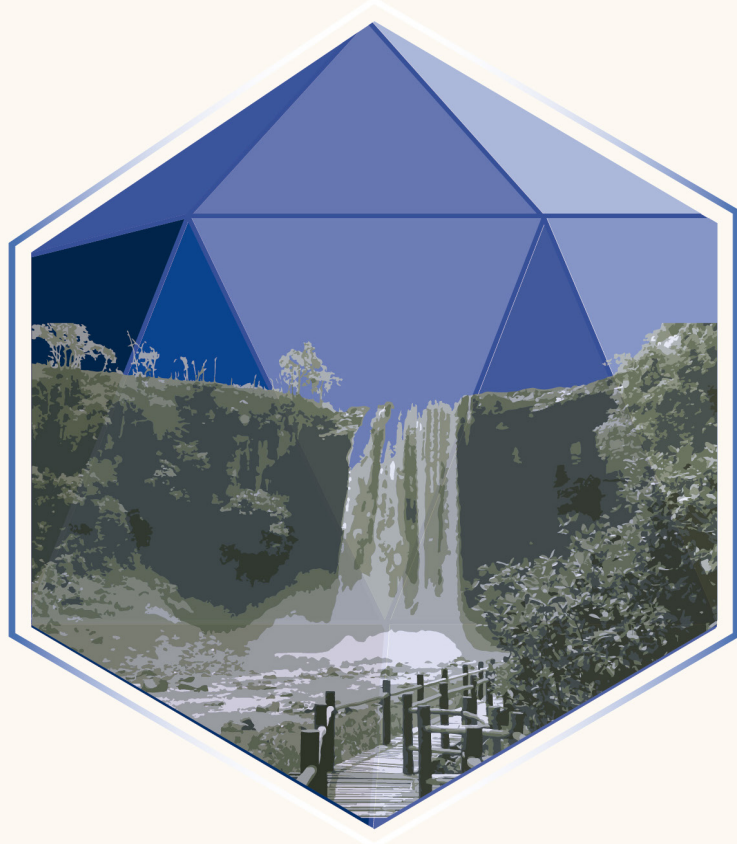


# VIRUS

Reviews and Research



## XXVII Congresso Brasileiro de Virologia

XI Encontro de Virologia do Mercosul

18 a 21 de setembro de 2016

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# Virus Reviews and Research

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Annals of the XXVII Brazilian Congress of Virology & X Mercosur Meeting of Virology  
September, 18 - 21, 2016, Pousada dos Pireneus Resort, Pirenópolis, Goiás, Brazil

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### **Secretary Office Hours**

September, 18<sup>th</sup> - 1:00 p.m. - 8:30 p.m.

September, 19<sup>th</sup> - 8:30 a.m. - 8:00 p.m.

September, 20<sup>th</sup> - 8:30 a.m. - 8:00 p.m.

September, 21<sup>st</sup> - 7:30 a.m. - 1:00 p.m.

### **Name Badge**

Name Badges will be required for access in all activities, including lunch.

### **Media Desk (for lecturers only)**

The media desk will be opened as scheduled for the secretary of the meeting. Data - files with presentations - must be delivered at the media desk at least 2 hours before the scheduled time for the presentation. Please note that personal computers will not be allowed in presentation room. Presentations will be copied and made available to members of SBV after the meeting at the institutional homepage unless not authorized by the speakers.

### **Certificates**

Certificates of attendance will be available on line at <http://www.sbv.org.br/congresso> 15 days after the end of the meeting.

### **Poster Presentations**

The posters must be displayed from 10:00 a.m. until the end of the session, and then removed.

POSTER SESSION 1: MONDAY – 19 SEPTEMBER, 6:30 - 8:00 P.M.

- Human Virology
- Basic Virology
- Environmental Virology

POSTER SESSION 2: TUESDAY - 20 SEPTEMBER, 6:30 – 8:00 P.M.

- Immunobiologicals in Virology
- Plant and Invertebrate Virology
- Veterinary Virology

## XXVII Brazilian Congress of Virology - Scientific Program

| TIME                 | ACTIVITY   |
|----------------------|--|
| Sunday, September 18 | <b>Round Table 1 - Enteric viruses / Ita e Alaor Room</b> <ul style="list-style-type: none"> <li>• <b>Adriana Luchs</b>, Instituto Adolfo Lutz, São Paulo, Brazil – “Epidemiology of noroviruses: challenges and developments”</li> <li>• <b>Alejandro Andrés Castello</b>, National University of Quilmes, Quilmes, Argentina – “Rotaviruses circulating in Argentina during the last years. Could massive vaccination in Brazil influence genotype frequencies and strain characteristics?”</li> <li>• <b>Mariela Martínez Gómez</b>, FIOCRUZ, Rio de Janeiro, Brazil – “Monitoring the genetic diversity of human Rotavirus A strains in Brazil after vaccine introduction”</li> <li>• <b>Fernando Rosado Spilki</b>, FEEVALE, Rio Grande do Sul, Brazil (Chair)</li> </ul> |
|                      | <b>Round Table 2 - Invertebrate virus diversity / Noemi Jaime Room</b> <ul style="list-style-type: none"> <li>• <b>Daniel Mendes Pereira Ardisson de Araújo</b>, UFSM, Rio Grande do Sul, Brazil – “Insect viruses in Brazil”</li> <li>• <b>Cintia Bittar Oliva</b>, UNESP, São Paulo, Brazil - “Culex flavivirus diversity”</li> <li>• <b>João Trindade Marques</b>, UFMG, Minas Gerais, Brazil - “Surveillance of insect viromes using virus-derived small RNAs”</li> <li>• <b>Bergmann Morais Ribeiro</b>, UnB, Distrito Federal, Brazil (Chair)</li> </ul>   |
|                      | <b>Round Table 3 - RNA viruses cell biology / Principal Room</b> <ul style="list-style-type: none"> <li>• <b>Ronaldo da Silva Mohana Borges</b>, UFRJ, Rio de Janeiro, Brazil – “Unveiling the role of flavivirus NS1 protein on host cell regulation”</li> <li>• <b>Patricia Garcez</b>, UFRJ, Rio de Janeiro, Brazil – “Zika virus impairs brain development”</li> <li>• <b>Luis Lamberti Pinto da Silva</b>, USP, São Paulo, Brazil – “Mechanisms of Oropouche virus assembly in mammalian cells” (Chair)</li> </ul>  |
|                      | <b>Round Table 4 - Emergent viruses in veterinary / Cavalhadas Room</b> <ul style="list-style-type: none"> <li>• <b>Leila Sabrina Ullmann</b>, UNESP, São Paulo, Brazil - “Exploring the virome of diseased horses”</li> <li>• <b>Eduardo Furtado Flores</b>, UFSM, Rio Grande do Sul, Brazil – “HoBi-like pestivirus infection”</li> <li>• <b>Ana Carolina Diniz Matos</b>, UFMG, Minas Gerais, Brazil – “Bluetongue: the emergence of clinical disease in Brazil”</li> <li>• <b>Zélia Inês Portela Lobato</b>, UFMG, Minas Gerais, Brazil (Chair)</li> </ul>   |
| 7:00 - 9:00 P.M.     | <b>Opening Ceremony - CONFERENCE 1 / Principal Room</b> <ul style="list-style-type: none"> <li>• <b>Charles M. Rice</b>, The Rockefeller University, New York, United States – “Hepatitis C and beyond: Never a dull moment”</li> </ul>  |
| 9:00 - 11:00 P.M.    | Cocktail reception and visit to exhibits / <b>Centro de Convenções Luciano Peixoto Hall</b>  |

| TIME                 | ACTIVITY   |   |
|----------------------|--|---|
| Monday, September 19 | <b>CONFERENCE 2 / Principal Room</b> <ul style="list-style-type: none"> <li>• <b>Pedro Fernando da Costa Vasconcelos</b>, Instituto Evandro Chagas, Pará, Brazil – “Zika virus in the Americas: Early epidemiological and genetic findings”</li> </ul> |   |
|                      | 10:00 - 10:30 A.M.   | Coffee break and visit to exhibits / <b>Centro de Convenções Luciano Peixoto Hall</b>   |
|                      | 10:30 - 12:00 A.M.   | <b>Round Table 5 - Viral diagnosis and treatment / Noemi Jaime Room</b> <ul style="list-style-type: none"> <li>• <b>Isabel Guedes Mello</b>, Butantan, São Paulo, Brazil – “Antivirals: mechanisms of action”</li> <li>• <b>Celso Francisco Hernandes Granato</b>, UNIFESP, São Paulo, Brazil – “The use of laboratory tests in diagnosis and monitoring of infected hepatitis virus types B and C patients”</li> <li>• <b>Menira Souza</b>, UFG, Goiás, Brazil – “Viral detection and molecular characterization”</li> <li>• <b>Paula Rahal</b>, UNESP, São Paulo, Brazil (Chair)</li> </ul> <b>Round Table 6 - Plant viruses vector interactions / Principal Room</b> <ul style="list-style-type: none"> <li>• <b>Renate Krause Sakate</b>, UNESP, São Paulo, Brazil - “Virus transmission by Brazilian native and invasive species of Bemisia tabaci”</li> <li>• <b>Jesús Navas-Castillo</b>, IHSM-UMA-CSIC, Algarrobo-Costa, Málaga, Spain - “Differential transmission of criniviruses and begomoviruses by whiteflies”</li> <li>• <b>William M. Wintermantel</b>, USDA, Salinas, United States - “Understanding the connection between gene expression in the whitefly and the biology of crinivirus transmission”</li> <li>• <b>Tatsuya Nagata</b>, UNB, Brasília, Brazil (Chair)</li> </ul> |

| TIME              | ACTIVITY  |
|-------------------|---|
| 10:30 - 12:00 A.M | <b>Round Table 7 - Environmental virology / Ita e Alaor Room</b> <ul style="list-style-type: none"> <li>• <b>Ana Cláudia Franco</b>, UFRGS, Rio Grande do Sul, Brazil - "Giant viruses in environmental samples"</li> <li>• <b>Fernando R. Spilki</b>, FEEVALE, Rio Grande do Sul, Brazil - "In the name of Poseidon, what's in Olympic waters?"</li> <li>• <b>Célia R. M. Barardi</b>, UFSC, Santa Catarina, Brazil - "New insights in Environmental Virology: approaches for infectivity and disinfection evaluation" (Chair)</li> </ul>  |
|                   | <b>Round Table 8 - Virus cell interaction / Cavalhadas Room</b> <ul style="list-style-type: none"> <li>• <b>Daniele da Glória de Souza</b>, UFMG, Minas Gerais, Brazil - "Dengue virus requires the CC-chemokine receptor CCR5 for replication and infection development"</li> <li>• <b>Renato Santana de Aguiar</b>, UFRJ, Rio de Janeiro, Brazil - "Clinical Neuropathogenesis and Immuneactivation of arboviruses (Zika, Chikungunya and Dengue)"</li> <li>• <b>Luciana Jesus da Costa</b>, UFRJ, Rio de Janeiro, Brazil - "HIV-1 nef inhibits protease activity of viral particles" (Chair)</li> </ul>  |
| 12:00 - 1:00 P.M. | <b>Mini-course 1 / Noemi Jaime Room</b> <ul style="list-style-type: none"> <li>• <b>Fernando Melo</b>, UNB, Distrito Federal, Brazil - "Next generation sequencing technologies for viral metagenomic analyses"</li> </ul>  |
|                   | <b>Mini-course 2 / Cavalhadas Room</b> <ul style="list-style-type: none"> <li>• <b>Antônio Augusto Fonseca Júnior</b>, LANAGRO, Minas Gerais, Brazil - "Viral phylogeny and sequence analysis"</li> </ul>   |
|                   | <b>Mini-course 3 / Principal Room</b> <ul style="list-style-type: none"> <li>• <b>Vitor Bortolo de Rezende</b>, BD Biosciences - "Uses of flow cytometry in virology"</li> </ul>  |
|                   | <b>Mini-course 4 / Ita e Alaor Room</b> <ul style="list-style-type: none"> <li>• <b>Luciana Jesus da Costa</b>, UFRJ, Rio de Janeiro, Brazil - "Viral replication mechanism"</li> </ul>   |
| 12:00 - 2:00 P.M. | Lunch break   |
| 2:00 - 3:30 P.M.  | <b>Oral presentations:</b> <ul style="list-style-type: none"> <li>• Session 1 – <b>Human / Principal Room - Chair: Eurico de Arruda Neto</b></li> </ul> <b>30 - HEPATIC MIRNA PROFILE IN DENGUE HEMORRHAGIC FEVER AND ASSOCIATION WITH APOPTOSIS REGULATION, VASCULAR INJURY AND INFLAMATION</b><br>Oliveira, L.F.; Vianez, J.L.G.; Pagliari, C.; Carvalho, L.V.; Silveira, T.S.; Telles, A.L.; Cardoso, J.F.; Vasconcelos, J.M.; Moreira-Nunes, C.A.; Burbano, R.M.R.; Nunes, M.R.T.; Santos, E.J.M.<br><b>179 - MUTATIONS PROFILE IN HIV TRANSCRIPTASE REVERSA AND PROTEASE GENES IN HIV/HBV AND HIV/HCV COINFECTED PATIENTS</b><br>Grotto, R.M.T.; Cantão, N.M.; Fogaça, L.; Wolf, I.; Almeida, R.; Cruz, A.A.; Barbosa, A.N.; Silva, G.F.; Valente, G.T.; Pardini, M.I.M.C.; Grotto, R.M.T.<br><b>207 - EFFICIENT PRODUCTION OF GP64 FREE HIV-1 VIRUS-LIKE PARTICLES (VLPS) USING BACULOVIRUS EXPRESSION SYSTEM</b><br>Chaves, L.C.S.; Ribeiro, B.M.; Blissard, G.W.<br><b>234 - IN SITU EVIDENCE ON INFLUENZA VIRUS INFECTION OF LYMPHOID CELLS IN HUMAN TONSILLAR TISSUES</b><br>Castro, I.A.; Martins Junior, R.B.; Jesus, B.L.S.; Pontelli, M.C.; Prates, M.C.; Silva, M.L.; Carezni, L.R.; Tamashiro, E.; Anselmo-Lima, W.T.; Arruda, E. |
|                   | <ul style="list-style-type: none"> <li>• Session 2 – <b>Veterinary / Noemi Jaime Room - CHAIRS: Marcos Bryan Heinemann and João Pessoa Araújo Junior</b></li> </ul> <b>9 - RECONSTRUCTION OF THE SPATIAL DISPERSION OF INFECTIOUS BRONCHITIS VIRUS: IBV FINDS ITS ROOTS</b><br>Saraiva, G.L.; Vidigal, P.M.P.; Pereira, C.G.; Figueiredo, J.F.; Campo, A.J.; Fietto, J.L.R.; Bressan, G.C.; Silva Júnior, A.; Almeida, M.R.<br><b>62 - SYSTEMIC AND MUCOSAL ANTIBODY RESPONSES INDUCED BY A VACCINE OF INACTIVATE AVIAN INFECTIOUS BRONCHITIS VIRUS (IBV) ENCAPSULATED IN CHITOSAN NANOPARTICLES</b><br>Lopes, P.D.; Okino, C.H.; Casagrande, V.M.; Pavani, C.; Fernando, F.S.; Tamanini, M.L.F.; Montassier, M.F.S.; Lopez, R.F.V.; Montassier, H.J.<br><b>74 - GENOMIC CHARACTERIZATION OF A NOVEL HUMAN INFLUENZA A(H1N2) VARIANT DETECTED IN BRAZIL</b><br>Resende, P.C.; Born, P.S.; Matos, A.R.; Motta, F.C.; Caetano, B.C.; Debur, M.C.; Riediger, I.; Brown, D.; Siqueira, M.M.   |

| TIME   | ACTIVITY  |
|--|---|
| 2:00 - 3:30 P.M.   | <b>80 - GENETIC CHARACTERIZATION OF INFLUENZA VIRUSES CIRCULATING WITHIN BRAZILIAN SWINE BETWEEN 2009 AND 2016</b><br>Schaefer, R.; Gava, D.; Nelson, M.I.; Haach, V.; Ciacci-Zanella, J.R.; Cantão, M.E.   |
|  | <b>85 - NEONATAL PIG MORTALITY ASSOCIATED WITH SENECAVIRUS A</b><br>Gava, D.; Lorenzetti, M.P.; Haach, V.; Driemeier, D.; Joshi, L.R.; Mohr, K.A.; Diel, D.G.; Caron, L.; Morés, N.; Morés, M.A.Z.; Schaefer, R.  |
|  | <b>143 - BRAZILIAN BATS AS CARRIERS OF VIRUSES WITH ZONOTIC POTENTIAL</b><br>Simas, P.V.M.; Barnabé, A.C.S.; Caserta, L.C.; Martini, M.C.; Durões Carvalho, R.; Fellippe, P.A.N.; Ferreira-Neto, D.L.; Beck, R.M.; Nascimento, G.M.; Jacomassa, F.A.F.; Moraes, A.P.; Miller, M.E.; Arns, C.W.  |
|  | <b>166 - EVALUATION OF SEROLOGICAL AND VIREMIC PROFILE FOR PORCINE CIRCOVIRUS TYPE 2 IN NATURALLY INFECTED PIGS FROM FARROW-TO-FINISH FARMS IN MINAS GERAIS STATE, BRAZIL</b><br>Dias, A.S.; Rehfeld, I.S.; Gallinari, G.C.F.; Costa, A.G.; Guedes, M.I.M.C.; Lobato, Z.I.P.  |
|  | • Session 3 – Basic / Ita e Alaor Room - Chair: Luis Lamberti Pinto da Silva  |
|  | <b>75 - IDENTIFICATION OF CELL PROTEINS THAT INTERACT WITH HUMAN RESPIRATORY SYNCYTIAL VIRUS M2-1 PROTEIN</b><br>Araujo, C.L.; Eléouët, J.F.; Ventura, A.M.   |
|  | <b>79 - TROPOMIOSIN INTERACTION WITH HUMAN RESPIRATORY SYNCYTIAL VIRUS MATRIX PROTEIN</b><br>Dias, T.D.; Oliveira, A.P.; Ogawa, J.K.; Eléouët, J.F.; Ventura, A.M.  |
|  | <b>168 - EVALUATION OF APOPTOTIC MECHANISMS MEDIATED BY UNFOLDED PROTEIN RESPONSE PATHWAY IN JURKAT CELLS STIMULATED WITH HIV-1 TAT PROTEIN</b><br>Campestrini, J.; Costa-Junior, A.O.; Pinto, A.R.   |
|  | <b>214 - RESPIRATORY SYNCYTIAL VIRUS MRNA TRANSCRIPTOME REVEALS SURPRISING PROFILES DURING ONE-STEP REPLICATION CYCLE</b><br>Jesus, B.L.S.; Cardoso, R.S.; Criado, M.F.; Souza, M.M.; Oliveira, A.S.; Prates, M.C.M.; Ventura, A.M.; Arruda, E.   |
|  | <b>215 - OROPOUCHE VIRUS ASSEMBLY IN MAMMALIAN CELLS REQUIRES THE ACTIVITY OF HOST ESCRT PROTEINS</b><br>Barbosa, N.S.; Mendonca, L.L.R.; Criado, M.; Arruda, E.; da Silva, L.L.P.  |
| • Session 4 – Plant and Invertebrates / Cavalhadas Room - Chairs: Alice Nagata and Tatsuya Nagata  |   |
| <b>102 - MOLECULAR CHARACTERIZATION OF GRAPEVINE ENAMO-LIKE VIRUS, A NOVEL PUTATIVE MEMBER OF THE GENUS ENAMOVIRUS</b><br>Silva, J.M.F.; Fajardo, T.V.M.; Al Rwahnih, M.; Blawid, R.; Nagata, T.   |   |
| <b>117 - IDENTIFICATION AND FUNCTIONAL ANALYSES OF THE COTTON BLUE DISEASE RESISTANCE LOCUS</b><br>Fausto, A.K.S.; Moura, M.O.; da Franca, T.S.; Romanel, E.; Vaslin, M.F.S.   |   |
| <b>118 - DICER-LIKE PROFILE EXPRESSION DURING VIRAL INFECTION IN SUSCEPTIBLE AND RESISTANT COTTON</b><br>Moura, M.O.; Fausto, A.K.S.; da Franca, T.S.; Romanel, E.; Vaslin, M.F.S.   |   |
| <b>175 - ARRACACIA XANTHORRIZA (MANDIOQUINHA-SALSA): A RESERVOIR OF PLANT VIRUS</b><br>Orlilio, A.F.; Inoue-Nagata, A.K.; Nagata, T.; Madeira, N.R.; Resende, R.O.; Blawid, R.   |   |
| <b>209 - THE COMPLETE GENOME SEQUENCE OF A NOVEL BETABACULOVIRUS ISOLATED FROM MOCIS SP. REVEALS AN ANCIENT GENOME EXPANSION AND A TENDENCY IN NOCTUID-INFECTING BETABACULOVIRUS</b><br>Ardisson-Araújo, D.M.P.; Melo, F.L.; Sosa-Gómez, D.R.; Ribeiro, B.M. |   |
| <b>244 - STUDY OF BEGOMOVIRUS DIVERSITY IN TOMATO PLANTS USING NEXT-GENERATION SEQUENCING</b><br>Rêgo, C.M.; Nakasu, E.Y.T.; Blawid, R.; Nagata, T.; Inoue-Nagata, A.K.  |   |
| 3:30 - 4:00 P.M.   | Coffee break and visit to exhibits / <b>Centro de Convenções Luciano Peixoto Hall</b>   |
| 4:00 - 5:00 P.M.   | <b>CONFERENCE 3 / Principal Room</b><br>• <b>Santiago F. Elena</b> , Instituto de Biología Molecular y Celular de Plantas, València, Spain - “Evolutionary and systems biology of RNA virus emergence”  |
| 5:00 - 6:30 P.M.   | <b>Round Table 9 - Update to arboviral diseases / Principal Room</b><br>• <b>Mauricio Lacerda Nogueira</b> , FAMERP São Paulo, Brazil – “Lessons from zika virus infection in São Paulo state” (Chair)<br>• <b>Paolo Marinho de Andrade Zanotto</b> , USP, São Paulo, Brazil – “A Zika virus-associated microcephaly case with background exposure to STORCH agents”<br>• <b>Renato Santana de Aguiar</b> , UFRJ, Rio de Janeiro, Brazil – “Zika outbreak: more questions than answers” |
| 6:30 - 8:00 P.M.   | <b>Poster Session 1 and Visit to Exhibits / Centro de Convenções Luciano Peixoto Hall</b><br>• Human Virology;<br>• Basic Virology;<br>• Environmental Virology   |

| TIME               | ACTIVITY   |
|--------------------|--|
| 9:00 - 10:30 A.M.  | <b>Round Table 10 – Animal coronaviruses / Ita e Alaor Room</b> <ul style="list-style-type: none"> <li>• <b>Paulo Eduardo Brandão</b>, USP, São Paulo, Brazil – “Mutant spectrum and molecular markers in Feline Coronavirus”</li> <li>• <b>Luiz Gustavo Bentim Góes</b>, USP, São Paulo, Brazil – “Coronavirus in bats”</li> <li>• <b>Hélio Montassier</b>, UNESP, São Paulo, Brazil – “Molecular epidemiology and evolution of avian infectious bronchitis virus”</li> <li>• <b>Marcos Bryan</b>, USP, São Paulo, Brazil (Chair)</li> </ul>  |
|                    | <b>Round Table 11 – Respiratory viruses - Noemi Jaime Room</b> <ul style="list-style-type: none"> <li>• <b>Edison Durigon</b>, USP, São Paulo, Brazil – “RSV mutations: implications for molecular diagnosis and resistance to neutralization”</li> <li>• <b>Nancy Bellei</b>, UNIFESP, São Paulo, Brazil – “Role of polyomavirus in severe respiratory disease in hospitalized patients”</li> <li>• <b>Eurico de Arruda Neto</b>, USP, São Paulo, Brazil – “Respiratory virus infection of lymphoid tissues”.(Chair)</li> </ul>   |
|                    | <b>Round Table 12 – Dengue virus vaccine development - Principal Room</b> <ul style="list-style-type: none"> <li>• <b>Eric Plennevaux</b>, Sanofi-Pauster, Paris, France – “Global strategic program management head for Dengue virus”</li> <li>• <b>David McIntosh</b>, Takeda Vaccines Inc. – “Takeda’s Dengue Vaccine Candidate: Program Update”</li> <li>• <b>Flavio Guimarães Fonseca</b>, UFMG, Minas Gerais, Brazil (Chair)</li> </ul>  |
|                    | <b>Round Table 13 – Plant virology and phytopathology / Cavalhadas Room</b> <ul style="list-style-type: none"> <li>• <b>Santiago F. Elena</b>, Instituto de Biología Molecular y Celular de Plantas, València, Spain - “Resistance to RNA virus based on the expression of amiRNAs: promises and disappointments”</li> <li>• <b>Maité Vaslin de Freitas Silva</b>, UFRJ, Rio de Janeiro, Brazil - “Identification and molecular characterization of the locus cbd associate to Cotton blue disease resistance”</li> <li>• <b>Francisco Murilo Zerbini</b>, UFV, Minas Gerais, Brazil – “Finding the needle(s) in the haystack: investigating within-host begomovirus populations with NGS”</li> <li>• <b>Renato de Oliveira Resende</b>, UNB, Distrito Federal, Brazil (Chair)</li> </ul>  |
| 10:30 - 11:00 A.M. | Coffee break and visit to exhibits / <b>Centro de Convenções Luciano Peixoto Hall</b>  |
| 11:00 - 12:00 A.M. | <b>CONFERENCE 4 / Principal Room</b> <ul style="list-style-type: none"> <li>• <b>Concepta Margaret McManus Pimentel</b>, Diretora de Relações Internacionais da CAPES - “The role of Capes for internacionalization of Brazilian Universities”</li> </ul>  |
| 12:00 - 1:00 P.M.  | <b>Mini-course 1 / Noemi Jaime Room</b> <ul style="list-style-type: none"> <li>• <b>Fernando Melo</b>, UNB, Distrito Federal, Brazil - “Next generation sequencing technologies for viral metagenomic analyses”</li> </ul>   |
|                    | <b>Mini-course 2 / Cavalhadas Room</b> <ul style="list-style-type: none"> <li>• <b>Antônio Augusto Fonseca Júnior</b>, LANAGRO, Minas Gerais, Brazil - “Viral phylogeny and sequence analysis”</li> </ul>  |
|                    | <b>Mini-course 3 / Principal Room</b> <ul style="list-style-type: none"> <li>• <b>Vitor Bortolo de Rezende</b>, BD Biosciences - “Uses of flow cytometry in virology”</li> </ul>   |
|                    | <b>Mini-course 4 / Ita e Alaor Room</b> <ul style="list-style-type: none"> <li>• <b>Luciana Jesus da Costa</b>, UFRJ, Rio de Janeiro, Brazil - “Viral replication mechanism”</li> </ul>  |
| 12:00 - 2:00 P.M.  | Lunch break  |
| 2:00 - 3:30 P.M.   | <b>Oral presentations:</b> <ul style="list-style-type: none"> <li>• Session 5 – Human / <b>Principal Room - Chair: Eurico de Arruda Neto</b></li> </ul>  |
|                    | <b>11 - INCREASED PRO-INFLAMMATORY CYTOKINES IN AMNIOTIC FLUID FROM ZIKA VIRUS ASSOCIATED MICROCEPHALY</b><br>Ornelas, A.M.M.; Pezzuto, P.; Silveira, P.P.; Melo, F.O.; Ferreira, T.A.; Oliveira-Szejnfeld, P.S.; Leal, J.I.; Amorim, M.M.R.; Cardoso, C.C.; Nixon, D.F.; Tanuri, A.; Melo, A.S.; Aguiar, R.S.<br><b>156 - ACTIVATION OF INTRINSIC COAGULATION PATHWAY AND LIPID METABOLISM IN DENGUE VIRUS PATHOGENESIS</b><br>Coelho, S.V.A.; Vellasco, L.; Marques J.R.E.T.A.; Scharfstein, J.; Arruda, L.B.<br><b>206 - IDENTIFICATION AND SELECTION OF DENGUE VIRUS SPECIFIC PEPTIDES FOR DIFFERENTIAL DIAGNOSTIC TESTS</b><br>Versiani, A.F.; Mendes, T.A.O.; Bartholomeu, D.C.; Nogueira, M.L.; da Fonseca, F.G.<br><b>254 - SALIVA AS THE BIOLOGICAL SAMPLE OF CHOICE FOR THE MOLECULAR DIAGNOSIS OF ZIKA</b><br>Monteiro, D.C.S.; Mejía, M.C.C.; Abdalla, L.F.; Santos, J.H.A.; Almeida, T.P.A.; Corado, A.L.G.; Souza, V.C.; Nascimento, V.A.; Naveca, F.G.<br><b>256 - ETIOLOGY OF THE ACUTE FEBRILE ILLNESS IN THE AMAZON STATE BRAZIL, DURING THE EMERGENCE OF ZIKA VIRUS</b><br>Nascimento, V.A.; Monteiro, D.C.S.; Silva, M.S.; Souza, V.C.; Corado, A.L.G.; Naveca, F.G. |

| TIME             | ACTIVITY   |
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|                  | <p><b>258 - SOROPREVALENCE DENGUE IGG IN PATIENTS IN A PROSPECTIVE COHORT STUDY OF SÃO JOSÉ DO RIO PRETO</b><br/>Silva, R.A.; Silva, G.C.D.; Zini, N.; Kanazawa, T.; Estofolete, C.F.; Watanabe, A.S.A.; Terzian, A.C.B.; Nogueira, M.L.</p> <ul style="list-style-type: none"> <li>• Session 6 – <b>Environmental / Ita e Alaor Room - Chair: Célia R. M. Barardi</b></li> </ul> <p><b>14 CORROSION AND BIOFILM REDUCED BY ECOPHAGES IN A PILOT ANAEROBIC SYSTEM</b><br/>Dias, R.S.; Oliveira, M.D.; Silva, J.D.; Bicalho, K.M.; Sousa, M.P.; Santos, V.V.C.M.; Silva, E.D.; Akamine, R.N.; Silva, C.C.; de Paula, S.O.</p> <p><b>49 - ENTERIC PATHOGENS SURVIVAL, PERCOLATION AND LEACHING IN BIOFERTILIZED SOILS USING SWINE DIGESTATE</b><br/>Fongaro, G.; García-González, M.C.; Hernández, M.; Kunz, A.; Barardi, C.R.M.; Rodríguez-Lázaro, D.</p> <p><b>54 - ROTAVIRUS AND OTHER HUMAN ENTERIC VIRUSES IN GASTROPODS</b><br/>Gularte, J.S.; Staggemeier, R.; Demoliner, M.; Heck, T.M.S.; Heldt, F.H.; Ritzel, R.G.F.; Henzel, A.; Spilki, F.R.</p> <p><b>88- DETECTION AND MOLECULAR CHARACTERIZATION OF GEMCIRCULAR VIRUS FROM ENVIRONMENTAL SAMPLES IN BRAZIL</b><br/>Assis, M.R.S.; Vieira, C.B.; Fioretti, J.M.; Rocha, M.S.; Almeida, P.N.; Miagostovich, M.P.; Fumian, T.M.</p> <p><b>177 - ADENOVIRUS INVESTIGATION BY MOLECULAR ANALYSIS IN PUBLIC WATER SUPPLY NETWORK</b><br/>Ferreira, C.S.; Sa-Oliveira, J.C.; Resque, R.L.; Ferreira, C.L.; Silva, E.S.; Muller, E.C.A.</p> <p><b>229 - CONSTRUCTED WETLANDS AS AN ALTERNATIVE SYSTEM TO REMOVE ENTERIC VIRUSES FROM WASTEWATER</b><br/>Moresco, V.; Magri, M.E.; Sezerino, P.H.; Barardi, C.R.M.</p> <p><b>265 - VIRAL STUDY IN UNTREATED AND TREATED SEWAGE WATER</b><br/>Moriya, N.M.N.; Blawid, R.; Silva, J.M.F.; Batista, L.F.; Nagata, T.</p> <ul style="list-style-type: none"> <li>• Session 7 – <b>Basic / Noemi Jaime Room - Chair: Luciana Jesus da Costa</b></li> </ul> <p><b>26 - THE ASIAN-AMERICAN VARIANT OF HUMAN PAPILLOMAVIRUS TYPE 16 EXHIBITS HIGHER ACTIVATION OF MAPK AND PI3K/AKT SIGNALING PATHWAYS, TRANSFORMATION, MIGRATION AND INVASION OF PRIMARY HUMAN KERATINOCYTES</b><br/>Hochmann, J.; Sobrinho, J.S.; Villa, L.L.; Sichero, L.</p> <p><b>174 - ACTIVATION AND DEATH PATHWAYS INDUCED BY DENGUE VIRUS IN INFECTED AND BYSTANDER ENDOTHELIAL CELLS</b><br/>Papa, M.P.; Slongo, J.; Arruda, L.B.</p> <p><b>192 - SCREENING TESTS TO EVALUATE THE EFFECTIVENESS AND TOXICITY OF TEN ANTIVIRAL DRUG CANDIDATES DEVELOPED BY BIOISOTHERM</b><br/>Fonseca, V.W.P.; Menegatti, R.; Costa, L.J.</p> <p><b>205 - THE NON-GLYCOSYLATED HRSV PROTEINS M AND N ARE ADDRESSED TO THE VIRAL ASSEMBLY SITE THROUGH THE SECRETORY PATHWAY</b><br/>Cardoso, R.S.; Jesus, B.L.S.; Carvalho, A.N.; Criado, M.; Viana, R.M.M.; Milani, E.R.; Viana, R.M.M.; Prates, M.; Rosales, R.; Ventura, A.M.; Silva, L.L.P.; Arruda, E.</p> <p><b>213 - HUMAN TONSIL EXPLANTS SUPPORT RHINOVIRUS INFECTION EX VIVO</b><br/>Martins Junior, R.B.; Gagliardi, T.B.; Criado, M.F.; Cardoso, R.S.; Jesus, B.L.; Silva, M.L.; Carenzi, L.R.; Tamashiro, E.; Valera, F.; Lima, W.; Arruda, E.</p> <p><b>243 - DELETION OF THE M SEGMENT NON STRUCTURAL PROTEIN (NSm) OF OROPOUCHE VIRUS AFFECTS VIRUS ASSEMBLY AND THE ARCHITECTURE OF VIRAL FACTORIES</b><br/>Cardoso, R.S.; Barbosa, N.S.; Acrani, G.O.; da Silva, L.L.P.; Arruda, E.</p> <ul style="list-style-type: none"> <li>• Session 8 – <b>Plant and invertebrates / Cavalhadas Room - Chair: Bergmann Ribeiro</b></li> </ul> <p><b>6 - TRANSLATIONALLY CONTROLLED TUMOR PROTEIN IS NECESSARY FOR AN EFFICIENT POTYVIRUS REPLICATION</b><br/>Bruckner, F.P.; Laliberté, J.F.; Alfenas-Zerbini, P.</p> <p><b>28 - VIROME IN ORNAMENTAL PLANTS FROM DISTRITO FEDERAL, BRAZIL</b><br/>Brant, P.M.; Nagata, T.; Pereira-Carvalho, R.C.</p> <p><b>72 - SEQUENCING OF THE COTTON ANTHOCYANOSIS VIRUS BY SMALL RNA DEEP SEQUENCING AND ITS SIVRNAS PROFILE IN COTTON</b><br/>Santos, R.O.; Fausto, A.K.S.; Andrade, R.; da Franca, T.S.; Giband, M.; Vaslin, M.F.S.</p> <p><b>99 - dsRNA DEEP SEQUENCING REVEALS FIVE VIRAL SPECIES IN COMMON BEANS</b><br/>Alves-Freitas, D.M.T.; Melo, F.L.; Faria, J.C.; Ribeiro, S.G.</p> |
| 2:00 - 3:30 P.M. |  |
| 3:30 - 4:00 P.M. | Coffee break and visit to exhibits / <b>Centro de Convenções Luciano Peixoto Hall</b>  |
| 3:30 - 5:00 P.M. | <p><b>CONFERENCE 5 - Microcephaly and Zika Virus / Principal Room</b></p> <ul style="list-style-type: none"> <li>• <b>Paulo Zanotto</b>, USP, São Paulo, Brazil – “Sequencing of Zikavirus from fetuses with microcephaly in Brazil”</li> </ul>  |

|                       | TIME             | ACTIVITY   |
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| Tuesday, September 20 | 5:00 - 6:30 P.M. | <p><b>Helio Gelli Pereira Award Oral Presentations / Principal Room</b></p> <ul style="list-style-type: none"> <li><b>Chair: Maurício Lacerda Nogueira</b> <ul style="list-style-type: none"> <li>Session 9 – <b>Human Virology / Noemi Jaime Room - Chair: Paula Rahal</b></li> </ul> </li> </ul> <p><b>2 - DETECTION OF THE EMERGING ROTAVIRUS G12P[8] GENOTYPE AT HIGH FREQUENCY IN BRAZIL IN 2014: SUCCESSIVE REPLACEMENT OF PREDOMINANT STRAINS</b><br/>Luchs, A.; Cilli, A.; Morillo, S.G.; Gregorio, D.S.; Souza, K.A.F.; Vieira, H.R.; Fernandes, A.M.; Carmona, R.C.C.; Timenetsky, M.C.S.T.</p> <p><b>35 - PREVALENCE AND VIROLOGICAL CHARACTERISTICS OF HEPATITIS B VIRUS INFECTION AMONG MEN WHO HAVE SEX WITH MEN IN CENTRAL BRAZIL: A RESPONDENT-DRIVEN SAMPLING</b><br/>Oliveira, M.P.; Silva, A.M.C.; Andrade, A.A.; Santana, E.B.R.; Freitas, N.R.; Matos, M.A.D.; Lopes, C.L.R.; Spitz, N.; Araujo, N.M.; Martins, R.M.B.</p> <p><b>41 - SAPOVIRUS IN CHILDREN WITH ACUTE GASTROENTERITIS ATTENDED AT HOSPITAL IN GOIÂNIA, GOIÁS</b><br/>Silva, T.N.; Dábilla, N.A.S.; Fiaccadori, F.S.; Cardoso, D.D.P.; Sousa, T.T.; Almeida, T.N.V.; Leite, R.A.; Souza, M.</p> <p><b>157 - SEROLOGICAL EVIDENCE OF CIRCULATION OF ALPHAVIRUS (VENEZUELAN EQUINE ENCEPHALITIS VIRUS AND UNA VIRUS) IN PARAGUAYAN POPULATION (2012-2013)</b><br/>Cardozo, F.M.; Konigheim, B.; Albrieu-Llinás, G.; Rivarola, M.E.; Aguilar, J.; Rojas, A.; Páez, M.; Guillén, Y.; Diaz, L.A.; Vallejos, M.A.; Herebia, L.; Recalde, M.L.; Contigiani, M.S.; Mendoza, L.P.</p> <p><b>212 - HIGH RATES OF DETECTION OF HUMAN RHINOVIRUS AND LACK OF ADENOVIRUS AND BOCAVIRUS SHEDDING IN ASYMPTOMATIC PATIENTS POST TONSILLECTOMY</b><br/>Martins Junior, R.B.; Prates, M.C.M.; Biasoli, B.; Rocha, L.P.; ARAGON, D.C.; Silva, M.L.; Tamashiro, E.; Valera, F.; Lima, W.; Arruda, E.</p> |
|                       | 6:30 - 8:00 P.M. | <p><b>Poster Session 2 and Visit to Exhibits / Centro de Convenções Luciano Peixoto Hall</b></p> <ul style="list-style-type: none"> <li>Immunobiologicals Virology;</li> <li>Plant and Invertebrate Virology;</li> <li>Veterinary Virology</li> </ul>  |

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| Wednesday, September 21  | 8:00 - 9:00 A.M.  | <p><b>CONFERENCE 6 / Principal Room</b></p> <ul style="list-style-type: none"> <li><b>Claudio L. Afonso</b>, USDA, Georgia, United States – “Exotic and emerging avian viral diseases”</li> </ul>                               |
|  | 9:00 - 9:30 A.M.  | Coffee break and visit to exhibits / <b>Centro de Convenções Luciano Peixoto Hall</b>   |
|  | 9:30 - 12:00 A.M. | SBV Business Meeting / <b>Principal Room</b>  |
|  | 12:00 - 1:00 P.M. | <p><b>Mini-course 1 / Noemi Jaime Room</b></p> <ul style="list-style-type: none"> <li><b>Fernando Melo</b>, UNB, Distrito Federal, Brazil - “Next generation sequencing technologies for viral metagenomic analyses”</li> </ul> |
|  |                   | <p><b>Mini-course 2 / Cavalhadas Room</b></p> <ul style="list-style-type: none"> <li><b>Antônio Augusto Fonseca Júnior</b>, LANAGRO, Minas Gerais, Brazil - “Viral phylogeny and sequence analysis”</li> </ul>                  |
| <p><b>Mini-course 3 / Principal Room</b></p> <ul style="list-style-type: none"> <li><b>Vitor Bortolo de Rezende</b>, BD Biosciences - “Uses of flow cytometry in virology”</li> </ul>        |                   |   |
| <p><b>Mini-course 4 / Ita e Alaor Room</b></p> <ul style="list-style-type: none"> <li><b>Luciana Jesus da Costa</b>, UFRJ, Rio de Janeiro, Brazil - “Viral replication mechanism”</li> </ul> |                   |   |

## *Hélio Gelli Pereira Award*

### **Hélio Gelli Pereira Award**

#### **PHYLODYNAMICS OF INFLUENZA A(H3N2) IN SOUTH AMERICA, 1999-2012**

Born, P.S.; Siqueira, M.M.; Faria, N.R.; Resende, P.C.; Motta, F.C.; Bello, G.

#### **CHARACTERIZATION OF NOVEL INTRAGENOTYPE RECOMBINATION EVENTS AMONG NOROVIRUS PANDEMIC GII.4 VARIANTS**

Siqueira, J.A.M.; Bandeira, R. da S.; Justino, M.C.A.; Linhares, A. da C.; Gabbay, Y.B.

#### **DIVERSITY OF BETA-PAPILLOMAVIRUS AT ANOGENITAL AND ORAL ANATOMICAL SITES**

##### **OF MEN: THE HIM STUDY**

Nunes, E.M.; Sudenga, S.L.; Gheit, T.; Tommasino, M.; Baggio, M.L.; de Ferreira, S.; Galan, L.; Silva, R.C.; Campbell, C.M.P.; Ponce, E.L.; Giuliano, A.R.; Villa, L.; Sichero, L.

Principal Room  
5:00 p.m. - 6:30 p.m.

## Oral Presentation

Monday, September 19

Principal Room - Chair Eurio de Arruda Neto  
2:00 p.m - 3:30 p.m

### SESSION 1 – Human Virology

#### HV30 - HEPATIC MIRNA PROFILE IN DENGUE HEMORRHAGIC FEVER AND ASSOCIATION WITH APOPTOSIS REGULATION, VASCULAR INJURY AND INFLAMMATION

Oliveira, L.F.; Vianez, J.L.G.; Pagliari, C.; Carvalho, L.V.; Silveira, T.S.; Telles, A.L.; Cardoso, J.F.; Vasconcelos, J.M.; Moreira-Nunes, C.A.; Burbano, R.M.R.; Nunes, M.R.T.; Santos, E.J.M.

#### HV179 - MUTATIONS PROFILE IN HIV TRANSCRIPTASE REVERSA AND PROTEASE GENES IN HIV/HBV AND HIV/HCV COINFECTED PATIENTS

Grotto, R.M.T.; Cantão, N.M.; Fogaça, L.; Wolf, I.; Almeida, R.; Cruz, A.A.; Barbosa, A.N.; Silva, G.F.; Valente, G.T.; Pardini, M.I.M.C.; Grotto, R.M.T.

#### HV207 - EFFICIENT PRODUCTION OF GP64 FREE HIV-1 VIRUS-LIKE PARTICLES (VLPS) USING BACULOVIRUS EXPRESSION SYSTEM

Chaves, L.C.S.; Ribeiro, B.M.; Blissard, G.W.

#### HV234 - IN SITU EVIDENCE ON INFLUENZA VIRUS INFECTION OF LYMPHOID CELLS IN HUMAN TONSILLAR TISSUES

Castro, I.A.; Martins Junior, R.B.; Jesus, B.L.S.; Pontelli, M.C.; Prates, M.C.; Silva, M.L.; Carezzi, L.R.; Tamashiro, E.; Anselmo-Lima, W.T.; Arruda, E.

Noemi Jaime Room - Chairs: Marcos Bryan Heinemann and João Pessoa Araújo Junior - 2:00 p.m - 3:30 p.m

### SESSION 2 – Veterinary Virology

#### VV9 - RECONSTRUCTION OF THE SPATIAL DISPERSION OF INFECTIOUS BRONCHITIS VIRUS: IBV FINDS ITS ROOTS

Saraiva, G.L.; Vidigal, P.M.P.; Pereira, C.G.; Figueiredo, J.F.; Campo, A.J.; Fietto, J.L.R.; Bressan, G.C.; Silva Júnior, A.; Almeida, M.R.

#### VV62 - SYSTEMIC AND MUCOSAL ANTIBODY RESPONSES INDUCED BY A VACCINE OF INACTIVATE AVIAN INFECTIOUS BRONCHITIS VIRUS (IBV) ENCAPSULATED IN CHITOSAN NANOPARTICLES

Lopes, P.D.; Okino, C.H.; Casagrande, V.M.; Pavani, C.; Fernando, F.S.; Tamanini, M.L.F.; Montassier, M.F.S.; Lopez, R.F.V.; Montassier, H.J.

#### VV74 - GENOMIC CHARACTERIZATION OF A NOVEL HUMAN INFLUENZA A(H1N2) VARIANT DETECTED IN BRAZIL

Resende, P.C.; Born, P.S.; Matos, A.R.; Motta, F.C.; Caetano, B.C.; Debur, M.C.; Riediger, I.; Brown, D.; Siqueira, M.M.

#### VV80 - GENETIC CHARACTERIZATION OF INFLUENZA VIRUSES CIRCULATING WITHIN BRAZILIAN SWINE BETWEEN 2009 AND 2016

Schaefer, R.; Gava, D.; Nelson, M.I.; Haach, V.; Ciacci-Zanella, J.R.; Cantão, M.E.

#### VV85 - NEONATAL PIG MORTALITY ASSOCIATED WITH SENECAVIRUS A

Gava, D.; Lorenzetti, M.P.; Haach, V.; Driemeier, D.; Joshi, L.R.; Mohr, K.A.; Diel, D.G.; Caron, L.; Morés, N.; Morés, M.A.Z.; Schaefer, R.

#### VV143 - BRAZILIAN BATS AS CARRIERS OF VIRUSES WITH ZOOLOGICAL POTENTIAL

Simas, P.V.M.; Barnabé, A.C.S.; Caserta, L.C.; Martini, M.C.; Durões-Carvalho, R.; Fellippe, P.A.N.; Ferreira-Neto, D.L.; Beck, R.M.; Nascimento, G.M.; Jacomassa, F.A.F.; Moraes, A. P.; Miller, M.E.; Arns, C.W.

#### VV166 - EVALUATION OF SEROLOGICAL AND VIREMIC PROFILE FOR PORCINE CIRCOVIRUS TYPE 2 IN NATURALLY INFECTED PIGS FROM FALLOW-TO-FINISH FARMS IN MINAS GERAIS STATE, BRAZIL

Dias, A.S.; Rehfeld, I.S.; Gallinari, G.C.F.; Costa, A.G.; Guedes, M.I.M.C.; Lobato, Z.I.P.

Ita e Alaor Room - Chair: Luis Lamberti Pinto da Silva - 2:00 p.m - 3:30 p.m

### SESSION 3 – Basic Virology

#### BV75 - IDENTIFICATION OF CELL PROTEINS THAT INTERACT WITH HUMAN RESPIRATORY SYNCYTIAL VIRUS M2-1 PROTEIN

Araujo, C.L.; Eléouët, J.F.; Ventura, A.M.

#### BV79 - TROPOMIOSIN INTERACTION WITH HUMAN RESPIRATORY SYNCYTIAL VIRUS MATRIX PROTEIN

Dias, T.D.; Oliveira, A.P.; Ogawa, J.K.; Eléouët, J.F.; Ventura, A.M.

#### BV168 - EVALUATION OF APOPTOTIC MECHANISMS MEDIATED BY UNFOLDED PROTEIN RESPONSE PATHWAY IN JURKAT CELLS STIMULATED WITH HIV-1 TAT PROTEIN

Campestrini, J.; Costa-Junior, A.O.; Pinto, A.R.

#### BV214 - RESPIRATORY SYNCYTIAL VIRUS MRNA TRANSCRIPTOME REVEALS SURPRISING PROFILES DURING ONE-STEP REPLICATION CYCLE

Jesus, B.L.S.; Cardoso, R.S.; Criado, M.F.; Souza, M.M.; Oliveira, A.S.; Prates, M.C.M.; Ventura, A.M.; Arruda, E.

#### BV215 - OROPOUCHE VIRUS ASSEMBLY IN MAMMALIAN CELLS REQUIRES THE ACTIVITY OF HOST ESCRT PROTEINS

Barbosa, N.S.; Mendonca, L.L.R.; Criado, M.; Arruda, E.; da Silva, L.L.P.

**SESSION 4 – Plant and Invertebrates Virology****PIV102 - MOLECULAR CHARACTERIZATION OF GRAPEVINE ENAMO-LIKE VIRUS, A NOVEL PUTATIVE MEMBER OF THE GENUS ENAMOVIRUS**

Silva, J.M.F.; Fajardo, T.V.M.; Al Rwahnih, M.; Blawid, R.; Nagata, T.

**PIV117 - IDENTIFICATION AND FUNCTIONAL ANALYSES OF THE COTTON BLUE DISEASE RESISTANCE LOCUS**

Fausto, A.K.S.; Moura, M.O.; da Franca, T.S.; Romanel, E.; Vaslin, M.F.S.

