and sanitary control. DNA was extracted from frozen tissue and submitted to the polymerase chain reaction (PCR), targeting the polymerase gene and P4b. Phylogenetic analysis was conducted by the MEGA 6.0, using the *neighbor-joining* method, and the evolutionary distances were computed using the *Kimura 2-parameter* model. The *Avipoxvirus* DNA was detected in the tissue sample collected from a domestic fowl. Phylogenetic analysis revealed that the amplified segment of the P4b and polymerase gene clustered in clade E. The sequence's similarity with APV isolated in Hungary in 2011, and Mozambique in 2016, was 99.2%. The late detection and description of this clade in the country could be, at least in part, due to the recent and scarce molecular characterization studies of APVs in Brazil. Furthermore, APVs are known to infect different species of wild birds, many of which are migratory. Thus, the introduction of new viruses through migratory wild birds is also a possibility. However, these hypothetical scenarios must be confirmed by further studies. Finally, epidemiologic monitoring of APV in domestic and wild-living birds is necessary for a better understanding of many aspects related to the occurrence, host range and genetic diversity of APVs in Brazil.

Financial Support: CAPES.

Keywords: APV, domestic fowl, fowlpox, P4b gene, polymerase gene

Hélio Gelli Pereira Award





FAECAL VIROME ANALYSIS OF WILD ANIMALS FROM BRAZIL

Matheus A. Duarte^{1,2}, João M. F. Silva², Clara R. Brito¹, Danilo S. Teixeira¹, Fernando L. Melo³, Bergmann M. Ribeiro², Tatsuya Nagata², Fabrício S. Campos⁴

¹Faculdade de Agronomia e Veterinária, Universidade de Brasília, Brasília-DF, ²Departamento de Biologia Celular, Instituto de Biologia, Universidade de Brasília, Brasília-DF, ³Departamento de Fitopatologia, Instituto de Biologia, Universidade de Brasília, Brasília-DF, ⁴Laboratório de Bioinformática e Biotecnologia, Campus de Gurupi, Universidade Federal do Tocantins, Tocantins-TO

Abstract

The Brazilian Cerrado fauna shows very wide diversity and can be a potential viral reservoir. Therefore, animal's susceptibility to some virus can serve as early warning signs of potential human virus diseases. Moreover, the wild animal virome of this biome is unknown. Based on this scenario, high-throughput sequencing contributes a robust tool for the identification of known and unknown virus species in this environment. In the present study feces samples from cerrado birds (*Psittacara leucophthalmus*, *Amazona aestiva* and *Sicalis flaveola*) and mammals (*Didelphis albiventris*, *Sapajus libidinosus* and *Galictis cuja*) were collected at the Veterinary Hospital, University of Brasilia. Viral nucleic acid was extracted, submitted to random amplification and sequenced by Illumina HiSeq platform. The reads were de novo assembled and the identities of the contigs were evaluated by Blastn and tblastx searches. Most viral contigs analyzed were closely related to bacteriophages. Novel archaeal viruses of the Smacoviridae family were detected. Moreover, sequences of Adenoviridae, Anelloviridae, Circoviridae, Caliciviridae and Parvoviridae families were identified. Complete and nearly complete genomes of known anelloviruses, circoviruses and parvoviruses were obtained as well as putative novel species. We demonstrate that the metagenomics approach applied in this work was effective for identification of known and putative new viruses in feces samples from Brazilian Cerrado fauna.