**PIV118 - DICER-LIKE PROFILE EXPRESSION DURING VIRAL INFECTION IN SUSCEPTIBLE AND RESISTANT COTTON**

Moura, M.O.; Fausto, A.K.S.; da Franca, T.S.; Romanel, E.; Vaslin, M.F.S.

**PIV175 - ARRACACIA XANTHORRIZA (MANDIOQUINHA-SALSA): A RESERVOIR OF PLANT VIRUS**

Orflio, A.F.; Inoue-Nagata, A.K.; Nagata, T.; Madeira, N.R.; Resende, R.O.; Blawid, R.

**PIV209 - THE COMPLETE GENOME SEQUENCE OF A NOVEL BETABACULOVIRUS ISOLATED FROM MOCIS SP. REVEALS AN ANCIENT GENOME EXPANSION AND A TENDENCY IN NOCTUIDINFECTIONING BETABACULOVIRUS**

Ardisson-Araújo, D.M.P.; Melo, F.L.; Sosa-Gómez, D.R.; Ribeiro, B.M.

**PIV244 - STUDY OF BEGOMOVIRUS DIVERSITY IN TOMATO PLANTS USING NEXT-GENERATION SEQUENCING**

Rêgo, C.M.; Nakasu, E.Y.T.; Blawid, R.; Nagata, T.; Inoue-Nagata, A.K.

**SESSION 5 – Human Virology****HV11 - INCREASED PRO-INFLAMMATORY CYTOKINES IN AMNIOTIC FLUID FROM ZIKA VIRUS ASSOCIATED MICROCEPHALY**

Ornelas, A.M.M.; Pezzuto, P.; Silveira, P.P.; Melo, F.O.; Ferreira, T.A.; Oliveira-Szejnfeld, P.S.; Leal, J.I.; Amorim, M.M.R.; Cardoso, C.C.; Nixon, D.F.; Tanuri, A.; Melo, A.S.; Aguiar, R.S.

**HV156 - ACTIVATION OF INTRINSIC COAGULATION PATHWAY AND LIPID METABOLISM IN DENGUE VIRUS PATHOGENESIS**

Coelho, S.V.A.; Vellasco, L.; Marques, J.R.E.T.A.; Scharfstein, J.; Arruda, L.B.

**HV206 - IDENTIFICATION AND SELECTION OF DENGUE VIRUS SPECIFIC PEPTIDES FOR DIFFERENTIAL DIAGNOSTIC TESTS**

Versiani, A.F.; Mendes, T.A.O.; Bartholomeu, D.C.; Nogueira, M.L.; da Fonseca, F.G.

**HV254 - SALIVA AS THE BIOLOGICAL SAMPLE OF CHOICE FOR THE MOLECULAR DIAGNOSIS OF ZIKA**

Monteiro, D.C.S.; Mejía, M.C.C.; Abdalla, L.F.; Santos, J.H.A.; Almeida, T.P.A.; Corado, A.L.G.; Souza, V.C.; Nascimento, V.A.; Naveca, F.G.

**HV256 - ETIOLOGY OF THE ACUTE FEBRILE ILLNESS IN THE AMAZON STATE BRAZIL, DURING THE EMERGENCE OF ZIKA VIRUS**

Nascimento, V.A.; Monteiro, D.C.S.; Silva, M.S.; Souza, V.C.; Corado, A.L.G.; Naveca, F.G.

**HV258 - SOROPREVALENCE DENGUE IGG IN PATIENTS IN A PROSPECTIVE COHORT STUDY OF SÃO JOSÉ DO RIO PRETO**

Silva, R.A.; Silva, G.C.D.; Zini, N.; Kanazawa, T.; Estofolete, C.F.; Watanabe, A.S.A.; Terzian, A.C.B.; Nogueira, M.L.

**SESSION 6 – Environmental Virology****EV14 CORROSION AND BIOFILM REDUCED BY ECOPHAGES IN A PILOT ANAEROBIC SYSTEM**

Dias, R.S.; Oliveira, M.D.; Silva, J.D.; Bicalho, K.M.; Sousa, M.P.; Santos, V.V.C.M.; Silva, E.D.; Akamine, R.N.; Silva, C.C.; de Paula, S.O.

**EV49 - ENTERIC PATHOGENS SURVIVAL, PERCOLATION AND LEACHING IN BIOFERTILIZED SOILS USING SWINE DIGESTATE**

Fongaro, G.; García-González, M.C.; Hernández, M.; Kunz, A.; Barardi, C.R.M.; Rodríguez-Lázaro, D.

**EV54 - ROTAVIRUS AND OTHER HUMAN ENTERIC VIRUSES IN GASTROPODS**

Gularte, J.S.; Staggemeier, R.; Demoliner, M.; Heck, T.M.S.; Heldt, F.H.; Ritzel, R.G.F.; Henzel, A.; Spilki, F.R.

**EV88 - DETECTION AND MOLECULAR CHARACTERIZATION OF GEMCIRCULARVIRUS FROM ENVIRONMENTAL SAMPLES IN BRAZIL**

Assis, M.R.S.; Vieira, C.B.; Fioretti, J.M.; Rocha, M.S.; Almeida, P.N.; Miagostovich, M.P.; Fumian, T.M.

**EV177 - ADENOVIRUS INVESTIGATION BY MOLECULAR ANALYSIS IN PUBLIC WATER SUPPLY NETWORK**

Ferreira, C.S.; Sa-Oliveira, J.C.; Resque, R.L.; Ferreira, C.L.; Silva, E.S.; Muller, E.C.A.

**EV229 - CONSTRUCTED WETLANDS AS AN ALTERNATIVE SYSTEM TO REMOVE ENTERIC VIRUSES FROM WASTEWATER**

Moresco, V.; Magri, M.E.; Sezerino, P.H.; Barardi, C.R.M.

**EV265 - VIRAL STUDY IN UNTREATED AND TREATED SEWAGE WATER**

Moriya, N.M.N.; Blawid, R.; Silva, J.M.F.; Batista, L.F.; Nagata, T.

Noemi Jaime Room - Chair: Luciana Jesus da Costa - 2:00 p.m - 3:30 p.m

**SESSION 7 – Basic Virology**

**BV26 - THE ASIAN-AMERICAN VARIANT OF HUMAN PAPILLOMAVIRUS TYPE 16 EXHIBITS HIGHER ACTIVATION OF MAPK AND PI3K/AKT SIGNALING PATHWAYS, TRANSFORMATION, MIGRATION AND INVASION OF PRIMARY HUMAN KERATINOCYTES**

Hochmann, J.; Sobrinho, J.S.; Villa, L.L.; Sicheo, L.

**BV174- ACTIVATION AND DEATH PATHWAYS INDUCED BY DENGUE VIRUS IN INFECTED AND BYSTANDER ENDOTHELIAL CELLS**

Papa, M.P.; Slongo, J.; Arruda, L.B.

**BV192 - SCREENING TESTS TO EVALUATE THE EFFECTIVENESS AND TOXICITY OF TEN ANTIVIRAL DRUG CANDIDATES DEVELOPED BY BIOISOTHERMISM**

Fonseca, V.W.P.; Menegatti, R.; Costa, L.J.

**BV205 - THE NON-GLYCOSILATED HRV PROTEINS M AND N ARE ADDRESSED TO THE VIRAL ASSEMBLY SITE THROUGH THE SECRETORY PATHWAY**

Cardoso, R.S.; Jesus, B.L.S.; Carvalho, A.N.; Criado, M.; Viana, R.M.M.; Milani, E.R.; Viana, R.M.M.; Prates, M.; Rosales, R.; Ventura, A.M.; Silva, L.L.P.; Arruda, E.

**BV213 - HUMAN TONSIL EXPLANTS SUPPORT RHINOVIRUS INFECTION EX VIVO**

Martins Junior, R.B.; Gagliardi, T.B.; Criado, M.F.; Cardoso, R.S.; Jesus, B.L.; Silva, M.L.; Carezzi, L.R.; Tamashiro, E.; Valera, F.; Lima, W.; Arruda, E.

**BV243 - DELETION OF THE M SEGMENT NON STRUCTURAL PROTEIN (NSM) OF OROPOUCHE VIRUS AFFECTS VIRUS ASSEMBLY AND THE ARCHITECTURE OF VIRAL FACTORIES**

Cardoso, R.S.; Barbosa, N.S.; Acrani, G.O.; da Silva, L.L.P.; Arruda, E.

Cavalladas Room - Chair: Bergmann Ribeiro - 2:00 p.m - 3:30 p.m

**SESSION 8 – Plant and invertebrates Virology**

**PIV6 - TRANSLATIONALLY CONTROLLED TUMOR PROTEIN IS NECESSARY FOR AN EFFICIENT POTYVIRUS REPLICATION**

Bruckner, F.P.; Laliberté, J.F.; Alfenas-Zerbini, P.

**PIV28 - VIROME IN ORNAMENTAL PLANTS FROM DISTRITO FEDERAL, BRAZIL**

Brant, P.M.; Nagata, T.; Pereira-Carvalho, R.C.

**PIV72 - SEQUENCING OF THE COTTON ANTHOCYANOSIS VIRUS BY SMALL RNA DEEP SEQUENCING AND ITS SIVRNAS PROFILE IN COTTON**

Santos, R.O.; Fausto, A.K.S.; Andrade, R.; da Franca, T.S.; Giband, M.; Vaslin, M.F.S.

**PVI99 - DSRNA DEEP SEQUENCING REVEALS FIVE VIRAL SPECIES IN COMMON BEANS**

Alves-Freitas, D.M.T.; Melo, F.L.; Faria, J.C.; Ribeiro, S.G.

Ihadas Room - Chair: Bergmann Ribeiro - 2:00 p.m - 3:30 p.m

**SESSION 9- Human Virology**

**HV2 - DETECTION OF THE EMERGING ROTAVIRUS G12P[8] GENOTYPE AT HIGH FREQUENCY IN BRAZIL IN 2014: SUCCESSIVE REPLACEMENT OF PREDOMINANT STRAINS**

Luchs, A.; Cilli, A.; Morillo, S.G.; Gregorio, D.S.; Souza, K.A.F.; Vieira, H.R.; Fernandes, A.M.; Carmona, R.C.C.; Timenetsky, M.C.S.T.

**HV35 - PREVALENCE AND VIROLOGICAL CHARACTERISTICS OF HEPATITIS B VIRUS INFECTION AMONG MEN WHO HAVE SEX WITH MEN IN CENTRAL BRAZIL: A RESPONDENT-DRIVEN SAMPLING**

Oliveira, M.P.; Silva, A.M.C.; Andrade, A.A.; Santana, E.B.R.; Freitas, N.R.; Matos, M.A.D.; Lopes, C.L.R.; Spitz, N.; Araujo, N.M.; Martins, R.M.B.

**HV41 - SAPOVIRUS IN CHILDREN WITH ACUTE GASTROENTERITIS ATTENDED AT HOSPITAL INGOIÂNIA, GOIÁS**

Silva, T.N.; Dábilla, N.A.S.; Fiaccadori, F.S.; Cardoso, D.D.P.; Sousa, T.T.; Almeida, T.N.V.; Leite, R.A.; Souza, M.

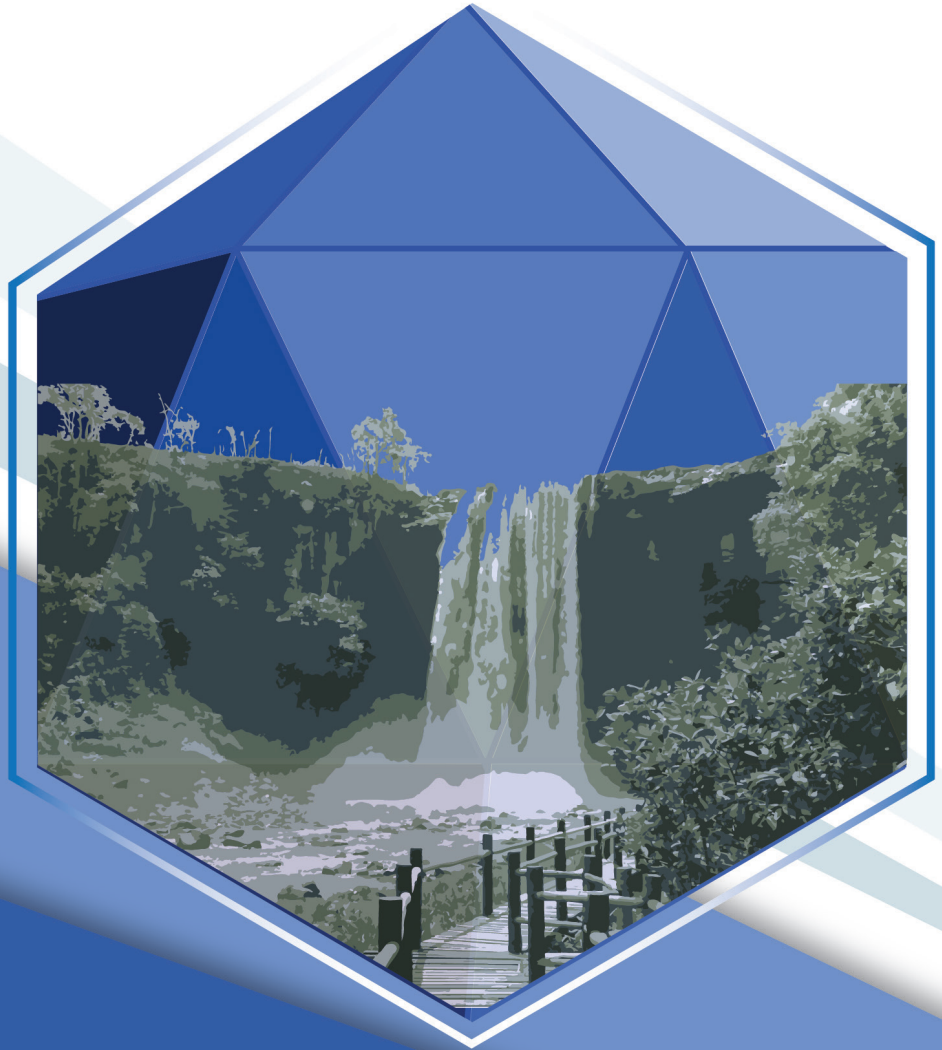
**HV157 - SEROLOGICAL EVIDENCE OF CIRCULATION OF ALPHAVIRUS (VENEZUELAN EQUINE ENCEPHALITIS VIRUS AND UNA VIRUS) IN PARAGUAYAN POPULATION (2012-2013)**

Cardozo, F.M.; Konigheim, B.; Albrieu-Llinás, G.; Rivarola, M.E.; Aguilar, J.; Rojas, A.; Páez, M.; Guillén, Y.; Diaz, L.A.; Vallejos, M.A.; Herebia, L.; Recalde, M.L.; Contigiani, M.S.; Mendoza, L.P.

**HV212 - HIGH RATES OF DETECTION OF HUMAN RHINOVIRUS AND LACK OF ADENOVIRUS AND BOCAVIRUS SHEDDING IN ASYMPTOMATIC PATIENTS POST TONSILLECTOMY**

Martins Junior, R.B.; Prates, M.C.M.; Biasoli, B.; Rocha, L.P.; Aragon, D.C.; Silva, M.L.; Tamashiro, E.; Valera, F.; Lima, W.; Arruda, E.

***HELIO GELLI PEREIRA AWARD***



**PHYLODYNAMICS OF INFLUENZA A(H3N2) IN SOUTH AMERICA, 1999–2012**

Born, P.S.; Siqueira, M.M.; Faria, N.R.; Resende, P.C.; Motta, F.C.; Bello, G.

1. *Respiratory Viruses and Measles Laboratory, Oswaldo Cruz Institute/Fiocruz, Av. Brasil 4365, 21040-360 Rio de Janeiro, RJ, Brazil*
2. *Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, United Kingdom*
3. *AIDS and Molecular Immunology Laboratory, Oswaldo Cruz Institute/Fiocruz, Av. Brasil 4365, 21040-360 Rio de Janeiro, RJ, Brazil*

The limited influenza A(H3N2) genetic data available from the Southern Hemisphere (particularly from Africa and Latin America), constrains the accurate reconstruction of viral dissemination dynamics within those regions. Our objective was to describe the spatial dissemination dynamics of influenza A(H3N2) within South America. A total of 469 sequences of the HA1 portion of the hemagglutinin gene (HA) from influenza A(H3N2) viruses sampled in temperate and tropical South American countries between 1999 and 2012 were combined with available contemporary sequences from Australia, Hong Kong, United Kingdom and the United States. Phylogenetic analyses revealed that influenza A(H3N2) sequences from South America were highly intermixed with sequences from other geographical regions, although a clear geographic virus population structure was detected globally. We identified 14 clades mostly ( $\geq 80\%$ ) composed of influenza sequences from South American countries. Bayesian phylogeographic analyses of those clades support a significant role of both temperate and tropical regions in the introduction and dissemination of new influenza A(H3N2) strains within South America and identify an intensive bidirectional viral exchange between different geographical areas. These findings indicate that seasonal influenza A(H3N2) epidemics in South America are seeded by both the continuous importation of viral variants from other geographic regions and the short-term persistence of local lineages. This study also supports a complex metapopulation model of influenza A(H3N2) dissemination in South America, with no preferential direction in viral movement between temperate and tropical regions.

**CHARACTERIZATION OF NOVEL INTRAGENOTYPE RECOMBINATION EVENTS AMONG NOROVIRUS PANDEMIC GII.4 VARIANTS**

Siqueira, J.A.M.; Bandeira, R. da S.; Justino, M.C.A.; Linhares, A. da C.; Gabbay, Y.B.

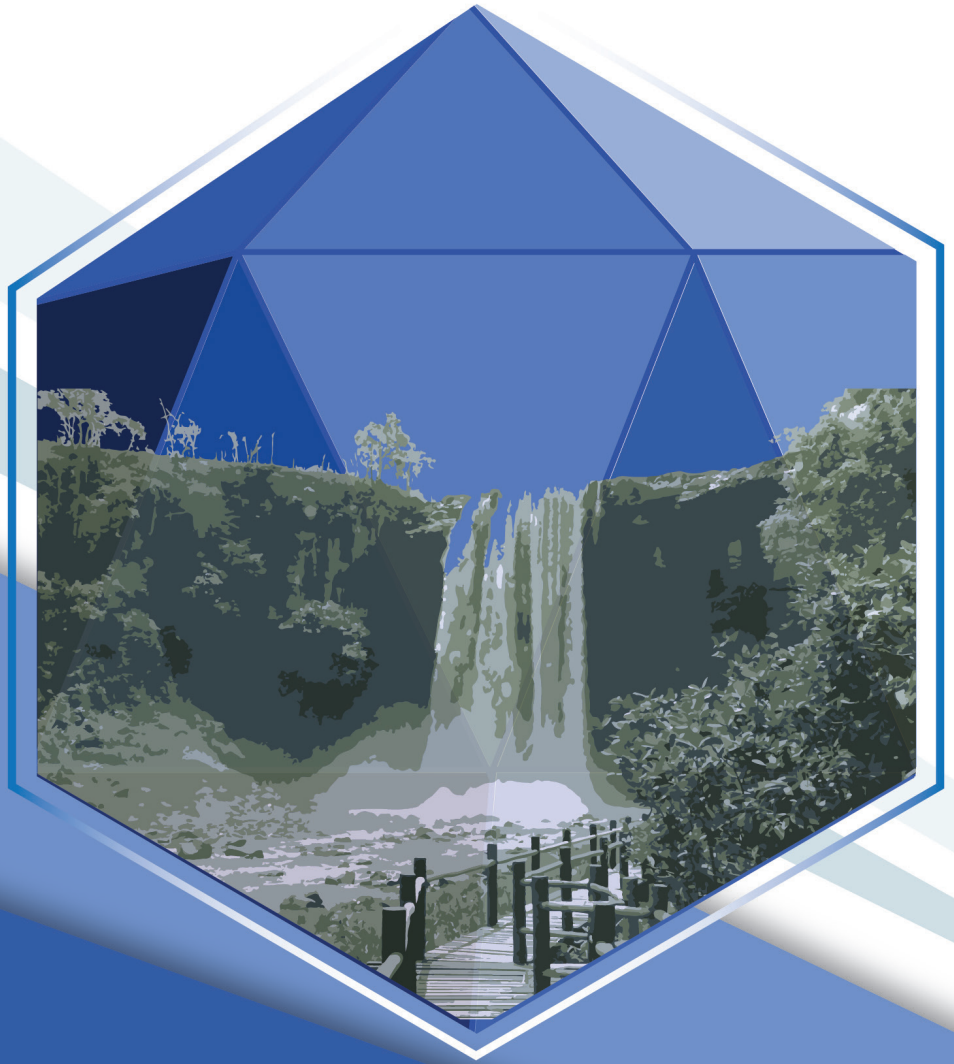
Recently, there has been an increase in the number of children hospitalized due to norovirus infection in Brazil. This is due both to the occurrence of more severe norovirus-related gastroenteritis cases after the introduction of the rotavirus vaccine and an increase in the tools for the detection of the disease. This pathogen is transmitted by the fecal-oral route, and the illness is characterized by diarrhea, vomiting, nausea and abdominal cramps. The genome of the virus is organized into three open reading frames showing strong mutation rates. Additionally, homologous recombination events, which can increase the virulence of the virus and lead to genotyping mistakes in molecular epidemiological studies, frequently occur. The purpose of this study was to describe two recombination events among different GII.4 variants that infected children who were hospitalized for severe acute gastroenteritis during distinct periods of time in Belém, Brazil. The recombination among the variants US95\_96/Kaiso\_2003 and Den Haag\_2006b/Yerseke\_2006a were observed in May 2003 and February 2009, respectively. In both cases, the association between the dominant variant at that point in time and another that was circulating at a low frequency in the population of Belém was demonstrated. Interestingly, the position of the breakpoint of the recombination event in the genome was the polymerase gene and was located at the nucleotide positions 4.834 and 5.002, which is an unusual location for the occurrence of recombination as other studies have previously reported the junction region as a breakpoint. In this study, both recombinant variant strains were related to severe cases of diarrhea that lead to hospitalization, demonstrating the viral evolution of GII.4 in response to selective pressures, which ultimately lead to the emergence of novel viral types in the pediatric population. The cases discussed here reinforce the need for continuous norovirus surveillance. To our knowledge, these two GII.4 variant

**DIVERSITY OF BETA-PAPILLOMAVIRUS AT ANOGENITAL AND ORAL ANATOMIC SITES OF MEN: THE HIM STUDY**

Nunes, E.M.; Sudenga, S.L.; Gheit, T.; Tommasino, M.; Baggio, M.L.; de Ferreira, S.; Galan, L.; Silva, R.C.; Campbell, C.M.P.; Ponce, E.L.; Giuliano, A.R.; Villa, L.; Sichero, L.

Our goal was to describe prevalence of  $\beta$ -HPVs at three anatomic site samong 717 men from Brazil, Mexico and US enrolled in the HPV Infectionin Men(HIM) Study.  $\beta$ -HPVs were genotype dusing Luminex technology. Overall, 77.7%, 54.3% and 29.3% men were positive for any  $\beta$ -HPV atthegenitals, analcanal, and oralcavity, respectively. Men from US and Brazil were significantly less like lyto have  $\beta$ -HPV at the anal canal than men from Mexico. Older men were more like lytohave  $\beta$ -HPV at the anal canal compared to younger men. Prevalence of  $\beta$ -HPV at the oral cavity was significantly associated with country of origin and age. Currents mokers were significantly less like lytohave  $\beta$ -HPV in the oral cavity than men who never smoked. Lack of associations between  $\beta$ -HPV and sexual behaviors may suggest other routes of contact such as auto inoculation which need to be explored further.

# *ORAL PRESENTATION*



### HV30 - HEPATIC MIRNA PROFILE IN DENGUE HEMORRHAGIC FEVER AND ASSOCIATION WITH APOPTOSIS REGULATION, VASCULAR INJURY AND INFLAMMATION

Oliveira, L.F.; Vianez, J.L.G.; Pagliari, C.; Carvalho, L.V.; Silveira, T.S.; Telles, A.L.; Cardoso, J.F.; Vasconcelos, J.M.; Moreira-Nunes, C.A.; Burbano, R.M.R.; Nunes, M.R.T.; Santos, E.J.M.

1. INSTITUTO EVANDRO CHAGAS
2. UNIVERSIDADE DE SÃO PAULO
3. FUNDAÇÃO ONCOCENTRO DE SÃO PAULO
4. FACULDADE DE MEDICINA DE SÃO JOSÉ DO RIO PRETO
5. UNIVERSIDADE FEDERAL DO PARÁ
6. UNIVERSIDADE FEDERAL DO CEARÁ

Dengue is the most prevalent arbovirolosis in the world caused by Dengue virus (DENV) and is present in all continents, for more than three decades has been a constant public health concern and often fatal by dengue hemorrhagic fever (DHF). The pathogenesis of dengue is closely related to the host immune response, reaching exacerbated inflammation and transient autoimmunity. All tissues are affected, which liver is one of the most important in severe conditions, due its intense viral replication and its significant role in metabolism. The study of microRNAs (miRNA) as regulatory elements of metabolism and immune response during infection is crucial to understanding the regulatory mechanisms of gene expression on DENV infection, and can help in diagnostic development of anti-viral therapies. We sequenced the miRNoma in MiSeq platform (Illumina) to identify the miRNA profile expressed in formalin-fixed paraffin-embedded (FFPE) liver tissue. Ten DHF fatal cases were compared to five control cases by differential expression analysis performed in edgeR, followed by target gene prediction in TargetScan and enrichment analysis of functional pathways in DAVID v6.7, this results were visualized in a gene-pathway network built in Cytoscape. Eight miRNAs exhibited differential expression in DHF FFPE liver, miR-126-5p (logFC = 3,09; FDR = 0,00675), a regulatory molecule of endothelial cells, and miR-133a-3p are up regulated in dengue. The others miRNA were down regulated in DHF: miR-122-5p (logFC = -6,59; FDR < 0,00000001), a liver-specific miRNA, miR-146a-5p, interferon regulator, miR-10b-5p, miR-204-5p, miR-148a-5p and miR-423-5p. Functional

analysis of KEGG pathways and GO terms with predicted target genes of over expressed miRNA found regulatory pathways of apoptosis and immune response, involving MAPK gene, RAS, CDK and FAS; immune response pathways showed NF- $\kappa$ B, CC and CX families, IL and TLR. The same analysis with target genes of downregulated miRNAs also identified in most pathways of apoptosis and biosynthetic pathways of metabolism. In our knowledge, this is the first description of the liver miRNA profile in DHF, the results together show a feasible relationship of miR-126-5p, miR-122-5p and miR-146a-5p with liver pathogenesis of DHF, through endothelial repair and vascular permeability regulation, control of homeostasis and liver expression regulation of inflammatory cytokines.

### HV179-MUTATIONS PROFILE IN HIV TRANSCRIPTASE REVERSA AND PROTEASE GENES IN HIV/HBV AND HIV/HCV COINFECTED PATIENTS

Grotto, R.M.T.; Cantão, N.M.; Fogaça, L.; Wolf, I.; Almeida, R.; Cruz, A.A.; Barbosa, A.N.; Silva, G.F.; Valente, G.T.; Pardini, M.I.M.C.

UNIVERSIDADE ESTADUAL PAULISTA

The use of antiretroviral combinations has demonstrated highly effectiveness in controlling of the progression of HIV infection and increased of the patients' survival. Although in the last years several advances have been achievement in the antiHIV therapeutic these drugs can be have yours activities reduced due to development of drug resistance. HIV drug resistance is consequence of the mutations in genes that encodes viral enzymes, representing the major obstacle to successful therapeutic. The resistance mutations in reverse transcriptase (RT) and protease (PR) genes have already been described and there are several laboratory tests to detected them. However, all tests and algorithms to interpretation of the test's results are based in information obtained from HIV monoinfected patients. In the moment there are no studies about the HIV RT and PR resistance mutations in patients coinfecting with hepatotropic virus, mainly, Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV). Then, the goal of this study was evaluate genetic sequences that encoded HIV RT and PR in HIV/HBV and/or HIV/HCV coinfecting patients assisted in the specialized health services in Botucatu city, Sao Paulo State, Brazil. Samples from 86 patients infected by HIV

were included in this study. The patients were divided in two groups: G1 (52 patients HIV monoinfected) and G2 (34 patients HIV/HBV and/or HIV/HCV coinfecting patients). RNA or DNA viral isolated from plasma was used as source to genotyped the HIV RT and PR genes using automatic sequencing. The consensus sequence obtained were analyzed using the Genotypic Resistance Interpretation Algorithm of Stanford University (HIVdb) and the subtyping was performed by REGA HIV1 Subtyping Tool and by RIP 3.0 program. The sequences were used to generate phylogenetic trees using SeaView. The results showed that although G1 presented more resistant's mutations than G2 the mutations' profile in G1 and G2 was similar. On the other hand, the phylogenetic trees showed clusters of the sequences from coinfecting patients (HIV/HBV and HIV/HCV) well separated of the clusters built from HIV monoinfected sequences. These results suggest that the hepatotropic virus presence seems to be provided selective pressure in HIV strains.

#### **HV207 - EFFICIENT PRODUCTION OF GP64 FREE HIV-1 VIRUS-LIKE PARTICLES (VLPs) USING BACULOVIRUS EXPRESSION SYSTEM**

Chaves, L.C.S.; Ribeiro, B.M.; Blissard, G.W.

1. UNIVERSIDADE DE BRASÍLIA
2. BOYCE THOMPSON INSTITUTE, CORNELL UNIVERSITY

Virus like particles (VLPs) are composed of viral capsid proteins that self-assemble into particles resembling natural virions. The baculovirus expression vector system (BEVS) is a powerful tool that has been widely used to produce VLPs in insect cells. However, purified VLPs samples from insect cells are known for being contaminated with baculovirus Budded virus (BV) particles. Besides that, these VLPs can have some host insect proteins expressed on their surface. The enveloped VLPs assembly depends on the capsid (or matrix) formation and then membrane enclosure for budding from a host cell membrane. Through this mechanism, enveloped VLPs can incorporate host proteins, which is the case of the baculovirus envelope protein GP64 that is expressed on the surface of insect cells during the baculovirus infection. During budding of BV and enveloped VLPs, the GP64 protein is captured and displayed on the surface of these

particles. Since VLPs are usually produced for vaccine development, the contamination with other virus particles or immunogenic proteins is a concern. In this work, we showed that VLP and BV particles cannot be separated by ultracentrifugation in sucrose gradient. Thus, to block BV production during VLP assembly, a recombinant baculovirus containing the gag HIV1 gene but lacking the baculovirus gp64 gene (vGAGHIV1 GP64 null) was constructed. This recombinant baculovirus was then used to infect Sf9 cells and shown to correctly produce HIV1 VLPs without BVs particles. GP64 protein was not detected in these cells, confirming the correct deletion of the gp64 gene. Furthermore, the presence of the GAG protein was detected and it was possible to see HIV1 VLPs produced from cells infected with this GP64 defective recombinant virus. Therefore, this work describes, for the first time, a new way of producing VLPs in insect cells without the contamination of baculovirus BV particles or proteins.

#### **HV234 - IN SITU EVIDENCE ON INFLUENZA VIRUS INFECTION OF LYMPHOID CELLS IN HUMAN TONSILLAR TISSUES**

Castro, I.A.; Martins Junior, R.B.; Jesus, B.L.S.; Pontelli, M.C.; Prates, M.C.; Silva, M.L.; Carezzi, L.R.; Tamashiro, E.; Anselmo-Lima, W.T.; Arruda, E.

RIBEIRAO PRETO MEDICINE SCHOOL,  
UNIVERSITY OF SAO PAULO

Influenza viruses cause more than two million annual episodes of seasonal acute respiratory infections (ARI) and approximately 500,000 deaths worldwide. Evidence from several models indicate that depending on virus strain and host immune status, acute infections by seasonal influenza A virus may reach sites other than the respiratory tract, such as kidneys, intestines, mucosal lymphoid tissues and lymph nodes. Infection of lymphoid cells by influenza virus raises the possibility that these cells could represent potential sites of persisting infection. In the present study influenza A virus nucleic acids and antigens were searched for in tissues of palatine tonsils and adenoids removed from 102 patients who underwent surgery for tonsillar hypertrophy, in the absence of ARI symptoms. A qRT-PCR screening revealed that tonsillar tissues from 7 of 102 patients (6.9%) were positive for influenza A virus. Of those, formalin-fixed, paraffin-embedded tonsillar tissues

were analyzed by immunohistochemistry with antibody to the influenza A virus nucleoprotein (NP). Strong staining was seen in tissue sections from 6 patients: 4 adenoids and 3 palatine tonsils. Staining was observed mostly on epithelia, both on the surface and within the crypts, but also in interfollicular lymphoid cells. Serial immunoperoxidase labeling and erasing (SIMPLE) of tissue sections with antibodies to cytokeratin confirmed infection of epithelial cells. Interestingly, staining with antibodies to CD3, CD4, CD8, CD11c and CD14 strongly indicated that CD3+CD8+ lymphocytes and CD11c+CD14 cells could harbor influenza virus. To confirm T CD8+ lymphocyte susceptibility to influenza virus, peripheral blood mononuclear cells from healthy donors were infected with Influenza A H1N1(2009) and the results confirmed the in situ findings on tonsil tissue sections. Further investigation is underway to understand the meaning of influenza A virus detection in lymphoid cells on human tonsils removed from people in the absence of symptoms of acute respiratory infections, a finding that suggests that human hypertrophic tonsils may be reservoirs of influenza virus for shedding and transmission in the community.

#### **VV9 - RECONSTRUCTION OF THE SPATIAL DISPERSION OF INFECTIOUS BRONCHITIS VIRUS: IBV FINDS ITS ROOTS**

**Saraiva, G.L.; Vidigal, P.M.P.; Pereira, C.G.; Figueiredo, J.F.; Campo, A.J.; Fietto, J.L.R.; Bressan, G.C.; Silva Júnior, A.; Almeida, M.R.**

*UNIVERSIDADE FEDERAL DE VIÇOSA*

Infectious bronchitis virus (IBV) is a Coronavirus associated with a highly contagious disease that primarily affects the upper respiratory tract of chickens, in addition to the epithelial cells of the urogenital and gastrointestinal tracts. Since its identification in the 1930s, IBV has achieved worldwide distribution, becoming endemic in most chicken-producing countries. This study aimed to reconstruct the IBV spatial dispersal routes, through phylogeography analysis in order to understand biological events along the evolutionary history of IBV. The databases included 256 and 142 complete sequences of S1 and N genes, respectively, obtained from GenBank from 24 countries. The Relaxed Random Walk (RRW) method was used to test hypotheses about the spatial dispersion of the IBV, inferring, visualizing

and reconstructing its dispersion pattern. This analysis was conducted in the Beast software version 2.2.1 with 50,000,000 generations and sampling frequency of 1,000. Parameter convergence was analyzed using Tracer version 2.2.1 and 10% of generated trees were discarded to produce the consensus tree using TreeAnnotator version 2.2.1. The consensus tree file generated was loaded into the Spread application to generate a kml format file that was then read by Google Earth to obtain the graphs of the IBV dispersion patterns over time. The results indicate that analysis of the N gene may reflect the earliest events and analysis of the S1 gene reflects the latest events of IBV evolution. Therefore, an integrated study of these genes may be a useful tool for the analysis of full evolutionary history of the IBV. Our hypothesis is that the point of origin of all current strains of IBV may be China. After dispersion throughout Asia, IBV reached the United States and dispersed from there to different continents, ultimately achieving worldwide distribution. This hypothesis is consistent with the theory of virus-host coevolution, since previous researches suggest that domesticated birds were taken from a single center of domestication in Asia to various locations, with dispersal routes converging in the Americas; and oldest traces of poultry are associated with bones found in China. This study also highlights a significant influence of the anthropic action on the dispersion of animal infectious agents around the world.

**VV62 - SYSTEMIC AND MUCOSAL ANTIBODY RESPONSES INDUCED BY A VACCINE OF INACTIVATE AVIAN INFECTIOUS BRONCHITIS VIRUS (IBV) ENCAPSULATED IN CHITOSAN NANOPARTICLES**

**Lopes, P.D.; Okino, C.H.; Casagrande, V.M.; Pavani, C.; Fernando, F.S.; Tamanini, M.L.F.; Montassier, M.F.S.; Lopez, R.F.V.; Montassier, H.J.**

1. *FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNIVERSIDADE ESTADUAL PAULISTA, CAMPUS JABOTICABAL*
2. *EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA*
3. *FACULDADE DE CIÊNCIAS FARMACÊUTICAS, UNIVERSIDADE DE SÃO PAULO, CAMPUS RIBEIRÃO PRETO*

Relevant vaccine failures are favoring the continuous occurrence of outbreaks of IBV infection in Brazil. The most likely reason is the low effectiveness of the available commercial vaccines to confer crossimmunity against the emerging IBV variants. Another constraint is related to the inability of the current inactivated vaccines to provide a strong activation of immune responses at the respiratory mucosa that is the primary site of IBV infection. Thus, the objective of this study was to evaluate the systemic and mucosal antibody responses induced by an inactivated vaccine formulated with a BR-variant IBV encapsulated in chitosan nanoparticles and administered to SPF chicks, by oculonasal route, and comparing the antibody responses with those induced by a conventional inactivated vaccine prepared with oil adjuvant, administered intramuscularly. A set of 160 SPF chicks, divided into six groups was used. Four groups of chicks were vaccinated with different vaccine protocols, associating or not with a previous dose of attenuated live vaccine (strain H120). Four weeks later, the chicks were challenged with the homologous strain. Two other groups were kept as positive control (PC; infected chicks) and negative control (NC; uninfected and unimmunized). Serum and tear samples were collected of chicks at one day before infection and at 1, 5 and 11 days postinfection (dpi). The levels of anti IBV IgG were measured in tear and serum samples and the levels of antiIBV IgA were measured only in tear samples, using the sandwich ELISA concanavalin A technique. The chicks vaccinated with live attenuated vaccine followed by vaccination with the nanoparticles vaccine (L+Nano)

or oil adjuvant (L+Oil) developed higher levels of anti-IBV IgG antibodies in serum and tear samples, either in pre and postchallenge periods, and there was a marked increase in IgG levels at 11 dpi compared to other groups. The tear antiIBV IgA levels were higher at 5 dpi in chicks vaccinated with nanoparticle vaccine (Nano) compared to L+Oil and NC chicks groups. Additionally, IgA reached the highest levels at 11 dpi in PC, Nano, L+Nano and L+Oil groups when compared to the NC group. In conclusion, the nanoparticle vaccine administered by oculonasal route was capable to induce high levels of mucosal and systemic antibody responses and the nanoparticles of chitosan prove to be a potent mucosal adjuvant for mass use in veterinary vaccines due to their easier administration and non invasiveness.

**VV74 - GENOMIC CHARACTERIZATION OF A NOVEL HUMAN INFLUENZA A(H1N2) VARIANT DETECTED IN BRAZIL**

**Resende, P.C.; Born, P.S.; Matos, A.R.; Motta, F.C.; Caetano, B.C.; Debur, M.C.; Riediger, I.; Brown, D.; Siqueira, M.M.**

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Influenza A(H1N2) virus has been described to infect human, avian and especially swine populations over the years. In contrast to the widespread circulation of seasonal H1N1 and H3N2 influenza A viruses, the H1N2 subtype has been observed sporadically in humans. In this study, we report the detection and characterization of a H1N2 variant (H1N2v) strain with a genomic combination not previously reported in humans. The virus A/Parana/720/2015 (H1N2v) was identified from a nasopharyngeal aspirate collected on November 26th, 2015, from a 16 years old female patient from a rural area from Castro city, Paraná, located in the Southern region of Brazil. Castro has approximately 67,000 inhabitants and a strong agricultural center for dairy cattle, poultry and pigs. The patient did not present any risk factor for influenza and had influenza like illness with an onset of symptoms on November 23rd, 2015. Direct contact with pigs was not reported in the epidemiological investigation form. She did not receive previous antiinfluenza vaccine, her clinical outcome was uneventful and no antiviral treatment was necessary. Basic Local Alignment Search Tool (BLAST) was performed for each gene segment

sequenced and revealed strong identity with an H1N2 genome detected in swine in Brazilian Santa Catarina Southern State, in 2011 (9799%). The human viruses with more identity with this novel H1N2v were a 2003 H1N2 human lineage for HA gene (95%), a 1998 H3N2 human seasonal lineage for NA (93%), and H1N1pdm09 lineage for the other genes (9899%). Phylogenetic reconstructions strengthens the BLAST findings and suggests a recent human introduction of this Brazilian H1N2v strain, from swine, once these similar swine strains were detected around 300 kilometers distance where the human case occurred. Regarding analyses of genetic markers associated to antivirals resistance, this novel virus presented the S31N marker in M2 protein, which confers resistance to adamantane antiviral class, as H1N1pdm09 viruses. To date, no further H1N2 human cases have been detected, however other samples from this region and period are being investigated to verify their occurrence. This finding highlights the importance of influenza surveillance in humans and animals and their interface, especially during influenza season when infectivity is high. Surveillance should be focused on geographical areas where human-animal contact is frequent to ensure early detection of influenza variants.

#### **VV80 - GENETIC CHARACTERIZATION OF INFLUENZA VIRUSES CIRCULATING WITHIN BRAZILIAN SWINE BETWEEN 2009 AND 2016**

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Although Brazil has one of the largest pig populations in the world (~ 41 million pigs), very few and scattered information about influenza A virus (FLUAV) infection in pigs prior 2009 is available. Since 2009, with the introduction of H1N1 pandemic (H1N1/2009) virus in pig farms, influenza virus diversity has increased via reassortment between cocirculating viruses, including H1N1/2009. As a result of the increased influenza surveillance efforts in pigs, we have found that H1N1/2009, humanlike H1N2 and H3N2 FLUAVs

are widespread in Brazilian pig herds. From 2009 to 2016 (July), a total of 1952 nasal swabs and 1871 sera collected from nursery and growing pigs, and 165 lung tissue samples collected from suckling, nursery and fattening pigs from 171 pig farms located in the southern, midwest and southeast regions of Brazil were submitted to ELISA, HI assay, RTqPCR, virus isolation and genomic sequencing. Swine from all tested farms had antibodies to FLUAV. Seventyfive percent (75.2%) of sera tested by ELISA were positive for FLUAV antibodies. The HI analysis revealed specific antibodies for H1N1/2009, H1N2 and H3N2/2015 in pig sera from 24 out of 48 of the tested pig farms. Antibodies against two or more influenza virus subtypes were detected in pigs in seven of those 24 farms. Influenza A virus was detected by RTqPCR in 306 (14.45%) of the 2117 tested samples (nasal swabs and lungs). Virus isolation of the influenza positive samples by RTqPCR was performed by the inoculation of lung tissue supernatant or nasal swab samples into MDCK cells or into SPF embryonated chicken eggs and resulted in 162 virus isolates. Complete and partial sequences of 58 FLUAVs were obtained by genetic sequencing and together with RTPCR subtyping results, revealed 23 H1N1/2009, 15 H1N2 and seven H3N2 FLUAVs. The sequence analysis showed that the HA genes of subtypes H3N2 and H1N2 are most closely related to human seasonal H3N2 and H1N2 viruses that circulated in humans in the 1990s and early 2000s, respectively. A novel N1 gene closely related to a human influenza virus that circulated in 2007 was detected in three H1N1 viruses isolated in 2014 and 2015. These findings highlight the importance of humant-swine transmission in the evolution of influenza virus diversity in swine in Brazil and represent a challenge for the design of effective crossprotective vaccines.

**VV85 - NEONATAL PIG MORTALITY ASSOCIATED WITH SENECAVIRUS A**

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Senecavirus A (SVA) is an emerging picornavirus that has been associated with outbreaks of vesicular disease in swine. In 2015, neonatal mortality affecting piglets of 07 days of age correlated with SVA, was reported in Brazil. Here, we present an investigation carried on during 2015/2016 in five farrow-to-finish swine operations in Southern Brazil showing an increased neonatal mortality and also vesicular disease that have been associated to SVA infection. Piglets were lethargic and had a watery diarrhea. The mortality rate increased in 23% and in some littermates a 100% of mortality was observed. Despite of a relatively fast onset of wasting syndrome progressing to mortality, all herds recovered to baseline mortality levels within 410 days. Piglets were necropsied and tissue samples were collected for histopathology, RTPCR for SVA detection targeting the VP1 VP3 region, and for viral isolation in H1299 cell culture. Genome sequences of VP1 gene of five SVA isolates were compared to other SVA sequences available on GenBank. Necropsy of six piglets revealed empty stomach and mesocolonic edema. In general, it was observed enlargement and edema of inguinal lymph nodes, pulmonary edema, ascites and ulcerative lesions on the snout and coronary band. Microscopic lesions were characterized by necrotic epidermitis and dermatitis of coronary band, mild enteritis with villus degeneration on small intestine, marked mesocolon edema and multifocal hemorrhage with lung edema. Senecavirus A was detected by RTPCR in tonsil, lung, liver, intestine and coronary band. SVA was isolated in cell culture from tonsil, lung, intestine and coronary band from piglets of all farms. Sequence comparisons based on a region of the VP1 gene (541 base pairs) revealed that the Brazilian isolates characterized here share 96-99% of nucleotide (nt) identity with contemporary Brazilian isolates, 95-98% nt identity with US and 90-

93% nt identity with the prototype strain SVV001. SVA was associated with neonatal mortality based on RT-PCR, virus isolation and sequencing results. The genetic analysis shows the diversity of the Brazilian SVA isolates and that more studies are needed to demonstrate if there are differences between SVA from neonatal mortality and vesicular cases. SVA is clinically and economically important due to its resemblance with vesicular diseases, so the diagnosis tools are critical to confirm the initial investigation.

**VV143 - BRAZILIAN BATS AS CARRIERS OF VIRUSES WITH ZOOONOTIC POTENTIAL**

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Bats are animals of importance to veterinary and epidemiological surveillance. Since SARS, MERS virus and coronavirus ancestors of all mammals have been identified in bats, they have been highlighted in the Coronaviridae family evolution. *Tadarida brasiliensis* is the bat species most widely distributed in the Americas, has colony numerous and cohabits with humans. The aim of this study was to identify Coronavirus in asymptomatic bats of a colony in the city of Campinas, S.P. Brazil using metagenomic and next generation sequencing analysis. This analysis was performed using oral and anal swabs of 10 *T. brasiliensis* bat specimens collected in 2011. Samples were submitted to pre-treatment (filtration and DNase/Proteinase K reaction) and the RNA extraction was conducted with a QIAamp Viral RNA Mini Kit. From an equimolar pool, the RNA library was prepared and it was submitted to RNASeq in HiSeq 2500 Sequencing System (Illumina), paired-end (2x 100bp). The genome assembly was made with 2 platforms, Metavelvet and Metavir 2, and the annotation with UniRef 90, ViPR e CoVDB databases. We obtained 345,409,110 reads, of which 76.47% had Q>30 score. Metavelvet assembled 10,742 scaffolds and the similarity analyses identified 98 viral and 35 Coronavirus matches.

Metavir 2 assembled 9,179 scaffolds, 827 viral, 44 ssRNA and 6 Alphacoronavirus matches. In the Coronavirus Database, it was identified 3 matches with PEDV, 2 with HCoVNL63. The 4936\_Scaffold\_0, assembled with Metavir 2, represented a hypothetical Coronavirus genome containing 24,688bp and presented similarities with human Coronavirus (NL63 - NC\_005831.2; 229E - NC\_002645.1; OC43 - NC\_005147.1; HKU1 - NC\_006577.2; SARS - NC\_019843.3; MERS - NC\_004718.3; Human enteric - NC\_012950.1) and also with strains of importance in veterinary health (PEDV - NC\_003436.1; FIV - NC\_002306.3; IBV - NC\_001451.1). Can conclude that metagenomic and NGS analysis were sensitive, fast and efficient in the virus detection and the assembly's platforms were complementary. Several Coronavirus of human and animal health importance were identified and these results contributed to the understanding of molecular ecoepidemiology of these viral agents.

**VV166 - EVALUATION OF SEROLOGICAL AND VIREMIC PROFILE FOR PORCINE CIRCOVIRUS TYPE 2 IN NATURALLY INFECTED PIGS FROM FARROW-TO-FINISH FARMS IN MINAS GERAIS STATE, BRAZIL**

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Porcine circovirus type 2 (PCV2) is an important pathogen associated with systemic disease in swine worldwide. Vaccine against the disease was first available in 2004 and since then, it has been used in piglets and sows as an efficient control measure. However, profile of viral circulation in the herds has been changing over the time, suggesting that PCV2 is infecting different ages in the production system, despite of vaccination. The objective of this study was to evaluate the profile of PCV2 circulation in farrowtofinish farms using vaccines against the virus. Serum samples were collected from May to August 2012 from eight farrowtofinish farms using vaccine against PCV2 in the herds. At each farm, blood samples were randomly collected from 20 animals in each production cycle category: breeding animals (sows and gilts), farrowing (2-3 weeks), nursery (4-7 weeks), grower (8-14 weeks), and finishing pigs (15-16 weeks), totaling 100 samples/farm and 800 animals in the study. Serum samples were submitted to real time PCR and

immunoperoxidase monolayer assay (IPMA) assays to quantify viral genome loads and to detect antibodies anti-PCV2, respectively. Serological profiles varied between the studied farms and three of them had breeding females with antibody titers lower than farrowing piglets. Since breeding females are usually vaccinated late in the pregnancy to provide passive antibodies to neonates through colostrum, these results were not expected and suggest natural infection in the farrowing age. Overall, means of antibody titers decreased over the age and pigs from growing and finishing categories had the lowest means, suggesting that these animals were more susceptible to viral infection than other categories at the production system. Four farms were negative by real time PCR and the positive farms had PCV2 detected in most of the categories, suggesting that the virus was circulating in these farms, despite the use of vaccine as a disease control method. Profile of PCV2 circulation has changed in the herds over the years since the virus was first reported as an important pathogen associated with systemic disease. At that time, PCV2 associated disease affected mainly pigs at nursery age, since they were more susceptible due to the decrease in passive immunity. Our results showed that growing and finishing pigs are now more susceptible to PCV2, suggesting that the viral infection profile is changing in Minas Gerais herds, despite of vaccination.

**BV75 - IDENTIFICATION OF CELL PROTEINS THAT INTERACT WITH HUMAN RESPIRATORY SYNCYTIAL VIRUS M2-1 PROTEIN**

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Human respiratory syncytial virus (HRSV) is one of the leading causes of acute respiratory illnesses in children six months to 2 years of age. So far there is no effective drug or vaccine approved against this virus. In this work we focused on the interactions between the HRSV M21 protein, fundamental for viral genome transcription, and cellular proteins of HEK293T cell line. Using the amino acid sequence of M21 (HRSV A2 strain, GI: 3089381) we asked for M21 gene synthesis with optimization of codons for expression in human cells. This gene was sub cloned into the pCDNAFLAG vector, generating

pcDNAFLAGM21 that expresses M21 protein with FLAG peptide fused to its amino terminus. FLAGM21 expression allows a co immunoprecipitation strategy with the highly efficient antiFLAG antibodies to identify M21 interacting proteins. The functionality of this vector was shown by transfection in HEK293T cells and western blotting detection by antiFLAG and anti M21 antibodies. In pcDNAFLAGM21 transfected cells, we did an immunoprecipitation with antiFLAG coupled beads (pcDNAFLAG transfection as control) and identified the cellular coimmunoprecipitated proteins by mass spectrometry. The M21 interacting protein highest score was for a cytoplasmic isoform of polyA binding protein. A validation will be performed with specific antibodies for this and other identified proteins. In the laboratory we have previously worked with HRSV nucleoprotein (N) and phosphoprotein (P), optimized for mammalian expression. They are also components of the replication complex and form inclusion body like structures when coexpressed in a cell. We asked if coexpressing N, P and FLAGM21, FLAGM21 would be incorporated onto these structures. This was done by transfection of the respective expression vectors followed by immunofluorescence assay. Interestingly the result shows a NP M21 colocalization in inclusion bodies. This allow us to propose a coimmunoprecipitation of this complex using the anti-FLAG coupled beads strategy to analyze what cellular components are added up with the higher complexity of this viral replication related structure. These data have the potential to contribute for elucidation of the virus replication process and identify new therapeutic targets.

#### **BV79 - TROPOMIOSIN INTERACTION WITH HUMAN RESPIRATORY SYNCYTIAL VIRUS MATRIX PROTEIN**

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The Human respiratory syncytial virus (HRSV) is one of the most important pathogens of the respiratory tract, causing respiratory illness particularly in newborns and babies. The genome of HRSV encodes eleven proteins, and is essential to understand its relationship with the host, to characterize the interactions between those proteins and cellular components. In a previous work we found

an evidence of interaction of the viral protein matrix (M) with the cellular protein tropomyosin isoform 3 (Tm3) by coimmunoprecipitation. Both genes were synthesized with codon sequence optimized for expression in bacteria. These genes were cloned in the pET and pGEX vectors and we present results showing in vitro interaction of the purified HisM and GSTTm3 proteins. We also show by confocal immunofluorescence that Tm3 and M colocalize in HRSV Hep2 infected cells, reinforcing that these proteins interact in vivo. We asked then if a variation in expression level of Tm3 would have some effect on HRSV replication. We did experiments with siRNAs targeting Tm3 and didn't found changes in virus replication. On the other direction, we did experiments to overexpress Tm3 transfecting its cDNA cloned in a mammalian expression vector. Interestingly we observed a consistent inhibition of HRSV replication in Hep2 cells with Tm3 overexpressed. Previous data from the literature report that the arrangement of actin microfilaments is affected by HRSV infection. Since tropomyosin associates and stabilizes these microfilaments we could show a similar phenomena by confocal microscopy labeling the microfilaments with anti Tm3 antibody comparing Hep2 infected and noninfected cells. We also observed that in cells with overexpressed Tm3 the microfilaments structure is more resistant to HRSV replication effect. Our working hypothesis is that the interaction of M with Tm is a driven force for the viral particle budding, and that greater stabilization of the filaments by Tm excess hinders the fluidity necessary for the budding of viruses in the cell membrane.

#### **BV168 - EVALUATION OF APOPTOTIC MECHANISMS MEDIATED BY UNFOLDED PROTEIN RESPONSE PATHWAY IN JURKAT CELLS STIMULATED WITH HIV-1 TAT PROTEIN**

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HIVpositive individuals usually have a high depletion of CD4 T lymphocytes. The death of cells infected or not by the HIV is a result, among many other factors, of apoptosis mediated by viral proteins. The Unfolded Protein Response (UPR) is one of the cellular pathways that regulates cell survival or cell death. UPR also regulates the Endoplasmic Reticulum (ER) stress caused by the accumulation of misfolded or unfolded protein,

by blocking the cell protein translation, increasing expression of chaperones that assist in protein folding and lead misfolded proteins for the degradation pathway associated with the ER. When the ER stress is prolonged, as in the case of viral infections, the UPR induces apoptosis through the expression of proapoptotic molecule CHOP. With the aim to investigate the involvement of UPR pathway in cell death induced by the Tat protein of HIV1, Jurkat cells were stimulated with different concentration of the Tat HIV1 subtype C (50, 100 and 200nM) for 24, 48 and 72 hours. The evaluation of apoptosis rate was performed by staining cells with Annexin V FITC and propidium iodide and acquired in the FACSVERSE Flow Cytometry (BD). The total RNA was also extracted, quantitated and the cDNA synthesized was used for qPCR in order to quantify the levels of transcripts of genes which encode proteins of the UPR as well genes whose proteins are related with apoptosis activation. It was observed that after 72 hours of stimulation with 200nM of Tat protein there was a significant increase in apoptosis rate in Jurkat cells (10%,  $p < 0.005$ ), indicating that Tat protein most likely exerts a biologic effect which triggers the apoptosis pathway. Cells stimulated also show significant changes in transcription profile of genes encoding proteins of the UPR pathway such as eIF2 $\gamma$ , IRE1, CHOP, BIP, ATF4, GADD34 and NOXA. These results indicate that the Tat protein induces cellular changes that lead to ER stress and activation of the UPR pathway. The increase in transcription of CHOP indicate that ER stress can be involved in the process of cell apoptosis. Other experiments will be performed in order to elucidate the involvement of CHOP in the induction of apoptosis and Western blot analysis is undergoing to confirm the increase in protein expression of key molecules involved in ER stress and apoptosis. Cytochrome c release and activation of caspase 3, 7 and 12 will be performed to evaluate the role of ER in the activation of the intrinsic apoptosis pathway.

#### **BV214 - RESPIRATORY SYNCYTIAL VIRUS MRNA TRANSCRIPTOME REVEALS SURPRISING PROFILES DURING ONE-STEP REPLICATION CYCLE**

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Human respiratory syncytial virus (HRSV) is a leading cause of acute respiratory infection (ARI), mainly bronchiolitis and pneumonia in children and the elderly. It is widely accepted that HRSV mRNA transcription are produced in quantities that follow a descending gradient from the 3' to 5' of the negativestranded template. This has been assumed based on assays done with other viruses of the family Paramyxoviridae. In the present study the kinetics of mRNA transcript accumulation for all HRSV genes was evaluated in comparison to that of mRNAs actively engaged for translation in polysomes. In addition, certain HRSV gene products were quantitated at different times post infection in HEp2 cell cultures. For quantification of HRSV RNAs, realtime RTPCR assays were developed and standardized by the SyberGreen strategy for all 10 HRSV genes. Protein expression analysis was done by immunofluorescence and western blot. As expected, mRNA quantities by real time RT-PCR increased over time during the viral replication cycle. However, when the quantities of individual viral mRNAs were compared among themselves, there was no decreasing pattern of viral gene transcription. Surprisingly, the same overall pattern of quantities of mRNAs for virus gene was nearly uniform over time in cells infected with high MOI. Strikingly, large relative quantities of mRNA for SH and M22 proteins were consistently found. The analyses of viral RNAs engaged in polysomes showed that the translation profile was similar to that found for the overall detection of viral RNAs. Western blot and immunofluorescence analysis for viral proteins for which antibodies are available were in agreement with this, revealing that viral protein F was present in larger quantities than M over time post infection. These results are novel and may help to understand the relative importance of different HRSV gene products in the replication of this agent in different tissues and cell types.

**BV215 - OROPOUCHE VIRUS ASSEMBLY IN MAMMALIAN CELLS REQUIRES THE ACTIVITY OF HOST ESCRT PROTEINS**

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Oropouche virus (OROV) is a Bunyavirus that can cause Oropouche fever in humans, a febrile illness that can lead to meningitis. Because little is known about OROV replicative cycle this study aimed to describe the intracellular pathway and host factors involved in OROV assembly in HeLa cells. Toward this goal, cells were inoculated with OROV (MOI = 1) and the dynamics of viral onestep replication cycle was monitored at different time points postinfection (p.i.). A quantification by TCID<sub>50</sub> assay showed that during the first hours intracellular viral titers were continuously reduced and barely detected at 6 h p.i., indicating virus eclipse. This was followed by a rapid increase in viral titers in cell lysates and culture supernatants, reaching peak levels at 24h p.i. Accordingly, viral proteins were detected by immunoblot in cell lysates at 9 h p.i and in culture supernatants at 24 h p.i. After 9h p.i. large vesiclelike structures enriched in OROV proteins were detected by immunofluorescence at a pericuclear region, indicating viral factories. These factories contained early endosome protein and the endoplasmic reticulum (ER) resident membrane protein. A transGolgi marker showed a dispersed pattern throughout the cytoplasm and also colocalized with the viral factories. In contrast, a cis-Golgi marker did not colocalize at any time point p.i. with these factories suggesting that Golgi apparatus may not be the main site of viral assembly. ImmunoEM analysis of infected cells revealed large multivesicular bodies structures (MVBs) that contained virus particles and were often associated with the ER. This data prompted us to verify a possible role for the ESCRT (Endosomal Sorting Complexes Required for Transport) machinery in viral replication. Knockdown of Tsg101/ESCRTI and Alix led to a strong reduction in OROV production (40% ±13.3% and 35%±14.8%, respectively) and compromised the formation of prominent viral factories, as intracellular OROV staining remained restricted to small puncta dispersed throughout the cytoplasm. The superexpression of a dominant negative form of the

AAATPase Vps4A, which disrupts the MVB pathway, led to an enlargement in the area of the viral factories (146%±63.2%), where the Vps4A mutant accumulated. Together our data presents new insights into cell compartments and host factors involved in OROV biogenesis indicating that OROV requires the host MVB pathway with the recruitment of the ESCRT machinery for a proper virus formation.

**PIV102 - MOLECULAR CHARACTERIZATION OF GRAPEVINE ENAMO-LIKE VIRUS, A NOVEL PUTATIVE MEMBER OF THE GENUS ENAMOVIRUS**

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The genus Enamovirus, family Luteoviridae, consists of one recognized viral species, Pea enation mosaic virus 1 (PEMV1) and two putative members, Alfalfa enamovirus1 (AEV1) and Citrus vein enation virus (CVEV). We encountered a novel Enamovirus, tentatively named Grapevine enamolike virus (GELV), in a *Vitis vinifera* 'Cabernet Sauvignon' vine in an experimental field in the municipality of Bento Gonçalves, Brazil. The symptoms in this host were those of severe grapevine leafroll disease and reddish leaves. To characterize the viromes of this sample, dsRNA was extracted from 30g of bark scrapings. Sequencing data was generated from a cDNA library that was constructed by Macrogen. The Illumina HiSeq2000 platform was used to generate about 20 million reads. CLC Genomics Workbench software was used for quality trimming and de novo contig assembly from the reads. All contigs were analyzed using NCBI's Blastx program against the viral RefSeq database. Bioinformatic analysis indicated that the longest contig (6206 bp, GenBank accession code KX645875) shares only 49% identity with PEMV1 (Query coverage 25%, E value: 9e130) thus indicating that GELV is a distinct member of the genus Enamovirus based on established criteria. To confirm the high throughput sequencing (HTS) results, dsRNA and total RNA were extracted from fresh plant material and screened by RTPCR using the specific primers (SetF: 5'TTCCCTTGGGAGACTCGGTTCTAT3' and SetR: 5'AAACATGACCACCGTCTCATAGC3'). The resulting amplicon (735 bp) was cloned, sequenced and

determined to be 99% identical with corresponding sequences generated by HTS. Grafttransmissibility of GELV was confirmed by grafting source vines onto P1103 cultivar rootstock (13+/16) and confirming the infection by RTPCR. This is the first report of a virus in the family Luteoviridae infecting grapevines. Further investigation using HTS lead to the discovery of this novel virus in three different samples of grapevines (cvs. CG 90450, Semillon, and Cabernet Franc) and using RTPCR in another sample (cv. Malvasia Longa). To gain insight into the virus organization and evolution, the 6206 bp contig was subjected to further bioinformatics analysis. Five ORFs were predicted and analyzed for conserved elements. Field surveys and biological studies are currently underway to determine the prevalence of GELV in Brazil, evaluate its potential natural spread, and assess its effect on vine performances and wine quality.

#### **PIV117 - IDENTIFICATION AND FUNCTIONAL ANALYSES OF THE COTTON BLUE DISEASE RESISTANCE LOCUS**

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Cotton blue disease (CBD) is a major cotton disease in Brazil. It is transmitted by *Aphis gossypii* and its causal agent is the Cotton leaf roll dwarf virus (CLR DV). CBD resistance is controlled by one single dominant locus, however nothing is known about it. Previously, we mapped the *Cbd* resistance locus, identifying two ORFs, named *Cbd1* and *Cbd2*, respectively. The promoter region of these two genes is bidirectional and presented many cis elements related to: salicylic acid, auxin responses and biotic and abiotic stress. *CBD1* is a low density lipoprotein receptor (LDL) and *CBD2*, an arginyl tRNA transferase (ATE) implicated in the Nend rule leading target specific proteins to degradation by 26S proteasome. The expression of GhCBD1 and GhCBD2 in organs of five cotton cvs (two susceptible, two resistant and one showing middle resistance to CLR DV) showed no differences that could justify their responses in infection. Studying GhCBD1 relative expression during viral infection we observed that its expression is 9to-

189x induced in all points (24 hpi, 5, 15 and 25dpi) in susceptible cv. However, its expression suffer just a slight variation in the resistant cv. along the infection. By the other side, GhCBD2/ATE was suppressed 24hpi (7x reduced) and induced at 5dpi (12x more expressed) in susceptible cvs. An opposite pattern was seen in the resistant cv (4x more expressed at 24 dpi and 5x reduced at 5 dpi). Investigation of similar effects in *Arabidopsis thaliana* Col. under CLR DV infection showed a significant increase (8x) of AtATE1 after CLR DV infection. *Arabidopsis pATE1:GUS* revealed an increase GUS activity in shoot and root apical meristems as well in young leaves compared with noninfected plants. 35S:ATE1 plants blocked viral infectivity. Thus, the data suggest that to prevent CLR DV spreading, the expression of CBD2/ATE must be induced in 24hpi. So, CBD2/ATE could act by inhibition of the replication and/or viral spread. In this sense, we saw that the CLR DV's movement protein (ORF4) has the necessary amino acids to be an ATE target. We suggests, CBD2/ATE can be a candidate protein to generate resistance, possibly, leading viral movement protein to 26S proteasome for degradation via the Nend rule.

#### **PIV118 - DICER-LIKE PROFILE EXPRESSION DURING VIRAL INFECTION IN SUSCEPTIBLE AND RESISTANT COTTON**

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RNA silencing is an important antiviral defense mechanism in plants. RNA silencing ou RNAi pathway is triggered when viral doublestrand intermediary RNAs (dsRNA) are generated during viral replication in the first steps of virus infection. Plant Dicerlike (DCL) ribonucleases, specially DCL2 and 4, recognize these dsRNA strands and dicers then, producing 21–24 nucleotide short interfering RNAs (siRNAs). The viral siRNAs are incorporated into RISC complexes, which recognizes and destroys siRNA complementary target RNAs. *Arabidopsis thaliana* presents four DCLs (DCL1–4), however, the cotton DCLs have not yet been characterized. The objective of this study is characterize

the DCLs of commercial cotton, *Gossypium hirsutum*, and analyze their expression profile during viral infection. Based on *Gossypium raimondii* cotton 2n ancestral species genome, primers were designed to amplify the distinct cotton DCLs (1, 2a, 2b, 3a, 3b e 4). Previously, we have identified a duplication of DCL3 in *G. raimondii*, which has never been shown before in eudicots plants. So, we looked for the presence of the 6 putative DCL (including DCL3 duplication) in 4n commercial cotton. Firstly, the cotton DCLs expression profiles was analyzed in distinct organs (leaf, stem, root and flower) by qRT-PCR in plants from two cvs.: FM and DO. FM cotton cv is susceptible to the cotton blue viral disease (CBD), and DO cv is resistant. CBD is an important cotton disease for Brazil and it is caused by the Cotton leafroll dwarf polerovirus (CLR DV). We observed that GhDCL4 (the DCL mainly responsible for antiviral defense in plant) is more expressed in resistant cultivars in the stem, root and flower than in the same organs of the susceptible FM. Expression levels of the 6 DCLs were also analyzed during CLR DV viral infection in FM and DO plants. For that, systemic leaves were collected 24 hpi and 5, 15 and 25 dpi and analyzed in pools of 35 leaves. As mock, plants from both cvs inoculated with aviruliferous aphids were used. DCL expression analysis showed that most of the DCLs genes were downregulated 24 hpi in the susceptible plants, except DCL1 and DCL2a. However, all the DCLs showed increased levels of expression at 5 dpi. In the cultivars DO, all DCL genes have shown increased expression levels at 24 hpi and 5dpi. Upregulation of DCL genes in cvs DO seems to be helping the plant in the viral defense and systemic distribution of small RNAs, improving the plant ability to face the virus infection.

**PIV175 - ARRACACIA XANTHORRIZA (MANDIOQUINHASALSA): A RESERVOIR OF PLANT VIRUS**

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Arracacha (*Arracacia xanthorrhiza*), known as mandioquinhasalsa in Brazil, is vegetatively propagated, and therefore it probably accumulates degenerative pathogens such as viruses. Arracacha plants with viral symptoms are frequently found in Brazil, and so far, only two potyviruses have been reported in the country. Thus,

to perform a survey on viruses occurring in this crop and to evaluate the losses they cause, appropriate tools is essential, enabling detection and characterization of the viruses. Nextgeneration sequencing is the most advanced technique for studying viral metagenomics. The major aim of this study was to apply metagenomic approach to characterize the viral biodiversity in arracacha plants in Brazil. To this extent, in 2015 plants were collected from different areas: arracacha germplasm collection of Embrapa Vegetables (Brasília) and green belt in São Paulo. Leaf tissues were subject to viral enrichment by ultracentrifugation on a 20% sucrose cushion followed by nucleic acid extraction (AllprepDNA/RNA Kit, Qiagen). RNA was sequenced through Illumina HiSeq 2000 platform. 21,048,084 million reads were generated by the joint data analysis after adapter and quality trimming using Trimmomatic. Assemble was performed with four different tools, SPAdes, Velvet, MEGAHIT and ABySS, in order to explore the data efficiently and to identify the complete set of viral species. All assembled contigs were submitted to Blastx searches against the RefSeq viral database. A total of 1442 (SPAdes), 6502 (Velvet), 1568 (Megahit), 7388 (ABySS) contigs produced significant hits with viral sequences. With our sequencing pipeline we were able to identify at least 10 new plant virus species infecting arracacha plants, possible members of the families: Closteroviridae, Rhabdoviridae, Betaflexiviridae, Luteoviridae, Endornaviridae, Secoviridae and Tymoviridae; as well as plant viruses that have been already described belonging to the family Potyviridae and Betaflexiviridae. To validate these results, specific primers were designed from the most conserved gene of the 10 species. Arracacha plants from the main producing areas of Brazil were analyzed and plants were found infected with up to 9 different species. This work reveals arracacha plants as a reservoir of plant viruses and represents a first step towards discovering and characterizing novel viruses, in order to increase the effectiveness and reliability of control measures against viruses in arracacha crops.

**PIV209 - THE COMPLETE GENOME SEQUENCE OF A NOVEL BETABACULOVIRUS ISOLATED FROM MOCIS SP. REVEALS AN ANCIENT GENOME EXPANSION AND A TENDENCY IN NOCTUID-INFECTING BETABACULOVIRUS**

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3. EMBRAPASOJA

In this report, we described the genome of a baculovirus isolated from the insect pest, *Mocis* sp. The genome is 134,272 bp in length with a G+C content of 38.2%. Based on the concatenated sequence of the 37 baculovirus core proteins, we found that the virus is a betabaculovirus closely related to other noctuid-infecting betabaculovirus including *Pseudaletia unipuncta* granulovirus, *Helicoverpa armigera* granulovirus, and *Xestia c nigrum* granulovirus. We called this novel species by *Mocis* sp. granulovirus (*MospGV*). Only three ORFs were found to be unique to *MospGV* and several auxiliary genes were found including *iap3*, *iap5*, *broa*, *brob*, and three enhancins. The virus genome lacked both chitinase and cathepsin. Interestingly, when we analyzed the enhancins, we found that the betabaculovirus genes were acquired from alphabaculovirus and underwent several duplications during evolution. Duplication also happened to an endonuclease-like gene. Moreover, genomic and gene content analyses revealed both a strict collinearity and gene expansion into the genome of the *MospGV* related species. Betabaculovirus genome sequencing is of importance to the field as few genomes are publicly accessible. *Mocis* sp. is a secondary pest of maize crops in Brazil and other cultures. Certainly, both discovery and description of novel baculoviruses may lead to development of green and safe pesticides in order to counteract and effectively control crop damage-causing insect population.

**PIV244 - STUDY OF BEGOMOVIRUS DIVERSITY IN TOMATO PLANTS USING NEXT-GENERATION SEQUENCING**

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Tomato (*Solanum lycopersicum*) is one of the most economically important vegetables in the world, being widely cultivated in Brazil. However, the yield is substantially impaired by the occurrence of many diseases, particularly those of viral etiology. Begomoviruses (family Geminiviridae) cause one of the most important diseases in tomatoes, occurring at high frequency rates in the major growing regions of the country. Currently, the use of moderately resistant cultivars is the most effective method for controlling this disease. The aim of this work was to study the begomovirus diversity in susceptible (BSP 0031, BSP 0034 and Sheena) and resistant (TY 2006, Tinto, BRS Sena and Candieiro) cultivars using NextGeneration Sequencing. Twenty samples of each cultivar showing typical symptoms of begomovirus infections were collected in Luziânia GO. After confirming the viral infection by PCR using universal primers for begomovirus, viral circular DNA was amplified by rolling circle amplification (RCA). RCA products of susceptible and resistant cultivars were pooled and sequenced on an Illumina platform. Two libraries were produced, one for susceptible samples (DNAsus, with 20,158,352 reads) and another for resistant samples (DNares, with 20,205,324 reads). All reads were trimmed in Geneious software (Q20) and assembled using the Velvet algorithm (71 kmer), resulting in 1,129 contigs for DNAsus and 1,668 for DNares. Following a MegaBLAST analysis (max. Evalue=1e20) against a geminivirus RefSeq database, DNAsus contigs shared high identity (> 91%) with Tomato severe rugose virus (ToSRV, 753 matched contigs), Tomato mottle leaf curl virus (ToMoLCV, 110 contigs), Bean golden mosaic virus (BGMV, 14 contigs), Euphorbia yellow mosaic virus (EuYMV, 31 contigs) and Sida micrantha mosaic virus (SiMMV, 81 contigs) sequences. On the other hand, DNares contigs presented identity only with ToSRV (1000 contigs), ToMoLCV (12 contigs) and SiMMV (2 contigs). In addition, two contigs of DNares library share < 85% identity with Centrosema yellow spot virus (CeYSP), indicating that a new

begomovirus species might be present in the sample. The results indicate a difference in the population composition of begomoviruses in the field, with lower diversity in resistant than in susceptible plants. Analyses of the viral genomes will be conducted to verify whether the isolates are undergoing a genetic variation process due to the selective pressure imposed by the use of resistant plants.

#### **HV11 - INCREASED PRO-INFLAMMATORY CYTOKINES IN AMNIOTIC FLUID FROM ZIKA VIRUS ASSOCIATED MICROCEPHALY**

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3. UNIVERSIDADE FEDERAL DE SÃO PAULO
4. THE GEORGE WASHINGTON UNIVERSITY

Recent advances in the understanding of neuropathogenesis associated to Zika infection have led to descriptions of neonatal microcephaly cases. However, any of these reports evaluated the humoral immune response during ZIKV infection. We investigated 27 cytokines, chemokines, adhesion molecules and growth factors in the amniotic fluid (AF) of pregnant women with confirmed diagnose of Zika and neonate microcephaly. All pregnant women enrolled in this study presented Zika infection symptoms in the first trimester of pregnancy confirmed by PCR testes from amniotic fluids collected during ultrasoundguided transabdominal amniocentesis. The microcephaly was confirmed through intrauterine ultrasound and neonate circumference measurement. We observed a remarkable increase of the inflammatory cytokines IL-6, IL15, IL8, MCP1, G CSF in the amniotic fluid of Zika positive pregnant women with neonate microcephaly. In contrast, we observed lower levels of IFN  $\gamma$ , IL5, IL-13, Eotaxin, RANTES and PDGF compared with ZIKV negative controls. The inflammatory microenvironment caused by ZIKV infection could in part determine the differentiation, proliferation, migration and survival of neural progenitor cells. The cytokine changes we

observed in AF from ZIKV women could partially explain neurodevelopmental defect. The downregulation of MCP1 or IL6 promotes the differentiation of human amniotic fluid derivedmesenchymal progenitor cells (MePR2B) to a neuroglial phenotype, and the increase in MCP1 and IL6 levels could be a developmental blockade to differentiation of derivedmesenchymal progenitor cells to mature neuroglial cells in the fetus, contributing with microcephaly. Higher levels of IL6, IL8 and MCP-1 and decreased levels of IL5, IL13, Eotaxin and PDGF are associated with undifferentiated MePR2B cells. In contrast, we found elevated GCSF in the AF of ZIKV positive pregnant women, and a previous study had suggested that this growth factor acts as an autocrine protective signaling mechanism in response to neural injury. Our findings support the relevance of immune activation in the neuropathogenesis of ZIKVassociated microcephaly cases. Finally, the inflammatory response in the ZIKV infected uterine environment should be further investigated, since proinflammatory cytokines could also damage neuron cells, interfere with fetal development and be used as biomarker candidates associated with a poor outcome in Zika infected pregnant women.

#### **HV156 - ACTIVATION OF INTRINSIC COAGULATION PATHWAY AND LIPID METABOLISM IN DENGUE VIRUS PATHOGENESIS**

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Dengue virus (DENV) infection induces increased vascular permeability and plasma leakage, which is related to exacerbated inflammation and hemostasis dysregulation. Activation of the intrinsic coagulation or contact pathway promotes the release of bradykinin (BK), which has vasodilation and hypotensive action, and is an inflammatory modulator in infectious diseases. This pathway is triggered by activation of factor XII by anionic polymers, such as dextran sulfate (DXS) or polyphosphates (PolyP) derived from activated platelets, culminating in conversion of prekalikrein (PKa) to kalikrein, and cleavage of kininogen, thus producing BK.

Increased BK levels are detected in individuals submitted to plasma apheresis with DXS to remove low density lipoproteins (LDL), indicating that contact pathway may be regulated by lipid metabolism. Here, we investigated the status of contact pathway and LDL content in the plasma of dengue patients with different clinical outcomes, by evaluating a kinetic hydrolysis of FITC-conjugated PKa substrate, in the presence or absence of DXS. Plasmas from patients with classic dengue or dengue with complications showed diminished activation of contact system, and this inhibition was detected since early infection. On the other hand, plasma from severe dengue patients presented an extensive activation of this pathway. These data suggest that severe disease may be associated to enhanced contact activation and increased plasma BK levels, whereas patients with mild dengue seem to present inhibitory elements in plasma, contributing to protection of the system and control of hemostasis and vascular damage. Preliminary RMN data suggested that the samples showing higher activation of contact pathways presented lower LDL content, indicating a potential crossregulation between lipid metabolism and contact pathway. We then investigated whether DXS affect lipid content and virus replication in endothelial cells infected with DENV. Addition of DXS to DENVinfected endothelial cells removed membrane sterols and inhibited the release of viral particles, as demonstrated by amplex red assay and plaque titration, respectively. Since DXS may aggregate LDL, and given that lower levels of plasma LDL was previously associated to severe dengue outcome, we believe that lower LDL levels allow increased activation of contact pathway and BK release, what may then contribute to vasodilation and plasma leakage, while limiting virus replication.

#### **HV206 - IDENTIFICATION AND SELECTION OF DENGUE VIRUS SPECIFIC PEPTIDES FOR DIFFERENTIAL DIAGNOSTIC TESTS**

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*LABORATÓRIO DE VIROLOGIA BÁSICA E APLICADA*

Dengue is one of the most important infectious diseases in Brazil and the early diagnosis is a determining factor for disease outcome, particularly for those afflicted with the most severe forms of infections. Meanwhile,

the co circulation of other flavivirus as Yellow fever virus (YFV), Saint Louis Encephalitis virus (SLEV) and especially Zika virus (ZIKV) highlights the importance of the development of differential diagnostic tests able to segregate acute febrile illnesses that are known to have serological crossreactivity with Dengue. The goal of this work is to identify conserved and polymorphic linear Dengue virus (DV) epitopes which could be used for ELISA or lateral flow chromatography. To this end, we aligned predicted viral proteomes based in genome sequences of the four DV serotype and performed an in silico epitope mapping. We developed a script in Perl integrating alignment and prediction information to identify potential serotypespecific epitopes. We excluded epitopes what are also present in the ZIKV and YFV genomes. A total of 15 peptides were found to be polymorphic among DV serotypes and 9 peptides were found to be conserved among all serotypes. A peptide array containing the predicted epitopes was prepared on a cellulose membrane. The reactivity of the peptides was tested using sera from rabbits monoinfected with each dengue serotype. Seven peptides were considered reactive with the test sera and not reactive with sera from noninfected rabbits, of which three were selected for soluble synthesis. After that, we perform a screening ELISAs for the selected three peptides with 80 DV positive human sera, 20 DV negative human sera, 6 YFV positive human sera and 12 ZIKV positive mouse sera. None of the three peptides were recognized by YFV and ZIKV positive sera, differently from the full recombinant DV envelope protein which was recognized by the heterologous sera. The best peptide showed 82% of sensibility and 87% of specificity in ELISA tests. These in silico and in vitro analyzes allowed the selection of three peptides, conserved among all DV serotypes, that present potential as dengue specific antigens not recognized by antibodies against other relevant and co-circulating flaviviruses

**HV254 - SALIVA AS THE BIOLOGICAL SAMPLE OF CHOICE FOR THE MOLECULAR DIAGNOSIS OF ZIKA**

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The Zika virus (ZIKV) is an Arthropodborne virus belonging to the Flaviviridae family, genus Flavivirus. It is mainly transmitted by the bite of infected female *Aedes* mosquitos. This virus is considered an emerging pathogen since 2007 when an outbreak was reported at the Yap Island in the Federated States of Micronesia. At the beginning of 2015, ZIKV was identified in autochthonous cases in Americas. Currently, Brazil is experiencing a massive outbreak, with all states reporting cases. The success of RNA detection for the diagnosis of viral infections has a direct relationship to the correct choice of body fluids, at the appropriate time post infection (p.i.). Different studies reported the detecting of ZIKV in blood and saliva until ten days after onset of the symptoms, while others reported urine as an alternative specimen in cases with more than ten days p.i.. Therefore, the aim of this study was to evaluate the best biological sample for the diagnosis of ZIKV infection, during the acute phase of illness. Between February and April 2016, three types of biologicals samples (serum, urine, and saliva) were collected from patients suspected of ZIKV infection, attended at Hospital Adventista de Manaus, a sentinel unit for the ZIKV surveillance in the Amazonas state, Brazil. Samples were sent to the Laboratory of Infectious Diseases Ecology in the Amazon, at Leônidas and Maria Deane Institute – Fiocruz Amazônia. The serum, urine, and saliva from 74 randomly selected patients were tested by RTqPCR, according to a previously described protocol. Among the tested samples, 50% (n=37) were positive in serum, 17.6% (n=13) in urine and 75.7% (n=56) in the saliva. Only seven patients (9.5%) were positive to ZIKV in the three specimens, and 11 were negative in all samples. Statistical analysis support that the number of positives samples in saliva is significantly higher than in serum (McNemar's test  $p=0.0005$ , OR 5.5, CI 1.89 – 15.96). Furthermore, the median of saliva Ct values was significantly lower (KruskalWallis test serum vs. saliva  $p=0.0034$ ; urine vs. saliva  $p=0.0025$ ).

Our results strongly suggest that saliva is the best body fluid for the molecular detection of ZIKV RNA during the acute phase. Furthermore, saliva collection is secure and non invasive, requiring nearly no training. The findings of this study suggest that the adoption of saliva testing might improve the molecular diagnosis of Zika, increasing the number of laboratorial confirmed cases.

**HV256 - ETIOLOGY OF THE ACUTE FEBRILE ILLNESS IN THE AMAZON STATE BRAZIL, DURING THE EMERGENCE OF ZIKA VIRUS**

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Arthropodborne viruses (arboviruses) are important infectious agents, notably for people living in tropical and subtropical regions around the planet. Arboviruses infections may cause an acute febrile illness with symptoms frequently related to viral infections, or other infectious agents, which may disguise the emergence of new human pathogens. Recently, Brazil faced the emergence of two important arboviruses, the Chikungunya virus (CHIKV) and the Zika virus (ZIKV) that led to outbreaks along with the Dengue virus (DENV). This epidemiological situation strengthens the necessity to conduct the differential diagnosis for these arboviruses. From October 2015 to February 2016, the Laboratory of Infectious Diseases Ecology in the Amazon, at Leônidas and Maria Deane Institute – Fiocruz Amazônia was responsible for the molecular diagnosis of Zika virus in the Amazonas State, as a request of the state health surveillance authorities. During this period, a total of 423 samples, 130 from males and 293 from females, including 73 pregnant women, all suspected of arbovirus infection were submitted to an RTqPCR protocol for ZIKV, CHIKV, and DENV testing. Additionally, negative samples were further processed for the detection of Mayaro (MAYV) and Oropouche (OROV) viruses, by an RTqPCR protocol previously developed by our group. Moreover, selected samples were also submitted to viral isolation in C6/36 or Vero cells and nucleotide sequencing. We identified 140 ZIKV positive samples (33.1%), 37 in pregnant women, 14 in children and 5 in the elderly. From the six Zika RTqPCR positive

samples submitted to viral isolation, one was isolated in C6/36 cells. In the experiments for the detection of other arboviruses, we obtained two positive samples for DENV, two for CHIKV, one for MAYV and six for OROV, all from patients that live in Manaus, with no travel history. These data indicate that in addition to ZIKV, DENV, and CHIKV; MAYV and OROV are also circulating in Manaus. This data is of particular concern since these viruses also have the potential to cause outbreaks, worsening the current epidemiological situation at least in the Amazonas State. The results of the present study indicate a need to increase the surveillance programs for other arboviruses, especially in places with close contact with extensive forest areas, as observed throughout the Amazon region.

#### **HV258 - SOROPREVALENCE DENGUE IGG IN PATIENTS IN A PROSPECTIVE COHORT STUDY OF SÃO JOSÉ DO RIO PRETO**

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Dengue is a viral infectious disease and one of the most important arboviral diseases in the world. The virus is maintained in an urban transmission cycle: human mosquito human. Dengue studies, often only consider the cases reported without grouping data on past epidemics. Prospective studies offer the advantage of determining the true incidence of a disease in a cohort for information on relative risk and absolute, the spectrum of clinical outcomes, risk factor analysis for severe disease development and spatial and temporal diversity in transmission serotype specific of Dengue virus. The aim of this study was to evaluate the seroprevalence of DENV in population by ELISA for antidengue IgG in 1481 patients enrolled in a prospective cohort study in São José do Rio Preto / SP. The results showed that in 1082 patients (73.06%) were positive, 15 were inconclusive (1.01%) and 384 patients (25.93%) were negative for dengue. Of the patients who reported not having DENV (506 patients, 34.17%), 414 patients, 27.95 (%) had antibodies to DENV. Among women, 59.08% were positive, while among men 40.20% were positive. The family income of the patients is R \$ 2,106.00 on

average. The data showed that at least 73.06% of studied patients in this cohort had contact with the DENV at least once in life, since the class of IgG antibodies shown this throughout the patient's life. According to literature, regions with lower financial conditions are more affected. São José do Rio Preto is hyperendemic to dengue and data presented highlights the importance of this region for the surveillance and control of dengue, as well as the importance in maintaining basic patient care, surveillance and control of dengue, improving notification and control of the disease, early diagnosis and care for severe cases of dengue. Moreover, it is very important to raise awareness about the need to keep control of vectors and their breeding, mainly in the case of *A. aegypti*.

#### **EV14 - CORROSION AND BIOFILM REDUCED BY ECOPHAGES IN A PILOT ANAEROBIC SYSTEM**

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Iron corrosion in an anoxic environment, like industrial pipelines, cause large economic losses; and are highly influenced by microorganisms, especially sulphate-reducing bacteria, which cause the iron deposition in pipelines inner. BRS are ubiquitous anaerobic microorganisms that uses iron as final electron acceptor, with consequent hydrogen sulphate production. It is estimated that 50% of the total mineralized carbon in oceanic floor was converted by BRS, which take a central role in carbon and sulfur cycle. 7 distinct phylogenetic groups divide BRS, among them 5 are found in Eubacteria domain and 2 in Archaea. Due the high diversity of BRS, in this work we evaluated an Ecophage cocktail for biofilm control and consequent corrosion by BRS in a pilot anaerobic system (loop). The system began operating with 77 specimens that were removed throughout the experiment to evaluate the biofilm and the corrosion degree. After 7 days bacterial cultures grown in selective media (BRS and heterotrophic) were inoculated in the system and then nutrient solution was added in order to promote bacterial growth. All parameters were

observed for about 1 month after the bacteria injection until the bacterial culture reach the order of  $10^4$  by the MPN method. At this time, the phage cocktail (final titer in  $10^{10}$ ) was inoculated containing five phages of Siphoviridae and Myoviridae families, all isolated using bacteria *Escherichia coli* species. The samples were collected throughout the experiment and evaluated by optical profiler, on day: 1, 4, 7, 12, 22, 34, 37 and 41, being the day 34, 37 and 41 after phages inoculation. One day after adding the phages in the system, little reduction in roughness of the specimens could be observed, reaching baseline levels about 7 days after inoculation. The data demonstrate the effectiveness of nonspecific phages to biofilms and corrosion control in a pilot system. As previously described in a lab scale experiment, phages evolution by host range expansion (HRE) could explain those results, since it is a closed system and the results could be better observed about 7 days after inoculation.

#### **EV49 - ENTERIC PATHOGENS SURVIVAL, PERCOLATION AND LEACHING IN BIOFERTILIZED SOILS USING SWINE DIGESTATE**

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2. INSTITUTO TECNOLÓGICO AGRARIO DE CASTILLA Y LEÓN, VALLADOLID, SPAIN
3. AIRTON KUNZ EMBRAPA SUÍNOS E AVES

Enteric pathogens present in biofertilizers can be accumulated in the soil, affecting water and foods. In this context, the present study evaluated the stability, percolation and leaching of enteric pathogens in clay and sandy soils after biofertilization with swine digestate, using the bacteriophage PhiX174, mengovirus (vMCo), *Salmonella enterica* Thiphymurium and *E. coli* as biomarker models. Viruses were quantified by plaque assay technique (PFU) and bacteria by colony forming units (CFU). The stability of these enteric microorganisms was evaluated up to 120 days using sentinel chambers (Eppendorf LidBac membrane lids, Eppendorf, Germany). Each sentinel chamber was filled with biofertilized soils and allocated vertically in clay and sandy soil microcosms (1020 cm of depth). For percolation assay PVC tubes (60 cm length × 30 cm diameter), were closed with a cap at the bottom,

and kept in horizontal position. Afterwards, soils in the PVC tubes were biofertilized by spraying with swine effluent (corresponding to 50m<sup>3</sup>/ hectare) derived from mesophilic biodigestor containing  $5.3 \times 10^7$  CFU mg<sup>-1</sup> of *S. Typhimurium*,  $4.8 \times 10^7$  CFU mg<sup>-1</sup> of *E. coli*,  $6.2 \times 10^5$  PFU mg<sup>-1</sup> of vMCo and  $3.4 \times 10^5$  PFU mg<sup>-1</sup> of PhiX. To estimate the percolation of the enteric microorganisms it was collected 1 g of soil sample at depths of 10, 20, 30, 40 and 50 cm by performing holes of 1 cm in diameter in the PVC tubes using a sterile probe. Samples were collected at 0, 0.12, 0.24, 0.5, 1, 2, 4, 8, 15 and 20 days after biofertilization. The vMCo and PhiX174 stability in clay soil was significantly lower ( $p=0.002$ ) than in sandy soil (2log<sub>10</sub> of difference), and PhiX174 showed the faster percolation and leaching in sandy soil (3.4log<sub>10</sub>) than clay soil (2.2log<sub>10</sub>). *E. coli* proved to be a good microbial biomarker of depth contamination and leaching in clay and sandy soils, while bacteriophages showed potential to be biomarkers of enteric pathogens persistence in both soils. These results can contribute to the development of predictive models of enteric pathogens behavior in different soils, as well for water and food contamination by biofertilization, considering the risks management and mitigation in the swine digestate recycles.

#### **EV54 - ROTAVIRUS AND OTHER HUMAN ENTERIC VIRUSES IN GASTROPODS**

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UNIVERSIDADE FEEVALE

Worldwide, the principal causes of waterborne diseases are related to viral infections. In this context the enteric viruses, those that infect the gastrointestinal tract, won special attention about their use in monitoring water pathogens. Within this group we highlight the rotavirus (RV), human adenovirus (HAdV), enterovirus (EV) and the hepatitis E virus (HEV). They are mainly introduced into water bodies through anthropogenic activities, as the launch of domestic effluents. *P. canaliculata* snails and water samples were collected bimonthly for one year (October/2014 August/2015) from 4 wetlands dispersed along of the Sinos River basin. The waters were concentrated from the adsorptionelution method. The snails were removed from shells and the body was completely macerated. One gram of tissue was diluted in 1 mL Eagle's minimal essential minimum (EMEM)

homogenized and centrifuged, the supernatant was used for viral detection. The snail hemolymph was drained from the mantle region. Real time polymerase chain reaction (qPCR) targeting HAdV hexon gene, and conventional polymerase chain reaction was used for RV, EV and HEV. Twenty six percent (19/72) of the samples tested were positive for HAdV, including water, hemolymph and gastropod tissues. Positive samples were tested for the presence of RNA viruses. RV was detected in 11% (2/19) of samples, while EV and HEV were absent. HAdV and RV were detected, suggesting fecal contamination, which may hamper the ecosystem services provided by these wetlands. These results also indicate that the snails have the ability to bioaccumulate enteric viruses.

#### **EV88 - DETECTION AND MOLECULAR CHARACTERIZATION OF GEMYCIRCULARVIRUS FROM ENVIRONMENTAL SAMPLES IN BRAZIL**

**Assis, M.R.S.; Vieira, C.B.; Fioretti, J.M.; Rocha, M.S.; Almeida, P.N.; Miagostovich, M.P.; Fumian, T.M.**

*FUNDAÇÃO OSWALDO CRUZ*

Gemycircularvirus (GemyCV) is a group of viruses which has been recently proposed as a new viral genus detected in fecal and environmental samples around the world. GemyCVs have been detected in human blood, brain tissue, cerebrospinal fluid, and stool sample. In the present study, we demonstrate for the first time, through molecular detection and characterization, the presence of GemyCVs in environmental samples from Brazil. Our results show a percentage of positivity ranging from 69 (25/36) to 97 % (35/36) in river water samples collected in Manaus, Amazon region, and wastewater from a wastewater treatment plant located in Rio de Janeiro, respectively, revealing GemyCVs as an important environmental contaminant.

#### **EV177 - ADENOVIRUS INVESTIGATION BY MOLECULAR ANALYSIS IN PUBLIC WATER SUPPLY NETWORK**

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In Brazil, the quality control of the water distributed by supply systems has been done by laboratory

identification of bacteria from the coliform group. However, other pathogenic microorganisms such as protozoa, cyanobacteria and enteric virus, associated with waterborne diseases have not been effectively eliminated from the water supply through conventional treatment. Among the viruses, adenoviruses are highlighted, which are present in the environment and represent great risk to public health; contaminating rivers, groundwater and water for human consumption. The objective of this study was to evaluate the quality of the water by detection of adenoviruses in the water supplied to the population from Macapá by the public supply system CAESA. Water samples from the Amazon River captured for treatment and from the outputs of the treated water distribution reservoirs supplied by the Company of water in the city of Macapá were analyzed, 14 points over the months of May, June and July, totalizing 42 samples. The investigation of adenovirus in water was based on the concentration (ultrafiltration) by adsorption-elution technique in unpolarized membrane, followed by the nucleic acid extraction using Mini Kit (RTP Molecular Stratec), and detection of genetic material by conventional PCR and nested PCR. Among the 42 samples examined so far, none (0/42) revealed the presence of human adenovirus in both samples, from the river and from the distribution network. Therefore, the implementation of this molecular analysis method in the evaluation of the water distributed to the population in the city of Macapá quality showed no contamination by adenoviruses, but contributed to the training of human resources in molecular biology field focused on the monitoring of water quality and basic sanitation in Amapá, which provide subsidies to control the prevalence of waterborne disease of viral etiology in the population and in effective contribution to the Ministry of Environment and State and Municipal Health secretariats databases. The project transcends in importance by being the first survey conducted in the state, evaluating the presence of virus in water by genomic amplification technique.

**EV229 - CONSTRUCTED WETLANDS AS AN ALTERNATIVE SYSTEM TO REMOVE ENTERIC VIRUSES FROM WASTEWATER**

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Wetlands systems are designed and constructed to utilize the natural functions of wetland vegetation, soils and their natural microbial populations to remove pathogens present in surface water, groundwater or wastewater. The presence of different pathogenic microorganisms, highlighting the high concentration of enteric viruses, is a challenge regarding their removal using wetlands for wastewater treatment. The aim of this study was to evaluate the presence of human rotavirus (RV) in two different configurations of constructed wetlands (CW). The configurations evaluated in this study consisted of: 1) one vertical saturated flow CW (VSF); and 2) one vertical flow CW (VF) followed by an horizontal flow CW (HF) (hybrid system). This wetland is located at UFSC being fed by raw sewage from the university neighborhood using *Thypha domingensis* as a macrophyte plant. Five samples were collected monthly representing the whole system: i) raw sewage in the system entrance; ii) wetland entrance (effluent primarily treated in a septic tank); iii) VSF exit; iv and v) VF and HF exits, from the hybrid system. In order to choose the best concentration method, a pool of the samples collected in the different stages of the wetland was spiked with a known amount of RV RotaTeq vaccine strain followed by concentration using skimmed milk flocculation or PEG methodology. Rotavirus recovery was evaluated by plaque assay (infectivity) and genome quantification by RTqPCR. PEG concentration showed a higher viral recovery when compared with skimmed milk flocculation method being the percentage of infectious virus recovery 7.67% and 0.68% respectively for PEG and skimmed milk flocculation. By RTqPCR, the

viral genome recovery was 49.8 % and 11 % by PEG and flocculation method, respectively. The samples collected from April to June, 2016 were then concentrated by PEG methodology adding murine norovirus (MNV1) as internal control. No reduction of RV genome copies were observed along the different stages of the wetland treatment system, being detected an average of 1.0 x 10<sup>5</sup> gc/ml in both CWs configurations. The percentages of MNV1 recovery (internal control) ranged from 1.2 to 10%, respectively for the raw sewage and VSF exit samples. A secondary treatment using UV light will be further evaluated in order to improve RV inactivation in the exit samples.

**EV265 - VIRAL STUDY IN UNTREATED AND TREATED SEWAGE WATER**

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Contamination of human pathogen in wastewater is an important matter, especially where sanitation is not ideal condition. Next Generation Sequencing (NGS) recently has been applied in several viral metagenomes (viromes) studies. Besides describing gene diversity, it is a helpful tool to analyze virome in sewage water. Despite the interest in wastewater treatment, Brasilia city lacks a census study and information aiming to assess the pollution in Paranoa lake. For this purpose, virome in untreated and treated wastewater was investigated using highthroughput sequencing technology (NGS). Untreated and treated wastewater samples were collected at the treatment station of wastewater in Brasília, Brazil and the samples were maintained on ice for transport to the laboratory. At first, bacterial and other debris were removed from the samples by low speed centrifugation. The resulting supernatant was collected and subjected to ultracentrifugation with 20% sucrose cushion. The pellet was resuspended and total RNA was extracted using ZR Soil/Fecal RNA MicroPrep kit (Zymo Research). The total RNA was treated by RiboZero rRNA removal kit for bacteria (Illumina) and the cDNA construction was performed using TruSeq Stranded Total RNA Library Prep Kit (Illumina). For treated water sample, the yield of RNA was very low, so

it was necessary to amplify cDNA by SMARTer Universal low RNA library kit (Clontech). The cDNA libraries from both samples were sequenced using Illumina HiSeq 2000 with the condition of 100 base paired end. The NGS reads were trimmed by Trimmomatic (<http://www.usadellab.org/cms/index.php?page=trimmomatic>) and the contigs were assembled using Megahit (<https://github.com/voutcn/megahit>). The assembled contigs were analyzed by BlastX against RefSeqVirus using Geneious Software v.8.1 (BioMatters). In untreated water, we could find human pathogens as Aichi virus, Human astrovirus, Norovirus GI and GII, Rotavirus A, Human picobirnavirus and Enterovirus. However, these viruses were not found in treated water. This result is very indicative that the treatment process is effective to eliminate such viruses.