Keywords: BFDV, CAV, Adenoviridae, Psittacine adenovirus 3, Chapparvovirus, Gyrovirus, Norovirus, Smacoviridae

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PRODUCTION OF YFV AND HIV VIRUS LIKE PARTICLES (VLPS) USING BACULOVIRUS EXPRESSION SYSTEM AND INSECT CELLS

Roberta C. Cahú¹, Fabricio S. Morgado¹, Bruno Milhomem¹, Lorena C.S. Chaves², Bergmann M. Ribeiro¹

¹Departamento de Biologia Celular, Universidade de Brasília – Brasília, DF, Brasil;

²Boyce Thompson Institute, Ithaca, NY

Abstract

The Yellow Fever Virus (YFV) is a member of the Flaviviridae family (Flavivirus genus) of single stranded RNA viruses that infects humans and are transmitted by mosquitoes. YFV is an endemic virus in Brazil and is a major threat to the public health, especially in low income areas. This virus encodes a polyprotein that is cleaved, producing the three structural proteins that make up the infective virions. The envelope protein (E) is the most important structural antigen because it binds to cellular receptors and is responsible for the fusion of viral envelope and host membrane. In this work, we designed a baculovirus based expression vector to produce YFV E protein coupled to Human Immunodeficiency Virus GAG proteins for the generation chimera Virus Like Particles (VLPs) in lepidopteran insect cells. E protein expression and VLP formation was demonstrated by a combination of western blotting, immunofluorescence microscopy, electron microscopy and low pH membrane fusion assays. This VLPs may serve as antigens for subunit vaccine development and antibody detection. Future in vivo immunological assays in mammals will be conducted to assess the efficacy of the VLPs as a possible vaccine against YFV.

Keywords: Flavivirus; baculovirus, VLPs, Yellow fever virus



INFLUENCE OF PHAGE vB_EcoM-UFV13 ON BIOFILM FORMED BY CONSORTIUM P48SEP

Adrielle Jéssica do Carmo¹, Roberto Sousa Dias², Clara Nogueira Laguardia², Larissa Cristina Araújo², Deborah Romaskevis Gomes Lopes¹, Jéssica Duarte da Silva¹, Maíra Paula de Sousa¹, Sérgio Oliveira de Paula²

¹Department of Microbiology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil;

²Department of General Biology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil.

Abstract

Control of microbiologically induced corrosion (MIC) is a challenge for the oil exploration sector. MIC is the result of electrochemical reactions, directed by microorganisms, mainly sulphate-reducing-bacteria (SRB), which accumulate on the surface of the ducts forming biofilms. SRB use sulfate as the final electron receptor, leading to production of hydrogen sulfide, compounds highly reactive, corrosive and toxic. The main form of control of SRB is the injection of biocides, however, these chemicals cannot penetrate the biofilm matrix and reach the microorganisms that cause MIC, besides being expensive, requiring continuous application and can generate resistant bacteria. This study evaluated the potential of phage vB_EcoM-UFV13 in preventing biofilm formation by microbial consortium P48SEP, consisting of SRS. Through scanning electron microscopy, chemical analysis and cell count in the biofilm showed the phage's ability to prevent biofilm development, reduced the main biomolecules responsible for biofilm adhesion and stabilization, and the number of living cells. In addition, the presence of the virus was able to alter the gene expression pattern of the analyzed genes, as well as the relative abundance of some proteins related to biofilm formation and cell stress response. This study shows for the first time the phage's ability to prevent the formation of biofilm from SRB, being a promising alternative for the control of biocorrosion in oilfields.

Keywords: PHAGE vB_EcoM-UFV13, biofilm, microbiologically induced corrosion



PRODUCTION AND PROTOTYPING OF AN ENZYME-LINKED IMMUNOASSAY FOR DIAGNOSIS AND SURVEILLANCE OF CHIKUNGUNYA

Bagno, F. F.^{1,2}; Godoi, L. C.^{1,3}; Figueiredo, M.M.¹; Sérgio, S.A.R.¹; Carvalho, G. P.⁴, Fonseca, M.S.P.^{1,3}; Da, Fonseca, F. G.^{1,2}.

¹Centro de Tecnologia de Vacinas (CT Vacinas), BH-Tec, UFMG. Belo Horizonte, MG, Brasil.

²Laboratório de Virologia Molecular e Aplicada, Depto de Microbiologia, ICB/UFMG, Belo Horizonte, MG, Brasil. ³Colégio Técnico da UFMG (COLTEC), Belo Horizonte, MG, Brasil.