**BV26 - THE ASIAN-AMERICAN VARIANT OF HUMAN PAPILLOMAVIRUS TYPE 16 EXHIBITS HIGHER ACTIVATION OF MAPK AND PI3K/AKT SIGNALING PATHWAYS, TRANSFORMATION, MIGRATION AND INVASION OF PRIMARY HUMAN KERATINOCYTES**

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Asian American (AA) HPV16 variants are associated with higher risk of cancer. Abnormal activation of intracellular signaling play a critical role in cancer development and progression. Our aim was to elucidate mechanisms underlying the higher oncogenic potential attributed to AA variant. We evaluated activation of MAPK and PI3K/AKT pathways in primary human keratinocytes (PHKs) transduced with E6/E7 of three HPV 16 variants: EP, AA, E350G. Phenotypes examined included migration, anchorage independent growth and invasion. AA PHKs presented the highest levels of active proteins involved in all cascades analyzed: MAPK ERK, MAPKp38 and PI3K/AKT. AA PHKs were more efficient in promoting anchorage independent growth, and in stimulating cell migration and invasion. MEK1 inhibition decreased migration. The mesenchymal phenotype marker vimentin was increased in AA PHKs. Our results suggest that MEK1, ERK2, AKT2 hyperactivation influence cellular behavior by means of GSK3b inactivation and

EMT induction prompting AA immortalized PHKs to more efficiently surpass carcinogenesis steps.

**BV174 - ACTIVATION AND DEATH PATHWAYS INDUCED BY DENGUE VIRUS IN INFECTED AND BYSTANDER ENDOTHELIAL CELLS**

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Dengue virus (DENV) infects endothelial cells, leading to cellular activation and death, which may contribute to the amplification of inflammation and vascular injury. Here, we investigated the mechanisms of endothelial cell death induced by DENV, using a human brain microvascular endothelial cell line (HBMEC). Cells were infected with DENV2 and death markers in DENV-infected and bystander cells were evaluated at different time points by flow cytometry and western blot. DENV2-infected HBMECs showed decreased viability after 48/72h p.i., evidenced by diminished mitochondrial metabolism, increased Annexin V (AnV) and Propidium Iodide (PI) staining, and release of LDH in the supernatants, demonstrating that both apoptosis and necroptosis markers were detected in the cultures. Separate analysis of infected (DENV+) and bystander cells (DENV-) demonstrated that, at 72h p.i., the great majority of live cells were DENV+, whereas DENV- cells were mostly AnV+PI+, suggesting that bystander, non infected cells were mostly affected at this time point. Western blot analysis demonstrated an increase in caspase 8 and 9 activation at 24h p.i., and increased RIPK1 expression at 72h p.i., indicating that apoptosis was triggered at early infection, and might be followed by necroptosis. Culture of infected HBMECs with caspase inhibitors decreased apoptosis in DENV+ and DENV- cells, whereas blocking of RIPK1 increased the frequency of late apoptotic cells in the DENV population only; indicating that necrosis was actually triggered in bystander cells, counteracting apoptotic pathways. Importantly, inhibition of cell death resulted in increased frequency of DENV+ cells, indicating that this might be a mechanism to control viral dissemination. We then investigated whether supernatants obtained from DENV-infected HBMECs would induce death of non infected cells. Cells were infected for 48h, the supernatants we harvested, inactivated by U.V. radiation, and cultured with non-infected HBMECs. Indeed, increased cell death was

observed, indicating that secreted mediators induced by DENV infection might promote death of bystander cells. Interestingly, RIGI silencing on DENV-infected inhibited the death of bystander cells. These results indicate that RIGI activation triggered by DENV infection induce the secretion of mediators that affect the survival of bystander cells, contributing to endothelial lesion, and controlling virus dissemination.

#### **BV192 - SCREENING TESTS TO EVALUATE THE EFFECTIVENESS AND TOXICITY OF TEN ANTIVIRAL DRUG CANDIDATES DEVELOPED BY BIOISOTERISM**

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Since the first report of AIDS, there is a constant search for therapies that prevent the transmission and/or the replication of HIV. Currently, the combined use of viral protease inhibitors, reverse transcriptase inhibitors and/or inhibitors of the viral Integrase, known as highly active antiretroviral therapy (HAART), is the most effective therapy against AIDS. However, HAART is not able to completely stop the viral replication and therefore to cure HIV infection. So it is urgent to search for new drugs with antiviral potential against HIV, thus increasing the therapeutic arsenal against AIDS. Our work aims at conducting screening tests to evaluate the effectiveness and toxicity of ten candidates to antiviral drugs, developed by bioisoterism. All the compounds were designed based on the NNRTI Delavirdine, in which the A and B subunits were substituted by the phenilpirazole group and in the D subunit, the pyrimidine was substituted by a phenyl. In our preliminary results, the maximum nontoxic concentration of each substance was determined in Hek293T, TZM and MOLT human cell lines. To test the antiviral potential of each compounds, first Hek293T cells were transfected with HIV1 infectious clone NL43 and 5 hours later incubated with the maximum nontoxic concentration of each compound. Supernatants from these cultures were collected 24 hours later and tested for levels of viral infectivity by titration in TZMbl indicator cells. In this assay, as expected for inhibitors of Reverse Transcriptase, none of the compounds inhibited viral production and infectivity in the transfected cells. Next, we performed assays

infecting TZMbl susceptible cells with HIV1 in a M.O.I. of 0.5 and incubated in the presence of the substances after the virus adsorption step. In this assay, almost all the substances inhibited the viral replication varying from 62,8 - 92,3% of inhibition, except the drug 182, which stimulated the viral replication by 37,1%. The inhibitory concentration of 50% of the viral replication (IC50%) of each substance in both TZM bl and MOLT cells was also evaluated and for the most potent compounds IC50% were 0.0183, 0.0444, 0.0586 and 0.0657  $\mu$ M. Together, these results suggest the potential of these substances developed by bioisoterism in inhibiting the early stages of HIV1 replication. Experiments will be performed in order to precisely determine the mechanism of action of these compounds as expected for the mechanism of action of Delavirdine.

#### **BV205 - THE NON-GLYCOSILATED HRSV PROTEINS M AND N ARE ADDRESSED TO THE VIRAL ASSEMBLY SITE THROUGH THE SECRETORY PATHWAY**

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Human respiratory syncytial virus (HRSV) is the most relevant cause of respiratory infection in children worldwide. Despite its importance in public health, some aspects of the mechanisms of the trafficking of viral structural proteins remain unclear. In the present study, immunofluorescence was used to understand how the virus matrix (M) and nucleocapsid (N) proteins, which are nonglycosylated, are addressed to inclusion bodies in Hep2 cells (MOI=3). M and N proteins followed similar intracellular trafficking routes as compared to the glycosylated fusion (F) viral protein. Moreover, M and N proteins colocalized with two key elements of the secretory pathway: transGolgi network46 (TGN46) and sorting nexin2 (SNX2). Viral proteins M and N appear to be involved in the recruitment of cell proteins to the inclusion bodies, as shown for Glucose transporter 1 (Glut1). The data suggest that HRSV M and N proteins follow the secretory pathway, initiating in

early endosomes, as indicated by the colocalization with TGN46 and SNX2. In addition, these host cell proteins accumulate in inclusion bodies that are viral factories, and can be part of budding viral progeny. Therefore, HRSV M and N proteins, even though they are not glycosylated, take advantage of the secretory pathway to reach virus inclusion bodies. Confocal images suggest that SNX2, which is known for its membranedeforcing properties, could play a pivotal role in HRSV budding.

#### **BV213 - HUMAN TONSIL EXPLANTS SUPPORT RHINOVIRUS INFECTION EX VIVO**

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Rhinovirus (RV) is the causative agent of common colds and the most frequent cause of asthma exacerbations in children and adults. RV is frequently detected within hypertrophic human tonsils, indicating that the virus can infect epithelium and lymphoid cells from adenoid and palatine tonsils. In the present study, tridimensional cultures of explants of hypertrophic tonsils were infected ex vivo with RV. Small tonsil explants (3 mm<sup>3</sup>) were obtained by mincing surgical specimens with razor blades, extensively washed in cold Hank's balanced salt solution to remove blood and debris. Explants were placed apical side up in the upper chamber of Transwell culture inserts in 100mm well dishes at 5% CO<sub>2</sub> and 37°C in a humid atmosphere, and RPMI medium was added to the lower chamber of the transwell, maintaining an airliquid interface. Explants from tonsils found to be negative for picornaviruses by qPCR were infected around day 7 with RV16. Five microliters of HRV16 (106 TCID<sub>50</sub>/ml) were inoculated on the apical (epithelial) side, with care to prevent spillage into the media. After overnight incubation, the tissue was washed three times with non-supplemented RPMI in order to remove the excess virus, and fresh medium was replaced. Tissue was incubated at 37°C and 5% CO<sub>2</sub> for another 3 days and then fixed in Carnoy's fixative. Immunohistochemistry with antibody for the VP2 capsid protein of HRV16 showed detection in stratified squamous epithelium and in few lymphoid cells in extrafollicular regions. The findings suggest that tonsil explants sustain RV infection ex vivo. Studies are

underway to trace the infection pathway of fluorescence-tagged RV by intravital microscopy in tonsillar explants.

#### **BV243 - DELETION OF THE M SEGMENT NON STRUCTURAL PROTEIN (NSM) OF OROPOUCHE VIRUS AFFECTS VIRUS ASSEMBLY AND THE ARCHITECTURE OF VIRAL FACTORIES**

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Oropouche virus (OROV) is an arbovirus in the family Bunyaviridae that was isolated for the first time from a dead sloth in the early 1960s, and has caused more than 30 outbreaks in the Amazon region infecting more than half million people. OROV is transmitted by the bite of the midge *Culicoides paraensis* and causes an acute febrile disease. OROV genome is composed of three singlestranded RNAs (L, M e S) of negative polarity, that encode 3 structural proteins, plus the polymerase and 2 other nonstructural proteins - NSm and NSs. NSm is a product from the maturation cleavage of the M polypeptide precursor, and studies with other bunyaviruses have shown that NSm plays important roles in the assembly and morphogenesis of virus particles in tubular structures associated with viral factories. However, nothing is known about the functions of NSm in OROV replication. To address this question, a plasmid-based reverse genetics approach was taken, based on fulllength cDNA copies of the three OROV genome segments to generate a recombinant OROV lacking the entire NSm protein (rOROV<sup>ΔNSm</sup>) and a wild type (rOROV<sup>wt</sup>). Successful rescue of recombinant viruses were confirmed by indirect immunofluorescence (IF) and sequencing. To analyze the morphological changes in organelles, HeLa cells monolayers were infected with rOROV<sup>ΔNSm</sup> and rOROV (MOI=1). Cells were fixed with paraformaldehyde at 0h, 12h, 18h and 24h post infection (pi), and stained by IF with mouse polyclonal anti OROV antibody. Dual labeling experiments were done with rabbit monoclonal anticalexin, antiTGN46, anti HRS or antigiantin. Cell nuclei were stained with DAPI and slides were examined by confocal microscopy. Our results show that at 12h pi rOROV<sup>wt</sup> is predominantly located at the endoplasmic reticulum, while rOROV<sup>ΔNSm</sup> is diffusely dispersed through the cytoplasm. Disruption of

the transGolgi network was delayed in rOROV?NSm as indicated by diffusion of TGN46 at 24h pi and formation of inclusions bodies. No significant differences were noted in the cisGolgi network and in endosomes. The results indicate that OROV NSm plays an important role in OROV assembly. Financial support: FAPESP, CAPES, CNPq.

#### **PIV6 - TRANSLATIONALLY CONTROLLED TUMOR PROTEIN IS NECESSARY FOR AN EFFICIENT POTYVIRUS REPLICATION**

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The Translationally Controlled Tumor Protein (TCTP) is a ubiquitously distributed protein in eukaryotes. It is involved in the regulation of basic processes such as cell cycle progression, cell growth, stress protection and apoptosis. Increase expression of its mRNA is observed during the early stages of tomato (*Solanum lycopersicum*) infection by the potyvirus Pepper yellow mosaic virus. Downregulation of its mRNA reduces virus accumulation in both tomato and *Nicotiana benthamiana* plants. Aiming to understand the role of TCTP in potyvirus infection, *N. benthamiana* plants silenced for TCTP by VIGS were agroinoculated with Turnip mosaic virus (TuMV). Western blot analysis showed that silenced plants accumulated fewer viruses than control plants. Also, the effect of TCTP overexpression in infection was analyzed in plants expressing TCTP transiently. As expected, TCTP overexpression increases TuMV accumulation when compared to control plants. To analyze TCTP subcellular localization in infection context, TCTP fused to GFP was coexpressed with TuMV engineered to express the viral protein 6K2 fused to mCherry and imaged by confocal microscopy. 6K2 is a membrane-associated protein implicated in the formation of vesicles involved in both virus replication and movement. TCTPGFP partially colocalized with 6K2-induced vesicles and with the perinuclear globular structure that is typically formed during potyvirus infection. Cellular fractionation showed that TCTP is mainly present in soluble fraction, but it is also present in membranous fraction in both infected and healthy plants. Since it colocalizes with some vesicles and is membrane associated, it could interact with 6K2.

However, TCTP did not coimmunoprecipitate with 6K2-GFP in infected plants. To find out if TCTP is involved in potyvirus replication, protoplasts of silenced and control plants were infected with TuMV and TuMVVNN, a non-replicative TuMV mutant. Quantification of viral accumulation in both pulls of protoplasts showed that TuMV accumulation decreases in silenced cells, suggesting an involvement in virus replication. Taken together, these results show that TCTP is a plant factor necessary for an efficient infection by different potyvirus and may be involved in virus replication.

#### **PIV28 - VIROME IN ORNAMENTAL PLANTS FROM DISTRITO FEDERAL, BRAZIL**

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The growing ornamental plant production in the Federal District (DF), Brazil, is facing with the increase of diseases. The research towards detection of viral species occurring in ornamentals are still few not only in DF but also in Brazil. Next Generation Sequencing (NGS) approach makes it possible to explore the identification of emerging or unknown virus species without having any background for the causative agents. The main objective of this work was to identify and analyze the viral diversity present in different ornamental plant species by NGS. For this purpose, plant samples showing viral (like) symptoms were collected at an important production and distribution center: Nursery I of NOVACAP, Brasília (Companhia Urbanizadora da Nova Capital do Brasil). The plant species used were: *Coreopsis lanceolata*, *Epipremnum pinnatum*, *Impatiens hawkeri*, *Jasminum nitidum*, *Streptosolen jasmonii*, *Pachystachys lutea*, *Pinanga kuhlii*, *Anthurium lindmanianum*, *Pelargonium* sp. and *Neomarica candida*. Leaf samples of each species were stored at freezer 80 °C and total RNA was extracted individually. For NGS, two grams of each plant sample were mixed and used as a pooled sample. The viral semipurification procedure was applied for this pooled sample, then the total RNA was extracted from this preparation. Total RNA was sent to Macrogen Inc. (Seoul, Korea) for the DNA library construction and posterior NGS sequencing by Illumina HiSeq 2000. The NGS of pooled sample resulted in 45,449,068 reads and a high number of contigs as well in each assembler used, Velvet (1.2.09), AbYSS (1.9.0) and SPAdes (3.7), with 1,657,028,

2,882,857 and 95,736 contigs respectively. Blastx search of these contigs with viral reference genomes resulted in 4,981 contigs detected as viral sequences. The category of these sequences varied from bacteriophages to plant viruses. In plant viruses, some viral families of DNA (Caulimoviridae) and RNA viruses (Tombusviridae, Luteoviridae, Rhabdoviridae, Potyviridae and Umbravirus) were more evident. Viral genomes were assembled in silico using the Geneious (R9) software, resulting in six possible new viral species, including three from Rhabdoviridae, one from Potyviridae, one from Tombusviridae and one from Umbravirus. With these results, we can assume that important entities of plant virus are present in ornamental plants produced in the DF, which can be a risk to ornamental production as well to other crops distributed around the area.

#### **PIV72 - SEQUENCING OF THE COTTON ANTHOCYANOSIS VIRUS BY SMALL RNA DEEP SEQUENCING AND ITS SIVRNAS PROFILE IN COTTON**

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Small RNAs or siRNAs (interfering RNAs) are small RNA molecules originated when plants and animals are infected by viruses. After virus entry into the cell, its genome is released and recognized by cellular proteins called Dicerlike. These proteins fragment viral genome producing small interfering viral RNA (siRNA), sequences that exhibit at approximately 2124 nucleotides (nts). The sequences of the siRNA are complementary to the viral genome. Total siRNA from Cotton anthocyanosis virus (CAV) infected plants were sequenced by deep sequencing in order to obtain the complete sequence of the CAV genome. The disease caused by CAV is restrict to Brazil, where is called "Vermelhão do algodoeiro". Symptoms are the intense reddening of leaves and stems. Until now, its agent causal was not known at molecular level. CAV was describing in Brazil in 1961 at Brazil by Santos and collaborators as belonging to the Luteoviridae family, Polerovirus genus. Polerovirus have

ssRNA + genomes with seven ORFs. In a previous work we sequenced part of CAV genome corresponding to viral capsid (ORF3) and part of its replicase (ORF2) and observed a high homology between these ORFs and ORFs 2 and 3 from Cotton leafroll dwarf virus (CLRDV) responsible for Cotton blue disease, reaching more than 90% identity. Using siRNA libraries obtain through deep-sequencing performed in Illumina platform at FASTERIS Co., Geneva, Switzerland, almost complete genome of CAV was mapped using SearchSmallRNA software. The analyzes showed that siRNA generated during the process of infection range from 1826 nts, with siRNA of 22 nts as the most abundant, followed by 24 nts. Some small genomic portions were not covered by mapping (gaps) corresponding to less than 5% of the genome. For gaps sequencing, sets of primers were design for reverse transcription followed Reaction Polymerase Chain (RT-PCR) and subsequent sequencing by Sanger. CAV genome has about 6000 nucleotides. Mapping results were validate by Sanger nucleotide sequencing. Alignment of the CAV ORFs nucleotide and amino acids sequences with other members of Luteoviridae family confirmed that it is a Polerovirus.

#### **PIV99 - DSRNA DEEP SEQUENCING REVEALS FIVE VIRAL SPECIES IN COMMON BEANS**

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Common bean (*Phaseolus vulgaris* L.) is an economically important leguminous crop cultivated worldwide. Viral pathogens play a significant role in reducing the productivity and quality of this crop. Transgenic bean golden mosaic virusresistant common bean plants were recently developed in Brazil. However, field experiments with transgenic lines presented diverse types of symptoms, probably due to infection by RNA virus. To investigate which viruses were present in these plants, we performed highthroughput sequencing from preparations enriched for viral dsRNA. Leaves from transgenic BGMVresistant common bean breeding line CNFCT16207 showing severe crinkling were collected in Goiás, Brazil. dsRNA extraction was conducted using

STEPhenol and cellulose column protocol. Pooled dsRNA samples were paired-end sequenced using MiSeq Illumina® high performance platform. Sequencing results were analyzed in CLC Genomics Workbench and Geneious® program software for contig construction and comparison with viral sequences in public database and gene annotation. A total of 27,897 contigs were assembled from 13,780,310 reads obtained in the Illumina sequencing. Six viral RNA genomes were recovered and identified as Cowpea mild mottle virus (CpMMV; Carlavirus, Betaflexiviridae), Bean rugose mosaic virus (RNA 1 and RNA 2 BRMV; Comovirus, Secoviridae), two species of the genus Endornavirus, Phaseolus vulgaris endornavirus 1 and 2 (PvEV1 and PvEV2; Endornavirus, Endornaviridae), and a new Cytorhabdovirus (Rhabdoviridae). The size of the viral contigs ranged from 3.7 to 14.8 kb. Based on the consensus sequences obtained through next generation sequencing, specific primers were designed for each virus species identified. Primers were used in PCR reaction to recover virus-derived fragments, confirming the presence of all viruses in the plants. Large scale sequencing technology and advanced bioinformatics platforms have allowed the discovery of new viral species and four other RNA viruses in common bean plants from the state of Goiás, being an attractive tool for studying viral diversity in plants. Additionally, dsRNA enriched samples permitted recover the RNA genomes in the replicative form, selecting specifically RNA viruses. This is the first report of a Cytorhabdovirus infecting common bean plants.

#### **HV2 - DETECTION OF THE EMERGING ROTAVIRUS G12P[8] GENOTYPE AT HIGH FREQUENCY IN BRAZIL IN 2014: SUCCESSIVE REPLACEMENT OF PREDOMINANT STRAINS**

**Luchs, A.; Cilli, A.; Morillo, S.G.; Gregorio, D.S.; Souza, K.A.F.; Vieira, H.R.; Fernandes, A.M.; Carmona, R.C.C.; Timenetsky, M.C.S.T.**

*INSTITUTO ADOLFO*

The continuum characterization of circulating RVA genotypes is essential to understand how vaccine introduction could impact virus epidemiology. In the present study, an unexpected rapid changing pattern of RVA genotypes distribution in Brazilian population during three followed seasons is described. From January/2012 to December/2014, a total of 3441 fecal

specimens were collected from collaborating centers across Southern, Southeastern and Midwest Brazil, and likely to be representative of Brazilian population. All specimens were screened for RVA using ELISA, and genotyped by RTPCR. Differences in proportions were tested using Chi Squares. A p value of less than 0.05 was considered statistically significant. RVA was detected in 19.7% (677/3441). G3P[8] remained prevalent in 2012 (37.6%, 69/185) and 2013 (40.1%, 74/186) ( $\chi^2=0.107$ ,  $p=0.743$ ), but declined markedly in 2014 (3.5%, 10/281) ( $\chi^2=71.770$ ,  $p=0.000$ ). G12P[8] was second highest strain in 2012 (22.7%, 42/185), decrease rapidly in 2013 (2.7%, 5/186) ( $\chi^2=26.224$ ,  $p=0.000$ ) and re-emerged as the predominant genotype in 2014 (86.6%, 243/281) ( $\chi^2=118.299$ ,  $p=0.000$ ). From July/2014, G12P[8] was the single genotype detected in all regions studied. The present study raised the hypothesis of a possible G12 outbreak being in progress. Nationally, the Hospital-based Information System surveillance data confirmed the long term decline in gastroenteritis hospitalization observed in Brazil after RVA vaccine introduction. Nevertheless, the sharp increase in diarrhea hospitalization prevalence from 2013 to 2014 observed in Southern and Southeastern regions is consistent with what appears to be an outbreak of G12P[8]. Furthermore, in 2014, the FIFA World Cup was held in Brazil, and the introduction a novel RVA strain was a real threat, given large numbers of visitors from areas with ongoing G12P[8] genotype transmission. Moreover, this event occurred right before the beginning of the RVA seasonality in the country. Worldwide, the emergence of genotype G12P[8] as an epidemiologically important strain could raise new concerns for RVA vaccine development. However, despite the possible emergence of new strains, vaccination has been shown to reduce the disease incidence of RVA infection and remain below prevaccination levels. Continued surveillance is needed to verify the effectiveness of the Rotarix™ vaccine in Brazil together with potential emergence of unusual genotypes.

### **HV35 - PREVALENCE AND VIROLOGICAL CHARACTERISTICS OF HEPATITIS B VIRUS INFECTION AMONG MEN WHO HAVE SEX WITH MEN IN CENTRAL BRAZIL: A RESPONDENT-DRIVEN SAMPLING**

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2. FACULDADE DE ENFERMAGEM/ UNIVERSIDADE FEDERAL DE GOIÁS
3. INSTITUTO OSWALDO CRUZ/ FUNDAÇÃO OSWALDO CRUZ

Men who have sex with men (MSM) are at increased risk of exposure to hepatitis B virus (HBV) compared with the general population. This study aims to determine the prevalence of HBV current infection (HBsAg carriers) and virological characteristics in a sample of MSM in Brazil. A cross-sectional study was conducted among MSM in the City of Goiânia, Central Brazil. From March to November 2014, participants were recruited using respondent-driven sampling (RDS). After signing the consent form, participants were interviewed and a blood sample collected. All samples were tested for HBV serological markers and HBV DNA. Nucleotide sequences of the amplified regions were determined by direct sequencing. Sequences were aligned and edited using SeqMan II, Clustal W and BioEdit. MEGA program was used to determine the HBV genotypes and subgenotypes by phylogenetic analysis, and also to identify mutations in the HBV genome. Of the 522 samples, five (0.6%; 95% CI: 0.21.6) were HBsAg and HBV DNA positive. Of these, two (Y431 and Y494) were successfully amplified for full-length HBV genome, one (Y513) for PreS/S, BCP (basal core promoter) and Pre C/C, one (Y02) for PreS/S, and one (Y413) for S gene region. Phylogenetic analysis of the S gene showed that all isolates belonged to HBV genotype A, subgenotypes A1 (n=3) and A2 (n=2). These results were further confirmed by analysis of other amplified genomic regions. Additionally, sequence analysis revealed that all HBV isolates had the T131N amino acid substitution in the S region (associated with persistence of the HBV as well as with vaccine escape). In the BCP and PreC/C regions, we found the double mutation A1762T/G1764A

(responsible for decreased HBeAg expression and has been linked to HBV oncogenesis) in sample Y431, G1862T/G1888A (characteristic of subgenotype A1) in Y494, and G1862T (genotype specific HBV/A1) in Y513. No mutations were detected in X and overlapping HBV polymerase regions. In conclusion, HBV DNA was found in all HBsAg-positive MSM, showing that they have active hepatitis B and a higher potential for HBV transmission. The genotype A identified in this study population corroborating the greater circulation of this genotype in Brazil. The presence of mutations on HBV isolates indicates the need for expert assistance and monitoring of HBV DNA-positive individuals to prevent progression to more severe diseases.

### **HV41 - SAPOVIRUS IN CHILDREN WITH ACUTE GASTROENTERITIS ATTENDED AT HOSPITAL IN GOIÂNIA, GOIÁS**

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INSTITUTO DE PATOLOGIA TROPICAL E SAÚDE PÚBLICA

Sapovirus (SaVs) are classified in the Caliciviridae family, and together with noroviruses (NoVs) are important causing acute gastroenteritis (AGE). SaV have been mainly detected in samples from AGE outbreaks, involving especially children and the elderly. The SaVs can be transmitted by the fecal-oral route through person-to-person contact, by ingestion of food or contaminated water and fomites. The respiratory route has been speculated for NoVs; however, the presence of SaV had not yet been investigated in samples from the respiratory tract. The objectives of this study were to evaluate the positivity rate for SaVs and the viral loads in clinical samples of children under six years of age, in association with symptoms presented by these children. Therefore, 204 samples were obtained from 102 children (a stool sample and a nasopharyngeal swab from each child) aged 0-65 months (mean 17 months). Samples were collected from May 2014 to May 2015 in Materno Infantil Hospital. Stool samples and nasopharyngeal swabs were extracted using a commercial kit (Qiagen Hilden, Germany), and screened by an RT-qPCR Taqman assay, with specific primers and probe targeting SaVs genogroups I, II and IV. To determine the viral load of

the samples a standard curve using serial dilutions of a recombinant plasmid was constructed. A positivity rate of 18.6% (19/102) was observed in fecal samples from children, with a mean viral load of  $5.12 \times 10^9$ . The virus was also detected in 36.2% (37/102) of nasopharyngeal swab samples, with a mean viral load of  $2.21 \times 10^9$ . Also, 7.8% (8/102) of the children were positive for the virus in both samples, with mean viral load in fecal samples of  $1.21 \times 10^{10}$  and of  $4.65 \times 10^9$  in nasopharyngeal swabs. Regarding the symptoms, 89% (17/19) of children were positive for SaV in fecal samples, and 94% (35/37) of the children who were positive in nasopharyngeal swabs had diarrhea. Vomiting was the most common symptom presented by 87% of the children that were positive in both samples (fecal and nasal swab). Data show the occurrence of SaV at high viral loads in the studied population. We also report, for the first time, the presence of SaV in samples from the respiratory tract; however, further studies are needed to better elucidate this finding.

#### **HV157-SEROLOGICAL EVIDENCE OF CIRCULATION OF ALPHAVIRUS (VENEZUELAN EQUINE ENCEPHALITIS VIRUS AND UNA VIRUS) IN PARAGUAYAN POPULATION (2012-2013)**

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The Alphavirus genus includes viral species that produce encephalitis in horses and humans, as well as febrile illness with rash and arthralgia in humans. Among the producers of encephalitis are found epizootic subtypes of Venezuelan Equine Encephalitis Virus (VEEV) complex. This complex also includes enzootic subtypes that, although they doesn't cause disease in horses (except subtype IE), it can cause them in humans. The Rio Negro Virus (RNV VEEV subtype VI), that circulates in

Argentina, where it was associated with undifferentiated febrile illness. The Mayaro (MAYV), Chikungunya and Una (UNAV) viruses belong to Semliki Forest virus complex, being recognized MAYV activity in Central and South American countries, with recent activity in Mexico. In addition, the UNAV has been detected in several countries in South America. The present study aimed to determine RNV, MAYV and UNAV seroprevalence by plaque reduction neutralization test (PRNT) in 650 samples of Paraguayan individuals mainly from Central Department, period 2012-2013. Seroprevalence for RNV was 5.8%, and for UNAV it was 0.46%. No neutralizing antibodies against MAYV were detected in the studied population. The 50.1% of neutralizing antibody titers against RNV were high (equal to or greater than 1/640), which would indicate a recent virus circulation. In addition, it was observed a seroprevalence increment tendency as age increases, which suggests an endemic behavior of this virus. These results represent the first indication of RNV and UNAV circulation in Paraguay, and these data will serve as the basis for future studies to search potential hosts and vectors of these viruses in the region.

#### **HV212 - HIGH RATES OF DETECTION OF HUMAN RHINOVIRUS AND LACK OF ADENOVIRUS AND BOCAVIRUS SHEDDING IN ASYMPTOMATIC PATIENTS POST TONSILLECTOMY**

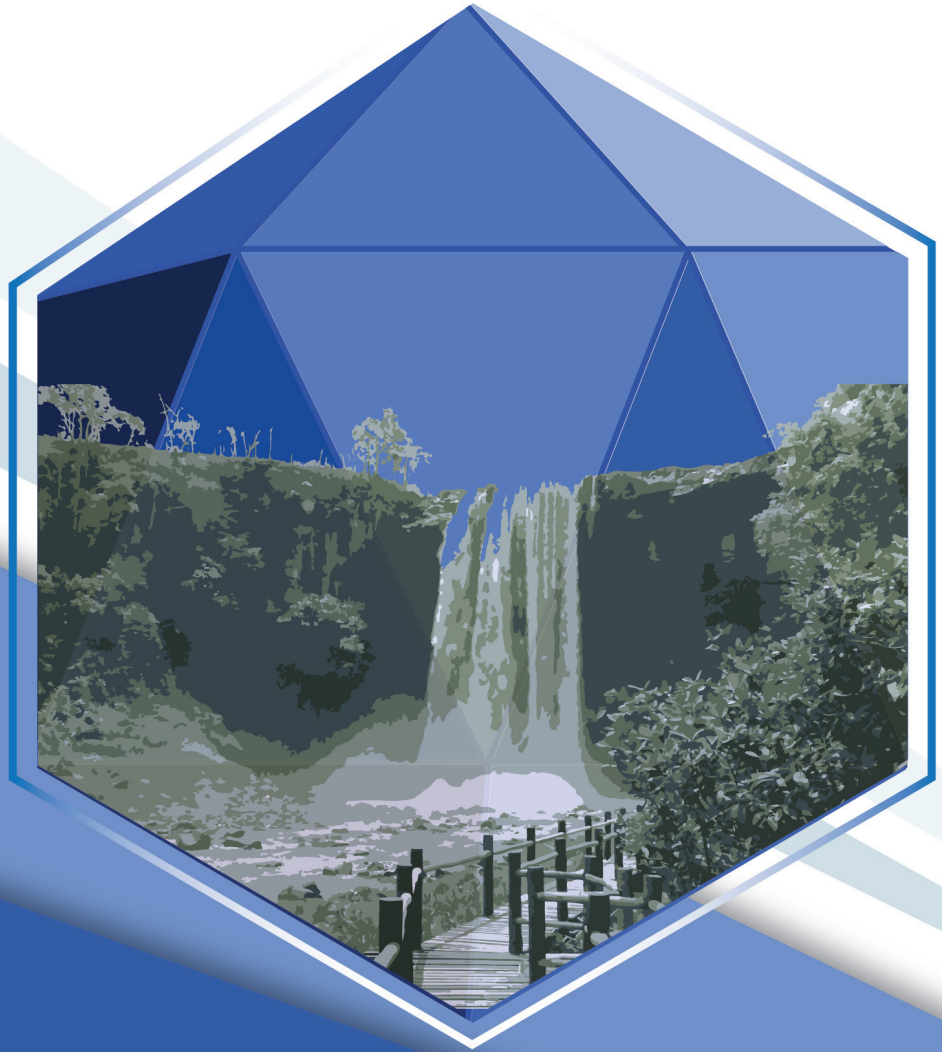
Martins Junior, R.B.; Prates, M.C.M.; Biasoli, B.; Rocha, L.P.; Aragon, D.C.; Silva, M.L.; Tamashiro, E.; Valera, F.; Lima, W.; Arruda, E.

FACULDADE DE MEDICINA DE RIBEIRÃO PRETO

Several studies have shown respiratory viruses infecting patients with chronic or recurrent tonsillar hypertrophy. Recent studies by our group revealed high frequencies of respiratory viruses (97%) in samples from lymphoid tissues and nasopharyngeal secretions (NS) in the absence of signs and symptoms of acute respiratory infection (ARI). We managed to obtain NS from 85 of those children (mean age 6 years) in posttonsillectomy followup visits (mean time = 4.2 years), in the absence of ARI symptoms. At the time of tonsillectomy, the overall frequency of virus detection in NS from those 85 children was 71.7% (61/85). The overall frequency of virus detection posttonsillectomy in NS collected from the

same children in the absence of ARI symptoms dropped to 58.8%. Rhinovirus (RV) was the most frequently detected virus, in 38 of the subjects (44.7%), followed by enterovirus (EV) in 7 (8.2%), human metapneumovirus (HMPV) in 6 (7%), human respiratory syncytial virus HRSV in 3 (3.5%) and human coronavirus HCoV in 1 (1.1%). The previous virus detection rates in NS at the time of tonsillectomy for the same 85 children were: RV in 27 (31.7%), human adenovirus (HAdV) in 19 (22.3%), EV in 18 (21.7%), HRSV and HMPV in 10 (11.7%) each, followed by HBoV, HCoV and influenza virus in rates lower than 10%. Except for RV, the virus detection rates in NS were generally lower. RV was the agent most frequently detected overall, and also the viral agent most frequently detected in coinfection (5 cases, 5.8%): 3 with HMPV and 2 with EV. Tonsillectomy reduced the frequency of virus codetection in NS, which was 70% at the time of tonsillectomy. The most striking result was the absolute lack of detection of HAdV or HBoV in asymptomatic patients post tonsillectomy. The findings strongly indicate that tonsillectomy significantly reduces asymptomatic shedding of HAdV and HBoV in NS.

# ***BASIC VIROLOGY - BV***



**BV8 - HISTOPATHOLOGICAL EVALUATION OF MUSCULAR DAMAGE INDUCED BY MAYARO VIRUS USING ANIMAL MODEL**

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The Mayaro virus is an alphavirus of the *Togaviridae* family, endemic in South American countries. It mainly affects people in touch with forest areas, because it is a sylvatic cycle virus. The Mayaro fever is characterized as an acute self-limiting illness, but it can sometimes present as severe and debilitating as a result of the development of long-lasting crippling arthritis. Now a days there is no specific treatment for arthritis and arthralgia induced alphavirus. The aim of this study was to evaluate the histological damage induced by Mayaro virus using a murine animal model. For this we used BALB / c mice with fifteen days old. The animals were infected in the left rear footpad, the control group received only phosphate buffer (PBS). At 3, 7, 10, 15 and 20 days three animals were sacrificed and the organs and tissue of interest were removed and fixed. They were then embedded in paraffin, cut into 5µm sections and stained with hematoxylineosin (HE). The analysis of muscle sections of both, hind limbs and forelimbs, at 7 days post infection showed the presence of inflammatory infiltrates and winding muscle fibers. However, damage analyses in other tissues and in other days are yet to be made and also other histological techniques such as immunohistochemistry to determine target cells in the affected tissues.

**BV13 - RNA INTERFERENCE ANTIVIRAL THERAPY AGAINST HERPETIC ENCEPHALITIS**

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2. UNIVERSITY OF SÃO PAULO RIBEIRÃO PRETO

Introduction: Herpetic encephalitis (HSE) is an acute encephalitis caused mainly by Herpes simplex virus 1 (HSV1) with an annual incidence of 14 cases/million of inhabitants. Nowadays, HSE treatment has encountered difficulties such as utilization of antivirals with elevated toxicity, metabolic side effects and HSV1 resistance. An alternative to antivirals is the use of small interfering RNA

(siRNA) as viral replication inhibitor. In this work, siRNA targeting UL39 region was evaluated for HSE treatment in vivo. Methods: BALB/C mice were inoculated via intranasal with HSV1 and treated with siRNA:RVG9R antiHSV1. Mice were divided in experiments to evaluate the kinetics of HSV1 replication inhibition, number of administered doses (one or two doses) of siRNA:RVG9R antiHSV1 and treatment with siRNA:RVG9R antiHSV1 combined with acyclovir in HSE experimental model. Besides that, HSE clinical signs, mortality and viral replication inhibition in brain and trigeminal ganglia were evaluated to measure siRNA therapy. Results: In kinetics experiment, treated group demonstrated reduction of HSE clinical signs and a significant virus replication inhibition varying from 43.6% to 99.9% in brain and from 53% to 98% in trigeminal ganglia. Animals treated with two doses of siRNA showed prolonged survival time, reduction of HSE clinical signs and viral replication inhibition in brain (67.7%) and trigeminal ganglia (85.7%). Also, animals treated with siRNA:RVG-9R antiHSV1 combined with acyclovir demonstrated reduction of HSE clinical signs and survival of 100%, as well as viral replication inhibition in brain (83.2%) and trigeminal ganglia (74.5%). Conclusions: These findings demonstrated that siRNA was capable of reducing HSE clinical signs, prolonging survival time and inhibiting HSV1 replication in mice. Thus, siRNA can be a potential alternative to standard HSE treatment especially to extend survival time and reduce the clinical signs of HSE in vivo.

**BV19 - MOLECULAR CHARACTERIZATION OF A INOVIRUS THAT INFECTS RALSTONIA SOLANACEARUM**

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2. CENTRO NACIONAL DE PESQUISA DE HORTALIÇAS

Viruses that infect bacteria are the most abundant organisms on the planet, and can have its genome DNA or RNA. Currently, due to increasing resistance of bacteria to antibiotics, phages can be an important biocontrol tool, which is known as phage therapy. An important phytopathogenic bacterium to be controlled is the *Ralstonia solanacearum*, which causes a disease known as bacterial wilt, an infection that affects more than 50 plant families and is responsible for the annual loss of

several crops such as, tomatoes, potatoes, eggplant and banana. This study aims to characterize a bacteriophage at a molecular level isolated from a *R. solanacearum* from Ceará, Brazil. Morphological characterization of the viral particle was performed by transmission electron microscopy, the particules are flexuous and elongated with aprox. 2,000nm. The viral genome has aprox. 8.000nt. The genome sequence and the morphological characterization suggest that the virus belongs to the Inoviridae family, genus Inovirus, which multiplies in a pseudo lysogenic manner, establishing a close relationship with the host. Biological characterization of the virus showed that isolates of *Ralstonia solanacearum* infected by these viruses, still maintained the ability to colonize its host, but was no longer pathogenic. The virus isolated here showed a high sequence similarity with other Inovirus TypeRSM, that reduce the virulence of their hosts. Studies of virus host interaction in this pathosystem will be useful to better understand the pathogenicity mechanisms of *R. solanacearum*.

#### **BV25 - PREDICTION OF THE FLUCTUATION OF THE CASE NUMBERS OF CHIKUNGUNYA FEVER IN FEIRA DE SANTANA - BAHIA, USING TECHNIQUES OF MACHINE LEARNING**

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*INSTITUTO EVANDRO CHAGAS*

The chikungunya virus (CHICKV) is a member of the family Togaviridae, genus Alphavirus. The vectors are the mosquitoes of the genus *Aedes*, especially *A. aegypti*. The first report of autochthonous transmission was in September 2014, with reported cases in Amapá and Feira de Santana. One of the possible applications of the techniques of machine learning (ML), devised in this study is the development of statistical models able to predict, using various variables, when a future outbreak may occur, or even predict fluctuations of the case numbers a disease already installed, allowing to assist the development of strategies for combating epidemics. The objectives of this work were to determine whether selected predictive variables are able to determine the fluctuation in the number of CHIKV cases in Feira de Santana; determine the best technique of ML for the problem at hand; determine which environmental variables are most useful to predict the number of cases; adjust the parameters of

the models to minimize the prediction errors; apply the best models to predict the number of cases in a time period after the one contemplated in the data set. The predictive variables used were the confirmed cases of CHIKV, climatological data and number of searches for the word "chikungunya" on the Internet through the Google Trends (GTRENDS) tool. The data was collected from 09/2014 to 09/2015, organized and normalized using the statistical package R. Training and testing of the model were performed using the package caret. It was observed that the variables mean temperature, maximum temperature and GTRENDS combined showed the best results, being able to predict the fluctuation in the number of cases with low error (RMSE=11.25). We evaluated the increase/decrease of cases, and the results were even better (100% specificity and 100% sensibility). We could observe the importance of climatic variables in the dispersion of vectorborne diseases, corroborating other studies, which show that the climate plays a fundamental role in the life cycle of the vectors. We can also see the importance of the use of keyword search data via the web, today being a manner widely used to search for information, and which can help us in monitoring of diseases. We argue that this digital surveillance approach can be as effective as and cheaper than the traditional techniques employed by the Ministry of Health for epidemiological surveillance.

#### **BV61 - VIRTUAL SCREENING STUDY OF COMPOUNDS AGAINST PROTEIN C MAYARO VIRUS FOR IDENTIFICATION OF ANTIVIRAL**

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*UNIVERSIDADE FEDERAL DE SÃO JOÃO DEL REI*

The Mayaro fever, caused by the Mayaro virus (MAYV) is a sublethal disease to humans, and symptoms are quite similar to another arboviruses like dengue, chikungunya and yellow fever. Symptoms of arthralgia associated with infection by MAYV can cause a highly disabling disorder, similar to that caused by Chikungunya virus. In recent years, they have been widely documented outbreaks in metropolitan areas and, to date, there is no therapy available. Therefore, this study aimed to perform the Virtual Screening method using the threedimensional model of the C protein of MAYV as a target for the

screening and development of new antiviral drugs. Previously, the threedimensional model of C protein of MAYV was constructed using C protein of Aura virus complexed with dioxane (PDB 4AGJ\_A) as template. Initially, the dioxane was transferred to C protein of MAYV through Discovery Studio 3.1 software. Then, in the AutoDock program was built a box centered in this ligand to define the region in which the molecule would be anchored. In order, to validate the method, we carried out the redocking, consisted to anchor the dioxane in the region delimited by the box and superimpose the conformation obtained for that ligand to crystallized conformation. The molecules obtained from the ZINC database and literature had their charge calculated to physiological pH and threedimensional structure defined by the MarvinSketch 15.8.24 program. After preparation, the molecules were anchored protein C using the framework Octopus 1.0. The box was defined as a cube with dimensions of 14x14x14 Å and coordinates X, Y and Z 23833, 11281 and 9142, respectively. The superimposition of conformations of dioxane provides the RMSD value of 1.92 Å, validating the methodology. The value obtained for the binding energy of dioxane was 2.8 kcal/mol. A total of 590 molecules were anchored C protein, and 6 molecules belonging to the same chemical class, show favorable binding energy of around 7.0 kcal/mol. These results show promising antiviral molecules against MAYV, since they have affinity to a protein site which is believed to interact with E2 protein to promote viral budding.

#### **BV64 - COMPUTATIONAL PREDICTION OF CD4+ TCELL EPITOPES IN HUMAN PAPILOMAVIRUS PROTEOME**

**Batista, M.V.A.; Prado, F.O.; Rocha, P.A.S.; Matos, A.S.**

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Human papillomavirus (HPV) is the major cause of cervical cancer worldwide. Tumors associated with HPV in the cervical region are common and constitute a serious public health problem. Until now, there are approximately 200 HPV types already known. However, only 18 HPV types are associated with malignant transformation, which are called as highrisk HPVs. Although there are a tetravalent vaccine available, it cannot prevent against all highrisk HPV types. In this way, wider spectrum vaccines should be developed in order to prevent HPV infections.

The epitope prediction is a useful tool for vaccine design because we could assess structural properties, cross-reactions and recognition by the immunoglobulins in a cheaper and faster way, working with large amounts of data so that various experimental stages of vaccine development can be abbreviated. Therefore, this study aimed to predict CD4+ Tcell epitopes in HPV proteome that could be used for human immunization. In order to do this, a local database was created for all HPV protein sequences retrieved from Protein/NCBI database, along with information regarding their physicochemical and structural characteristics. Using IEDB Analysis Resource, epitopes for these proteins were predicted using MHCII Binding Predictions tool according to the most frequent HLA-A alleles. The analysis showed that 34 epitopes were highly immunogenic, and they presented high identity with other HPVs. These epitopes are intrinsically associated with the response of more than 70% of the HLA-A alleles in the world population. Finally, these epitopes were mapped into the 3D structure of HPV proteins, confirming their accessibility on the protein surface. Therefore, it was possible to predict a promising CD4+ Tcell epitope set in HPV proteome with high immunogenicity that may present a response in >70% of individuals worldwide, being a strong candidate for an epitopebased vaccine with high capacity to activate immune response and the possibility of crossprotection among different HPV types. These promising results can serve as basis for further studies to develop synthetic peptides and test their immunological response, which could lead to new prophylactic strategies to prevent cervical cancer.

#### **BV91 - EVALUATION OF THE CORAL EXTRACT OF MUSSISMILIA BRAZILIENSIS AS AN INHIBITOR OF HTLV1 REPLICATION**

**Carvalho, L.D.; Martins, C.P.S.; Reis, J.K.P.; Kassar, T.; Resende, C.F.; França, J.P.; Melo, S.R.G.; Marin, L.J.; França, L.P.; Franco, G.M.; Dantas, A.N.G.; Pellizoni, T.A.; Souza, G.O.S.**

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Human T cell lymphotropic virus type 1 (HTLV1) is known to be a major agent of severe and fatal lymphoproliferative disease named adult Tcell leukemia/lymphoma (ATLL), and HTLV1 associated myelopathy/tropical spastic paraparesis (HAM/TSP), a neuroinflammatory disease.

Moreover, HAM/TSP is very common in Brazil, and until the present time, there is no consensus on the specific treatment for HAM / TSP). For this reason, researchers have been attempting to isolate and characterize new extracts which can inhibit HTLV1 replication and infection. *Mussismilia braziliensis* (phylum Cnidarian, class Anthozoam, family Mussidae) is endemic in Brazil and is found along the coast of Bahia state. The potential antimicrobial activity of several cnidarian species against different microorganisms has been shown previously by others. However, *M. braziliensis* has never been tested as an antiviral agent. The present study aimed to investigate the potential antiviral effect of *M. braziliensis* coral extract in MT2 cell lines permanently infected with HTLV-1. Coral extract from *M. braziliensis* was obtained and dissolved in RPMI. To perform cell viability assay, HTLV1 infected (MT2 cells) and not infected (Jurkat cells) were incubated in the presence or absence of coral extract (cell control) at concentrations of 10, 30, 80 or 100mg using MTT method. No cytotoxicity was observed in any concentration of the extract tested. The mRNA levels of the viral genes *tax/rex* and *gag/pol* were evaluated using realtime PCR. The results demonstrated that *M. braziliensis* extract was able to inhibit the expression of *tax/rex* mRNA at concentration of 80 mg with significant p value ( $p < 0.05$ ). Antiviral activity on *gag/pol* mRNA was not observed at any concentration tested ( $p > 0.05$ ). Hence, coral extract from *M. braziliensis* may inhibit viral replication in permanently HTLV1infected MT2 cells, reducing expression of viral mRNAs of *tax/rex* and, it may be useful for development of new treatment for HTLV1 infection.

#### **BV95 - A NOVEL ENTROPYBASED COMPUTATIONAL TOOL TO IDENTIFY MOLECULAR MARKERS AND DESIGN PRIMERS FOR VIRAL DETECTION AND GENOTYPING**

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Phylogenetic analyzes of molecular sequences are an integral part of many modern molecular and evolutionary biology studies. In phylogenetic analysis, a genomic region that presents high mutational rate are affected by the saturation effect, and a region with

no variation does not present enough phylogenetic signal in order to proper group together the studied taxa. So, a relevant task in phylogeny is to find the most phylogenetic informative regions in a genome that presents a balanced quantity of sequence variation. In this context, Shannon entropy has been shown to be a good measure in order to find those informative regions. In addition, in many viral epidemiological and population genetics studies, the number of samples analyzed could be enormous, which makes the whole genomic approach very expensive. Therefore, the entropy approach has been applied with success in finding genomic markers with satisfactory phylogenetic signal that could be used in larger studies. Recently, some attempts have been made to develop degenerate primer design tools. One of them uses the entropy measure to position the primers, but the problem is that the user has to inform the region that has to be amplified. Therefore, we present a novel entropybased computational tool that selects phylogenetic informative genomic regions coupled with degenerate primer design applied to the detection and genotyping of viral samples. This tool identified proper phylogenetic markers and proposes suitable degenerate primers to amplify and sequence them, which could be used in different viral epidemiological and population-based genetic studies. To evaluate the tool, we selected 12 different types of Bovine papillomavirus (BPV) and we have identified a genomic region in L1 gene that was suitable for phylogenetic analysis. Thereafter, multiple sequence alignment using the algorithm implemented in software MEGA5 was performed. It was calculated the average value of entropy, site to site, and low entropy regions were selected. Subsequently, primers were designed and developed in conventional PCR. Four different concentrations of new primers were tested and all BPV samples were detected. Thus, new primers were designed from the entropy method, which showed very good sensitivity and specificity for the detection of BPV DNA. In conclusion, this tool could be implemented to increase the efficacy of different viral diagnostic methods.

**BV105 - SYNTHETIC AMINOCHALCONES INHIBIT HEPATITIS C VIRUS REPLICATION**

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Hepatitis C is a liver disease caused by the hepatitis C virus (HCV) and about 170 million people are estimated to be infected worldwide. The current therapy available to the chronically infected patients is based on interferon $\alpha$ , ribavirin and direct acting antivirals (DAAs). However, this treatment is expensive, presents several side effects and the antiviral resistance has been documented, demonstrating the need of new therapeutical approaches. In this context, natural compounds have demonstrated medical interest due to their biological activities. Among them, flavonoids are a class of plant secondary metabolites with several properties, including antiHCV activity. The addition of an amino group into the chemical structure of the precursors chalcones results in the aminochalcones. This subclass of compounds has previously showed to possess antiseptic, antifungal, antitumor, and antimalarial activities. Here we evaluated the antiviral effects of 35 synthetic aminochalcones on HCV replication by using a subgenomic replicon system of HCV (genotype 2a SGRFeo JFH1). Huh7.5 cells stably harboring SGRFeoJFH1 were treated with compound for 72 h and cell viability (MTT) and replication (luciferase assay) were analyzed. The results demonstrated that the compounds D15 and D18 at nontoxic concentration inhibited 35 and 70% of HCV replication, respectively. The favorable ratio of cytotoxicity to antiviral potency (SI = CC50/EC50) was evaluated for both compounds (Selective Index - SI, D15 1.0; D18 4.9) by calculating the ratio of cytotoxic concentration of 50% (CC50 - D15 = 9.4  $\mu$ M; D18 = 10.0  $\mu$ M) and effective concentration of inhibition of 50% (EC50 D15 = 9.2  $\mu$ M; D18 = 2.0  $\mu$ M). In conclusion, the aminochalcones D15 and D18 exhibited antiHCV activity. Further analysis will be performed to investigate the action of these compounds on the other steps of the HCV replicative cycle.

**BV113 - INVESTIGATION OF ARBOVIRUS IN CHIROPTERA**

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The arbovirus, are characterized by being maintained in nature in cycles involving hematophagous arthropod vectors and a wide range of hosts. These hosts are often vertebrates, especially mammals and birds. Among mammals, it is believed that various zoonosis originates on bats. They present a broad geographical distribution, being able to fly long distances, being often come into direct or indirect contact with the human population. Lately, such vertebrates, have received increasing attention as an important source for the emergence of zoonosis and possibly as viral reservoirs. Among the arbovirus, there are many representatives of the genus Flavivirus and Alphavirus, being responsible for important epidemics such as Dengue virus, Zika virus and Chikungunya virus. This study aims to investigate the presence of arbovirus of the Flavivirus and Alphavirus genus, in bats, based on the importance of the analysis of potential viral reservoirs for zoonosis control. Also, to provide information that can contribute to the epidemiological surveillance of high impact diseases in public health. Bats were collected from São José do Rio Preto (São Paulo) and Barreiras (Bahia), were euthanized, followed by removal of the liver of each individual. The RNA was extracted and after quantification and quality analysis, cDNA was synthesized. PCR for the endogenous gene betaactin was performed for all samples in order to evaluate the cDNA quality. Samples were tested for Flavivirus and Alphavirus by PCR and nestedPCR using specific primers. Finally, the nestedPCR products were analyzed on one percent agarose gels. Fiftyseven bats samples tested so far were negative for the presence of Flavivirus and Alphavirus. The results indicate that the animals examined were not infected with arbovirus at the time of collection, indicating that they probably do not constitute a reservoir for these viruses in the studied

areas. Serological tests are required to determine whether the animals had previous arbovirus infection that did not become persistent.

#### **BV131 - CHARACTERISATION OF CEREBRAL INJURIES AND MICROGLIAL ACTIVATION DURING INFECTION INDUCED BY CARAJAS VIRUS**

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The Carajas virus (VCJS) is a Rhabdoviridae family member, genus Vesiculovirus. It was isolated from sandflies (*Lutzomyia* spp) caught in the Serra Norte (Carajás region, Pará State, Brazil), in 1983. Although its isolation occurred more than two decades ago, little is known about its neuropathological features. OBJECTIVES: The aim of this study was to characterize the experimental neuropathology induced by VCJS in adult mice, after intranasal inoculation. MATERIAL AND METHODS: VCJS infected animals were observed twice a day for clinical signs followed by histological and immunohistochemistry procedures to detect viral antigens and activated microglia at the 5th, 10th and 15th postinoculation days (d.p.i.). RESULTS: VCJS infected mice showed bristling, hunched posture, hyperemic conjunctiva, hind limb paralysis, circular unintentional movements and weight loss. Sixty percent of the individuals died between 14 and 16 d.p.i. Histopathological increased as disease progressed from 5th to 15th postinoculation. At 5th d.p.i. it was observed only discrete leukocyte infiltrate and vascular congestion, whereas at 15 d.p.i, midbrain and diencephalon areas around the ventricles, cerebral aqueduct and vessels vicinity, showed mixed leukocyte infiltrate, endothelial proliferation and vascular ectasia, lytic necrosis, pyknotic nuclei, cariorrexis, small hemorrhagic foci, capillary congestion and in some cortical regions, meningeal inflammation was observed. Immunohistochemistry detected viral antigens in the same regions as described above, especially at later stages. Cortical changes were also apparent in the frontal, temporal and entorhinal areas, hippocampus and olfactory bulb. The greatest degree of microglial activation occurred at 10 d.p.i. More intense morphological signs of microglial activation coincided with the same regions where the viral antigens and cellular lesions were detected. CONCLUSION:

These results indicate that after intranasal inoculation, VCJS induces acute encephalitis in adult mice which is associated with intense histopathological changes in the brain parenchyma and meninges. Morphological microglial activation and inflammatory histopathological changes were fatal to 60% of infected animals.

#### **BV133 - INHIBITION OF ZIKA VIRUS, CHIKUNGUNYA VIRUS REPLICATION AND DERIVED FROM NATURAL PRODUCT EXTRACTS**

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Viruses transmitted by arthropods (commonly called arboviruses) normally circulating in nature through biological transmission between susceptible vertebrate hosts and bloodfeeding arthropods, such as mosquitoes. Several studies have shown a wide difficulty of reaching an accurate diagnosis of arboviral diseases that currently have had major impact on health, such as infection Zika virus and Chikungunya. Moreover, as there is no effective treatment or vaccine available, and such viruses may be associated with serious diseases such as microcephaly in case of infection Zikavirus, as well as severe muscle and joint injuries with prolonged recovery time for Chikungunya. The aim of this study was to assess inhibition of marine natural product extracts and others. To perform the tests, 96well plates containing  $2.0 \times 10^4$  vero cells were maintained at 37°C with 5% CO<sub>2</sub>, in Modified Dulbecco Medium supplemented with 5% fetal bovine serum, 2mM L glutamine, plus 100µg of Penicillin and Streptomycin. Initially the cells were exposed to increasing concentrations of 8 different extracts evaluated for obtaining CC50. Subsequently, infected cells were isolated and Zika and Chikungunya at an MOI of 0.01 and treated with different concentrations of the extracts evaluated, 0.5, 1.0, 5.0, 10.0, 20.0 and 40.0µg/ml respectively. The extracts evaluated, we found that at least 4 of the extracts were able to inhibit significantly both Zika as the Chikungunya dose dependent manner. Interestingly the four best results are seaweeds, as in the case of *Osmundaria* and *Caulerpa* who had EC50 of 1.4µg/mL and 4.2µg/mL to Zika virus and 1.82µg/mL

and 1,98µg/ml for Chikungunya. Both extracts showed indices of promising selectivity, 420/288 for *Osmundaria* and 174.2/369.7 *Caulerpa* to Zika and Chikungunya respectively. The results of our group showed large substances development possibilities with antiviral potential for Chikungunya and Zika, demonstrating the possibility of reducing the aggravation of frames and severity of these infections.

### **BV136 - ANALYSIS OF A PROTOCOL FOR ZIKA VIRUS CULTURE IN VITRO**

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**Introduction.** Zika virus (ZIKV) is a RNA virus that belongs to the Flaviviridae family, like dengue and yellow fever viruses. After the ZIKV outbreak in Brazil and in the context of a global emergency, researchers all over the world, especially in Brazil, needed to standardize diagnostic methods for this virus. With this purpose, the ability to grow the virus in vitro as an option to provide diagnosis in cases of doubt is a useful tool. Further, with reports of microcephalic babies and brain damage associated with ZIKV, it was necessary to start studies to clarify the physiopathology of ZIKV, in order to bring a better understanding of how the virus is causing these problems. With this purpose, we started to expand ZIKV in cell culture to be distributed for research laboratories interested in basic and applied research on it. This was an important support for the research community interested in ameliorate the current knowledge of ZIKV. In this context, we present results obtained with the culture of one of the first Brazilian isolates of ZIKV obtained from Evandro Chagas Institute/PA (ZIKVIEC) as a contribution for the begging of efforts on ZIKV in Brazil. **Methods.** ZIKV was grown in Vero cell line and C6/36 cell line in three subcultures (T1T3). Quantitative Reverse TranscriptionPCR (qPCR) was used to follow all the subcultures, along with titration by Plaque reduction neutralization test (PRNT). Immunofluorescence with monoclonal antibodies for Zika virus was also used for virus characterization. **Results.** Cytopathic effects

were better seen in Vero cells than in C6/36 cells from the third subculture (T3), however the C6/36 showed more intense fluorescence when compared with Vero cell. Syncytia were seen within 4 days in Vero cells, while in C6/36 cells syncytia were seen in 6 to 10 days. C6/36 cells were, on the other hand, more efficient to produce quantitatively the ZIKV. The C6/36 subcultures cycle thresholds (Ct) by qPCR were T1=17.26, T2=15.5 and T3=9.52. The Vero subcultures Cts by qPCR were T1=20.0, T2=19.89 and T3=14.17. The C6/36 subculture titers by PRNT were T1=6x10<sup>8</sup>, T2=7.5X10<sup>6</sup>, T3=4X10<sup>12</sup>. The Vero subculture titers by PRNT were T1=9.5x10<sup>4</sup>, T2=3x10<sup>2</sup>, T3=2x10<sup>4</sup>. **Conclusions.** The results showed that the growth of ZIKV in C6/36 cell line produces higher titers and lower Cts compared to Vero cell line in the same subculture, although Vero cells are better to detect cytopathic effects of ZIKV and formation of syncytia.

### **BV 140 - GENOTYPIC DIVERSITY OF CLINICAL ISOLATES OF CANTAGALO VIRUS**

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Vaccinia virus (VACV) is the prototypic species of the family Poxviridae and has been isolated from pustular lesions in dairy cows in Brazil for the last two decades. Cantagalo virus (CTGV) is a field strain of VACV and was first isolated in 1999 in Rio de Janeiro. Phylogenetic studies based on full genome sequences shows that CTGV shares a most recent ancestor with the VACV strain IOC used as smallpox vaccine in Brazil. After 1999, other outbreaks of CTGV or viruses similar to CTGV (CTGVlike) have been reported in several Brazilian states. Analysis of the whole genome sequences showed differences between CTGV1999 and other CTGVlike isolated afterwards in other regions. CTGV1999 has a deletion of about 4.5 kb in 5' region of the genome corresponding to the CPXV 77kDa and C9L virulence genes, but these genes are present in other few CTGV like that were examined. Nevertheless, only a few genome sequences of CTGVlike are available in public databases, which limit our understanding on the genetic diversity of the different clinical isolates of CTGVlike in Brazil. Therefore, the goal of this study is to detect major deletions in CTGVlike genomes, mainly the presence or absence of

the 4.5 kb deletion. PCR assays were performed with 64 clinical isolates identified as CTGVlike that were collected from milkers and cows between 1999 and 2013 in the North, CentralWest and Southeast regions of Brazil; nine samples presented the genetic pattern of CTGV1999 relative to the CPXV 77kDa/C9L deletion and 48 samples did not present the deletion. The data reveal a relationship of temporal and geographical disposition; all CTGVlike samples isolated until 2003 caused outbreaks in the Southeast Brazil and presented the CTGV1999 signature; most CTGVlike from 2006 to 2013 were collected in CentralWest or North regions and only 6 were isolated in Southeast Brazil; all of them had the CPXV 77kDa/C9L genes. Interestingly, 2 clinical isolates from RJ collected in January 2003 presented both signatures, suggesting a potential cocirculation of genetically distinct viruses. Another region at the 3' end of the genomes was analyzed and corresponded to the B16R/B17L genes, which are absent in the genome of Serro2 virus, a CTGVlike virus isolated in Minas Gerais in 2006. None of the 39 samples analyzed so far had the B16R/B17L deletion similarly to CTGV1999. Thus, the results suggest the existence of a genetic diversity in the Brazilian CTGV clinical isolates.

#### **BV142 - GENETIC AND BIOLOGICAL DIVERSITY OF VACCINIA VIRUS STRAIN IOC**

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Vaccinia virus strain IOC (VACVIOC; Orthopoxvirus, Poxviridae) was the vaccine strain used for the manufacture of the Brazilian smallpox vaccine by the Instituto Oswaldo Cruz-RJ until late 1970s. It confers crossimmunity against variola virus and possibly originated from the Beaugency strain imported from France in 1887. In this work, we investigate the genetic and biological features of VACVIOC, and its phylogenetic relationships with other VACV strains. Hereto, we initially isolated VACVIOC clones B141 and B388. Both clones showed similar virus production and spread in cell culture. However, intranasal infection of mice with clone B388 caused a transitory 10% weight loss, whereas infection with clone B141 did not. Furthermore, infection clone B141 did not cause any other clinical

signs, similarly to the effects of the licensed smallpox vaccine Acam2000. Infection with the parental VACVIOC caused weight loss at intermediate levels between both clones. Moreover, mice were fully protected from a lethal challenge with VACVWR. Genome sequencing revealed a 4.5kb deletion in the 3' inverted terminal repeat (ITR) junction of B388 genome. More importantly, orthologs of K3L and C3L genes were fragmented in B141 genome but intact in B388. To further investigate VACVIOC diversity, we isolated thirty additional clones. Amplification of the genome terminal regions of the 30 clones by long PCR revealed three different patterns: a 9 kbend similar to B141, a 4.5kb pattern at the 3' end similar to B388, and an 8kb end similar to clone A111 that was recently sequenced. The genomes of four clones were sequenced, and two had a 7.7kb deletion at the 5' variable region of the genome outside the region amplified by the long PCR. Nevertheless, the plaque phenotype varied greatly between clones sharing the same terminal region pattern, suggesting that these deletion patterns might not correlate with differences in plaque phenotype or virus production. VACVIOC clones branched within a novel, independent phylogenetic cluster formed also by the field strains of Brazilian vaccinia virus Cantagalo and Serro2, and the supposedly VACV ancestor horsepox virus. Interestingly, this novel cluster branched as the sister group to the American/Dryvax cluster, and not to the Eurasian cluster. A historical investigation was undertaken and suggested that both the Dryvax and the IOC strains were derived from the Beaugency strain, which would explain their phylogenetic relationship.

#### **BV144 - MOLECULAR AND BIOLOGICAL CHARACTERIZATION OF AN AURA VIRUS ISOLATE**

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Aura virus (AURAV) is a member of the Alphavirus genus, that encompasses different arthropod borne viruses (arboviruses), many of which are involved in the etiology of encephalitis or diseases whose main symptoms are fever, rash and arthralgia. Its genome is constituted of a positivesense singlestranded RNA of approximately 11,7 kb. Previous studies have shown a closer antigenic and phylogenetic relationship with Western equine encephalitis virus and Sindbis virus, however only a

single complete genome sequence is available in the GenBank database (NC\_003900.1). The first isolates of AURAV were identified in pools of mosquitoes that had been collected in the vicinity of the city of Belém (Brazil) and in the Misiones province (Argentina). There are no posterior accounts of new virus isolations and the available data indicate it does not have another known host, therefore it is considered nonpathogenic to humans and its distribution restricted to South America. During a work with a sample in which it had been previously identified a dengue virus serotype 3, we have identified phenotypes in insect and mammalian cell cultures that were not compatible with dengue virus infection. Using transmission electron microscopy and sequencing of nonspecifically amplified PCR products the identification of an AURAV was possible. Considering the scarce information on AURAV and the medical importance of other members of the same genus, the present work aimed at the genetic and biological characterization of this new isolate. The genetic analysis involved sequencing of the whole genome after amplification through RTPCR using sequence specific primers. When the sequence of the new isolate was compared to the only other complete sequence available many nucleotide and amino acid differences were observed throughout the genome. Moreover, with regard to the biological characterization, the C6/36 cell line seems to be more susceptible to AURAV infection than the BHK21 cell line. The complete characterization of this isolate will contribute to the knowledge on the basic biology of viruses belonging to this genus and possibly open avenues for its use as a biotechnological tool.

#### **BV148 - ANTIVIRAL EVALUATION OF VEGETABLE COMPOUNDS AGAINST HUMAN HERPESVIRUS 1 AND AICHI VIRUS**

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Viral infections are a serious worldwide public health problem and despite the emergence of prophylactic measures, such as vaccines and development of some antivirals, the treatment of viral infections remains a challenge, especially for the difficulty in developing

drugs capable of inhibiting viral replication without interfering with the host cell metabolism. In this context, it has been widely reported the use of natural products, mainly medicinal plants as a material for the antiviral drugs development. Thus, this study aims to evaluate the antiviral activity of compounds isolated from native medicinal plants of CentroOeste against human herpesvirus 1 and aichivirus as well as determine their action mechanisms. For this, five previously purified and identified substances extracted from *Campomanesia adamantium* and *Campomanesia xanthocarpa* were tested against herpesvirus humano 1 and aichivirus. The substances were prefractionated and purified by comparative and preparative thinlayer chromatography, and identified by spectroscopic analyzes. Vero cell cultures were performed and the cytopathic effects were observed by inverted microscope. The cytotoxic assay was performed by the MTT method and the antiviral activity was determined by the viral titer reduction using the statistical method of Reed & Muench, expressed in viral inhibition index (IIV) and percentage inhibition (PI). The index of selectivity (IS) was calculated as the ratio of CC50 and ED50. The tests showed that the substance 5,7 dihydroxy-6,8-dimethylflavanone was active against HSV1 presenting 99,8% of inhibition and 44,05 of IS. This substance also showed antiviral activity against aichivirus, with 99,8% of antiviral inhibition and 27,95 of IS. The remaining substances showed no antiviral effect. Before that, the study prove that one of the substances tested showed activity against HSV1 and aichivirus, allowing potential antiviral effect. According to the literature review, this is the first study related to antiviral activity of *Campomanesia* sp.

#### **BV152 - ANTIVIRAL BACTERIOCIN EVALUATION PRODUCED BY LACTOBACILLUS PLANTARUM ST8SH AGAINST HERPES VIRUS HUMAN TYPE 1**

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The viruses are present in a wide range of organisms and some of them are a public health problem. Considering that some drugs are available for the treatment of viral infections, health managers and vaccination programs have been reinforced to prevent human viral infections.

Natural compounds represent an abundant source of pharmacological activities, among them the antiviral, offering new options for drug therapies. From these compounds it is possible to extract active substances make structural changes, making them more effective and less toxic. It can also be used as a model for synthetic drugs with pharmacological activities similar to the originals. These substances have demonstrated significant effects as an antiviral peptide against different microorganisms, including viruses, making them candidates for antiviral drugs. This study aimed to carry out the antiviral analysis of the bacteriocin produced by *Lactobacillus plantarum* ST8SH (BacST8SH) against human herpesvirus 1. MATERIALS AND METHODS: BacST8SH semipurified in isopropanol different concentrations (20%, 40%, 60% and 80%) was quantified by fluorometric method (Qubit). All experiments were performed in Vero cell cultures and cytopathic effects were observed by microscopy. The cytotoxic effect of the semipurified bacteriocin was tested by MTT method and antiviral activity against herpes simplex virus 1 (HSV1) was determined by the reduction in the viral titer using the statistical method of Reed and Muench, expressed in inhibition index viral (IIV) and Percent Inhibition (PI). RESULTS: The antiviral assays showed that the semipurified bacteriocin ST8SH in isopropanol concentrations of 20%, 40%, 60% and 80% showed PI 93.7%; 96.8%; 99.9% respectively, and only BacST8SH80% showed no antiviral effect against HSV1. CONCLUSIONS: The study showed that the semipurified bacteriocin produced by *Lactobacillus plantarum* ST8SH showed antiviral effect with potential application. It will require further testing for statistical validation.

#### **BV158 - ANTIVIRAL ACTIVITY OF NACETYLL-CYSTEINE AND NUCLEOSIDE ANALOGS AGAINST ZIKA VIRUS**

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Zika virus (ZIKV) is a member of the Flavivirus genus within the Flaviviridae family and has a positive strand RNA genome of about 11,000 nucleotides. This virus can be transmitted to people primarily through the bite of an infected *Aedes* species mosquito (*Ae. aegypti* and *Ae. albopictus*) and vertically by placental passage during

pregnancy, being related as a cause of microcephaly and other severe fetal brain defects. As there is no specific vaccine or licensed drug to treat Zika virus infection and given the current epidemic situation, it has become a global public health problem. The aim of the present study was to evaluate the antiviral activity of the compounds N-acetylcysteine (NAC), 2'-C-methylguanosine (2'-C-MeG) and 2'-C-methylguanosine tryptamine phosphoramidate monoester (phosphoramidate monoester) against Zika virus. NAC is an antioxidant compound with known antiviral activity against seasonal human influenza A virus, 2'-C-MeG is a nucleoside analog inhibitor of viral RNA dependent RNA polymerase previously developed for hepatitis C virus and the monoester phosphoramidate compound is a tryptamine phosphoramidate nucleoside prodrug designed to enhance the intracellular delivery of monophosphorylated nucleoside analogs. Cytotoxicity of the compounds was measured by the neutral red uptake assay to verify the maximum nontoxic dose (MNTD). All the experiments were performed in triplicate. Vero cells were infected with ZIKV at a multiplicity of infection of 0.05. After adsorption period, cells were treated with 100  $\mu$ M of each compound and incubated for 72 hours, at 37°C in 5% CO<sub>2</sub> atmosphere. The cells were analyzed once a day for cytopathic effect (CPE) observation. Supernatant was harvested 72 h post infection and viral replication was detected by real time polymerase chain reaction (qPCR) assay. No cytopathic effects (CPE) were observed up to 48 h of infection, but at 72 h it was possible to notice more CPE on infected nontreated cells when compared to infected and treated cells. The treatment with 2'-C-MeG was capable to reduce 96,06% of viral replication, while phosphoramidate monoester and NAC treatment inhibited 99,99% of viral replication. These data suggest a tendency of viral replication inhibition by 2'-C-MeG and a most relevant antiviral activity of NAC and phosphoramidate monoester in Vero cells infected with ZIKV. Viral plaque reduction assay will be performed in order to complement these preliminary findings.

**BV170 - STUDY OF ANTIVIRAL ACTIVITY OF ESSENTIAL OIL OF PITANGA (EUGENIA UNIFLORA L.) ON HERPES SIMPLEX VIRUS ON TYPE 1 (HSV1)**

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Due to the increasing observed resistance on antivirals, natural products extracts have been studied as alternative products on current therapies. *Eugenia uniflora* L. (also known as Pitanga) had already been used on popular medicine due to its antioxidant, antitumor, analgesic, antiinflammatory and diuretic activities. Besides that, there are no studies, until the present moment, showing the potential antiviral activity of Pitanga essential oil for the treatment against herpetic pathologies. Objective: evaluate the antiviral activity of essential oil of *Eugenia uniflora* L. (Pitanga) on viral replication of herpes simplex virus type 1 (HSV1). Methods: After extracting the essential oil by the Clevenger method, the oil maximum nontoxic concentration (MNTC) was evaluated in Vero cells and three experiments in duplicate were done. Pretreatment of Vero cells with a MNTC of the essential oil, followed by the infection with different dilutions of HSV1. Viral inactivation method done by exposing the HSV1 to a MNTC of the oil and then infecting the Vero cells to see the presence or absence cytopathic effect (CPE) inhibition. Posttreatment where Vero cells were inoculated with HSV1 followed by exposure of the cells with a MNTC of Pitanga essential oil and evaluation for CPE. Results: The MNTC of Pitanga essential oil in Vero Cells was 156,25 µg/ml. We could notice that the Pitanga essential oil could inhibit replication of HSV1 through the different experiments used to evaluate the different replication phases. On pretreatment, a 50% viral replication inhibition was observed at the 10<sup>7</sup>/17 viral dilution, with similar results noticed on the viral inactivation test. On the posttreatment test, however, the dilution which showed 50% of inhibition of replication was 10<sup>7</sup>/19. The highest antiviral activity was seen when HSV1 was incubated with the oil before cell infection. Conclusion: Our findings showed that *Eugenia uniflora* essential oil could inhibit HSV1 replication regardless the treatment considering its possible use as an antiviral product.

**BV171 - DENGUE VIRUS INDUCED REACTIVE OXYGEN SPECIES AFFECTS CELL VIABILITY AND VIRAL REPLICATION IN HUMAN ENDOTHELIAL CELLS**

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Dengue virus (DENV) infection is associated to vascular alterations, including vasodilation and increased permeability, as a result of systemic inflammation and endothelial lesion. We have previously demonstrated that endothelial cells were permissive to DENV, which triggered activation of RNA sensors, leading to cytokine production and cell death. Virus sensing is related not only to cellular activation, but also to cellular stress responses, including mitochondrial stress and reactive oxygen species (ROS) production. These mediators, in turn, may regulate cellular activation and survival. Here, we investigated if DENV infection induced ROS production by endothelial cells, and whether these mediators would affect viral replication, endothelial activation, and cell death. We used human brain microvascular endothelial cells (HBMECs) as an endothelial cell model. The cells were infected with DENV2 (16681 strain) and ROS production was analyzed by immunofluorescence and flow cytometry. Virus replication was evaluated by qRT-PCR, flow cytometry and plaque assay. Cell death was evaluated by flow cytometry and XTT assay. Cytokine production was analyzed by qRT-PCR and ELISA. We observed that dengue infection on HBMECs results in increased ROS production, which was dependent on viral replication. ROS inhibition resulted in decreased viral load, prolonged cell survival, and was also associated to apoptosis of bystander cells. Interestingly, inhibition of ROS resulted in diminished cytokine secretion by HBMECs. These data suggest that DENV-induced ROS production in HBMECs may be an essential primary signal to virus replication, and to PRR-mediated cell activation. Sustained ROS production results in endothelial cell death, which may contribute to in vivo, vascular lesion.

**BV195-INFLUENZALIKEVIRUSANDPARAMYXOVIRUS SCREENING IN BRAZILIAN BAT**

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Bats are recognized as natural reservoirs of emergent viruses related to severe human disease outbreaks including Rabies, Nipah, Hendra and SARS coronavirus. Since the discovery of Hendra and Nipah emergent paramyxovirus (PAR) in late 90's in flying foxes bats from Australia and Asia, others batborne paramyxovirus have been identified in bats across the globe including bats species from Australia, Asia, Africa and South America. Moreover, bats have also been described as possible hosts of influenza virus and hantavirus, whose main reservoirs are rodents and birds respectively. The importance of the circulation of these virus in bats and its relationship to human infections has not been determined. Despite the great diversity of viruses recently detected in bats from different continents and the recent spillover events of virus from bats to humans, few studies had analyzed the occurrence and geographical distribution of influenzalike virus and paramyxovirus in Brazilian's bats. The present study aims to evaluate the occurrence and diversity of Influenzalike virus and Paramyxovirus in different bat species from Brazil. For that, intestine tissue from 533 bats (25 species and three families) from urban, continuous and fragmented forest areas were screened for Influenzalike virus and Paramyxovirus. The Total Nucleic Acid was extracted by automatized method

in EasyMag BioMerieux. Randomic cDNA synthesis was performed with High Capacity kit. cDNA samples were screened by PCR assay targeting the PB1 gene using primers developed by CII - Columbia University (New York, USA) to Influenzalike virus and by a Semi Nested PCR assay designed for the detection of the presence of viral RNA paramyxoviruses. PCR fragment was observed in electrophoresis analysis and the samples were purified and sequenced by Sanger method in 3130xl equipment. None sample was confirmed to Influenzalike virus and two samples was positive to Paramyxovirus. One Morbilliviruslike was detected in an insectivorous bat *Molossus rufus* and an Unclassified Paramyxovirus was found in one hematophagous bat *Desmodus rotundus*. This preliminary study report the absence of Influenzalike virus in bats from Atlantic Forest Biome, Brazil, and the presence of Paramyxovirus genotypes in bats commonly found in rural and urban area reinforcing the necessity of expanded and continuous surveillance of potential emergent virus in the bat fauna of this hot-spot biome.

**BV197 - CORONAVIRUSES DIVERSITY IN BATS FROM URBAN AND ATLANTIC FOREST FRAGMENTS OF SÃO PAULO STATE**

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7. DIVISÃO TÉCNICA DE MEDICINA VETERINÁRIA E MANEJO DA FAUNA SILVESTRE (DEPAVE3), SECRETARIA DO VERDE E MEIO AMBIENTE, PREFEITURA DO MUNICÍPIO DE SÃO PAULO, SÃO PAULO

Epidemiological and phylogenetic studies indicate that four out of six coronavirus (CoV) capable of infecting humans are the result of spill over events of virus from bats to humans. Over the past 13 years, two highly

pathogenic CoV were isolated from humans, CoVSARS (Severe Acute Respiratory Syndrome) and the CoV MERS (Middle East Respiratory Syndrome) with a mortality rate of 10 and 42%, respectively. Subsequent studies have identified CoV in bats all over the world including CoV with great genetic similarity and capable to use the same cell receptor of SARS and MERSCoV. Despite the great diversity of CoV in bats, the large number of bat species in Brazil and the classification of Atlantic Forest Biome (AFB) as a hotspot region for emergence of new infectious disease, studies about the occurrence and diversity of CoV in bats in Brazil are scarce. The present study aims to evaluate the diversity of coronavirus in bats from Urban and Forest Fragments of São Paulo State located inside the Atlantic Forest biome. Intestine samples from bats received by the Center of Zoonosis Control of São Paulo Municipality (N=132) and Oral/Rectal Swabs Samples collected from bats from Forest Fragments, inside or close to São Paulo Metropolitan area, were screened for CoV RNA (N=119). Shortly, total nucleic acid were obtained from 30mg of intestine tissue extracted in NucliSENS® easyMAG® automatic extractor (BioMerieux). cDNA was prepared using random primers and subjected to a modified pancoronavirus NestedPCR. We screened a total of 251 individuals of 31 distinct species including members of Phyllostomidae, Molossidae and Vespertilionidae bat family. Alphacoronavirus RNA was detected in one intestine sample obtained from CCZSP (*Phyllostomus discolor*) and 7 swabs samples from bats of Forest Fragments of SP state (*Artibeus lituratus*, *Glossophaga soricina* and *Sturnira lilium*) presenting a general prevalence of 0,7 and 5,9% respectively. ?CoV sequences obtained from bats of same genus presented high nucleotide sequence with sequences detected in other studies from bats of geographically distant regions. Similar results were previously reported for a variety of bat CoVs and are taken as evidence of coevolution of CoV genotypes and specific host genera. Our results demonstrate the need for expanded and continuing surveillance of CoVs in bat fauna, including those in the AFB regions of Brazil.

## **BV202 - MOLECULAR ANALYSIS OF NOROVIRUS SPECIMENS FROM CHILDREN ENROLLED IN A 1982-1986 COLLECTION SAMPLES IN BELÉM, BRAZIL: A COMMUNITYBASED LONGITUDINAL STUDY**

**Siqueira, J.A.M.; Júnior, E.C.S.; Linhares, A.C.; Gabbay, Y.B.**

*INSTITUTO EVANDRO CHAGAS*

Several molecular studies have shown a high degree of norovirus (NoV) genetic diversity, and although numerous genotypes are known to infect humans, genogroup II strains have remained dominant in most outbreaks of gastroenteritis (GE) and cases of GE at both hospital and community levels. Specimens were collected during a longitudinal, communitybased study carried out in the city of Belém, North Brazil, over 3 years (October 1982 to March 1986), where 20 children were followed up from birth to 3 years of age. A total of 229 samples were screened for NoV by Real Time PCR targeting polymerase gene (RdRp) and the positives were characterized by the regions B (RdRp) and C or D (VP1 gene). In case of a disagreement between the two regions genotyped, the junction region between ORFs 1/2 was considered to suggest a recombination event. Samples classified as GII.P4/GII.4 were analysed by P2 region to determine the current variants. Nucleotide sequences analyses were made by maximum likelihood method with 1000 bootstrap replicates. An overall positivity of 16.1% (37/229) was observed, including GI (16.2%6/37) and GII (83.8%31/37) genogroups. Cases of NoV reinfection in at least twomonth intervals were observed and 12 children developed at least one case of asymptomatic NoV infection. 48.6% (18/37) NoVpositive samples were subjected to nucleotide sequencing analysis targeting at RdRp gene: GI.P3 (n=1), GII.Pa (n=1), GII.Pc (n=1), GII.P4 (n=5), GII.P6 (n=5), GII.P7 (n=3), GII.P12 (n=1) and GII.P22 (n=1). The VP1 gene allowed the characterization of 14 (77.8%) samples of the 18 previously genotyped: GI.3 (n=1), GII.2 (n=1), GII.4 (n=4), GII.6 (n=4), GII.7 (n=1), GII.12 (n=1), GII.14 (n=1), GII.22 (n=1). In three cases were suggested recombination events (GII.P12/GII.2, GII.P7/GII.14, GII.Pa/GII.12) and four samples genotyped as GII.P4/GII.4 were analysed to identify variants, but any one showed contemporary counterparts. Three children developed consecutive NoV infections by different genotypes. The present report documents the importance of NoV as a

cause of childhood infection during a longitudinal study conducted more than 30 years ago, demonstrating prolonged shedding; high prevalence in controls; possible resistance to infections; and relationship between breastfeeding and susceptibility to infections at community level, besides a broad genetic diversity likewise it can be currently be observed.

### **BV203 - NATURAL HISTORY OF NOROVIRUS INFECTIONS IN CHILDREN FROM BELÉM, PARÁ: A COMMUNITYBASED LONGITUDINAL STUDY**

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Norovirus (NoV) are the most important pathogen when considered outbreaks of AGE in human populations. The genotype GII.4 is the most prevalent worldwide and is responsible by the majority of the global epidemics (pandemics) of viral aetiology. The objective of this study was to detect and characterize the infections by NoV that occurred in children followed up in the community from birth until the three years old, residents in neighbourhoods of low socioeconomic status from Belém, between 1982 and 1986. Fecal specimens were obtained during a communitybased longitudinal study whose total of 2.013 samples were collected. It was tested a subset of 216 fecal samples belonging to three children residents in the districts of Barreiro (n=69), Marco (n=77) and Terra Firme (n=70) of which were collected feces fortnightly or when they have diarrhea. Samples were tested by quantitative PCR (qPCR) using the kit Superscript III OneStep RTPCR Systems with Platinum Taq (Invitrogen) for the detection of GI and GII genogroups. Positive samples by qPCR were submitted to Seminested RTPCR reaction using primers JV13I/JV12Y (first step) and JV13I/G1 or JV12Y/NoroIIR (second step) for GI and GII, respectively. The positive ones were sequenced aiming the partial characterization of region A of the viral polymerase gene. The phylogenetic construction was performed using the method of NeighborJoining Kimura 2parameters, with bootstrap of 1000 replicates. The positivity of 14.3% (31/216) was observed, being 13% (28/216) for GII and 1.8% (4/216) for GI. One sample was positive for both GI and GII. It was possible to classify 60.7% (17/28) of GII and 100% (4/4) of GI, being observed the genotypes GII.P4

(58.8%), GII.P6 (11.8%), GII.Pa (5.8%), GIIinconclusive (11.8%) and GII.Pnew (11.8%). For genogroup I it was observed the genotypes: GI.P5 (25%), GI.P7 (25%), GI.Pd (25%) and GI.Pf (25%). The highest frequency of infection was detected in the age range of 6 to 12 months (p=0.0024) and it was not determined no seasonality for NoV in the period of study, showing peaks in November 1983, August 1984 and September 1985. These results demonstrated the diversity of NoV in children in the community circulating in the 1980s, causing mainly asymptomatic cases. In addition, it was observed that the GII.4 was the most prevalent genotype since that time. This study contributed to a better understanding of this pathogen in gastrointestinal infections.

### **BV222 - EVALUATION OF MYRCIARIA FLORIBUNDA IN VITRO ACTIVITY AGAINST ZIKA VIRUS**

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Zika virus (ZIKV) had an increase in the number of cases reported in the past few years. Diseases like microcephaly and GuillainBarre syndrome are commonly associated with the ZIKV infection. Due to its upsurge severity studies in the development of medicines to inhibit virus replication have become essential. In this sense, Myrciaria floribunda is a widely spread tree, especially in the north of Brazil, whose essential oil properties have been documented for antimicrobial, antiinflammatory and antitumor activities. The stem and leaf of M. floribunda were extracted with dichloromethane, ethyl acetate and hexane. To evaluate the antiviral activity of the extracts, VERO cells, growing in 24 wells plates with 1 x 10<sup>5</sup> cells/well density, were infected with ZIKV (1 x 10<sup>4</sup> PFU) using 0,1 MOI for one hour at 37°C in 5% CO<sub>2</sub> atmosphere. Afterwards, the cells were treated with the extracts in two concentrations, 10 and 30?g/mL, in 5% FBS medium. The cells were lysed by three freezing and thawing cycles, the supernatant was titrated by plaque essay. The plaque remained in an incubator for five days, subsequently the supernatant was removed and crystal violet was added. The inhibition percentage of M. floribunda hexane, dichloromethane and acetate leaf extracts at 30?g/mL was above 85%. On the other

hand, at 10<sup>7</sup>g/mL concentration, the ethyl acetate leaf extract resulted in 95% of inhibition. At the same concentration, the other substances presented lower inhibition percentage, being above 40%. The hexane and dichloromethane stem extracts at 10<sup>7</sup>g/mL concentrations inhibited the viral replication over 38% whereas the acetate stem extract inhibited 96% the production of viral particles. These results show that *M. floribunda* extracts may be useful as an antiviral against ZIKV, but further tests are needed.

#### **BV224 - SUSCEPTIBILITY OF HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS TO "IN VITRO" INFECTION WITH ZIKA VIRUS**

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Zika virus (ZIKV) is a member of the family Flaviviridae that became a major public health concern since the recognition of its association with microcephaly and other fetal damage. However, very little is known about the pathogenesis of this virus. To assess whether ZIKV can infect peripheral blood mononuclear cells (PBMCs), 5mL of peripheral blood was collected from healthy donors and cells were isolated using centrifugation in percoll density gradients. PBMC were infected after 2 hours in culture (MOI=1), and formalin fixed 24 hours post infection. Slides were incubated with primary antibodies (mouse polyclonal antibody against ZIKV and antibodies against CD3, CD4, CD8, CD11c, and CD20), following incubation with specific secondary antibodies. The nuclei were stained with DAPI and preparations were examined by confocal microscopy. The results indicated that CD20+ (B lymphocytes) and CD11c+ (monocytes) cells are seem to be susceptible "in vitro" to infection by ZIKV. On the other hand, ZIKV did not infect cells positive for CD3, CD4 or CD8, indicating that T lymphocytes are not susceptible. The present results open new possibilities of investigation of the biology of ZIKV infection of B lymphocytes and monocytes as a way to understand basic mechanisms of ZIKV pathogenesis. In addition, these findings suggest that immunofluorescence of peripheral blood smears may provide a way to make rapid diagnosis of ZIKV infections.

#### **BV236 - ALTERATIONS IN GENES OF DNA DAMAGE REPAIR PATHWAYS ARE CRITICAL FOR CERVICAL CANCER DERIVED CELLS TUMORIGENIC POTENTIAL: POTENTIAL ROLE OF TREX1 AND RPA1 IN CERVICAL CANCER ONSET AND PROGRESSION**

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Alterations in the dna damage repair mechanisms associated with the presence of hpv have been described in different experimental models. however, the effect of hpv on the expression of genes involved in theses pathways has not been analyzed in detail. in the present study, we compared the expression profile of 135 genes involved in dna damage repair pathways between primary human keratinocytes (phk), hpv-positive (siha and hela) and hpv-negative (c33a) cervical cancer derived cell lines. we observed that tumor derived cell lines exhibited major alterations in the expression profile of genes from several dna damage repair pathways. the expression of 18 was consistently altered in all three transformed cell lines compared to normal cells. interestingly, we identified 9 differentially expressed genes that distinguished hpv-positive tumor cell lines from c33a. gene expression data were confirmed by real-time pcr and western-blot. moreover, the expression of two selected genes, rpa1 and trex1, was silenced in tumor cells using lentiviral vectors expressing specific shrna. we observed that rpa1 and trex1 silencing greatly affect tumor cells clonogenic potential and anchorage independent growth ability. we observed that the effect of trex1 and rpa 1 silencing was dependent on both hpv16 e6 and e7 expression. furthermore, the effect of these genes silencing was more dramatic in human keratinocytes immortalized by hpv16 oncoproteins than in those acutely transduced with hpv oncogenes. finally, an analysis conducted in human cervical tissues samples showed that trex1 expression levels are upregulated in precancer lesions, squamous carcinoma and adenocarcinoma. our results support the presence of significant changes in the expression of genes involved in dna damage repair pathways in cervical cancer derived cell lines. besides, we show that some of these alterations may be important for tumor cells survival, tumorigenic potential and tumor establishment/progression.

**BV238 - ATM PATHWAY IS IMPORTANT FOR HUMAN PAPILLOMAVIRUS TRANSFORMED CELLS SURVIVAL**

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Human Papillomaviruses (HPV) are nonenveloped DNA viruses that infect epithelial cells. Persistent infection with some HPV types is the main risk factor for the development of cervical cancer. DNA repair machinery plays an essential role in several stages of the HPV life cycle and is crucial for tumor cells' survival. During malignant transformation, HPV E6 and E7 oncoproteins induce structural and numerical chromosome alterations and modulate DNA damage response. These observations suggest that cellular DNA repair machinery may play a dual role in both HPV biology and pathogenesis. In the present study, we sought to investigate the role of DNA repair proteins in cervical cancer derived cells biology. In order to achieve this goal, the expression of 189 genes was silenced in HeLa (HPV16) and SiHa (HPV18) cells as well as in primary normal human epidermal keratinocytes (NHEK) using lentiviral vectors expressing specific shRNA. The effect of gene silencing was determined by cell viability assay, cell growth analysis, clonogenic and soft agar colony formation test. We observed that ATM, BRCA1 and CHEK2 down-regulation decreased growth rate, clonogenic potential and cellular anchorage independent growth of HPV-transformed cervical cancer derived cell lines with no effect in normal keratinocytes. Treatment of cells with drugs that inhibit ATM and CHEK2 activity showed that tumor cells are more sensitive to the inhibition of these proteins than NHEK. Besides, we show that NHEK expressing HPV16 E6 alone or along with HPV16 E7 were more sensitive to these inhibitors than control NHEK or NHEK expressing only E7. Moreover, NHEK expressing E6 mutants defective for p53 degradation were less sensitive than NHEK expressing E6wt. Altogether, these results indicated that these genes are required for HPV transformed cells survival. Besides, our results suggest that this effect is related to HPV16 E6 oncoprotein expression and its capacity to degrade p53.

**BV259 - COCAL VIRUS INDUCES ENCEPHALITIS IN MICE BALB/C ADULTS AFTER INTRANASAL INOCULATION: HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS**

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The Cocal virus (COCV) belonging to the Rhabdoviridae family, genus Vesiculovirus. The COCV infection affects livestock including cattle and horses in South America, (including Argentina, Uruguay and Brazil). Humans were rarely infected. It is already known that in newborn mice, intranasal inoculation of COCV caused acute infection followed by death one day postinoculation, however, little is known about this viral encephalitis in adult mice. The aim of this study was to describe the neuropathological features of COCV adult mice encephalitis. To that end we investigated the distribution of viral antigens and microglial activation in the brain parenchyma of BALB/c adult mice, after intranasal inoculation. Histopathology (hematoxylineosin stained) and immunohistochemical assays to detect microglial morphological response were done at 3th and 6th day post inoculation (d.p.i.). RESULTS: COCV infection induced cortical perivascular edema and hemorrhagic foci, associated with signs of cell death by apoptosis and necrosis in the cortex and olfactory bulb, and leukocyte infiltrate in the cortex. Microglial cells with activated morphology were mainly found in the olfactory bulb, cortex and hippocampus. The viral antigens and activated microglia were first detected in olfactory bulbs and frontal cortices at 3th d.p.i., and in the hippocampus, brainstem, striatum and cerebellum at 6th d.p.i. CONCLUSION: Our results suggest that infection of the central nervous system of mice by the COCV, after intranasal inoculation, invade the central nervous system through the olfactory receptors causing encephalitis, with an intense inflammatory response and cell death leading to 100% mortality.

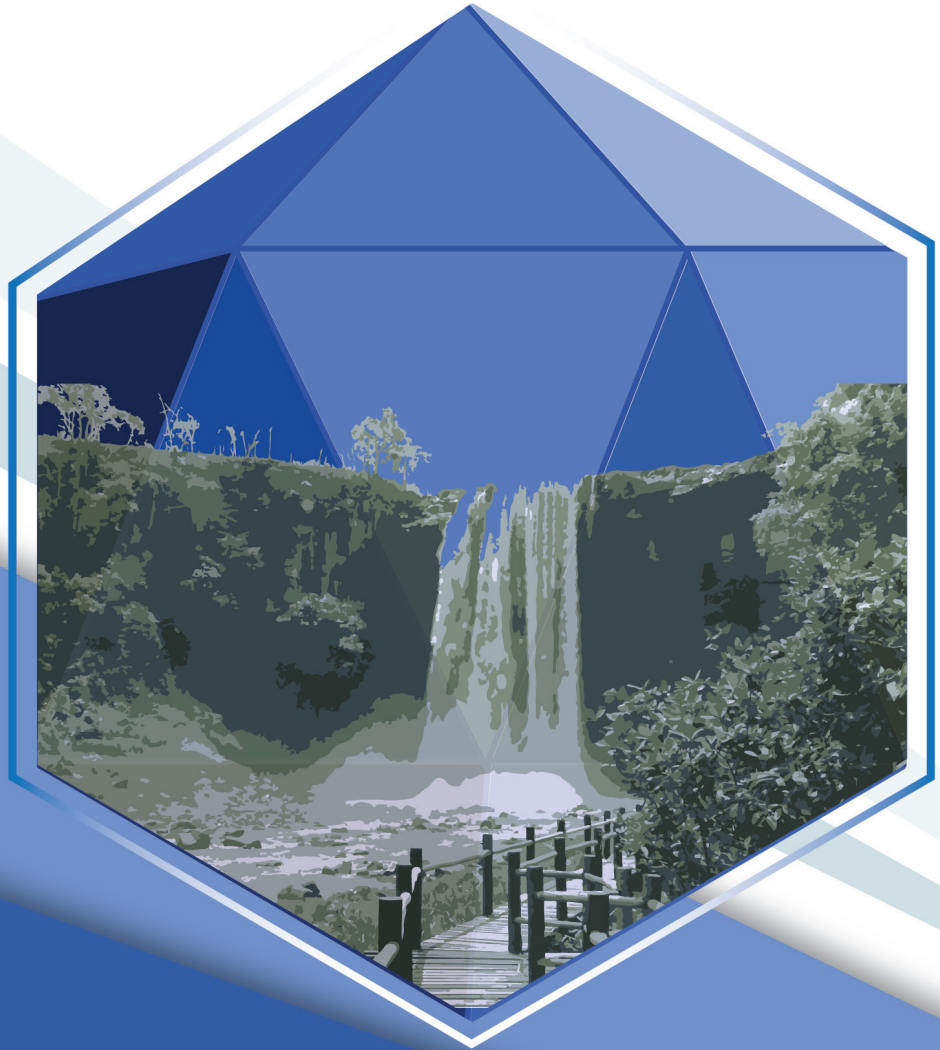
**BV274 - AUTOPHAGY INHIBITION BY A VACCINIA VIRUS VIRULENCE FACTOR****Schnellrath, L.C.; Damaso, C.**

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Vaccinia virus (VACV) encodes many virulence factors that are involved in the antiviral pathway triggered by interferons. During infection this response leads to an increased expression and activation of PKR (double-stranded RNA-dependent protein kinase), culminating in eIF2 $\alpha$  (alpha subunit of the eukaryotic translation initiation factor 2) phosphorylation and inhibition of cellular protein synthesis. The product of VACV059 gene is a VACV-encoded protein that antagonizes dsRNA formed as a result of viral convergent transcription. The absence of VACV059 causes host restriction and reduction of pathogenicity. Previous data showed that wild-type VACV does not induce apoptosis or autophagy. However, we recently observed that the absence of VACV059 during infection results in autophagy and apoptosis. Therefore, our goal is to evaluate how autophagy is induced during VACV infection in the absence of VACV059 and how it interferes with cell death through apoptosis. During wild-type VACV infection we observed that despite dsRNA production, activation of PKR/eIF2 pathway is blocked and we do not observe autophagy. PKR is involved in autophagy during infection with the mutant VACV through eIF2 $\alpha$  phosphorylation pathway. However, only eIF2 $\alpha$  phosphorylation, in the absence of PKR phosphorylation, already induces autophagy. In the absence of viral DNA replication and post-replicative phase there was no production of dsRNA, and we did not observe the induction of autophagy or apoptosis during infection with the mutant VACV. Complementing cells were capable of inhibiting autophagy and of rescuing viral replication during the infection with the mutant VACV. We silenced Beclin-1 and Atg7 and confirmed that the induction of autophagy occurred through the canonical pathway. The addition of a synthetic dsRNA to cells confirmed our results mimicking the effect of viral dsRNA produced during infection. When the autophagy pathway was impaired, we observed that infection with the mutant VACV led to early apoptosis and reduced cell viability when compared with autophagy-competent cells. Therefore, in these conditions, autophagy seems

to have a cytoprotective role. Financial support: CAPES, CNPq, FAPERJ and Pró-Defesa.