⁴Fundação Ezequiel Dias (FUNED), Belo Horizonte, MG, Brasil.

Abstract

Chikungunya virus is a re-emerging alphavirus that causes a disease characterized by febrile illness associated with arthralgia and may result in long term sequelae, including prolonged joint pain and chronic arthritis. In order to provide an accessible diagnostic tool to detect CHIKV infections, we successfully developed an indirect ELISA assay which could detect IgG antibodies against CHIKV from human sera. A total of 104 clinical samples were tested in this study in which we evaluate sensitivity, specificity, repeatability, reproducibility and stability of the developed method based on current recommendations for the development of bioanalytical products. The results demonstrated that the ELISA kit was able to detect IgG antibodies against CHIKV with high sensitivity (100%) and specificity (96%) compared with a commercially available reference kit. No cross-reactivity was found against positive sera for Dengue, Zika and rheumatoid factor. The repeatability and reproducibility assessments indicated a coefficient of variation (CV) <10%, and there was no distinct difference among three recombinant protein batches tested during the intra-assay study. Furthermore, the kit showed shelf stability of approximately 2.3 years. These data suggested that our ELISA assay has good repeatability, stability and shelf life, reaching required standards of commercially acceptable ELISA tests. The developed ELISA assay provides a convenient and specific method for the large-scale determination of CHIKV infections in human sera samples with high accuracy

Keywords: Chikungunya virus, Chikungunya Fever, arboviruses, diagnosis, ELISA, prototyping.

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MORPHOLOGIC AND GENOMIC ANALYSES OF NEW ISOLATES REVEAL A SECOND LINEAGE OF CEDRATVIRUSES

Rodrigo Araújo Lima Rodrigues^{1,2}, Julien Andreani¹, Ana Cláudia dos Santos Pereira Andrade², Talita Bastos Machado², Souhila Abdi¹, Anthony Levasseur¹, Jônatas Santos Abrahão², Bernard La Scola¹

Abstract

Giant viruses have been isolated and characterized in different environments, expanding our knowledge about the biology of these unique microorganisms. In the last 2 years, a new group was discovered, the cedratviruses, currently composed of only two isolates and members of a putative new family, “Pithoviridae,” along with previously known pithoviruses. Here we report the isolation and biological and genomic characterization of two novel cedratviruses isolated from samples collected in France and Brazil. Both viruses were isolated using *Acanthamoeba castellanii* as a host cell and exhibit ovoid particles with corks at either extremity of the particle. Curiously, the Brazilian cedratvirus is 20% smaller and presents a shorter genome of 460,038 bp, coding for fewer proteins than other cedratviruses. In addition, it has a completely asyntenic genome and presents a lower amino acid identity of orthologous genes (73%). Pangenome analysis comprising the four cedratviruses revealed an increase in the pangenome concomitant with a decrease in the core genome with the addition of the two novel viruses. Finally, phylogenetic analyses clustered the Brazilian virus in a separate branch within the group of cedratviruses, while the French isolate is closer to the previously reported *Cedratvirus lausannensis*. Taking all together, we propose the existence of a second lineage of this emerging viral genus and provide new insights into the biodiversity and ubiquity of these giant viruses.

Keywords: Cedratvirus, giant virus, NCLDV, new lineage, virion volume, genome length, pangenome

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DEVELOPMENT AND VALIDATION OF REVERSE TRANSCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP) FOR RAPID DETECTION OF ZIKV IN MOSQUITO SAMPLES FROM BRAZIL

Severino Jefferson Ribeiro da Silva¹, Marcelo Henrique Santos Paiva^{2,3}, Duschinka Ribeiro Duarte Guedes³, Larissa Krokovsky³, Fábio Lopes de Melo⁴, Maria Almerice Lopes da Silva⁴, Adalúcia da Silva¹, Constância Flávia Junqueira Ayres³, Lindomar J. Pena¹

¹Department of Virology, Oswaldo Cruz Foundation (Fiocruz), Recife, Pernambuco, Brazil.

²Agreste Academic Center, Federal University of Pernambuco (UFPE), Caruaru, Pernambuco, Brazil.

³Department of Entomology, Oswaldo Cruz Foundation (Fiocruz), Recife, Pernambuco, Brazil.

⁴Department of Parasitology, Oswaldo Cruz Foundation (Fiocruz), Recife, Pernambuco, Brazil

Abstract

The rapid spread of Zika virus (ZIKV) represents a global public health problem, especially in areas that harbor several mosquito species responsible for virus transmission, such as Brazil. In these areas, improvement in mosquito control needs to be a top priority, but mosquito viral surveillance occurs inefficiently in ZIKV-endemic countries. Quantitative reverse transcription PCR (qRT-PCR) is the gold standard for molecular diagnostic of ZIKV in both human and mosquito samples. However, the technique presents high cost and limitations for Point-of-care (POC) diagnostics, which hampers its application for a large number of samples in entomological surveillance programs. Here, we developed and validated a one-step reverse transcription LAMP (RT-LAMP) platform for detection of ZIKV in mosquito samples. The RT-LAMP assay was highly specific for ZIKV and up to 10,000 times more sensitive than qRT-PCR. Assay validation was performed using 60 samples from *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes collected in Pernambuco State, Brazil, which is at the epicenter of the Zika epidemic. The RT-LAMP had a sensitivity of 100%, specificity of 91.18%, and overall accuracy of 95.24%. Thus, our POC diagnostics is a powerful and inexpensive tool to monitor ZIKV in mosquito populations and will allow developing countries to establish better control strategies for this devastating pathogen.

Keywords: ZIKV, RT-LAMP, Mosquito, Brazil

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INTERPLAY BETWEEN THE ACTIVATION OF THE KALLIKREIN-KININ SYSTEM AND VIRUS REPLICATION DURING DENGUE VIRUS INFECTION

Sharton V. A. Coelho¹, Naiara M. Rust^{1,2}, Lucas Vellasco², Michelle P. Papa¹, Marli T. Cordeiro³, Ernesto T. A. Marques Jr ^{3,4}, Júlio Scharfstein², Luciana B. de Arruda¹

¹Departamento de Virologia, Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, RJ, Brazil; ²Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, RJ, Brazil; ³Fundação Oswaldo Cruz, Centro de Pesquisas Aggeu Magalhães, Recife, BA, Brazil; ⁴University of Pittsburgh, Pennsylvania, United States, USA.

Abstract

Exacerbated inflammation and altered hemostasis are hallmarks of dengue virus (DENV) infection and results in vascular permeability. The kallikrein-kinin system (KKS) is a branch of the intrinsic coagulation pathway that amplifies the coagulation process and generates the vasoactive and proinflammatory peptide bradykinin (BK). Activation of the BK receptor B2R was also associated to increased virus replication in a model of alphavirus infection. Here, we evaluated the activation of plasma KKS during DENV infection and investigated whether BK would affect virus replication. Plasma obtained from dengue patients showed lower kallikrein activity when challenged with a contact activator of KKS, in comparison to uninfected donors. This event was probably a consequence of the earlier consumption of the contact factors FXII and kininogen, detected in patients with different clinical forms of the disease. Treatment of DENV-infected human microvascular endothelial cells (HBMECs) with BK increased virus replication via B2R activation, which was associated with inhibition of virus-induced cell death and nitric oxide production. Importantly, inhibition of B2R decreased the viral load in the brains of DENV-infected mice. These results indicate that modulation of KKS by DENV may contribute to virus replication and point to inhibition of B2R as a potential therapeutic strategy to control DENV replication.

Keywords: DENV, bradykinin, endothelial cells, kallikrein-kinin system, contact pathway

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