# ***ENVIRONMENTAL VIROLOGY - EV***



**EV38 - WIDESPREADED CONTAMINATION BY HUMAN ADENOVIRUS IN SURFACE AND GROUNDWATER IN WATER COLLECTED FROM FARMS IN THE SINOS RIVER BASIN, BRAZIL**

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UNIVERSIDADE FEEVALE

Enteric virus are ubiquitous in the environment and their occurrence are attributed mainly to improper disposal of animal and agricultural waste and lack of sanitation facilities. Therefore, the rural areas are more likely prone to dispersal of waterborne diseases. Human adenovirus (HAdV) are members of Adenoviridae family and has been considered as an excellent indicator of fecal contamination of water matrices. HAdV are double-stranded DNA genomes nonenveloped viruses being relatively high resistant in the environment. The aim of this study was to detect and quantify the genome of HAdV in different water matrices, in order to determine sources of fecal pollution by human origin along the Rio dos Sinos river basin. A total of 86 groundwater samples were collected from springs and artesian wells. Another 38 surface water samples from stream, river and ponds were also surveyed. Samples were collected from November to December 2015, in 34 farms from 11 municipalities located along the Sinos River basin. Water samples were first concentrated by ultracentrifugation and genetic material were extracted using a commercial silica based kit. Realtime polymerase chain reaction (qPCR) using VTB2 primers for the conserved region of hexon gene of HAdVC was performed for viral detection and quantification. Of the analyzed farms, 47% (16/34) had between one and two points of contamination. In which, 20% (17/86) samples of groundwaters and in 13% (5/38) of surface water, the genome of HAdV was detected, ranging to  $9,40 \times 10^4$  to  $6,59 \times 10^7$  genomic copies /L. In only 18% (2/11) of the municipalities all samples were negative for HAdV. Generally HAdV contaminations occurs most often in urban areas, due to higher population density. However this can be overpassed by the lack of basic sanitation.

**EV42 - MAMMALIAN ADENOVIRUSES: DNA POLIMERASE GENE DIVERSITY IN SURFACE WATER FROM BELO STREAM IN CAXIAS DO SUL RS**

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Environmental water samples may harbour an immense microbiological diversity, since it may be contaminated by different sources of effluents. Adenoviruses (AdV) are often found in the aquatic ecosystems, since current sewage treatment methods are not fully effective into removal of viral particles. AdV can cause respiratory tract infections, conjunctivitis and gastroenteritis in human, and a plethora of clinical manifestations in other animal species. The Belo Stream is one of the affluent of the Caí River watershed, is inserted Caxias do Sul municipality (Brazil), being impacted by discharges of domestic and industrial effluents. The main goal of this study was to evaluate the AdV diversity in Belo Stream surface waters, both in concentrated and unconcentrated samples. Samplings were performed from March 2015 to April 2016 in Belo Stream in four sites: P1 and P2 in urban region, P3 in countryside and P4 in the final stretch of the river, which is mainly used for recreational purposes, totaling 55 samples. Concentrated samples (using ultracentrifugation method) and unconcentrated samples were subsequently submitted to nucleic acid extraction with a commercial kit (Biopur®). For screening the presence of AdVs, a partial sequence of the DNA polymerase (pol) gene was amplified by nested PCR aiming the detection of several AdV types from the genera Mastadenovirus and Atadenovirus. Sequencing was performed in all positive samples. In a total of 55 concentrated samples, AdV DNApol gene was detected in 43.6% (24), from these, different AdV species were found: human adenovirus (HAdV) species C (4;7.2%), D (6;10.9%), E (2;3.6%), and F (9;16.3%). AdV from other hosts were also found, including bovine adenovirus 3 (1;1.8%) and murine adenovirus 1 (2; 3.6%). From the unconcentrated samples, 23.6% (13/55) were positive for HAdV belonging to species C (6; 10.9%), D (1; 1.8%) and F (6; 10.9%). Thus, in the comparison of concentrated and unconcentrated samples, we achieved more positive samples when they were submitted to this step allowing also a wide diversity of AdV species, including other

hosts than human. It is important to state that when comparing both protocols it is not always possible to find the same diversity using only one of them mainly due to the interference of factors as volume, inhibitors, and the relation between the diversity and molecular detection.

#### **EV43 - EVALUATION OF DIFFERENT CONCENTRATION AND EXTRACTION METHODS FOR HUMAN ADENOVIRUS TYPE 5 IN WATER**

**Girardi, V.; Demoliner, M.; Pressi, G.; Ruskowski, L.; Albino, S.M.; Rigotto, C.; Fleck, J.D.; Spilki, F.R.**

UNIVERSIDADE FEEVALE

Viral analysis of water samples usually require techniques involving concentration steps allowing the increase of viral recovery indices. The extraction step is also an important factor on viral recovery, where nucleic acids losses might occur during the process. The main goal of this study was to establish and standardize concentration and extraction protocols effective for viral recovery from different water matrices. To standardize the concentration method, recovery rate of human adenovirus type 5 (HAdV5) was evaluated by ultracentrifugation protocol and compared with the adsorption-elution method from ultrapure and surface water artificially inoculated with viral suspensions at different concentrations ( $10^3$ – $10^8$  gc/5 $\mu$ L). The DNA was extracted from the samples previously concentrated by commercial kit (Biopur®). In the tests for methods' standardization for DNA extraction HAdV5 suspensions were used and surface water samples previously concentrated by ultracentrifugation and artificially inoculated with HAdV5 ( $2.20 \times 10^8$  gc/5 $\mu$ L) were also assayed. In the extraction tests three protocols were analyzed: 1) silica column commercial kit; 2) Heating (100°C 10 minutes) followed by proteinase K treatment (37°C 1 hour); 3) same as Protocol 2, followed by extraction using the commercial kit. Viral quantification was performed by real time PCR (qPCR) and the recovery rates were calculated based on the values achieved before and after concentration and the nucleic acids extraction steps. Apart from the artificial inoculation of different concentrations in the ultracentrifugation evaluation, the minimum recovery rates obtained were 848% and 42% respectively for surface and ultrapure water. In the adsorption-elution method the minimum recovery rates obtained were 37% and 87% in surface and

ultrapure water samples, respectively. Thus, for surface water matrix the ultracentrifugation method was more efficient, while for ultrapure water adsorption-elution method was more efficient. From the evaluation of the different extraction methods, the use of commercial kit showed the higher values of recovery rate, as follow: 1900% for viral suspension and 802% for surface water. Thus, in this study either for virus suspensions and environmental matrices samples evaluated, the steps of heating or treatment with proteinase K did not improve the further quantification of nucleic acids by qPCR.

#### **EV44 - ENVIRONMENTAL SURVEILLANCE OF HAV IN THE AURÁ RIVER HYDROGRAPHIC BASIN IN METROPOLITAN REGION OF BELÉM, PARÁ, BRAZIL**

**Morais, L.L.C.S.; de Paula, V.S.; Lima, M.O.; Aranha, D.P.; Pinto, W.V.M.; Silva, L.V.M.; Martins, R.P.G.; Vale, E.R.**

1. INSTITUTO EVANDRO CHAGAS
2. FUNDAÇÃO OSWALDO CRUZ

Hepatitis A, considered a serious public health problem, is an acute infectious disease caused by hepatitis A virus (HAV). Its transmission is mainly by fecal-oral route, and being directly related to the socioeconomic conditions of each region. This study aims to detect HAV particles in water samples from Aurá River hydrographic basin, where the main surface water sources that supply the metropolitan region of Belém are located, and may have been impacted by Aurá landfill in Belém, PA, correlating microbiological and physicochemical variables. Between February 2015 and April 2016, 40 samples of surface water were collected (flood and ebb) in five different points of collection distributed along the Aura River and Uriboquinha River, Pará. HAV search was based on the method of adsorption-elution in membrane filter, followed by RNA extraction with a commercial kit and reverse transcription with SSIIRT. Subsequently, the samples were subjected to nested-PCR. Quantification of coliforms was performed with Colilert kit 18. The physicochemical variables were measured with multiparameter probe and spectrophotometry. Statistical analysis were performed in softwares BioEstat 5 and R. Positive sample were purified with Pure link kit and were submitted sequencing with BigDye Terminator v3.1 Cycle Sequencing kit on the platform 3130xl Genetic analyzer. The HAV RNA was detected in 5% of the samples with

high similarity with Brazilian sequences highlighting the endemic circulation of VHA in the region. The thermotolerant coliform concentrations varied from 160 to  $3.87 \times 10^4$  MPN/100 mL, and *Escherichia coli* varied from 100 to  $1.21 \times 10^4$  MPN/100 mL, exceeding 70% of the cases according to the limits established by CONAMA 357/05. The logistic regression analysis showed that there was no association between the presence of HAV and physicochemical and bacteriological parameters of the water. The results showed the circulation of HAV in this region, providing additional information about the environmental epidemiology of HAV in Brazil, as well as the high degree of microbiological contamination has impacted those water bodies, showing the results of a deficient sanitation service.

#### **EV52 - ASSESSMENT OF ADENOVIRUS INFECTIVITY IN SURFACE WATER SAMPLES FROM ARROIO BELO, CAXIAS DO SUL RS**

**Girardi, V.; Albino, S.M.; Gras, C.K.; Posser, K.C.; Pressi, G.; Rigotto, C.; Spilki, F.**

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Human adenoviruses (HAdV) are often detected in drinking and recreational waters. The source of water contamination by HAdV is usually untreated or insufficiently treated domestic sewage. Consequently, the second leading cause of recreational waterborne diseases is HAdV. Human exposure to these pathogens is a public concern worldwide. Realtime PCR (qPCR) is commonly used for detected DNA virus in water, however this method is not able to discern infectious to defective particles. Use of integrated cell culture PCR (ICCPCR) allows inferring the presence of infectious viruses in the sample, even if they do not produce cytopathic effect in cell cultures, in a sensitive and specific manner. This study aimed to assess the presence, integrity and viability of HAdV in surface water (n=32) collected from four sites of Arroio Belo, Caxias do Sul, Brasil. Water samples were aseptically collected, concentrated using ultracentrifugation and HAdV genome quantified by qPCR after nucleic acids extraction, specific reactions were used to differentiate HAdVF and HAdVC species. Viral integrity and infectivity assays were performed respectively by DNase exposure previous to DNA extraction and ICCqPCR. Both nonconcentrated and concentrated samples were analyzed. The real time PCR

results showed prevalence of 37,5% (12/32) for enteric adenoviruses and 21,8% (7/32) for group C human adenovirus. ICCqPCR assays showed 12,5% (4/32) for HAdVF and 28,1% (9/32) for HAdVC. This study revealed the occurrence of HAdVs infectious particles in Arroio Belo waters, thus suggesting a risk to public and environmental health.

#### **EV83 - THERMAL STABILITY EVALUATION OF ADENOVIRUS SEROTYPE 5 AND HEPATITIS A VIRUS IN DIFFERENT STORAGE CONDITIONS**

**Souza, F.G.; Pressi, G.; Demoliner, M.; Manfro, I.; Posser, K.; Girardi, V.; Rigotto, C.; Fleck, J.**

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Temperature is one of the factors with the greatest influence on the stability of viruses, which may influence the maintenance of its integrity or its disintegration. Considering the possible temperature influence, the objective of this study was to evaluate the quantification and infectivity of HAdV and HAV suspensions, as well as the quantification of their genomes in several storage conditions. To this, in the first experiment HAdV5 and HAV (HM 175 strain) suspensions were submitted to different periods and temperatures of storage: 4°C (1, 5 and 10 days); 20°C (30, 60 and 90 days) and 80°C (60 and 90 days). The initial quantification of HAdV5, at 4°C, 20°C and 80°C, was  $1.01 \times 10^8$ ,  $8.24 \times 10^7$ ,  $9.36 \times 10^6$  cg/ $\mu$ L, respectively. To HAV these values were  $9.50 \times 10^7$ ,  $8.24 \times 10^8$  and  $1.54 \times 10^7$  cg/ $\mu$ L, at 4°C, 20°C and 80°C, respectively. In the second assay, the evaluation of thermal stability of nucleic acids previously extracted from HAdV5 and HAV suspensions with quantifications of  $1.58 \times 10^8/1.09 \times 10^6$  cg/ $\mu$ L, respectively, was performed allocating samples under different storage conditions: 4°C (1 day), 20°C (30 days) and 80°C (90 days). In all assays, samples were analyzed in duplicate. To evaluate the infectivity, ICCqPCR and ICCRTqPCR were performed in all samples. The qPCR analysis for HAdV5 was performed with SYBR green detection and oligonucleotides that amplify the hexon protein and for HAV quantification by RTqPCR, TaqMan probe and oligonucleotides to 5'UTR region were applied. The results showed that the storage at 4°C affected both viruses, reducing around 2 to 3 logs for HAdV5 and HAV, respectively. The infectivity of HAV declined around 4 logs after 10 days at 4°C, however in the evaluation of HAdV-

5 there were an increase of almost 2 logs in the same condition. We were not able to detect HAV genome after the period of 30 days at 20°C. The freeze at 80°C resulted in a decay of 1 log in the HAdV5 quantification when this was exposed for a period of 90 days. Considering these findings, the 80°C temperature was the best condition to maintain the infectivity of viral particles for longer periods. It is observed that temperature is one important factor to be considered to prevent losses in the viral genomes quantification. Then, in an ideal scenario, is indicated that genome extraction or infectivity assays of viral samples should be taken around the time of their evaluation and when it is not possible, they should be allocated in ultrafreezers.

#### **EV84 - METHODS FOR RECOVERY OF HEPATITIS E VIRUS (HEV) FROM FOOD SAMPLES**

**Souza, F.G.; Rigotto, C.; Henzel, A.; Demoliner, M.; Pressi, G.; Spilki, F.**

*UNIVERSIDADE FEEVALE*

Consumption of raw or undercooked wild boar or pig meat from infected animals is an important cause of humans hepatitis E (HEV) infections. HEV, a small nonenveloped RNA virus classified as member of Hepevirus genus within the Hepeviridae family, may spread through the food chain to humans from animal reservoirs and it's an emerging pathogen. The infections are usually asymptomatic affecting mainly adults, however in immunosuppressed patients and pregnant woman the mortality increases significantly. Given the importance of ensuring the safety of food the objective of the work was the standardization of extraction methods in experimentally inoculated food with HEV comparing three different lysis buffers. Six sausage and salami samples were experimentally inoculated with monkey feces containing 1,000 HEV genome copies/g. One hour after inoculation, samples were fragmented and rinsed using Eagle's Minimum Essential Medium (E MEM, pH 7,1), phosphate buffered saline (PBS, pH 7,0) or MilliQ water. After, RNA extraction, cDNA synthesis and PCR for amplification of ORF1 viral target were used equally for the 3 treatments. PBS and Distilled water allowed recovery of HEV only from sausage samples. On the other hand, EMEM allowed detection of HEV from all sausage and salami samples, demonstrating to be a better lysis buffer for further studies.

#### **EV86 - DETECTION, FEASIBILITY AND GENOTYPING OF ENTEROVIRUS AND ROTAVIRUS A IN SURFACE WATER FROM MOSQUEIRO ISLAND, BELÉM, PARÁ, BRAZIL, 2012 TO 2014**

**Alves, J.C.S.; Teixeira, D.M.; Wanzeller, A.L.M.; Alves, A.S.; Silveira, E.; Oliveira, D.S.; Smith, V.C.; Deus, D.R.; Morais, L.L.C.S.; Monteiro, J.C.; Siqueira, J.A.M.; Primo, E.G.; Soares, L.S.; Mascarenhas, J.D.P.; Tavares, F.N.; Gabbay, Y.B.**

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Enteric viruses are the major causes of waterborne diseases. These agents are present in the stools of infected individuals in large amounts. They remain viable and infective for months in the environment and may contaminate the water used for consumption and recreation. So, its monitoring is required, since bacteriological indicators used to assess the water quality do not have relation with viral contamination. In addition, some pathogens transmitted by water are fastidious, such as Enterovirus (EV) and Group A Rotavirus (RVA). The purpose of this study was to detect EV and RVA in surface water samples from four beaches (Paraíso, Murubira, Farol and Areião) located in Mosqueiro Island, Belém, Brazil. Water samples were collected monthly in the period of January 2012 to December 2014, with exception of July when it was collected fortnightly. Two liters of water were concentrated by adsorption-elution method in filtering membrane, followed by centrifugation to obtain a final volume of 2mL. RNA was obtained using the silica extraction. The seminested PCR with primers P2, P3 and P10 for EV, and Nested PCR using primers VP6F/VP6R and VP6NF/VP6NR for RVA were employed. The total positivity obtained was 28.2% (44/156), being 25% (11/44) related to EV, 56.8% (25/44) to RVA and 18.2% (8/44) to both viruses. These agents were detected in all the beaches studied, with the highest positivity on the Paraíso beach (33.3%). The greatest positivity occurred at high tide. A total of 26.5% (39/147) of the samples with *E. coli* acceptable concentration (<2000) were positive for EV and/or RVA. Two positive samples for RVA when submitted to cell culture showed infectivity. After EV phylogenetic analysis, 63.2% (12/19) were classified as nonpolio enterovirus, 15.8% (3/19) as polio 1 vaccine and in 21% (4/19) the sequence obtained did not show sufficient quality for analysis. These beaches

are contaminated through sewage galleries that flow directly in them, contributing with their contamination, and, consequently, exposing people who use these places, mainly susceptible children. These viruses are of great relevance in terms of public and environmental health, as they have an enormous potential spread due to their extended maintenance into the environment.

#### **EV87 - ENVIRONMENTAL SURVEILLANCE OF HUMAN ADENOVIRUSES IN A WASTEWATER TREATMENT PLANT**

**Assis, A.S.F.; Otenio, M.H.; Domigues, A.L.S.; Drumond, B.P.; Fumian, T.M.; Miagostovich, M.P.P.; Rosa e Silva, M.L.**

1. UNIVERSIDADE FEDERAL DE JUIZ DE FORA
2. EMBRAPA GADO DE LEITE
3. UNIVERSIDADE FEDERAL DE MINAS GERAIS
4. INSTITUTO OSWALDO CRUZ

Sewage treatment may be insufficient for the complete elimination of pathogenic microorganisms such as enteric viruses. Nevertheless, the fecal coliforms (FC) are widely used as indicators for evaluating the quality of the effluent in wastewater treatment plant (WWTP). Thus, the return of the treated effluent to the nature can provide significant harm to public health, considering the importance of the waterborne pathogens. Human adenoviruses (HAdV) are associated with sporadic cases and outbreaks of gastroenteritis. These agents are present in various types of aquatic environments and also have been described as the most prevalent enteric viruses in sewage. In this environmental surveillance study we investigated the HAdV presence in four different points of the sewage treatment, evaluated the impact of sewage treatment with activated sludge in HAdV viral load and in FC counting in a WWTP from Juiz de Fora/MG. For this purpose, raw sewage (n=24), primary sewage (n=24), sludge (n=24) and treated effluent samples (n=24) were collected bimonthly, between January and December 2014. The samples were concentrated using elution and skimmed milk flocculation procedure. Viral nucleic acids were extracted using a commercial kit and the viral load was determined using realtime PCR. The FC counting was determined monthly in each point. HAdV were detected in 85.4% (82/96) of the tested samples, with viral loads values ranging from 3.27E+02 to 2.42E+06 genome copies per milliliter. HAdV positivity rate in raw sewage, primary sewage, sludge and treated effluent

was 100%, 95.8%, 70.8% and 75%, respectively. Sewage treatment was able to reduce the FC counting into 2 logs in all samples analyzed. However, reductions of 12 logs in the HAdV viral load were observed in some, but not in all treated effluent samples. Although the secondary treatment was able to reduce the FC counting, it was not always efficient to HAdV removal in domestic sewage. Thus, more studies on the impact of sewage treatment in viral removal should be accomplished to establish new and effective wastewater management policies.

#### **EV92 - ABSENCE OF HEPATITIS A VIRUS (HAV) IN AFFLUENT AND EFFLUENT SAMPLES FROM SEWAGE TREATMENT PLANTS LOCATED IN NOVO HAMBURGO, BRAZIL**

**Manfro, I.D.; Pressi, G.F.; Demoliner, M.; Souza, F.G.; Hamerski, F.; Spilki, F.R.; Rigotto, C.; Fleck, J.D.**

UNIVERSIDADE FEEVALE

Hepatitis A virus (HAV) is responsible for the largest number of cases of acute viral hepatitis worldwide. HAV is a member of Hepatovirus genus, Picornaviridae family, and viral particles are composed by naked icosahedral capsids protecting a positive singlestranded RNA genome. HAV particles may remain viable in environment and food for long periods. It is estimated that 1.4 million people in the world suffer annually for hepatitis A, whose prevalence is related to sanitation and socioeconomic conditions. HAV is detected sporadically in environmental matrices and only one sample out of 100 was positive for HAV in surface waters from Novo Hamburgo (pop. approximately 250,000 inhab.) in a survey conducted in 2015. The goal of the present work was evaluate if HAV is also rare in affluent and effluent samples from two sewage treatment plants (STPs) of Novo Hamburgo, through the polymerase chain reaction real time (RTqPCR). In the period from November 10th 2015 to May 17th 2016, fortnightly collections were carried out from an STP running in parallel activated sludge and floating macrophytes, and another STP using a sequential treatment process consisting of UASB reactor, aerobic filter, activated sludge and anoxic reactor. Sixty six (n=66) samples were collected, being 22 raw sewage, 10 UASB reactor effluents, 12 effluents from activated sludge, 10 anoxic reactor residual waters and 12 floating macrophytes effluents. The samples were concentrated by ultracentrifugation method and

subjected to extraction of viral RNA. The cDNA was synthesized and subsequently submitted to molecular detection by RT qPCR, using primers targeting 5'UTR of HAV genome. Results were revealed using TaqMan® HAVspecific probes. HAV was absent throughout thus corroborating previous findings of a very low prevalence of the virus under environmental conditions in the Novo Hamburgo city.

#### **EV94 - OCCURRENCE OF NOROVIRUS IN RECREATIONAL WATER FROM MOSQUEIRO ISLAND, BELÉM CITY, PARÁ STATE, NORTHERN BRAZIL**

Teixeira, D.M.; Deus, D.R.; Smith, V.C.; Santos, D.S.A.S.; Alves, J.C.S.; Siqueira, J.A.M.; Bandeira, R.S.; Morais, L.L.C.S.; Gabbay, Y.B.

1. SEÇÃO DE VIROLOGIA - INSTITUTO EVANDRO CHAGAS/SVS/MS
2. PROGRAMA DE PÓSGRADUAÇÃO EM BIOLOGIA PARASITÁRIA NA AMAZÔNIA, UNIVERSIDADE ESTADUAL DO PARÁ
3. SEÇÃO DE MEIO AMBIENTE - INSTITUTO EVANDRO CHAGAS/SVS/MS

The precarious sewage infrastructure favors the viral particles spread in aquatic environments and causes public health problems. Noroviruses (NoV) and others enteric viruses has been described in sea water, lagoon and river, and are considered the major cause of gastroenteritis outbreaks worldwide. This study aimed to detect and genotyping NoV genogroups GI/GII on surface water samples from four beaches: Paraíso (PA), Murubira (MU), Farol (FR) and Areião (AR) located on the Mosqueiro Island, metropolitan region of BelémPa, from January/2012 to December/2014. The samples were collected monthly with exception of July when they occurred fortnightly. NoV particles were concentrated by adsorptionelution method in filtering membrane. Viral RNA was obtained by silica method and subjected to semi nested RTPCR using in the first step the primer pair JV13I/JV12Y and in the second the JV13I/GI and JV12Y/NoroR pairs for specific detection of GI and GII, respectively. Positive samples by semi nested RTPCR were analyzed for 5' end ORF2 region (capsid) by nested (GI) and seminested (GII) for identification of circulating genotypes, which were purified using commercial kit and subjected to molecular characterization. From 156 samples tested, 30.1% (47/156) were positive for NoV, and 63.8% (30/47) were classified as GI, 31.9% (15/47)

as GII and 4.3% (2/47) as GI+GII. This pathogen was identified in all the analyzed beaches and although the highest positivity was found on PA beach (38.5% 15/39), it was not observed significant difference from the others (FR/AR  $p=0.63$  and MU  $p=0.13$ ). Positive samples by semi nested RTPCR were retested for the 5' end ORF2 and NoV was detected in 29.8% (14/47). Of these, three were classified as GII.4 and one as GI.8. The low quality of the other sequences ( $n=10$ ) did not allow genotyping seven for GI and three for GII. Statistically, positivity found in the years of 20122013, was not influenced by rainfall ( $p=0.87$ ), also, no relation was verified comparing the periods of more and less rainy ( $p=0.06$ ), corresponding to the 1st and 2nd semesters, respectively. Of the positive samples for NoV, 91.5% (43/47) were detected in water with acceptable concentrations of Escherichia coli (<2000) by Brazilian resolution. This study provides evidence of a risk of acute gastroenteritis on users of recreational water, mainly children, and highlights the importance of including enteric viruses in the quality monitoring of recreational water.

#### **EV103 - ASSESMENTOF THE PRESENCE OF HUMAN ADENOVIRUS, SEROTYPES 40 AND 41 ON SURFACE WATERFROMRIO CAÍ WATERSHED IN RIO GRANDE DO SUL STATE, BRAZIL**

Oliveira, F.C.; Heck, T.M.S.; Staggemeier, R.; Ritzel, R.G.F.; Dutra, J.M.M.; Silva, L.G.A.; Schneider, T.; Almeida, S.E.M.

UNIVERSIDADE FEEVALE

By linking human presence and the precariousness of the collection system and treatment of industrial and domestic sewage we are faced with a big problem. The water quality has been compromised by this precarious system. As cities grow due to increased population density problems tend to increase. The concept of environmental quality is directly linked to quality of life and related to other factors, including biological. We can consider that humans are largely responsible for the pollution and spread of pathological microorganisms. Viruses are one example of microorganisms that cause these diseases. Among them we can mention the Human Adenovirus (HAdV), which are highly contaminating group responsible for several diseases such as gastroenteritis affecting millions of people around the world, this is mainly caused by the serotypes

40 and 41. They are eliminated in the faeces by infected persons. When they dispersed in the environment, to remain stable, adherent to environmental substrates for long periods and thus contaminate other individuals. Through its detection in the environment, we can say that there is an indicator of human fecal contamination. This study linked the presence of these viruses in the environment and the quality of surface water in parts of the lower stretch from Rio Caí in the state of Rio Grande do Sul. A total of 10 water samples were collected in June 2016 in 10 points. Concentrations were carried out by ultracentrifugation method and viral DNA extraction. Viral molecular detection occurred via qPCR, primers specific hybridization to the viral genome. The results show that 90% (9/10) of the samples confirm the presence of adenovirus serotype 40 or 41 in surface water. The viral load ranged from  $1.82 \times 10^6$  genome copies/L in point 8 to  $9.70 \times 10^6$  genome copies/L in point 9. According to the results, we can say that there is fecal contamination of human origin, present in surface water of the Rio Caí. And relate to the quality of water used by the population for various purposes such as consumption, hygiene and recreation, and the methods used today in accordance with Brazilian law are not suitable for viral elimination in the treated water. So in this way, the use of this water brings risk for the human health, becoming a major public health problem.

#### **EV104 - DETECTION OF HUMAN ADENOVIRUS AT SEDIMENTS SAMPLES FROM RIO CAÍ WATERSHED, RIO GRANDE DO SUL STATE, BRAZIL**

**Dutra, J.M.M.; de Oliveira, F.C.; Schneider, T.; Ritzel, R.G.F.; Silva, L.G.A.; Heck, T.M.S.; Staggemeier, R.; Almeida, S.E.M.**

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The Adenovirus belong to a heterogeneous group of viral agents denominated as enteric virus, making yourself steady in the gastrointestinal tract as in the environment, when eliminate with the faeces. His resistance allows him to contaminate the water and the soil for a long period of time. It can be used as a strong indicator of fecal contamination. The residual treatment lack and the industries effluents, had caused a dispersion and contamination of many ecosystems by pathological microorganisms, like the Adenovirus, able to infect humans and animals. Those virus have a high

resistance to many chemical compounds, and are spread at large number in the faeces, turning into a public health problem. The human Adenovirus (HAdV) type F serotype 41 it's one of the major precursor of infectious diarrhea in the world, becoming fatal for elders and children. The Rio Caí, located at the Rio Grande do Sul state, it has his water used for many functions, like irrigation, recreation and supply. However, it is one of the most polluted rivers in Brazil, because of degradation and environment problems, providing the proliferation of pathologic microorganisms. The HAdV has the capacity to connect with the sediment and percolate through the soil, hitting underground waters, and staying viable for more time at particulate material. With these information, this study have the objective to detect the human Adenovirus enteric type 41 on samples of sediment from the watershed of Rio Caí, with the goal of relate the region's environment quality, searching to understand the actions and effects that viral agents can cause. There were made 10 collects of sediment on different locations at down of the river stretch at June 2016. The samples were conserved until processed. The viral genomes were extracted with the commercial extraction kit Biopur, following the recommended methodology. The molecular detection was realized by the qPCR technician, using specific primers for the HAdV detection. The results showed 3 out of 10 samples of sediment were positive for the presence of the Adenovirus F 40/41. The HAdV's presence means that the sediment samples from Rio Caí, indicates faeces contamination, and shows the presence of an anthropology environmental impact, that, may compromise the population's life quality since the virus can reach underground waters or returning to the water column, infecting again the hydric resource.

**EV111 - DETECTION OF HUMAN ADENOVIRUS IN WATER AND SEDIMENT SAMPLES FROM PARANHANA RIVER IN RIO DOS SINOS WATERSHED, BRAZIL**

Heck, T.M.S.; Ritzel, R.G.F.; Oliveira, F.C.; Schneider, T.; Dutra, J.M.M.; Silva, L.G.A.; Röhnelt, N.M.S.; Jesus, L.F.; Nascimento, C.A.; Spilki, F.R.; Staggemeier, R.; Almeida, S.E.M.

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Paranhana river is situated in the centralnorthwest region of the Rio dos Sinos Watershed (RSW) in the state of Rio Grande do Sul (RS) and is one of the main tributaries of the Rio dos Sinos. Its springs are located on the border between the municipalities of São Francisco de Paula and Canela and its mouth in the city of Taquara, place of its confluence with the Rio dos Sinos, which receives possible contaminants carried along its route to the RSW. Enteric viruses are excreted in large amounts in human faeces and are considered good markers of environmental pollution. Among them, the Human Adenovirus (HAdV) to be doublestranded DNA and non enveloped remains infectious for longer in water and soil / sediment than a simple stranded RNA viruses. This pathogen is included in the "Contaminant Candidate List 4" of the United States Environmental Protection Agency (USEPA) for their health importance, frequent occurrence in many aquatic environments and due to disease outbreaks mainly in children under 4 years associated with fecaloral route, as gastroenteritis. It is very important the use of biomarkers that allow monitoring and identifying the source of contamination, and therefore the anthropic impact on river basins, such as the subbasin of Paranhana river, which contributes to the public water supply in the region. Contamination of water resources is influenced by soil/sediment due to potential adhesion/desorption of the viral particle returning the water column and contaminate water catchment sources particularly in regions where the system is poor or is devoid of sanitation. This study aims to assess the environmental quality through molecular detection of HAdV at different points from its spring to its mouth in the Rio dos Sinos. Bimonthly samples of water and sediment samples were performed at 12 points along the Paranhana river from May 2015 to March 2016, totaling 72 samples. Among the results in water, it was verified the presence of the viral genome in 87.5% (63/72) observing an average  $4.20 \times 10^7$  gc/L

with variation in viral quantifying  $1.07 \times 10^5$  to  $6.98 \times 10^8$  gc/L. In relation to the sediment, the viral genome was detected in 79.2% (57/72) on average  $1.58 \times 10^6$  gc/g, showing variation of  $1.22 \times 10^4$  to  $3.50 \times 10^7$  gc/g. From this study it is possible to realize a strong anthropic impact demonstrated by the presence of HAdV in the region of the sub basin of Rio Paranhana, an important source of water catchment for public supply of RSW.

**EV112 - ADENOVIRUS AS A TRACKER OF HUMAN FECAL CONTAMINATION IN PUBLIC RECREATIONAL WATERS IN THE STATE OF RIO GRANDE DO SUL, BRAZIL**

Heck, T.M.S.; Röhnelt, N.M.S.; Ritzel, R.G.F.; Oliveira, F.C.; Jesus, L.F.; Staggemeier, R.; Rigotto, C.; Nascimento, C.A.

UNIVERSIDADE FEEVALE

Enteric viruses have frequently been implicated in recreational waterrelated gastrointestinal (G.I.) disease and most infections can be contracted by ingestion of HAdV contaminated water or inhalation of droplets as a result of swimming, canoeing or other recreational use of sewagepolluted water may be viral in nature. Human adenoviruses (HAdVs) have been considered critical emerging viruses since the potential health risks associated with their waterborne transmission. Recent studies suggest HAdV as a marker of viral contamination in the environment, since its presence in water indicates human fecal contamination and present themselves as a better marker than bacterial indicators (*E. coli* and thermotolerant), that are currently used in Brazilian legislation for balneability assessment. Although HAdVs have frequently been identified in various environments such as waste water drinking waters, groundwater, surface waters and recreational waters, quantitative information for HAdV occurrence at public lake or river beaches is still insufficient. The overall goal of this study was to assess the HAdV presence in water samples from a river beach located at the Rio dos Sinos basin, by quantitative polymerase chain reaction (qPCR). Water samples were collected from João Martins Nunes beach, located in the county of Taquara, Rio Grande do Sul. One sample was collected weekly in sterile bottles of 500mL for a period of five weeks (November 19 to December 17, 2015). For the viral concentration, 36mL

of each sample were subjected to ultracentrifugation method followed by nucleic acids extraction through Spin kit Plus 250 (Biopur ®). The detection and quantification of the viral genome was performed from the qPCR (commercial SYBR ® Green Platinum kit qPCR SuperMixUDG, Invitrogen) using specific primers for the Hexon gene of HAdV. The results present 40% (2/5) of positive samples for HAdV from the first two consecutive samples of November with quantification of  $2.1 \times 10^6$  and  $1.5 \times 10^3$  gc/l (genomic copies/liter), respectively. In this preliminary study, the search of HAdV highlighted the need of viral evaluation in recreational waters to track the source of fecal contamination together with fecal bacteria identification.

#### **EV114 - ADENOVIRUS REMOVAL AND SHEDDING IN DRINKING WATER FROM CONVENTIONAL TREATMENT PLANTS**

Staggemeier, R.; Jesus, L.F.; Heck, T.M.S.; Röhnelt, N.M.S.; Nascimento, C.A.; Spilki, F.R.

UNIVERSIDADE FEEVALE

Contamination of water resources and the increase of waterborne diseases are directly related to sanitation and inadequate water treatment. Water treatment plants aims to reduce the impacts on health and the environment of biological and chemical agents present in raw water. Lately it has been questioned whether conventional water treatment processes are capable of removing microbial and chemical contaminants. The goal of this study was to evaluate the presence and quantification of human adenovirus C (HAdVC) in the four conventional water treatment stages in eight (8) conventional water treatment plants from Rio Grande do Sul comprising 8 municipalities along the Sinos River (Santo Antônio da Patrulha, Rolante, Esteio, Taquara, Três Coroas, Parobé, Campo Bom, and Nova Santa Rita). From May 2011 to May 2013 samples from raw, decanted, filtered, and treated (drinking) water were collected monthly, in a total of 832 samples. Adsorption-elution concentration method using a negatively charged membrane was performed. Viral nucleic acids were extracted with a commercial kit and quantitative real time polymerase chain reaction (qPCR) was performed using primers designed to amplify the hexon protein gene of HAdVC. Comparing different treatment steps 56% of raw water samples were positive for HAdV, 13% of decanted water, 23% of filtered water,

and 38% of treated water samples. The values ranged from  $2,14 \times 10^8$  maximum to  $2,89 \times 10^0$ , minimum. In all water treatment plants variation of HAdV removal was observed. When reductions in the viral load across the treatment were observed, they are below the 4logs required by many international standards. It was also noticeable samples in which there was an increase in the levels of HAdV especially after the filtration step. This finding may point to operational errors and misconducts and contamination during water treatment.

#### **EV116 - ADENOVIRUS AS HUMAN CONTAMINATION INDICATOR FECAL IN WATERSHED OF RIO TRAMANDAI, RS**

Ritzel, R.G.F.; Andriguetti, N.B.; Oliveira, F.C.; Heck, T.M.S.; Luz, R.B.; Gularte, J.S.; Rocha, C.M.; Heinzelmann, L.S.; Bianchi, E.; Staggemeier, R.; Almeida, S.E.M.

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2. UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Enteric Viruses are important etiological agents of infection of the gastrointestinal tract. The contamination is faecaloral route, one of the forms of propagation of such microorganisms are the effluents. From the enteric viruses, adenoviruses have a capsid which preserves the DNA molecule, nonenveloped, with an icosahedral form. The Adenoviruses are resilient to the environment and the usual water treatment. It has the ability to infect several human tissues, such as the gastrointestinal mucosa and cause respiratory infections, there is a bigger incidence of transmission occur in children under four years. According to the Tramandaí Committee, it's released every year four thousand of pollution load in Rio Tramandaí's watershed, adding to the untreated sewage, industrial waste, livestock and agriculture. The present study goal is to evaluate the fecal contamination by human Adenovirus ( HAdV ) in complex ponds of Tramandaí's watershed. Sixty samples were collected from 10 lakes between December / 2013 and May / 2014. The method used for water concentration was by adsorption / elution negative membrane was took after extraction of the viral DNA samples. Viral detection was obtained by quantitative polymerase chain reaction ( qPCR ). In the analyzed period, 58% ( 35/60 ) of samples were positive for HAdV. Evaluating seasons separately, 30 samples on the summer were tested, 20 (66.6 %)

were positive, and 30 samples were assessed on the autumn, only 15 showed the presence of virus (50 %). At the period December to February there is a seasonality season, increasing the number viral levels. However, the contamination of lakes it's eminent, demonstrating a significant antropic impact on Rio Tramandaí watershed.

#### **EV120 - ENTERIC VIRUSES IN SEDIMENT AND SURFACE WATER FROM URBAN AREAS IN THE RIO DOS SINOS WATERSHED, SOUTHERN BRAZIL**

Staggemeier, R.; Heck, T.M.S.; Ritzel, R.G.F.; Andriguetti, N.B.; Oliveira, F.C.; Spilki, F.R.; Almeida, S.E.M.

*UNIVERSIDADE FEEVALE*

Enteric viruses are considered good biological indicators of environmental pollution by human faeces. They may be deposited on the soil or water, are very resistant both in the gastrointestinal tract as in the environment for long periods, bringing risks to human health of those who consume the water coming from these contaminated sources. The anthropic action in particular has affected significantly the quality of soil and water, thus becoming important to evaluate the quality of these environmental matrices by using different biomarkers, and searching the source of environmental contamination to develop solutions that mitigate the human impact. The present work aims to assess the frequency of human enterovirus (EV), human adenovirus (HAdV), and group A rotavirus (RV) in soil and water samples from four streams in the municipalities of Campo Bom, Estância Velha, Novo Hamburgo and Portão, Rio dos Sinos Watershed (RSW), Rio Grande do Sul state, Brazil. Twelve bimonthly collections of water and sediment samples were carried out from September 2012 to July 2014 in 16 different sites from four streams in RSW, southern Brazil, totaling 192 samples of each matrix. Water samples were concentrated by adsorptionelution method, while the sediment was eluted, followed by viral DNA/RNA extraction. For RNA viruses (RV and EV) was performed one more step of reverse transcriptase. Molecular detection was performed using quantitative polymerase chain reaction (qPCR). Of the 192 samples collected from water, 79.2% showed positive for the presence of HAdV 2/5, 24.5% for HAdV 40/41, 34.4% for EV, and 12.5% for RV. Regarding the detection of nucleic acids in sediments, 63% of the samples were detected as positive

for HAdV 2/5, followed by HAdV 40/41 (35.9%), RV (26%), and EV (20.8%). The viral loads ranged from 102 gc/L up to 109 gc/L (water), and from 101 gc/g to 108 gc/g (sediment). This is the first report showing the description of viral genomes in water and sediment samples taken from the streams in RSW, contamination of waters and sediments in the region reflects the lack of sanitary sewage services, carelessness with domestic wastewater and intense urbanization.

#### **EV201 - ADENOVIRUS CAN BE DETECTED OVER TIME IN DIFFERENT FREQUENCIES ON HOSPITAL SURFACES, EQUIPMENT AND SUPPLIES**

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The contaminations caused by viruses in hospital facilities are often not reported due to lack of virological monitoring routine in most hospitals, that is restricted to bacterial indicators. Human Adenoviruses (HAdV) are common pathogens often associated with respiratory and gastrointestinal illness and/or conjunctivitis in young people and were selected to be used in the present study as a marker. These viruses belong to Adenoviridae Family and Mastadenovirus genus, are nonenveloped with doublestranded DNA genome and great resistance in the environment, even after cleaning, disinfection and sterilization. In the present study we evaluated the presence and viability of HAdV on fomites from a hospital in the Vale dos Sinos region, Brazil. Samples were collected with sterile swabs, from November 2015 to February 2016. The target sites were divided into two groups of samples: a) Group 1: total of 64 samples from the surfaces of hospital sections b) Group 2: total of 32 samples from the materials of autoclaving sterilization process. These samples were subjected to viral DNA extraction procedure with the commercial kit Biopur® and for quantification we applied the partial amplification of the HAdV hexon gene by polymerase chain reaction in real time (qPCR) using specific primers to identify HAdVC as follows: VTB2f sequences (5'GAGACGTA CTT CAGCCTGAAT3') and VTB2r (5'GATGAACCGCAGCGTCAA3'). To identify HAdVF we used specific primers VTB1F (5'GCCTGGGGAACAAG TTCAGA3') and VTB1r (5'GCGTAAAGCGCACTT TGTAAG3'). HAdV was

present in 14,06% (9/64) of the total samples of group G1 with variations in positivity rates over the months. From group G2, we were not able to detect any positive sample for HAdV. Positive samples were also evaluated by Infectivity by performing ICCqPCR assays, resulting in three possible samples, all from the same collection month (February). The viable samples were obtained from mouse and work table the of profissionais of uti and a patient clipboard interned in pediatrics. These results reveal contamination in hospital fomites, thus emphasizing the extreme importance of the effectiveness of the cleaning process, highlighting the need to improve virological monitoring in health facilities.

#### **EV227 - DETECTION OF KLASSEVIRUS IN WASTEWATER IN RIO DE JANEIRO, BRAZIL**

**Seglia, M.; Fioretti, J.M.; Ferreira, M.S.R.; Miagostovich, M.P.**

*FUNDAÇÃO OSWALDO CRUZ*

Enteric infections are responsible for clinical cases of acute diarrhea (AD), affecting millions of people worldwide, with a major impact in children less than 5 years in developing countries. Although 40% of all cases of diarrhea are of unknown etiology it is increasing the number of emerging virus associated with this disease. The main objective of our study was to demonstrate the presence of Klassevirus (KV) in sewage samples obtained from wastewater treatment plant (WWTP) in Rio de Janeiro. KV is an emerging virus described in 2009 and classified into Picornaviridae family. For this study, 52 wastewater samples obtained from June 2013 to May 2014. were previously concentrated by organic flocculation using skimmed milk method. For viral detection a quantitative realtime PCR (qPCR) using TaqMan® system was employed to amplify the region 3D. KV was detected in 84.6% of samples with viral concentration ranging from 3.93 to 1.77 x10<sup>3</sup> genome copy (gc) / reaction. A higher percentage of KV (11.36%) was detected in the months of summer and autumn, although the small number of samples does not allow inferring the seasonality of these viruses. The detection of KV in almost 85% of the samples studied shows the circulation of those emerging viruses in the metropolitan region of the state, which has not been recognized by the absence of diagnosis or by association with asymptomatic infections.

#### **EV246 - ENTERIC VIRUSES IN WATERS FROM 2016 OLYMPIC VENUES**

**Spilki, F.R.; Staggemeier, R.; Venker, C.A.; Heck, T.M.S.; Demoliner, M.; Ritzel, R.G.F.; Röhnelt, N.M.S.; Girardi, V.**

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Rio de Janeiro's inner and coastal waters are heavily impacted by human sewage pollution for decades. Even though authorities promised in their 2009 winning Olympic bid that cleaning the waterways were a legacy of the event. Enteric viruses, including human adenoviruses (HAdV), human enterovirus (EV) and group A rotavirus (RV) are more likely to be found in contaminated surface waters and have been the focus of many studies, because of their persistence in the environment its importance in public health. The present work aimed to assess the frequency and loads of EV, HAdV and F species, and RV in sediment and water samples from Guanabara Bay, Rodrigo de Freitas Lagoon, and beaches of Ipanema, Copacabana, Marina da Glória, Leblon and Barra da Tijuca, in water venues used during the 2016 Summer Olympics and by tourists attending the event. Sixteen monthly collections of water (and sediment samples were carried out from March 2015 to July 2016 in 12 different sites from Rio de Janeiro, Brazil, totaling 146 water samples and 45 sediment samples. Water samples (0,5l) were concentrated by ultracentrifugation method, while the sediment was eluted in minimum essential medium, both followed by viral DNA/RNA extraction. For RNA viruses (RV and EV) cDNA synthesis was performed one more step of reverse transcriptase. Molecular detection was performed using quantitative polymerase chain reaction (qPCR). All samples were further investigated by integrated cell culture PCR (ICC PCR) to check about the presence of HAdV infectious virus particles. From all water samples collected, 95.9% showed contamination with at least one viral target. Regarding the viruses individually, the following results were found (% for water and sediment respectively): HAdV and F (93.1% and 64.9%), RV (12.3% and 10%) and EV (26.7% and 8.8%). The most contaminated points were the Rodrigo de Freitas Lagoon, where Olympic rowing will take place, and the Marina da Glória, the starting point for the sailing races, in which adenoviral and rotaviral loads in samples ranged from 105 to 109 genome copies/L. RV and HAdV were also found in Copacabana and Ipanema sand and water samples. Many samples presented infectious particles at the first passage in A549 cell cultures, and up to 90% of all sites presented viable viruses.

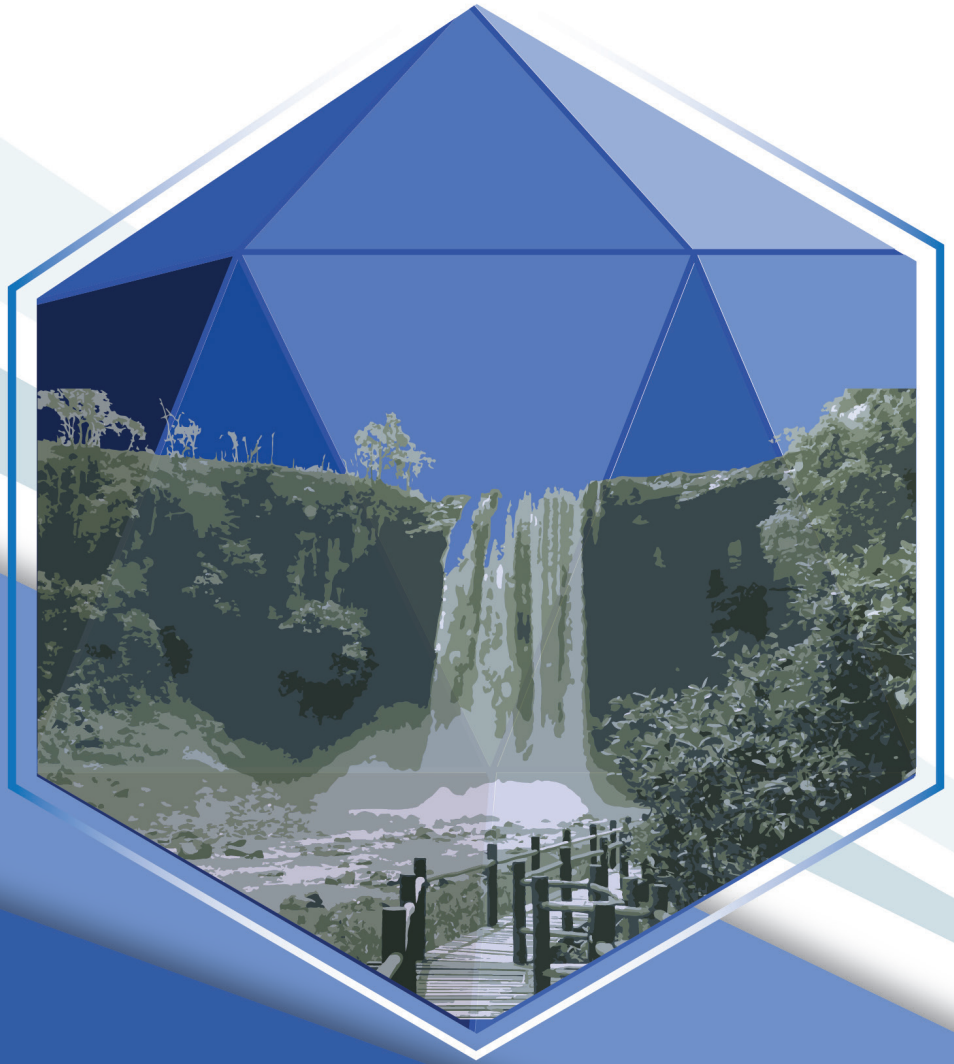
**EV253 - GENETICS CHARACTERIZATION OF METAVIRUS ASSOCIATED WITH AMAZON SOIL FUNGUS**

**Oliveira, R.R.; Barata, R.R.; Cardoso, J.F.; Oliveira, R.S.; Vasconcelos, J.M.; Lemos, P.L.; Franco Filho, L.C.; Nunes, M.R.T.**

*INSTITUTO EVANDRO CHAGAS/CENTRO DE INOVAÇÕES TECNOLÓGICAS*

The retrotransposons are present at various eukaryotic organisms, such as fungi. Currently, they are classified into two viral families: Pseudoviridae and Metaviridae. Between viral fungi species, only 11 are officially recognized (five from metavirus genus, three Hemivirus and three pseudovirus). The presence of reverse transcriptase is common in both families, but the presence and order of its protein domains is specific for each family. To detect the viruses presence in fungi isolated from soil samples of Environmental Protection Area of the Combu island (Belém – State of Pará), the genetic material of three fungi was extracted and sequenced using Ion PGM platform. The generated readings were assembled with Mira v.4 software and the contigs were compared with the non redundant sequences database of NCBI, using BlastX algorithm. The viruslike contigs were annotated and its coding regions were submitted to InterProScan (EMBLEBI) for the conserved protein domains prediction. The domains were compared with representative species sequences of Metavirus, Hemivirus and Pseudovirus by aligning the secondary structure using the PROMALS3D. Only one fungi showed viral contigs, (polyprotein of 3186 bp) with 9.5x coverage. The predicted polyprotein presented domains similar to Metavirus (Metaviridae Family), with a conserved polymerases domain followed by a Hlike ribonuclease. The complete polyprotein alignment showed 17.97% identity with the *Schizosaccharomyces pombe* virus Tf1, while the secondary structure region alignment of polymerases showed 28.05%. These identity values show a large difference between this metavirus and specimens database, arguing that a possible new retrotransposon for Metaviridae family.

# *HUMAN VIROLOGY - HV*



### **HV3 - OUTBREAK OF G2P[4] ROTAVIRUS GASTROENTERITIS IN A RETIREMENT COMMUNITY, BRAZIL, 2015: AN IMPORTANT PUBLIC HEALTH RISK?**

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2. CENTRO DE CONTROLE DE DOENÇAS
3. COORDENADORIA REGIONAL DE SAÚDE

Group A Rotavirus (RVA) gastroenteritis outbreaks in aged care facilities have been reported globally, and can represent an important public health risk. In Brazil, the frequency of RVA outbreaks among elderly in nursing homes is virtually unknown. The aim of the present study was to describe a RVA outbreak in a private residential care home in São Paulo, Brazil, using epidemiologic and molecular diagnostic methods. A descriptive clinical, epidemiological and environmental investigation was conducted. Stool samples were collected and screened for RVA, Norovirus (NoV), Enteric Adenovirus 40/41 (AdV 40/41) and Astrovirus (AstV) using ELISA, PAGE, RTPCR, qRTPCR, electron microscopy and sequencing methods. Because viral etiology was suspected from the outset, bacteria and protozoa were not searched for. Outbreak occurred during 26th-29th October, 2015, and affected 28 individuals (22 residents; 6 staff). The attack rate was 25.9% and 8.5% among residents (median age: 85.5 years) and staff (median age: 28 years), respectively. Symptoms were mild and hospitalization was not required. A female staff was identified as the index case. State of hygiene of the nursing home was assessed as suitable. RVA was detected in 87.5% (7/8) of the collected samples, and characterized as G2P[4] genotype with short dsRNA profile. NoV genogroup GII was detected in one sample (12.5%; 1/8) only by qRTPCR assay. This unique positive NoV sample was also positive for RVA, highlighting one case of mixed infection; however NoV was not linked to the presented outbreak. All samples were negative for AdV 40/41 and AstV. Genetic analysis of VP7 and VP4 genes demonstrated that the outbreak involved one single G2P[4] strain, suggesting a common source infection. G2P[4] strains also grouped within the lineages currently circulating in children worldwide, hinting that institutionalized elderly are susceptible to the same types of RVA as kids. Data

presented here suggests that RVA should be considered as a possible cause of gastroenteritis during outbreaks investigations in residential facilities, and raise the question if the current licensed RVA vaccines for children could also be helpful for the elderly. Our investigation also highlights the importance of a tight collaboration between nursing home staff, public health authorities and reference laboratories. As far as we are aware this is the first documented report of RVA outbreak in elderly care home in Brazil.

### **HV7 - INFECTION ASSESSMENT BY EBV AND H. PYLORI AMONG GASTRIC ADENOCARCINOMA PATIENTS IN PARA STATE, NORTHERN BRAZIL**

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1. UNIVERSIDADE FEDERAL DO PARÁ
2. INSTITUTO EVANDRO CHAGAS

Gastric adenocarcinoma accounts for about 95% of gastric cancer cases and it is a major public health problem in the State of Pará, Northern Brazil. Belém, the state capital, has already been in 11th place among the cities with the highest prevalence of the disease in the 1990s. Viral and bacterial infections may contribute to increase individual susceptibility to the gastric adenocarcinoma development. The infectious agents of major importance for the development of gastric cancer are Epstein-Barr Virus (EBV) and *Helicobacter pylori*. The objectives of this study were to investigate the prevalence of these agents in patients with gastric adenocarcinoma and the association of such infections with age, gender, histological type, location, stage and metastasis. A cross-sectional, observational and analytical study was conducted, enlisting DNA extracted from tumor tissue samples from 203 patients treated at the Ophir Loyola Hospital, during 1998 and 1999. Detection of EBV was carried out by using the qPCR test. The amplification of the EBNA1 gene region was performed with the commercial kit qPCRAlert EBV® (Nanogen). For detection of *H. pylori*, a PCR was performed, having as targets conserved regions of the urease A (ureA) and 16S ribosomal RNA (16S) genes, being considered as positive result if noticed the amplification for one or both targets. Among the tested tumor samples, 22.7% (46/203) were positive for both agents, 6.9% (14/203) only for *H. pylori*, 31% (63/203) only for EBV and

39.4 % (80/203) negative for both. Statistical analysis by simple logistic regression demonstrated that the presence of a pathogen has been associated with the presence of the other ( $p < 0.0001$ , OR = 4.1723 CI = 2.11 to 8.26). It was not possible to determine the direction of this association, that is, which agent produces favoring infection by the other. A relationship of coinfection with advanced age and presence of metastasis was observed, strengthening the hypothesis of a synergistic action by the two agents. These results reinforce the important role that EBV and *H. pylori* have in the development and progression of gastric adenocarcinoma.

#### **HV10 - DISTRIBUTION OF BETA-PAPILLOMAVIRUS AT ANOGENITAL AND ORAL ANATOMIC SITES OF MEN: THE HIM STUDY**

Nunes, E.M.; Sudenga, S.L.; Gheit, T.; Tommasino, M.; Baggio, M.L.; Ferreira, S.; Galan, L.; Silva, R.C.; Pierce Campbell, C.M.; Lazcano-Ponce, E.; Giuliano, A.R.; Villa, L.L.; Sichoero, L.

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3. HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO

Beta human papillomaviruses (?HPV) comprises viral types ubiquitously distributed throughout cutaneous surfaces from body since early infancy, suggesting these may be part of the commensal flora. Our goal was to describe the prevalence and distribution of 43 ?HPV types among samples collected at the same time point from the external genital skin, the anal canal, and the oral cavity from 717 men participating in the HPV Infection in Men (HIM) Study. ?HPV detection and genotyping was conducted using the Luminex technology. Overall, 77.7%, 54.3% and 29.3% men were positive for any ?HPV type at the genital, anal canal, and oral cavity, respectively. ?1 and ?2 species were the most prevalent at all sites, and HPV21, 22, 24, and 38 were the most frequent. Multiple ? HPV types were more commonly detected at the genital (ranged from 2 to 19 types detected) than at the anal canal (29) or oral cavity (211). Men from the US and Brazil were significantly less likely to have any ?HPV at the anal canal (adjusted OR (aOR)=0.55, 95% CI 0.38–0.80 to US and aOR=0.49, 95% CI 0.33 0.73 to Brazil) and oral cavity (aOR=0.39, 95% CI 0.26–0.60 to US and aOR=0.46, 95% CI 0.310.70 to Brazil) than men from

Mexico. Age was marginally associated with anal HPV prevalence, with older men (aOR=1.59, 95% CI 0.982.58 for 3144 years) being more likely to have any ?HPV at the anal canal compared to younger men (1830 years). Prevalence of any ?HPV at the oral cavity was strongly associated with age (aOR=1.79, 95% CI 1.092.96 for men ages 45–70 years compared to men 1830 years). Current smokers were significantly less likely to have ?HPV in the oral cavity than men who never smoked (aOR=0.50, 95% CI 0.320.80). The lack of association between specific sexual behaviors and ?HPV detection suggests digital contact and autoinoculation as surrogate routes of viral transmission.

#### **HV17 - DETECTION OF NOROVIRUS IN IMMUNOSUPRESSED PATIENTS SUBMITTED TO KIDNEY TRANSPLANT IN BELÉMPA**

Resque, H.R.; Lucena, S.S.; Silva, V.P.; Brasil Costa, I.; Migone, S.R.C.; Silva, A.B.; Viana, C.A.; Gabbay, Y.B.

1. HOSPITAL OPHIR LOYOLA
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Gastroenteritis is one of the most common complications observed in immunosuppressed individuals, either by clinical condition or by that undergoing organ transplant, and its cause is often unknown. Norovirus (NoV), a member of the Caliciviridae family, is a major cause of gastroenteritis outbreaks worldwide, involving people of all ages and is generally observed in closed environments such as cruise ships, hotels, restaurants and hospitals, due to its easy person-to-person transmission. NoV has already been described in studies involving transplant patients, worldwide and in Brazil. This study aims to detect NoV in the feces of kidney transplant recipient patients, with and without gastroenteritis, hospitalized, treated and followed up at the Ophir Loyola Hospital in BelémPA. Each patient included in this research is monitored for a period of 2 years, having their stool samples collected monthly for the first six months, then every two months for another six months and every three months in the last year, when possible. At the Evandro Chagas Institute/SVS/MS, the stool sample is initially tested by a commercial enzyme immunoassay (EIA). From May 2014 to April 2016, 69 patients were included in the project. Of the 275 samples collected until now, 7 (2.5%) tested positive for NoV by EIA. These positive samples belong to 5 patients, coded as PTR041 (1),

PTR046 (1), PTR055 (1), PTR060 (1) and PTR064 (3). Regarding patient PTR064, all 3 positive samples were collected in 2016, on February 15th, April 6th and April 29th, wherein the transplant occurred in February 8th. Due to the proximity of collection dates, molecular tests and nucleotide sequencing will be performed to clarify if this is a case of single infection with prolonged shedding or multiple infections by different NoV strains. With regard to symptoms, only patients PTR041 and PTR046 reported diarrhea episodes when questioned. The other patients were asymptomatic or had no data available. Therefore, it is concluded that NoV is circulating among immunosuppressed patients submitted to kidney transplant, corroborating some studies described in the literature. Lastly, it is worth mentioning that this is a pioneering study in Brazil, as regards the transplanted kidney recipient patients, and that it is still in progress, informing that molecular tests will also be performed, aiming for NoV genotyping and the research of other enteric viruses as well.

#### **HV20 - WHOLE TRANSCRIPTOME ANALYSIS REVEALS THAT ZIKA VIRUS HALTS CELL CYCLE PROGRESSION AND DISRUPTS NEURONAL DIFFERENTIATION IN HUMAN NEUROSPHERES**

Vianez Júnior, J.L. da S.G.; de Vasconcelos, J.M.; Garcez, P.P.; Nascimento, J.M.; da Costa, R.M.; Delvecchio, R.; Trindade, P.; Loiola, E.C.; Higa, L.M.; Cassoli, J.S.; Vitória, G.; Fuzii, H.T.; Sochacki, J.; Tanuri, A.; de Filippis, A.M.B.; Rehen, S.K.

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4. INSTITUTE OF BIOLOGY, FEDERAL UNIVERSITY OF RIO DE JANEIRO
5. INSTITUTE OF BIOLOGY, DEPT OF BIOCHEMISTRY AND TISSUE BIOLOGY, UNIVERSITY OF CAMPINAS
6. FEDERAL UNIVERSITY OF PARÁ

Brazil is facing an unprecedented growth in the number of microcephaly cases in babies. this phenomenon coincided with the recent zika virus (zikh) outbreak in this country. although the brazilian ministry of health was quick to recognize that zikh was probably the cause of microcephaly in newborns, the underlying mechanisms leading to the development of this pathology have not

been established. to tackle this problem at the molecular level, we employed whole transcriptome sequencing of human neurospheres derived from neural stem cells exposed to zikh isolated in brazil (asian genotype). differential gene expression analysis of control (mock) and zikh infected neurospheres generated a list of 26 down-regulated and 64 up-regulated genes. Among the up-regulated detected genes, the cyclin-dependent kinase inhibitor 1a (cdkn1a) and the glial fibrillary acidic protein gene (gfap) were found. cdkn1a prevents the activation of the cyclin e/cdk2 complex, acting as a regulator of cell cycle progression during g1 and gfap is a known marker of astrocytes. we also observed a decrease in the expression of the neurogenic differentiation 1 gene (neurod1), which is directly involved in the neurogenic program. Those findings suggest that zikh infection induces cell cycle arrest and inhibits the neuronal differentiation, resulting not only in the reduction of the size, but in a deeper disruption of the normal development of the human brain.

#### **HV21 - EVOLUTIONARY ANALYSIS AND CHARACTERIZATION OF WALIKE AND DS1LIKE G12P[6]ROTAVIRUS STRAINS RECOVERED FROM DIARRHEIC CHILDREN IN BRAZIL**

Bezerra, D.A.M.; Guerra, S.F.S.; Serra, A.C.S.; Fecury, P.C.M.S.; Sousa Junior, E.C.; Bandeira, R.S.; Junior, E.T.P.; Lobo, P.S.; Linhares, A.C.; Soares, L.S.; Mascarenhas, J.D.P.

INSTITUTO EVANDRO CHAGAS

The G12 genotype of group A rotaviruses (RVAs) has become the sixth most prevalent genotype associated with human infections. The genomic constellation designates a specific genotype that is assigned to each of the 11 segments GxP[x]IxRxCxMxAxNxTxExHx, wherein "x" defines the genotype of the VP7VP4 genes (VP6-VP1VP2VP3NSP1NSP2NSP3NSP4NSP5), culminating in three main genetic groups as follows: Walike, DS1 like, and AU1 like. The G12 genotype is associated with P[6], P[8] and P[9]. The most frequent constellation belongs to the Walike; however, the availability of gene sequences of the G12 genotype in combination with P[6] (especially those belonging to the DS1 constellation) is scarce. Therefore, the aim of the present study was to analyse the evolutionary dynamics of G12P[6].RVA strains of the Walike and DS1like constellations isolated from children

with diarrhoea in Brazil. For the present study, 30 G12P[6] samples were selected for partial analysis of the genes. The results obtained in the present study exhibited the Walike (16 samples), DS1like (13 samples) and Wa x DS1like constellations (1 sample). G12 was classified as belonging to lineage III and P[6] as belonging to lineage I. In relation to the evolutionary mechanisms, there was no evidence of recombination in the samples; however, reassortment was observed in a VP2 gene of swine origin in one sample, and intergenogroup reassortment events in the NSP4 gene were detected in another sample. The molecular clock of the VP6 gene indicated Walike G12 with ancestry related to G1P[8] and DS1like G12 related to G2P[4]. Generally, the results suggested that the VP7 and VP4 genes belonging to the DS1like constellation had genetic signatures that distinguished them from the Walike constellation; however, these mutations led to aminoacid changes that were not part of the antigenic sites described for these proteins. The study provides important insights into the G12P[6] genome of the Wa and DS1 constellations.

#### **HV34 - CYTOKINE PROFILE PATTERN AS PREDICTOR OF SEVERE DENGUE DISEASE IN PATIENTS AT RISK**

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Dengue is an arthropodborne emerging viral disease with clinical manifestations ranging from asymptomatic to severe forms including severe plasma leakage, bleeding and hypovolemic shock in affected individuals. Although patients with severe forms present a 'cytokine storm', with high levels of circulating cytokines and chemokines which may exacerbate DENV pathogenesis, a cytokine pattern is not well established. Herein, we investigated the potential of cytokine profile from dengue patients as a tool for early predicting dengue severity in dengue with warning signs (DwWS) and dengue without warning signs (DwoWS) patients whose may later develop severe dengue (SD). Plasma levels of 17 different types of cytokines, chemokines, adhesion molecules and growth factors were assessed by multiplex fluorescent microbead immunoassay in dengue patients at the 3 different phases of illness. The association between levels of cytokines and clinical parameters were analyzed. We observed a remarkable growing

trend of the cytokines and chemokines in accordance with the progression of the disease. The increased levels of IL1?, MIP1?, MCP1, TNF?, IL8, IL6 and IL10 was associated with dengue severe and dengue with warning signs. Furthermore, MIP1? was negatively correlated with platelets counts and hematocrit, enquanto IL4 was correlated with hematocrit. This cytokine profile pattern in dengue identified herein, from a Brazilian population and detected by a multiplex bead immunoassay, favour early prediction of SD in DwWS and DwoWS patients who are at risk of developing severe outcome. The increased levels of IL1?, MIP1?, MCP1, TNF?, IL8, IL17 and IL10 to a lesser extent at different phases of illness can indicate the disease progression related to more severe cases and contribute to the establishment of more attention and therapeutic/hospitalization procedures.

#### **HV46 - ANALYSIS OF BACTERIAL DIVERSITY IN HIV/HPV COINFECTED PATIENTS WITH CERVICAL INTRAEPITHELIAL LESIONS THROUGH NEXT-GENERATION SEQUENCING**

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The main factor associated with the development of cervical cancer is the infection by human papillomavirus (HPV). HPV is necessary, but not sufficient to cause cervical cancer. Several studies have shown an increase in bacterial diversity in HPVpositive cervical cancer patients and the association of specific bacteria in cervical intraepithelial lesions. However, little is known about cervical microbiome of HIVHPV coinfecting patients. HIV patients have a high prevalence of highrisk HPV and a greater chance of developing persistent HPV infection. The aim of this study is to evaluate the bacterial profiles of the cervical region of HIVHPV coinfecting patients, looking for a putative association of such profiles with cervical intraepithelial lesions. To achieve this, we are analyzing 140 HIV+ cervical smear samples from women followed at Instituto de Puericultura e Pediatria Martagão Gesteira (IPPMG), collected from 2010 to 2013. Samples have been categorized according to their collection point, CD4+ Tcell counts and cervical intraepithelial lesion. Total DNA was extracted, the bacterial 16S rRNA gene was amplified and libraries

were generated for nextgeneration sequencing, using an Illumina HiSeq 2500 platform. After sequencing, reads were processed and compared against the 16S Greengenes database. All bioinformatics analyses were carried out using an opensource bioinformatics pipeline for performing microbiome analysis named QIIME. A total of 89 samples have been analyzed to date. The most abundant genus found was *Lactobacillus*, representing 30% of the bacterial population in the samples. We also observed the presence of *Gadernella*, *Atopobium*, *Provotella*, *Streptococcus*, *Fusobacterium*, *Sneathia* and *Megasphaera*. These bacteria are described in other microbiome studies in HPV patients. Of note, the samples showed an unequal frequency distribution of bacteria in the different study groups. So far, it is not possible to associate specific bacteria with cervical intraepithelial lesion classifications. On the other hand, distinct profiles of bacterial communities have been observed according to lesions and to CD4+ Tcell counts, and further refinement of the conducted analyses is necessary to evidence the significance of those differences.

#### **HV50 - DIFFERENTIAL DIAGNOSTIC OF HUMAN PARVOVIRUS B19 INFECTION IN FULMINANT HEPATIC FAILURE PATIENTS**

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The parvovirus B19 (B19V) infection is usually acute and selflimited but also provokes a variety of clinical manifestations, as erythema infectiosum, transient aplastic crisis, chronic bone marrow failure in immunocompromised hosts and nonimmune hydrops fetalis. Recent studies have suggested that B19V may cause hepatitis in immunocompetent patients in the absence of coinfection with other hepatotropic viruses. The hepatic manifestations of B19V can range from biochemical changes until fulminant hepatic failure (FHF). The objective of this study was to establish a

real time PCR (qPCR) for detection and quantification of B19VDNA in order to evaluate the role of the B19V as etiological agent of acute and FHF hepatitis. DNA was extracted from clinical samples using QIAAmp DNA mini Kit (Qiagen) and qPCR was performed by Sybr Green and Taqman methodologies using primers for NS1 region (E1905/E1987) and a synthetic standard curve. To examine the suitability of qPCR, sera from patients with (n=17) or without (n=40) confirmed B19V infection were tested: antiB19V IgM+ (n=12), antiB19V IgM (n=6), B19VDNA+/HIV+ (n=5), antirubella IgM+ (n=9), antiDENV IgM+ (n=9), HIV+ (n=5), HAV+ (n=4), HBV+ (n=3), HSV1/2+ (n=4). After that standardization, serum and liver biopsy samples from 11 patients with FHF were tested. The standard curve parameters were for Sybr Green (R<sup>2</sup>=0.99; Efficiency=97%) and for Taqman (R<sup>2</sup>=0.99; Efficiency=95%). The detection limits of Sybr Green and Taqman were 4.32x10<sup>1</sup> and 1.83 copies/μL, respectively. None false positive or negative results were detected by Taqman. Using Sybr Green a false positive result from antiDENV IgM+ serum sample was observed. The Taqman assay was then used to test the samples from FHF patients. Among then, 8 liver biopsy and 6 serum samples were positive for B19VDNA, with viral loads ranging from 4.4 to 1.6x10<sup>2</sup> copies/μL in liver biopsy, and 3.5 to 1.1x10<sup>1</sup> copies/μL in serum. Among FHF patients with B19V DNA, one was coinfecting with HAV, one was coinfecting with HBV and six were hepatitis cases of unknown etiology (cryptogenic). In conclusion, the Taqman qPCR was more suitable for detection and quantification of B19V and was able to identify B19V coinfections with HAV and HBV. These results indicate that B19V should be considered in the differential diagnosis of cryptogenic and FHF hepatitis and demonstrate the importance of establishing sensitive and specific molecular methods to clarify these cases of infection.

### **HV51 - MULTIPLEX PCR FOR SIMULTANEOUS DETECTION AND QUANTIFICATION OF BETAHERPESVIRINAE FROM PATIENTS WITH FULMINANT HEPATITIS OF UNKNOWN ETIOLOGY**

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Cytomegalovirus (HCMV), Human Herpes Virus 6 (HHV6), human herpes virus 7 (HHV7) belong to the genus herpesvirus, family Herpesviridae, subfamily Betaherpesvirinae and have linear doublestranded DNA. The HCMV causes an infection similar to mononucleosis syndrome, while HHV6 and HHV7 cause roseola infantum, febrile seizures and other febrile syndromes in children. After infection, the Betaherpesvirus can remain latent until an immune deficiency host favors its reactivation. HHV6 and HHV7 have quite homologies, genetic and biological each other and are the main cause of sudden rash. The primary HCMV infection and/or HHV6 can also generally cause mild and selflimited hepatitis in immunocompetent patients. Complications resulting from HHV7 infection have also been shown to be a factor in organ transplantations. However, acute and fulminant forms of hepatitis may also be associated viruses of the family Herpesviridae. The poor prognosis of evolution for hepatitis in cases of herpes is associated with late diagnosis and delayed treatment with specific antiviral therapy. Therefore, the need for early diagnosis for initiating the therapy is evident. For virus detection and quantification was used the multiplex realtime PCR TaqMan. The virus quantification was performed using the synthetic standard curve. After optimization of reaction and standard curves were tested 21 serum and 14 liver samples from patients with fulminant hepatitis of unknown etiology by realtime PCR monoplex and multiplex. For HCMV 14.2% (3/21) were positive in serum, and 7.1% (1/14) were positive in liver, to HHV6 4.76% (1/21) in serum was positive and 42.8% (6/14) were positive in liver and for HHV7, 14.2% (2/14) were positive in liver and none of the serum samples was positive. All positive samples in realtime PCR was tested in conventional PCR for future sequencing. The samples that were positive in monoplex, also were positive in multiplex showing that it is possible to perform only one realtime PCR to detect and differentiate Betaherpesvirus.

Consideration of Betaherpesvirus in the differential diagnosis in fulminant hepatitis is important and early initiation of antiviral treatment may be lifesaving in this situation.

### **HV58-HIV1 GENETIC DIVERSITY AND ANTIRETROVIRAL DRUG RESISTANCE AMONG INDIVIDUALS FROM RORAIMA STATE, NORTHERN BRAZIL**

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The HIV1 epidemic in Brazil had spread towards the Northern country region, but little is known about HIV1 subtypes and prevalence of HIV strains with resistance mutations to antiretrovirals in some Northern states. HIV1 protease (PR) and reverse transcriptase (RT) sequences were obtained from 73 treatmentnaïve and experienced subjects followed between 2013 and 2014 at a public health reference unit from Roraima, the northernmost Brazilian state. The most prevalent HIV1 clade observed in the study population was the subtype B (91%), followed by subtype C (9%). Among 11 HIV-1 strains from treatmentnaïve patients, only one had a transmitted drug resistance mutation for NNRTI. Among 59 treatmentexperienced patients, 12 (20%) harbored HIV1 strains with acquired drug resistance mutations (ADRM) that reduce the susceptibility to two classes of antiretroviral drugs (NRTI and NNRTI or NRTI and PI). Other five (8%) harbored HIV1 strains with ADRM that reduced susceptibility to only one class of antiretroviral drugs (NNRTI or PI). No patients harboring HIV strains with reduced susceptibility to all three classes of antiretroviral drugs were detected. A substantial fraction of treatmentexperienced patients with (63%) and without (70%) ADRM had undetectable plasma viral loads (<40 copies/ml) at the time of sampling. Among treatmentexperienced with plasma viral loads above 2,000 copies/ml, 44% displayed no ADRM. This data showed that HIV1 epidemic in Roraima displayed a much lower level of genetic diversity than that described in other Brazilian states. The relatively high frequency of undetectable plasma viral load and the low overall

prevalence of ADRM observed among patients on treatment, also supports for a high rate of success of antiretroviral therapy in this northernmost part of the country.

#### **HV60 - THE OCCURRENCE OF ROTAVIRUS G12P[8] OF HOSPITALIZED CHILDREN AT REFERRAL HOSPITAL OF GOIÂNIA, GOIÁS**

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Acute gastroenteritis (AG) is important cause of morbidity and mortality in worldwide, especially in children up to five years old, whereas Rotavirus species A (RVA) is one of the major causative agents of the disease. In this context, since 2006, two vaccines Rotarix® and Rotateq® have been used for control and prevention of the infection caused by this virus. Both vaccines are used in Brazil; Rotarix® has been included in the National Immunization Program in 2006. Once the vaccination process is started, studies have shown the gradual reduction in AG cases, as well as the severity of symptoms associated with this disease. Thereby, the present study aimed to verify the occurrence of RVA in children up to five years old, with or without AG, hospitalized in a referral hospital for pediatric care in Goiânia, Goiás from 2014 to 2015. The study material consisted of 335 fecal samples, 134 samples from children with AG and 201 children without AG. RVA detection was done by polyacrylamide gel electrophoresis, and the characterization of genotype G (VP7) and P (VP4) was done after sequencing using the online automated genotyping tool RotaC. Of the total samples it was observed that nine (2.7%) were positive for RVA, of which eight were from children with AG. The characterization of the G and P genotypes showed that of the nine positive samples, four were G12P [8], one G12, two P [8] and the other could not be genotyped for G and P. Phylogenetic analysis of the sequences coding VP4 and VP7 genes showed that G12 and P[8] belong to the lineage III. The results showed the decline in RVA detection, and the occurrence of G12 and P [8] samples in the postvaccination period. These data reinforce the importance of the vaccination process. Additionally, the prevalence of samples G12 and P [8] indicate a tendency to fluctuation of genotypes RVA over time. In this

context, it is necessary for continued monitoring of RVA in the human population consideration the vaccination process.

#### **HV65 - PHYLOGENETIC ANALYSIS OF GROUP A ROTAVIRUS ISOLATES FROM BRAZIL EVIDENCES CHANGES IN GENOTYPE CONSTELLATIONS ASSOCIATED WITH THE VACCINE**

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Rotaviruses cause approximately 1.7 billion episodes of acute diarrhoea, leading to nearly 700,000 deaths worldwide annually. In March 2006, it was introduced in Brazil the monovalent vaccine Rotarix containing a single human genotype, G1P[8]. Traditionally, a dual classification system was established for Rotavirus A (RVA) based on two genes (VP4 and VP7). However, a new classification system has been proposed including all 11 genes. Therefore, the objective of this study was to evaluate the genomic diversity of RVA circulating in Brazil in the last 30 years in order to investigate possible changes in the prevalence of genotypes before and after the implementation of the vaccine according to the genomic constellation of RVA. In total, 820 gene sequences were obtained from the Virus Sequence Database. It was possible to recover 368 isolates of Brazilian human RVA from 1986 to 2011. Then, BLAST tool were used to obtain the sequence identity of the isolates. Sequence alignments were performed using ClustalW program. Phylogenetic analyses were performed under the GTR + I and HKY + G models of nucleotide substitution by using jModelTest2 program, and Maximum Likelihood phylogenetic trees were inferred for each gene using PhyML 3.0 program. It was possible to observe that VP4, VP7, NSP4, and VP6 gene sequences were more frequently found in the database. In relation to P genotype, we found that P[8] was more frequent than P[6] and P[4]. When it comes to the genotype G, G9 and G2 were more frequent than G1. The most frequent combinations were G2P[4], G1P[8], and G9P[8]. When we compared the genomic constellation of the vaccine Rotarix genotype (G1P[8]I1R1C1A1M1N1E1T1H1) with other isolates of the study we found that the VP2 gene is the most associated with genomic constellation of the vaccine, and the lowest was the G1 genotype. Most of the

genes are distributed phylogenetically according to the genotypes (NSP1, NSP2, NSP3, NSP5, VP1, VP2, VP6, and VP7). NSP2 and VP7 were distributed throughout the years, and VP3 and VP4 showed no pattern of distribution by year, location and/or genotypes. New genotypes are circulating in the country, so it is important to study the genomic constellation of Brazilian isolates in order to understand the genetic diversity of circulating isolates, and thus assess the further effectiveness of the implemented vaccine, serving as basis for possible future formulations.

#### **HV71 - IMMUNOCYTOCHEMISTRY CHARACTERIZATION OF RAP1 PROTEIN EXPRESSION IN SQUAMOUS CELL BLOCKS FROM CERVICAL CYTOLOGY IN LIQUID MEDIUM**

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The accurate early diagnosis of cervical cancer, relevant public health problem worldwide and in Brazil by cytology (Pap test), is hampered by its subjectivity and variability of false positive and false negative results, particularly on atypical squamous cell (ASC). Recent technical innovations, such as based liquid cytology and immunocytochemistry for with cell proliferation biomarkers, increased the expectation cervical cancer screening. However, the applicability of these innovations in early stages of epithelial dysplasia and atypia remains uncertain. Considering previous findings of our research group, which identified the RAP1 protein as a biomarker diagnosis of cervical dysplasia, this study aims to characterize the expression of RAP1 (compared to the expression of p16 and Ki67 biomarkers) by immunocytochemistry in cell blocks of cervical squamous cells, for possible applicability in screening for cervical cancer. For this purpose, 27 patients with benign cellular changes (ACB) and 7 patients with ASC diagnosis were collected in Hospital das Clínicas from UFMG. The results indicated that 85% of the samples of cell blocks were satisfactory for morphological analysis and also that the cytological technique reproduces the main parameters of the conventional cytology. Regarding its use for diagnosis, the cell block had a sensitivity of 38.46%, specificity of 90.47% and an interobserver variability with concordance rate of approximately 30%

for the ACB and ASC groups. The RAP1 expression was positive in most of the samples of the group "ACB" (15/27 or 55.56%) and most negative in the sample group "ASC" (4/7 or 57.14%) with a sensitivity of 16.66% and specificity of 75%. The immunocytochemical reactions for p16 and Ki67 showed predominance of negative or all negative staining in both groups. HPV DNA was detected in 9 (33.33%) of the 27 samples of the ACB group and in 4 (57.14%) of 7 ASC group samples. HPV16 was detected in 4 samples ASC group. Samples from the group ACB HPV16 were detected in 5 samples, HPV58 in 2 samples, HPV45, HPV1 sample, and 66 in one sample. We observed no relationship between the presence of HPV and immunostaining of RAP1 in both groups. In conclusion, cell blocks can be a ancillary tool to the Pap test for cervical cancer in screening and the expression of the RAP1 protein is increased in cervical cells in an inflammatory environment, with or without the presence of HPV.

#### **HV89 - MOLECULAR CHARACTERIZATION OF ENTEROVIRUS FROM CHILDREN WITH ACUTE GASTROENTERITIS IN BELEM AND METROPOLITAN REGION, PARÁ STATE**

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Acute gastroenteritis (AGE) remains an important public health problem and a common cause of morbidity and mortality among children and elderly in worldwide. It is estimated 35 billion cases of acute gastroenteritis and 1.52 million deaths occur each year in children under 5 years. A causative agent in approximately 40% of diarrheal cases still remain unknown etiology although more sensitive molecular methods are available. Currently, several reports highlighting enteroviruses as one of the viral etiologies of AGE have been also documented. Identify enterovirus detected in stool samples collected from children under 5 years old with AGE and presented diarrhea in Belém, Para State. 175 stool samples of children under 5 years of age presenting AGE collected during May/2010 and April/2011 were the object of study. Samples were previously screened by Real time PCR and all positive samples were inoculated for viral isolation using HEp2 and RD cell lines. Samples presenting cytopathic effect (CPE) were submitted

to viral RNA extraction, cDNA synthesis and PCR for amplification and partial nucleotide sequencing of VP1. Samples with no CPE were submitted to SemiNested PCR and nucleotide sequencing for viral identification. Sequences were edited, analyzed and compared to the GenBank database of NCBI. From 16 isolated samples in cell culture, 9 different enterovirus serotypes were identified [Coxsackievirus (CV) CVB3, CVB4, Enterovirus (EV) EVC99, EVC96, CVA5, CVA6, CVA13, Echovirus (E) E6 e E7]. Of the 30 analyzed fecal suspension samples (no CPE), 22 were amplified by Semi Nested PCR. It was possible to identify 9 samples with 8 different serotypes CVA5, CVA9, CVA10, CVB3, EV C99, E9, E15 e PV3. This study shows a high circulation and diversity of enterovirus serotypes circulating in childrens presenting AGE suggesting a possible association of enterovirus as etiologic agent. The findings are of great importance for future research and to establish a monitoring system for enterovirus, aiming to identify and monitoring the emergence of new variants/genotypes.

#### **HV90 - DETECTION OF ENTEROVIRUS IN CHILDREN FROM OUTSKIRTS OF BELÉM, PARÁ, IN THE PERIOD OF 1982 TO 1986**

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Acute gastroenteritis (AGE) is one of the most common diseases in humans and remains the leading cause of morbidity and mortality worldwide. It affects mainly children from developing countries, being responsible for 2530% of all deaths among children less than 5 years old. Recently, members of the Picornaviridae family, such as the enteroviruses (EV), have been considered as agents associated with cases of diarrhea in humans. Thus, the knowledge of the involvement of EV in sporadic cases and outbreaks of AGE is very important to establish the real impact of these agents in diarrheal diseases. The aim of this study was to detect EV infections in three children living in the outskirts of BelémPA, who were followed from birth until three years old. The study used 216 stool samples collected during a community-based longitudinal study conducted from October 1982

to March 1986. The children were regularly monitored, being the feces collected daily at the hospital and fortnightly after discharge or when presented diarrhea. Samples were tested by the quantitative reverse transcription polymerase chain reaction (RTqPCR) using the commercial ID AgPath™ OneStep RTPCR kit. Of the 216 samples tested, 137 (63.4%) were positive for EV, being 47/69 (68.1%) related to the first child; 44/70 (62.9%) to the second; and 46/77 (59.4%) to the last one. Samples with cycle threshold (ct) equal or less than 42 were considered positives. The mean ct of the positive samples was 32.1, and the second child had the lower values with ct varying from 16,1 to 40, indicating a higher viral load. Of the 216 samples, only 6.5% (14/216) were symptomatic, with 50% (7/14) of positivity. In the asymptomatic ones the positivity was 63.9% (129/202). This study demonstrated a high circulation of EV in fecal samples collected for over 30 years. The positive asymptomatic cases may be related to a constant contact with the virus through insufficient inoculum intake to cause disease in the host, as well as a prolonged excretion of the virus due to episodes of diarrhea experienced many weeks before. All positive samples will be tested in cell culture, because this technique is considered the gold standard for the virus detection and in the positive ones will be performed the molecular characterization of the positive fluids.

#### **HV93 - GENOTYPING OF HUMAN PAPILLOMAVIRUS AND ITS RELATIONSHIP WITH THE PRESENCE OF CERVICAL LESIONS IN WOMEN COINFECTED WITH HIV**

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Papillomaviruses are doublestranded DNA viruses with size of 55nm and icosahedral form. The HPV is a non enveloped and can induce squamous epithelial tumors in different anatomical locations. They belong to the Papillomaviridae family and have a genome of about eight thousand base pairs, protected by capsid proteins. More than 200 different types of human papillomavirus

(HPV) have been identified and classified into two groups, high risk and low risk, depending on their association with the development of cancer. The HPV genomic integration is a mechanism of persistent viral infections, which eventually develops in cancerization phase. This is typically a random process and can occur anywhere in the DNA of the host cell. In some cases, the integration can contribute to the development of cervical carcinoma, which is preceded by precursor lesions such as cervical intraepithelial neoplasia or squamous intraepithelial lesions. Chronic HPV infections, which are often characterized by high viral load, can be facilitated by coinfection with HIV, which reduces the likelihood of spontaneous elimination of HPV. On this basis we investigated the presence of HPV as well as their genotype in 80 samples, collected in two different years, of 40 patients coinfecting with HIV and diagnosed with cervical lesions. For this purpose we used the oligonucleotides PGMY09/11 and GP5+/6+ in the polymerase chain reaction (PCR) to detect the virus and sequencing reaction according to the Sanger method for HPV genotyping. As a result it was observed the presence of HPV in 65 samples (81.3%), where all these samples were successfully genotyped. The high risk were predominant between the HPV types detected (65%) specifically, the most frequent types were HPV56 (22%) following by HPV16 (17%). The results are in agreement with the literature that shows the presence of HPV in up to 88.4% of patients coinfecting with HIV as well as the prevalence of highrisk HPV types (60%), mostly HPV16, and thus can lead to occurrence or progression of cervical lesions.

#### **HV96 - DETECTION OF ADENOVIRUS IN SERUM SAMPLES FROM HOSPITALIZED CHILDREN WITH ACUTE GASTROENTERITIS IN BELÉM CITY, NORTHERN BRAZIL**

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The acute gastroenteritis (AGE) is characterized as a gastrointestinal tract infection, having as one of its viral

etioloical agents the adenovirus (AdV), Adenoviridae family, Mastadenovirus genus, composed of DNA double-strand, not enveloped, consisted of seven species (AG) and 57 distinct serotypes. The species F, serotypes 40 and 41 are the most prevalent in outbreaks and sporadic cases of GA. This work aimed to detect and genotype the AdV in serum samples from children hospitalized with acute gastroenteritis in Belém city, Brazilian Northern region. The specimens were collected from children hospitalized with GA in two clinics of Belém, from March/2012 to June/2015. Sera were initially analyzed for the presence of rotavirus and norovirus, and only the negatives ones were tested for AdV. The serum samples were only tested for AdV when the children had positive results in the feces. The QIAmp Viral RNA/DNA Mini Kit (Qiagen) was used for the nucleic acid extraction, according manufacturer's recommendation. The detection of AdV was made by PCR and NestedPCR employing the pair of primers Hex1deg/Hex2deg and Nehex3deg/Nehex4deg, respectively, besides the use of the 2X Reaction Mix (a buffer containing 0.4 mM of each dNTP, 3.2 mM MgSO<sub>4</sub>) and Platinum Taq DNA Polymerase (Invitrogen) to increase its sensibility. Positive samples in the PCR and/or NestedPCR that showed a good quality of amplified product were purified with the commercial kits QIAquick® PCR Purification and QIAquick® Gel Extration (Qiagen) according to the manufacturer's instructions. The samples were sequenced with the use of the kit Big Dye Terminator (v.3.1) (Applied Biosystems) in an automatic sequencer. A total of 111 (43%) children had their feces positive for AdV, and in 80 (72.1%) their serum were also tested for this virus, with a positivity of 63.7% (51/80). The nucleotide sequencing was done in 78.4% (40/51) samples with predominance of the species F (80% 32/40), followed by species C (12.5% 5/40), A (5% 2/40) and B (2.5% 1/40). The results demonstrated for the first time in Northern Brazil, the detection of this virus in serum of children with AGE, with a high percentage, suggesting the extra intestinal circulation of this virus. Also it was verified that the specie F, types 40/41 was the most prevalent, confirming the elevated circulation of this type in cases of AGE.

**HV97 - DETERMINATION OF A NOVEL PRIMER SET FOR HUMAN PAPILOMAVIRUS DETECTION BASED ON ENTROPY**

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Papillomaviruses are circular doublestranded DNA viruses that specifically infect skin or mucocutaneous epithelium of mammals, reptiles and birds, causing asymptomatic infections, benign and malignant lesions. The classification of papillomaviruses is based on the L1 gene sequence identity. However, several studies on Human papillomavirus (HPV) diversity make use of only 450 bp fragment in L1 in order to classify novel HPV types, subtypes, and variants. It has been observed that this fragment is not appropriated for detection and genotyping of HPV. So, the aim of this study was to develop and apply a novel computational tool based on entropy in order to identify phylogenetic informative genomic regions that could be used as markers for the detection and genotyping of HPV. To develop the method, a comparative analysis was performed to assess the genetic variability of L1 sequences from Alphapapillomavirus, Betapapillomavirus and Gammapapillomavirus genera. Shannon entropy was calculated. Informative sites were identified by using a cutoff of 1.0 bits of information. Phylogenetic trees were constructed based on those informative sites. Degenerate primers were designed to amplify this region in the largest possible number of HPV types. To test the efficiency of the primers, PCR tests were performed to detect HPV DNA and the confirmation of the amplified products were sequenced. Blast was used to compare the sequences with reference sequences deposited in GenBank. All samples tested positive for the presence of HPV. Different HPV types were tested and the primer set was able to detect them, which evidences high specificity. A test of sensibility was performed and we were able to observe that the primers were capable to detect HPV DNA in several dilution series. This result shows a high sensitivity of the developed marker. The degenerate primers proposed in this study proved to be effective in the detection of HPV DNA. Thus, we propose the utilization of a new molecular marker that are capable of detecting different viral types and it can assist in the improvement of HPV detection and genotyping.

**HV107 - PHYLOGEOGRAPHIC DISPERSION OF INFLUENZA VIRUSES ISOLATED IN AMAZON REGION**

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The flu or Influenza is a disease of the respiratory tract caused by the influenza virus, which has the potential to affect about 10% of the population each year. Influenza viruses belong to the genus Influenza A, B and C of the Orthomyxoviridae family. The viral particle is spherical and enveloped, where are inserted the hemagglutinin protein (HA) and neuraminidase (NA), the main targets of the host immune response, which gives high variability of the genes encoding such proteins. The influenza virus has a pattern of global distribution with distinct dispersion directions. In this context, the phylogeography has become an important epidemiological tool to predict the areas where the emergence and spread of infectious agents may occur from the construction dispersal routes analyzing phylogenetic data. In this context, this study aims to characterize genetically the Influenza virus strains isolated in the Amazon region between 2010 and 2015 and contribute to the molecular epidemiology generating information on entry routes and spread of these viruses in the region. Material and Methods: Isolated strains of influenza viruses were analyzed in MDCK cell culture through genetic characterization of the HA gene encoding into four main steps: a) extraction of viral RNA; b) complementary DNA synthesis; c) Amplification of cDNA by PCR and d) sequencing. Results: In the analyzed period 1,254 positive samples for influenza were found, being 1,050 (83.2%) influenza A and 212 (16.8%) influenza B. Of these, 222 were isolated in cell culture, of which 48 were sequenced. The analysis of seasonal profile showed that the peaks of influenza virus circulation were in March with 398 cases (31.53%), April with 263 (20.83%) and May with 198 cases (15.69%). As for the dynamics of spread, influenza B virus had the State of São Paulo as the place of entry in Brazil, Acre, Amazonas and Roraima acting as dispersal routes in the Amazon region. Regarding the virus A (H3N2) strains have emerged in the states of Acre and Pará. Pará has the largest number of leakage fluxes of

these strains. Conclusions: The peak of influenza virus circulation in the Amazon region occurred in the first six months of the year. The dynamics of influenza viruses circulating in the Amazon region indicates the states of Acre and Pará main routes of dispersal.

#### **HV108 - ANALYSIS AND CORRELATION OF THE EXPRESSION OF THE P16INK4A PROTEIN AND HPV DNA IN INDIVIDUALS WITH PENILE CANCER IN THE STATE OF GOIAS, BRAZIL**

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1. UNIVERSIDADE FEDERAL DE GOIÁS
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3. UNIVERSIDADE DE BRASÍLIA
4. HOSPITAL ARAÚJO JORGE

Penile carcinoma (PC) is a rare disease, however it is still considered a serious public health problem accounting for high morbidity and mortality in developing countries. Lack of hygiene, compounded by the persistence of phimosis in adulthood may promote infection with human papillomavirus (HPV). The expression of p16INK4a, a protein associated with tumor suppression, can be used as a marker for the presence of high risk HPV DNA. The upregulation of this protein is understood to be an attempt to stop uncontrolled cellular proliferation in response to HPV infection. The goal of this study was to estimate the prevalence of HPV DNA and evaluate the expression and correlation of p16INK4a with HPV DNA in patients with PC in Goiás, Brazil. This retrospective cohort study involved 93 patients with PC treated in the UroOncology service of Hospital Araujo Jorge (HAJ), a unit of the Association Against Cancer in Goiás (ACCG), from January 2003 to November 2015. This study was approved by the Research Ethics Committee of HAJ. The paraffin blocks containing the cancerous tissue fragments were subjected to extraction of viral DNA using a commercial kit (Promega Corporation, USA), subsequently subjected to polymerase chain reaction testing with short PCR fragment (SPF PCR) primers to detect HPV DNA. The marking of the p16INK4a protein was performed with polymer-based immunohistochemistry, using a commercial kit (Mach 4 Universal HRP Polymer Detection System – Biocare Medical, CA, USA). The slides were evaluated independently by two pathologists. Of

the 93 samples tested, 33 (35.5%) (CI 95%: 26.3 to 45.6) showed positive HPV DNA and 40 (43.0%) (CI 95%: 33.2 to 53.2) showed expression of p16INK4a. The correlation between the presence of HPV DNA and p16INK4a was 63.6% (CI 95%: 46.3 to 78.6). Although there is no expression of p16INK4a in 100% of cases positive for HPV DNA, there was statistical significance between the presence of viral DNA and expression of p16INK4a ( $p < 0.003$ ). Some studies suggest that the standard knowledge of the expression of the p16INK4a protein may be a useful marker for HPV activity in patients with penile cancer. The results of this study showed that there are significant differences between the expression of this protein in positive and negative HPV DNA samples.

#### **HV119 - ASSOCIATION BETWEEN CCR5DELTA32 AND HEPATITIS B IN PATIENTS FROM RORAIMA**

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Hepatitis B virus is a hepatotropic, noncytopathic, which causes acute or chronic disease to the liver. Many epidemiologic, viral and genetic factors bias the susceptibility and persistency of the HBV infection. The chemokine 5 receptor (CCR5) is a gene located in the p21.3 region from chromosome 3, altogether with its ligand, CCR5 has an important role in the immune response to viral infections, among them, the Hepatitis B. Chemokine receptors are proteins found on cell's surface. There by, the existence of a mutant allele of the CCR5 gene, which presents a deletion of 32 base pairs (CCR5delta32), leads to receptor expression decrease and dysfunction. HBV chronically infected patients are incapable of clearing the viral infection from hepatocytes, and probably, a down-regulated CCR5 leads to low immune cells recruitment to the infected hepatocytes. This study's objective was investigate the presence of the CCR5delta32 mutation in infected patients and health controls in Roraima State and establish its possible correlation with HBV infection. This project was approved by the COEP (protocol 1.134.36). A hundred twentytwo HBV chronic carriers and 79 health controls were tested. DNA was extracted and analyzed according to Farias et al. (2012). Fenotype and allelic frequencies were calculated and evaluated by Hardy-

Weinberg Equilibrium. Comparison among proportions and differences among groups were determinate given by Fisher's Exact Test. Our results show the following genotype frequency between patients and controls respectively: CCR5/CCR5 (95.04% e 94.94%), CCR5/CCR5delta32 (4.96% e 5.06%), CCR5delta32/delta32 (0%, in both), there are no significant difference among groups ( $p=0.6076$ ), as well as mutant allele (CCR5delta32) frequency in patients and control group, respectively, 2.4% and 2.5%, ( $p=0.6480$ ). No relation was found between the mutation and HBV risk (OR = 0.97, IC95% 0.263.58;  $p=0.76$ ). In conclusion, we did not find evidences that show association between polymorphism CCR5delta32 and HBV infection, we observed that among all analyzed samples no homozygosity to the polymorphism was found, which could probably be explained because of Roraima's mixed population.

#### **HV121 - CLINICAL AND LABORATORY ASPECTS IN INDIVIDUALS WITH CHIKUNGUNYA FEVER OF TWO STATES: AMAPA AND GOIAS**

**Koga, R.C.R.; Maia, A.P.V.M.; Barletto, J.S.; Fonseca, S.G.; Pfrimer, I.A.H.**

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2. UNIVERSIDADE FEDERAL DE GOIÁS

**Introduction:** Many researchers confirm that the Chikungunya virus (CHIKV), transmitted by mosquitoes arthropods, has spread globally and originated outbreaks in sites hosting vector species, becoming a significant challenge to public health. The aim of this study was to evaluate clinical and laboratory aspects of individuals with clinical findings of Chikungunya fever, residents in the states of Amapa and Goiás. **Methodology:** The study was conducted in Immunologic Center of Studies and Research of the Catholic University of Goiás in Goiania, and in Health Care Units of Macapa and Oiapoque AP cities. A total of 80 volunteers who agreed to participate signed a consent form (TCLE) and answered a questionnaire with clinical and sociodemographic data. It was performed the extraction of the RNA viral in samples, followed by quantitative detection of RNA CHIKV by Real Time PCR. It was also performed an immunoassay for IgM and IgG for CHIKV. The symptoms of the subjects were correlated with the results of serology. **Results:** No sample showed quantitative detection of viral RNA by RTPCR. However, 26 samples (32.5%) were positive for IgG and 3 of those

for IgM. In relation to the disease stage, 10 were in the acute stage, 04 in the subacute stage and 12 in chronic stage. Correlated serology results to symptoms, it was observed that all volunteers had fever in the acute stage, 90% headache, 70% arthralgia and 60% edema. While in the subacute stage was more frequent arthralgia, headache (100%), myalgia and edema. (75%), and in chronic stage were edema (100%), arthralgia (92%) and myalgia (75%). When compared participants with negative serology,  $n = 54$ , the more presented symptoms were exanthema ( $n = 39$ ) headache, fever ( $n = 35$ ) and arthralgia ( $n = 31$ ). **Conclusion:** The study focused on people with clinical findings for FCHIK. The most common symptom in the three stages was arthralgia, followed by edema and myalgia, fever was frequent only in the acute stage. All participants were negative by Real Time PCR, because the virus has a short duration in the body, and this methodology is very limited by the start time symptoms. However, 32.5% were positive for IgG serology. Individuals who did not show IgM or IgG despite having articular pain, they presented rash, headache and fever, symptoms that may be correlated with other arbovirus as Zika virus.

#### **HV127 - INFLAMMATORY PROFILE OF INDIVIDUALS INFECTED BY ZIKA VIRUS IN ACUTE AND CONVALESCENT STAGES**

**Pfrimer, I.A.H.; Barletto, J.S.; Maia, A.P.V.M.; Silva, P.A.N.; Koga, R.C.R.; Paiva, P.L.; Dias Neto, O.S.; Ribeiro, L.L.S.; Fonseca, S.G.;**

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**Introduction:** The Zika virus (ZIKV) is an arbovirus responsible for asymptomatic and symptomatic infections in human. The disease was considered benign until 2007, but from that period on there were complications such as microcephaly and GuillainBarre syndrome. Despite the numerous articles published recently, little is known about the inflammatory response to ZIKV. The aim of this study was to evaluate markers of the inflammatory response, Creactive protein (CRP) and ferritin in individuals positive to ZIKV in acute and convalescent stages. **Methodology:** Samples of 27 individuals were positive for ZIKV and negative for the chikungunya and dengue virus by Real Time PCR. A new blood sample was collected from six of these patients.

The control group consisted of 21 samples from Goiana Central of Serology and Immunohematology, which were subjected to the same analysis of the study subjects. Inflammatory proteins were quantified by turbidimetric method. The participants signed a consent form and answered a questionnaire with signs and symptoms and sociodemographic data. Statistical analysis was performed by GraphPad Prism by nonparametric test of Mann Whitney (PCR and ferritin in patients with Zika x controls, and CRP and ferritin levels in acute patients x convalescent) and parametric by r coefficient of Pearson (CRP and ferritin x amount of clinical manifestations). Results: CRP levels were significantly higher than in controls  $p < 0.0001$ , while ferritin levels were not significantly different  $p < 0.7552$ . When comparing the levels in acute and convalescent stages, the results were  $p < 0.0087$  and  $p < 0.8182$  for PCR and ferritin, respectively. The levels of CRP and ferritin and the number of clinical manifestations was not significant,  $p > 0.05$ . The main clinical manifestations presented by patients were rash (88.8%), headache (59.2%), arthralgia (55.5%) and myalgia (55.5%). Conclusion: CRP levels are increased in the acute stage of infection, and return to normality in convalescent stage. There was no association between abnormal levels of CRP and the number of clinical manifestations. No changes were observed in the levels of ferritin, both in the acute stages and convalescence in patients with Zika virus.

#### **HV128 - MONOCYTE SUBSETS IN HIV-1 POSITIVE PATIENTS USING OR NOT ANTIRETROVIRALS**

**Pfrimer, I.A.H.; Carvalho, A.E.S.; Morais, C.O.B.; Castro, F.O.F.; Borges, A.F.; Barletto, J.S.; Souza, L.C.S.; Fonseca, S.G.**

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3. HOSPITAL DAS CLÍNICAS UFG

Introduction: Monocytes may be divided into subsets based on the levels of CD14 and CD16 expression: classical (CD14<sup>high</sup>CD16), intermediate (CD14<sup>high</sup>CD16<sup>+</sup>) and nonclassical (CD14<sup>low</sup>CD16<sup>+</sup>). Objective: to evaluate the monocyte subsets in patients infected with HIV1 in use or not of Antiretroviral Therapy (ART). Methods: we analyzed four study groups, made up of: individuals not infected with HIV 1; individuals infected with HIV1 without the use of ART; individuals infected with HIV-

1 whose ART consisted of two Reverse Transcriptase Inhibitors Nucleoside (T1) and individuals infected with HIV1 whose ART consisted of two Reverse Transcriptase Nucleoside Inhibitors associated with one Reverse Transcriptase not Nucleoside Inhibitor (T2). Monocyte subsets were determined by flow cytometry and the statistical analyzes performed with the GraphPad Prism 6. Results: the CD14<sup>high</sup>CD16 subset presented low percentage in T1 and T2. In CD14<sup>low</sup>CD16<sup>+</sup> subset, as well as reduced in the mentioned groups, there was also a reduction in the patients who did not use ART. In CD14<sup>high</sup>CD16 monocytes, we see a low percentage of the control group. Conclusion: Our studies have shown that monocytes are reservoirs of HIV and spread the virus in the body. Furthermore, they are important targets for ART.

#### **HV129 - MOLECULAR EPIDEMIOLOGY OF NOROVIRUS IN CHILDREN HOSPITALIZED DURING A RANDOMIZED CLINICAL TRIAL IN BELÉM, NORTHERN BRAZIL**

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1. INICIAÇÃO CIENTÍFICA/FUNDAÇÃO AMAZÔNIA DE AMPARO A ESTUDOS E PESQUISAS
2. PROGRAMA DE PÓSGRADUAÇÃO EM VIROLOGIA/ INSTITUTO EVANDRO CHAGAS
3. INSTITUTO EVANDRO CHAGAS

Norovirus (NoV) have been identified as the major cause of gastroenteritis (GE) outbreaks globally. This virus affects all age groups, but the illness can be more severe in specific groups such as children and the elderly. The purpose of this study was to detect and genotype NoV in fecal samples collected of hospitalized children in BelémPará from January 2004 to August 2005, during a randomized clinical trial. All feces were initially tested for rotavirus. Later, 200 samples with negative results were screened by an enzymelinked immunosorbent assay (EIA) for NoV antigen detection. Samples that presented positive results were subjected to a seminested RT PCR for amplification and genotyping targeting the A region of Polymerase gene (RdRp) and the ones with positive results were characterized by C/D regions of the VP1 gene. Analysis from P2 region was held to a more accurate classification of GII.4 variants. In samples that showed possible recombinant events, the ORF1/2 junction region analysis was performed. The overall positivity to NoV was 26% (52/200), being 69.2% (36/52) of

them positive samples amplified, which 27 (75%) were characterized by the A region as: GII.P13 [n=1], GII.P21 [n=1], GII.P7 [n=2] and GII.P4 [n=23]. About the 23 GII.P4 samples, in 9 it was clearly possible classified as Hunter\_2004 by this region observing its position in the tree. The VP1 analyzes held in the 27 characterized samples showed 66.7% (18/27) of amplification, classified as GII.4 [n=9], GII.6 [n=1], GII.3 [n=1], GII.17 [n=1] by C region and GII.4 [n=6] by D region. Of the 10 samples classified as GII.P4/GII.4, 7 were characterized as variants, being 6 as Asia\_2003 and 1 as Hunter\_2004 by the P2 region. Thus, considering the results of both regions (A and P2) the replacement of variant Asia\_2003 by Hunter\_2004 was clearly observed in May 2005 in Belém city. Discrepancies were identified in 3 samples after comparison of the analyzed regions: GII.P6/GII7 [n=2] e GII.P13/GII.17 [n=1] which were confirmed as recombinant strains by the junction region. The monthly distribution analysis showed a higher prevalence of NoV infection in August 2004 (53.8% 7/13). This study showed a high positivity of NoV, demonstrating wide genetic diversity, with the presence of two variants and recombinant strains. These results will contribute to a greater understanding of NoV, providing a perspective on how was its molecular epidemiology at that period.

#### **HV130 - ADENOVIRUS IN FECAL AND NASOPHARYNGEAL SWAB SAMPLES FROM CHILDREN ATTENDED AT HOSPITAL IN GOIANIA GOIAS**

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Human adenoviruses (HAdVs) may cause several clinical syndromes, and are a major cause of respiratory and acute gastroenteritis (AGE), especially among children. However, data on viral load, in more than one clinical sample obtained from the same child, are still scarce. The aims of the present study were to evaluate the frequency of the HAdV, to determine the load viral in clinical samples, and to proceed molecular characterization of positive samples from children up to five years of age. For this, 200 children attended at Hospital Materno Infantil in Goiânia, Goiás; between March 2014 and July 2015, were included in the study. One fecal and one nasopharyngeal swab sample was obtained from

each child (total of 400 samples), who presented AGE and/or respiratory symptoms. The clinical samples (fecal and nasopharyngeal swabs) were submitted the DNA extraction by a commercial kit (QiagenHilden, Alemanha), and screened by RTqPCR (TaqMan) assay, with specific primers and probe targeting the hexon region of HAdV genome. To determine the viral load of the samples a standard curve using serial dilutions of a recombinant plasmid was constructed. The global frequency of HAdVs was 21% (42/200). Positivity in nasopharyngeal swabs was 9.5% (19/200), and in fecal samples 16% (32/200). A higher positivity was observed among children up to 24 months old, when compared to the positivity in older children. Regarding symptoms, 64.5% (129/200) of the children had at least one AGE or respiratory symptom, and 35.5% (71/200) of the population was asymptomatic. Among the children that had at least one of the respiratory symptoms, 54% (108/200) were positive for HAdV in fecal samples and 9.2% (10/108) were positive in nasopharyngeal swab samples. Also, 20.9% (22/105) of the children with AGE symptoms, were positive for HAdVs in fecal samples, and 4.5% (9/200) were positive in both clinical samples (fecal and nasopharyngeal swab). Mean viral loads in fecal and swab samples were 1.57E+ 13 copies/g and 8.79E+ 11 copies/mL, respectively. Data reveals higher HAdV positivity rate in fecal samples, when compared to positivity in nasopharyngeal swabs. High viral loads were observed in both fecal and nasopharyngeal swab samples from symptomatic and asymptomatic children. After sequencing the positive samples, we hope to be able to establish associations between HAdV positivity and loads, and type of HAdV with the age and symptoms presented by the children.

**HV132 - ANALYSIS OF NUCLEOTIDE CHANGES IN HUMAN PAPILLOMAVIRUS 6 E6 REGION IN CONDYLOMA ACUMINATUM SAMPLES**

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Condyloma acuminatum (CA) or genital warts are benign proliferative lesions founded in the skin or mucosa, that are mostly related to types 6 and 11 of human papillomavirus (HPV). Papillomaviruses infect epithelia of vertebrates and may cause diseases or remain asymptomatic. These viruses contain the genes E6 and E7, which are responsible for the host epithelium transformation, and can lead to the development of carcinomas. Due the importance of the E6 protein in the process of carcinogenesis related to HPV, the aim of this study was identify variants and nucleotide alterations presents in E6 of HPV6 detected in condyloma acuminatum samples. We tested 31 condyloma samples positive to HPV6, that were submitted to the Polymerase Chain Reaction (PCR) using a pair of specific oligonucleotide to the E6 region and the products were submitted to electrophoresis on agarose gel 1%. Positive products to the E6 region were submitted to cloning using the pJET1.2 vector. These products were purified and sequenced by the dideoxy fluorescent terminal method using the BigDye® Terminator v3.1 Cycle Sequencing Kit. The computer program Phred/Phap/consed available on website <http://www.biomol.unb.br/> was used to the sequences quality analysis and the electropherograms were analyzed using BioEdit software. The prototype and samples sequences alignment in both directions were performed in CLUSTAL W program. A total of 24 samples were positive to the E6 region. In this way, the HPV6vc variant was identified in 11 of the samples analyzed, while the HPV6a variant was founded in other 11 patients. All the HPV6a samples showed a nucleotide alteration of G>A on position 474 of the genome, and one of this samples showed one more alteration in the 369 position, occurring the nucleotide change T>G. The

two samples rest have to be tested again. Because the changes in E6 sequence can influence the expression of the protein, it is believed that there are differences in the lesions progression in patients with different variants of human papillomavirus.

**HV134 - THE HUMAN PLATELET ANTIGENS (HPA) 1, 3 AND 5 POLYMORPHISMS IN HIV INFECTED PATIENTS**

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Introduction: Although HIV host cells express CD4 molecule, major receptor associated with virus entry in your target cells as macrophages and lymphocytes; the HIV presence has already been described in cells without CD4 cellular surface marker, including platelets. Platelets do not express CD4, then others molecules on the platelet surface could be candidates to HIV platelets ligation. Besides that cellular adhesion molecules have already been associated with the entry of the other virus in your target cells as adenovirus, rhinovirus and echovirus. The integrins can be a example of cellular adhesion molecule that has a role of the receptor or coreceptor for several virus. In particular, the Human Platelet Antigens (HPA) 1, 3 and 5 polymorphisms, which resides in integrins of the platelets surface, have been associated with viral infections and progression of the viral infection. These associations have already demonstrated to HCV, fibrosis progression in Chronic Hepatitis C and Dengue. The purpose of this study was to evaluate a possible association of the HPA 1, 3 and 5 polymorphism with HIV presence using samples from HIV infected patients. Materials and methods: Genomic DNA isolated from 100 HIV infected patients (whole blood) was used to genotyped HPA 1 and 3 by PCR-SSP, and HPA5 by PCR RFLP. A control group previously described, from blood donors (without HIV), from same region of this study, was used to data analysis. Results: The results showed that the HPA1a/1b and 1b/1b were significantly ( $p=0,004$ ) more frequent in HIV infected than control group (without HIV). In addition, there were deviation in Hardy-Weinberg equilibrium to HPA1 system. The HPA 1b allele frequency was, significantly, higher than control group (without HIV) ( $p=0,0021$ ). Conclusions: The results here obtained showed, for the

first time, an association of the HPA 1b allele and the HIV presence and, suggest that the integrin beta3 present in GPIIb IIIa glycoprotein complex, which resides on platelet membrane can be involved in HIV platelet ligation in infected patients.

### **HV135 - HPV PCR DETECTION AND GENOTYPING IN PARAFFIN EMBEDDED TISSUES (PETS) OBTAINED FROM WOMEN WITH INVASIVE CERVICAL CARCINOMA**

Sousa, V.B.P.; Peixoto, L.R.; Duarte, W.H.; Aguiar, M.F.G.; Silva, K.A.; Tafuri, A.; Pascoal Xavier, M.A.; Fernandes, P.A.; Vago, A.R.

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Introduction: Cervical cancer, the third most common neoplasia among women worldwide is a severe disease that constitutes a significant public health problem, especially in development countries. Human papillomavirus (HPV) is considered the main etiologic agent for the development of cervical cancer and its precursor intraepithelial lesions. Highrisk (HR) HPV genotypes, namely HPV 16 and, to a lesser extent, types 18,45,56,31,33,35,51,52 and 58 are most frequently found in premalignant and malignant anogenital lesions, whilst lowrisk (LR) HPV types are with benign/condylomatous lesions. Paraffinembedded tissues (PETS) represent an unlimited source of material for analysis in relevant retrospective studies. Aims: This study aimed to investigate the prevalence of HPV infection and of the oncogenic HPV16, 18 and 31 types in 61 cervical PET biopsies obtained from women with invasive cervical cancer from Belo Horizonte city, Minas Gerais state, Brazil. Methodology: Five tissue sections of 57 µm obtained in microtome were submitted to the DNA extraction step. HPV DNA search was performed by using a sensible Nested PCR protocol with MY09/MY11 and GP5/GP6+ primers sets, which are able to respectively amplify a 450bp and a 150bp DNA fragments from the conserved L1 gene from HPV genome. An efficient Hemi-nested PCR protocol was employed for searching DNA-fragments from the 16, 18 and 31 HRHPVs and detecting HPV DNA fragments of 149bp, 177bp and 249bp amplified from these HPVs genomes, respectively. The integrity of DNA extracted from tissues was assessed by

PCR using the PC03/PC04 set primers which are able to amplify the 110bp DNA fragment from the human  $\beta$ -globin gene. Results: Among the 61 analyzed samples 48 (79%) showed positive amplification for the human  $\beta$ -globin gene. The HPV DNA detection was verified in 35 (58%) out of the 61 analyzed samples, with 21 (35%) cancer samples positive for HPV 16, 6 cervical tissues (10%) positive for HPV 18 and 5 cervical samples (8.5%) positive for HPV 31. Conclusion: Our results demonstrated a relatively high prevalence of HPV DNA infection among the analyzed cervical cancer biopsies. Additionally, a higher prevalence for HPV 16, followed by HPV 18 and HPV 31 could be observed among the studied samples. However the observed prevalence of HPV DNA and the HR types may be possibly underestimated, since the DNA integrity isolated from PETS might be damaged by a deficient DNA conservation of these archival tissue samples.

### **HV137 - DNA PREVALENCE OF THE HIGH RISK (HR) HPV58 IN CERVICAL SAMPLES OBTAINED FROM CERVICAL SAMPLES OBTAINED FROM WOMEN WITH CYTOPATHOLOGY ABNORMALITIES**

Peixoto, L.R.; Sousa, V.B.P.; Aguiar, M.F.G.; Duarte, W.H.; Lopes, L.V.A.; Fonseca, L.P.; Toppa, N.H.; Vago, A.R.

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Introduction: Cervical cancer is one of the most common causes of cancer-related death in women worldwide. Persistent infections with highrisk (HR) types of human papillomavirus (HPV) consist the main risk factor for the cervical cancer (SCC) and pre neoplastic lesions development, which are classified by means of cytopathology criteria as lowgrade (LSIL) or high-grade (HSIL) squamous intraepithelial lesions (SILs). According to phylogenetic and molecular epidemiology studies, the HR HPVs types 16,18,45,56,31,33,35,39,45,51,52,56 and 58 are closely associated with SCC and SILs progression. Very recently, a novel 9valent HPV L1 Viruslike Particle (VLP) vaccine (Merck) was developed, which is directed against the L1VLPs from types 6/11 (lowrisk), 16,18,31,33,35,45,52 and 58. Despite the epidemiological association to cervical cancer and

precursor lesions worldwide, there is a scarcity of data concerning the HPV58 prevalence among the worldwide women, including the Brazilian ones. Therefore, the purpose of this work was to investigate the HRHPV58 prevalence among women from Belo Horizonte city, MG state, Brazil. Methodology: The prevalence of HPV58DNA was evaluated by using a conventional PCR protocol and the E7CR3/585F set primers which are able to amplify a 375bp DNA fragment from the E6/E7 genes from the HPV genome. DNA samples were isolated from 187 liquid-based cytology (LBC) cervical samples obtained from patients from Belo Horizonte city and exhibiting cervical alterations. According to the cytological diagnosis, patients were diagnosed as presenting ASCUS (70), LSIL (111) and HSIL (6). Results: This study is the first to investigate the HPV58 type prevalence among women from Minas Gerais state. HPV58DNA was observed in 25 (13.4%) out of the 187 analyzed samples, with the follow prevalence verified among the lesion groups: 10% (7/70) from the ASCUS cases, 15.3% (17/111) from the LSIL samples and 16.6% (1/6) from the HSIL cases. Conclusions: Our results pointed out a relatively high incidence of HPV58 infection among the analyzed women, who were mainly diagnosed as exhibiting low-grade (ASCUS and LSIL) cervical lesions. By considering that most of women submitted to the cytology cervical screening were diagnosed as LSIL, our data emphasize the relevance of the HPV58 type inclusion in newly designed vaccines to effectively prevent this HR type dissemination among the sexually active women.

#### **HV138 - THE ABSENCE OF THE PROTECTOR EFFECT FROM HIV GWG VARIANT IN HIV/HBV AND HIV/HCV COINFECTED PATIENTS**

**Watanabe, T.; Massolini, V.M.; Barbosa, F.H.; Silva, G.F.; Barbosa, A.N.; Simões, R.P.; Ferrasi, A.C.; Pardini, M.I.M.C.; Grotto, R.M.T.**

UNIVERSIDADE ESTADUAL PAULISTA

The subtype B of Human Immunodeficiency Virus (HIV) is the most frequent virus in Brazil and it has a variant, called B'. This variant has been described in the country and it codes the amino acid sequence GWG instead of GPG on the tip of gp120 V3 loop; GPG is the most common motif among variants of subtype B. The GWG variant has been associated to a slower progression of HIV infection, giving a better prognosis. However, these studies were

performed in HIV monoinfected patients but there are no studies concerning GPG association in HIV coinfecting patients with Hepatitis C (HCV) and B Virus (HBV). Here, it was evaluated if the HIV B' variant presents the same "protector effect" in HIV coinfecting patients with HBV and/or HCV. Plasma viral RNA isolated from 649 HIV infected patients was used to HIV subtyping, to infer the tip of V3 loop and the HIV syncytium-inducing (SI) ability. The progression of HIV infection was assessed by clinical evaluation (aids presence and HIV risk factors) and laboratorial data (CD4 count, plasma viral load, time of infection by HIV, HCV and/or HBV presence) obtained from the patient's medical records. The results showed a significant statistic association between HIV B' variant and CD4 count and time of HIV infection in HIV monoinfected. Although no difference among this variables were founded in coinfecting groups. This data can suggest that the B' variant does not induce the protector effect due in HCV or HBV presence. The mechanism involving in this process is unknown.

#### **HV146 - HPA 1A/1B COULD BE CONSIDERED MOLECULAR PREDICTOR OF POORLY PROGNOSTIC IN CHRONIC HEPATITIS C**

**Santos, F.M.; Picelli, N.; Silva, G.F.; Ferrasi, A.C. ; Sarnighausen, V.C.R.; Pardini, M.I.M.C.; Grotto, R.M.T.**

UNIVERSIDADE ESTADUAL PAULISTA

Hepatocellular carcinoma (HCC) can be developed in patients with Chronic Hepatitis C. Although previous studies have already demonstrated that HCC developing in patients with chronic Hepatitis C is influenced by age, sex and alcohol abuse, host genetic polymorphisms have been associated with HCC presence. Previous studies already demonstrated that the levels of the some integrins can be altered in HCC. The Human Platelet Antigen (HPA) polymorphism that resides in integrins has already associated with HCV presence and, specifically, the HPA1 system was related with fibrosis progression in Chronic Hepatitis C. It is unknown if there are association between HPA1 polymorphism and HCC presence. The goal of this study was to evaluate the association between the HPA1 polymorphism and HCC presence in patients with chronic Hepatitis C. HPA genotyping was performed from 76 HCV infected patients by PCRSSP. There were no association between patients with and without HCC. There was significant

difference ( $p < 0.05$ ) in HPA 1 genotypic frequency distribution between patients with HCC and with lower (F1/F2) fibrosis degree but not with advanced fibrosis (F3/F4). The results suggest, for the first time, that the polymorphism HPA 1a/1b can be constitute a molecular marker of the poorly prognosis in chronic Hepatitis C.

**HV147 - THERAPEUTIC RESPONSE TO SECOND GENERATION DIRECT ANTIVIRALS FROM PATIENTS WITH CHRONIC HEPATITIS C. EXPERIENCE OF THE GASTROENTEROLOGY DIVISION OF THE BOTUCATU CLINICAL HOSPITAL, UNESP**

Santos, F.M.; Barreto, S.F.D.; Alho, M.J.O.; Assis, R.C.F.P.; Silva, C.N.; Poli, G.B.; Nunes, C.; Santos, F.M.; Silva, G.F.; Grotto, R.M.T.; Pardini, M.I.M.C.

UNIVERSIDADE ESTADUAL PAULISTA

The Hepatitis C affects 185 million people in the world. In Brazil, there are, approximately, 1.4 to 1.7 million of the persons infected by Hepatitis C Virus (HCV). The Hepatitis C course involve, in most f cases, a subclinical evolution, with about 80% of the patients with asymptomatic infection. This chronic infection can lead to cirrhosis and hepatocellular carcinoma. The therapeutic success in Chronic Hepatitis C is the achievement of the Sustained Virologic Response (SVR), defined as undetectable viral load after 12 or 24 weeks after finished the therapy. Nowadays, the Brazilian Ministry of Healthy provides the second generation of the DirectActing Antiviral Drugs (DAAs) to Chronic Hepatitis C treatment: Sofosbuvir, a polymerase inhibitor; Simeprevir, a protease inhibitor and Daclatasvir, NS5B inhibitor. The therapeutic success should be performed using the HCV RNA quantification (Viral Load) in plasma. The Brazilian protocol to confirm SVR involves to performed the Plasma Viral Load before treatment, 12 and 24 weeks after finished the therapy using qPCR assay. The goal of this study was evaluate the SVR and relapsed (patients with undetectable HCV RNA at the end of treatment but again detectable within the following 6 months) in patients assisted in Gastroenterology Division of the Botucatu Clinical Hospital. Samples from 38 patients with Chronic Hepatitis C that finished the treatment with second generation DAAs were included in this study. These patients were separated in two groups: G1 (26 patients treated with sofosbuvir and daclatasvir) and G2 (12 patients treated with sofosbuvir and simeprevir). The results showed that

in G1 96.1% of patients presented undetectable plasma viral load in the end of the treatment. In G2 75.0% of patients presented undetectable viral load in the end of the treatment. In G2, 8.3% of patients did not present the information about viral load in the end of the treatment. In relation to SVR, 34% of patients presented Plasma Viral Load undetectable 12 weeks after end of the treatment (SVR). On the other hand, in G2 75% of patients achieved SVR. The results here presented showed that the HCV new therapies with second generation DAAs are highly effective in controlling viral replication.

**HV149 - MOLECULAR EPIDEMIOLOGY OF RESPIRATORY SYNCYTIAL VIRUS STRAINS ISOLATED IN NORTHERN BRAZIL**

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Introduction: Respiratory infections are common causes of morbidity and mortality worldwide, representing a major public health problem due to its high incidence and easy dissemination in the community. Human Respiratory Syncytial Virus (HRSV) is an important pathogen associated with these infections, contributing approximately to 50% of the cases of pneumonia and 90% of cases of bronchiolitis in infancy. The HRSV belongs to Mononegavirales order, Pneumovirus family and Orthopneumovirus gender. Based on the genetic diversity of the F and G proteins, this virus is classified into two groups, HRSVA and HRSVB, which have 24 and 20 genotypes, respectively. In this context, studies have been conducted worldwide aiming to analyze the genetic diversity of HRSV and its circulation factors. Objective: Characterize genetically HRSV strains isolated in the Northern Brazil, between January 2015 and January 2016, and to determine the most frequent HRSV subgroup, identify current genotypes and describe the period of greatest circulation HRSV in this population. Material and Methods: 57 HRSV strains, isolated in cell cultures, were analyzed to the molecular characterization of the

G protein gene, in four main steps: a) extraction of viral RNA; b) G gene amplification by RTPCR; c) sequencing d) phylogenetic analysis. Results: The distribution of the 57 HRSV positive cases by age showed that the majority of patients, 38 cases (67%) had between zero and four years of age. The State of Amazonas had the highest number of positive cases, 23 (40.4%). The circulation of HRSV focus in the first months of the year, especially between the months of March and July, climate transition period in the study area. Viral subgroup was present in 45 (79%) specimens, of these, 40 were in the HVRS B subgroup and five to HRSV A subgroup. Conclusion: HRSV infections occurred predominantly in children aged zero to four years old. It was the HRSV circulation in all the studied States, with the exception of the states of Roraima and Amapá. The circulation of HRSV was related to the climate transition. Both HRSVB subgroups and HRSVA circulated in the period studied, with the Buenos Aires HRSVB genotype being the most common in the North of Brazil. The detection of ON1 genotype the HRSVA in this study configured in the first report of movement or circulation of this genotype in Brazil.

#### **HV150 - HUMAN CYTOMEGALOVIRUS IN PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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The Human Cytomegalovirus (HCMV) is an important cause of morbimortality in recipients of allogeneic hematopoietic stem cell transplantation (AloHSCT). However, there is not a consensus on which protocol to use for monitoring the infection by HCMV and, data on the frequency and clinical manifestations of the infection in this group population are quite variable among the distinct transplant centers in the world. Thus, the main objective of the present study was to proceed the monitoring of active HCMV infection in patients undergoing AloHSCT by three different methodologies: antigenemia (AGM), nestedPCR and realtime PCR (qPCR) and determine viral load, correlating active infection with the clinical manifestations and prognosis

of patients. For this, 21 patients undergoing AloHSCT were monitored (from pretransplant period 5 days prior to transplantation until one year after transplantation). For HCMV detection three methodologies were used: AGM, nestedPCR and qPCR, and for molecular detection a comparison was made between detection of HCMV in DNA extracted from peripheral blood leukocytes (PBL) and sera, in a paired manner. The results showed that the active HCMV infection was detected by at least one of three methodologies in 95.2% (20/21) of patients and 45% (9/20) of these were positive in pretransplantation period, having been observed good agreement between the results of AGM and qPCR ( $\kappa = 0.65$ ). Of the 20 patients positive for active HCMV infection, 85% (17/20) were positive for the three methods and only 15% (3/20) were positive for AGM and qPCR, and negative by nested-PCR. Regarding the type of clinical sample, molecular techniques showed higher sensitivity to the PBL over the sera. The main alteration of patients was pancytopenia and the main complication was graft versus host disease. Six patients died during the study period, however, it was not possible to confirm if HCMV active infection was directly associated with the cause of death. The obtained data reveal a high positivity index and the occurrence of HCMV syndrome in patients submitted to aloHSCT. We hope that the results may assist in the therapeutical measures, as well as in the methodology of choice and the type of clinical sample for detection of active HCMV infection, in order to contribute for the inclusion of HCMV monitoring is included in the routine testing of patients.

### **HV153 - GENETIC DIVERSITY OF THE INFLUENZA VIRUS B STRAINS CIRCULATING IN THE AMAZON REGION**

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2. FACULDADE METROPOLITANA DA AMAZÔNIA
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The Influenza B virus is one of the etiological agents of flu, is associated with epidemics throughout the world. It has two antigenic and genetically distinct lineages, known as Yamagata and Victoria. Constituting the viral particle are two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), and the genes encoding these proteins are those which are most selective pressure which induces occurring mutations that may cause strains capable of escaping the immune system and resistance to antiviral drugs. Thus, the genetic diversity of influenza B virus impacts the vaccine formulation and possible treatment failure. In order to assess the genetic diversity of circulating influenza B virus strains in the Amazon Region, from January 2013 to December 2015, was carried out the genetic characterization of the HA and NA genes. The methodology involved extraction of viral nucleic acid followed by RTPCR (polymerase chain reaction preceded by reverse transcription) for amplification of the HA and NA genes and their sequencing. The analysis of the circulation pattern showed that the peak activity of influenza B virus was concentrated in the first half of the years studied, except for the year 2013 in which a second peak was identified in the second half. The circulation of both Victoria and Yamagata lineages of influenza B strains were detected in 2013 and 2014, and the Victoria was more prevalent, thus being at odds with the vaccine strain that belonged to Yamagata lineage, since in 2015 only Yamagata lineage it was detected, supporting the vaccine strain. The analysis of aminoacidic changes in the HA changes were observed in both Victoria and Yamagata lineages. Among the replacements were found N141D and N144D (150 loop) in Victoria lineage strains and P123A, K131N, Q137K (120 loop) I165S (160 loop), T197K and T197A

(190 helix) in Yamagata lineage strains. Regarding the NA, the E105K substitution was detected in Victoria and D197N lineage and P139S in Yamagata lineage, both related resistance to neuraminidase inhibitors. The detection of influenza B virus was more evident in the first half of the year in the Amazon Region, consistent with the rainy season. There was disagreement circulating strain against vaccine strain in the years 2013 and 2014. Mutations were observed, which resulted in aminoacidic substitutions in antigenic sites of HA. There were aminoacidic substitutions in NA associated with resistance to antiviral used to treat influenza.

### **HV159 - PDGFA OVEREXPRESSION IN PLATELETS IS ASSOCIATED WITH ADVANCED HEPATIC FIBROSIS IN CHRONIC HEPATITIS C**

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UNIVERSIDADE ESTADUAL PAULISTA

Activation of hepatic stellate cells (HSCs) is the primary event that leads to fibrosis in chronic hepatitis C. TGF- $\beta$ 1 is the most profibrogenic cytokine for HSCs and PDGF the most potent mitogen, so both have essential roles during fibrogenesis. The aim of this study was to evaluate the expression profile of TGFB1 and PDGF mRNA in hepatic tissue and platelets from HCV carriers. Fortythree patients were evaluated according two groups depending on the fibrosis degree: G1 (n=19, F1 and F2) and G2 (n=24, F3 and F4). mRNA expression of those growth factors in hepatic tissue and platelets was evaluated by qPCR. Expression of PDGFA mRNA in platelets was significantly higher in G2. Although we did not find a significant association, TGFB1 and PDGF A mRNA was more expressed in platelets when comparing with hepatic tissue, differently from PDGFB that was more expressed in hepatic tissue. These results suggest that an alternative pathway for the development of fibrosis mediated by over expression of TGFB1 and PDGFA in megakaryocytes (platelets precursors), could be involved in fibrosis developing, contributing to the HSCs activation and, to the process of fibrogenesis.

### HV160 - INFLUENCE OF SNP 764C> G INTERFERON GAMMA GENE IN PATIENT RESPONSE TO TREATMENT OF THE HEPATITIS C GENOTYPE 3

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Infection by hepatitis C virus (HCV) is a major cause of cirrhosis and hepatocellular carcinoma, which induces the need for liver transplantation. In recent years, new factors are related to predicting success of HCV treatment, among them are the single nucleotide polymorphisms (SNPs) in many genes, particularly those related to the innate immune response of the patient. Interferon-gamma (IFN $\gamma$ ) is involved in the host response to HCV infection. It has been reported that 764C> G variant increased the efficiency of this gene promoter up to three times, improving the host's response to treatment for HCV. There is little information the influence of this SNP in patients infected with HCV 3 and in a country with mixed ethnicity like Brazil. The objective this project was verify the presence of the polymorphism 764C> G of the IFN $\gamma$  gene in patients chronically infected with HCV nonresponders to treatment with IFN and ribavirin. For this, were used 52 samples from patients positive for HCV, from these 31 positive for viral RNA (group I). The polymorphism site sequence was amplified using a high fidelity DNA polymerase, using specific primers. After amplification, the fragments were purified and sequenced by the Sanger method. The generated sequences were evaluated by PHRED software / phrap / CONSED (<http://www.bioinformatica.ucb.br/electro.html>). Statistical analysis was made using the BioEstat software version 5.3. The variant 764C> G was found in two samples of group I, as well as two of the group II, being the frequency of this polymorphism 6.45% in group I and 9.52% in group II. The comparative analysis of the groups, by  $\chi^2$ , showed no significant difference for the presence of the variant in each group ( $p = 0.1594$ ). The analysis is not consistent with the literature, which showed correlation of SNP 764C> G with spontaneous cure for HCV genotype 1 patients. Therefore, the polymorphism 764C> G of the IFN $\gamma$  gene cannot be used as predictive factor of response for viral genotype

patients type 3 in Brazil. However, it is important to highlight that the number of samples was low, which may interfere with the final result, cannot infer it with such certainty and therefore more studies are needed.

### HV161 - EVALUATION OF THE HLA CLASS I KILLER IMMUNOGLOBULINLIKE RECEPTORS (KIR) LIGANDS IN HIV/HCV COINFECTED PATIENTS

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UNIVERSIDADE ESTADUAL PAULISTA

The first immune defense against pathogens is the innate response, which natural killers cells have a fundamental role. These cells present in their surface receptors named KILLERIMMUNOGLOBULINLIKE RECEPTORS (KIR). These receptors (KIR) interact with HLA class I molecules presenting inhibitory or stimulatory effects. The HIV infection progression is dependent on viral and host factors. Polymorphisms in the KIR genes and ligands HLA class I (A, B and C), have been associated with HIV progression. Although the specific KIRHLA combination with HIV progression has already been well described in literature, there are no reports about this in patients with coinfection HIV/HCV. The goal of this study was evaluated the influence of the ligands KIR-HLA polymorphisms in the HIV infection progression in HIV/HCV coinfecting patients. Genomic DNA was isolated from 251 patients and used as source to genotype KIR genes by PCRSSP and HLA class I genes by sequencing. The patients included in this study were attended in Botucatu Medical School. The patients were separated in Group 1: HIV monoinfected (n=100), Group 2: HCV coinfecting, Group 3: HIV/HCV coinfecting patients. The information about HIV infection progression was obtained from patients medical reports. The results showed that ligands KIRHLA class I were more frequent in group 1, 2 and 3, where KIR2DL3C1/X (77%, 33%, 47%), KIR3DL1BW/X (53%, 47%, 60%), KIR2DS1C2/X (50%, 63%, 67%) respectively. (/X is the heterozygosis of the ligands KIRHLA with ligand non allele). The statistical analyzes showed no significant difference ( $p > 0.05$ ) among the groups (G1, G2, G3) according to KIRHLA class I ligands. Then, the HCV presence did not seem to affect the influence on combination in HLA class I KIR genes in HIV infection progression. Others genetic

polymorphisms can be evaluated to infer about the HIV infection progression in HIV/HCV patients.

#### **HV162 - DETECTION AND EPIDEMIOLOGIC PROFILE OF HUMAN ADENOVIRUS IN PEDIATRIC PATIENTS WITH ACUTE RESPIRATORY INFECTION**

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UNIVERSIDADE DE SÃO PAULO

Human Adenovirus (HAdV) belongs to the Adenoviridae family, genus Mastadenovirus, and has a genome of doublestranded DNA. HAdV is an important etiologic agent responsible for various diseases in adults and children, particularly respiratory tract infections, eye infections, gastroenteritis and hemorrhagic cystitis. Currently there are 68 known types of HAdV divided into seven species (HAdVA to HAdVG). In the case of acute respiratory infections, they are associated with HAdV B, C and E species. Acute respiratory infection in the lower tract is the fourth leading cause of death worldwide. In children under two years of age, Human Adenovirus is responsible for 5 to 15% of viruses that cause acute respiratory infection and 1 to 5% of all respiratory infections, which shows the importance of surveillance and monitoring of HAdV. In this context, 1129 nasopharyngeal aspirate samples from children under five who presented acute respiratory infection framework were collected in the University of São Paulo's University Hospital (HUUSP), in 2015. Samples were extracted by automated method and analyzed by real-time polymerase chain reaction for detection of HAdV. Preliminary results showed approximately 20% of the samples tested positive for the virus.

#### **HV165 - COMPARISON OF DIFFERENT CLINICAL SAMPLES FOR DETECTION OF ZIKA VÍRUS IN SYMPTOMATIC PATIENTS BY REALTIME PCR**

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Introduction: Zika virus (ZIKV) has been described recently causing problems for humans as a disease able to result in complications such as skin rash, arthralgia, myalgia and conjunctivitis. In addition, if a pregnant woman is infected with ZIKV may result in a natidead

or in some cases in infants with microcephaly, which is drawing the attention of the public health community. Because of that, there is an urge need for better diagnosis tools for health providers as well the identification of the most valuable clinical samples for diagnosis. Objective: Our aim was to compare different clinical samples and verify which one is more feasible for ZIKV diagnostic by RT PCR. Methodology: Samples of saliva, urine, semen, and serum of patiente which showed DENVlike symptoms, GuillanBarr or microcephaly, were extracted on the NUCLISENS® easyMag® platform (BioMerieux). The RTPCR reaction was carried out for ZIKV, DENV and CHIKV with RNA from each sample with a set of primers and probes with FAM as dye reporter for the probe, as previously described by Lanciotti et al., 2008; Wagner et al., 2004 and LU et al., 2012, respectively. Patients positive for ZIKV, were followed for 60 days. Results. We analyzed samples of 38 patients, which showed DENVlike symptoms, 1 patient with Guillan Barr and 5 newborn with microcephaly. Of these, 13.63% (6/44) were ZIKV positive and 6.81% (3/44) were DENV for at least one of the tested specimens. Of the 6 patients positive for ZIKV, 1 collected only serum and 5 colleted urine, Saliva and Serum and 2 of these five collected semen. Of the total patients, 3 had ZIKV detected in the serum and with a maximum of 8 days after the onset of symptoms, 5 had ZIKV in the urine with detection time 30 days, 2 patients were positive in salive for 30 days and 2 were positive in the semem for at least 60 days after the onset. A baby with microcephaly persisted until 56 days after the birth in urine e 64 in the serum. Conclusion: Data demonstrated that samples of semen and urine presents ZIKV for longer time, what make them the best option for ZIKV diagnosis, when possible.

### **HV173 - ANALYSIS OF EPIDEMIOLOGICAL PROFILE, SEASONAL AND MOLECULAR OF CHILDREN INFECTED WITH ADENOVIRUS HOSPITALIZED AFTER THE INTRODUCTION OF THE ROTAVIRUS VACCINE IN BELÉM, PARÁ**

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2. UNIFAP
3. UFPA

Gastroenteritis are the third cause of infant morbidity and mortality worldwide, especially among children under 5 years old. Adenoviruses (HAdV) are icosahedral nonenveloped virus, has 240 proteins "hexon" specific and doublestranded DNA. They belong to Adenoviridae family, Mastadenovirus genre, are distributed in 7 species (A to G) and 57 serotypes. Epidemiological studies found AdV in 214% of cases of acute childhood diarrhea in hospitals and clinical ambulatories. The main purpose of this study was to detect the presence, define the epidemiological profile and types of adenoviruses in stool samples from 842 children under three years of age, hospitalized with gastroenteritis and vaccinated against rotavirus; participants of the study "Rotavirus case control" and conducted by Instituto Evandro Chagas, from May 2009 to April 2011 in Belém, PA, CEP n. 0013.0.72.00011. ELISA and immunochromatography techniques were used for screening; and PCR and sequencing nucleotides for typing and molecular identification. The AdV were found in 7.2% (61/842) of the tested sample, with the enteric adenoviruses (ADE) being 50.8% (31/61) of the positive cases. The analysis on the gender of the infected children showed 7.7% (28/362) being female and 6.8% (33/480) were male. Positivity by age of the patients analyzed, detected a higher prevalence among those over 24 months of age, corresponding to 8.9% (16/178) of all positive cases. Regarding Ads temporal distribution, the month of June was the most prevalent, with 11.4% (8/70) of the total cases. The sequencing reaction characterized the species F as more prevalent in our region, accounting for 64.5% (29/45) of the sequenced samples, with the type 41 detected in 69% (20/29) of positive cases for this species and 31% (9/29) was characterized as type 40. The results of this study confirm the presence of these viruses in the city of Belém, demonstrating their

importance as a cause of gastroenteritis requiring hospitalization in children under 3 years; especially after the introduction of rotavirus vaccine in Brazil.

### **HV176 - PRESENCE AND GENOTYPING OF HUMAN PAPILLOMAVIRUS IN CONDYLOMA ACUMINATA LESIONS**

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Genital human papillomavirus (HPV) infection is the most common sexually transmitted disease. The HPV infection produces a wide range of disease presentations, from asymptomatic infection to benign genital warts to invasive cancer. It is extremely common and affects between 2% and 43% of the female population worldwide. Human papillomaviruses are members of the Papovaviridae family of epitheliotropic doublestranded DNA viruses and are considered tumor viruses because of their ability to immortalize normal cells. Currently more than 130 types of HPV have been identified; characterized as "lowrisk" types which are associated with genital warts and respiratory papillomatosis, or as "highrisk" which are associated with invasive cancer. Condylomata acuminata, are the most common virally transmitted STD, affecting 1.9 million Brazilians each year. It is most associated with the low risk HPV types 6 (70% of cases) and 11 (20% of cases) although, the highrisk HPV co infection is common. Therefore, the objective of this work was to improve the knowledge about the pathogenesis of the condylomata acuminata and thus establish more appropriate therapies for HPV infection and injury caused. On this basis we investigated the presence of HPV as well as their genotype in 44 genital lesions from 44 patients from Ribeirão Preto city and region; collected at the Clinical Hospital of Ribeirão Preto Medical School. For this purpose we used the oligonucleotides PGMY09/11 and GP5+/6+ in the polymerase chain reaction (PCR) to detect the virus and sequencing reaction according to the Sanger method

for HPV genotyping. As a result it was observed the presence of HPV in 43 samples (95.5%), where all these samples were successfully genotyped. The low risks were predominant between the HPV types detected (97.7%) specifically, the most frequent types were HPV6 (63.6%) following by HPV11 (20.4%). The HPV6 variants were also analyzed and showed the presence of HPV6vc and HPV6a in a half samples. The results are in agreement with the literature that shows the presence of HPV in condylomata acuminata lesions as well as the prevalence of low risk HPV types (60%), mostly HPV6. Genital HPV infection is the most common STD and is responsible for a wide range of conditions from benign warts to anal cancer. The obtained results can contribute to a better understanding about HPV infection and management and treatment of condylomata acuminata lesions.

#### **HV183 - HIGH FREQUENCY OF RESPIRATORY VIRUSES IN ASYMPTOMATIC AND SYMPTOMATIC CHILDREN ATTENDED IN PEDIATRIC HOSPITAL, GOIÂNIA GOIÁS**

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The acute respiratory infections (ARIs) are an important cause of morbidity and mortality worldwide. They are responsible for more than four million of deaths annually, affecting mainly children and elderly people. Children fewer than five years have about four to six ARIs per year and this is a common cause of hospitalization, mainly in developing countries. With regard to etiologic agents associated with respiratory infections, viruses have a prominent role. In Brazil, particularly in the West Central region, studies that evaluate the circulation of respiratory viruses in the pediatric population are scarce. Thus, this study aimed to investigate the occurrence of respiratory viruses in the pediatric population, from Goiânia Goiás. Between May/2014 and May/2015, 251 samples of nasal swabs were collected from children between zero and six years of age presenting or not respiratory symptoms, attended at the reference children's hospital in Goiânia. For the molecular screening, three Multiplex Nested-PCR protocols were performed. The first Multiplex Nested-PCR was performed using specific set of primers targeting the nucleoprotein gene for influenza virus (FLUA, B and C) and the F gene for respiratory syncytial

virus (RSVA and B). The second Multiplex Nested-PCR was performed using specific set of primers targeting the hemagglutinin neuraminidase gene for parainfluenza viruses (PIV14), the S gene for coronavirus (HCoV) and the 5'NCRVP4/VP2 genome region for rhinovirus (HRV) and enterovirus (EV). The third Multiplex Nested-PCR was performed using primers targeting the hexon gene for adenovirus (HAdV), the NP1/VP1/VP2 genome region for bocavirus (HBoV) and the matrix gene for human metapneumovirus (hMPV). It was observed a global detection rate of 35.9% (90/251), being rhinovirus (31%) and respiratory syncytial virus (27,4%) the most prevalent. Similar detection rate was observed among the groups symptomatic (37%) and asymptomatic (34,5%). It was observed codetection rate of 6,4% (16/251), mostly in samples of symptomatic patients (13/16) ( $p < 0,05$ ). The results reinforce the importance of respiratory viruses in children and contribute to further understanding of the epidemiological factors associated with different pathogens in our region. Thus, they open the way to new studies in the state of Goiás, also providing information to assist in the construction of control measures and more effective prevention of these infections.

#### **HV184 - VALIDATION OF A MOLECULAR PANEL FOR HSV AND ENTEROVIRUS IN CEREBROSPINAL FLUID FOR CLINICAL USE IN A PRIVATE HOSPITAL IN SAO PAULO, BRAZIL**

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HOSPITAL ISRAELITA ALBERT EINSTEIN

Viruses are common etiological agents of central nervous system (CNS) infections. A rapid molecular diagnosis is recommended to improve patient management. Clart Entherpex kit allows the simultaneous detection of these herpesviruses: Herpes Simplex Virus types 1 and 2 (HSV-1 and HSV2), Varicella Zoster Virus (VZV), Epstein Barr Virus (EBV), Human Cytomegalovirus (CMV), Human Herpes Virus 6, 7, 8 (HHV6, HHV7 and HHV8), as well as the most clinical relevant Enteroviruses (Poliovirus, Echovirus, Coxsackievirus), including enterovirus 71 (EV71). For the diagnosis of these virus infections in the CNS, we validated a laboratory test based on this kit for their detection in the cerebrospinal fluid (CSF). METHODS: Nucleic acids from cerebrospinal fluid (CSF) samples

were extracted using automated EasyMag system and submitted to a multiplex reverse transcription PCR DNA microarray test (CLART® ENTHERPEX kit) for human Herpesvirus and Enterovirus detection. Validation was conducted according to CAP guidelines. We evaluated accuracy, reproducibility and sensitivity. Accuracy was tested comparing obtained results with previous assays for the detection of single agents carried out in our laboratory or in references laboratories. Sensitivity was evaluated by dilutions of a commercial control with known concentration. RESULTS: For accuracy, we compared the results of 35 samples and obtained 97,2% correlation. Reproducibility was performed using two negative samples and two positive samples (HSV2 and enterovirus), tested in three different days by different persons. All the results were concordant. The test was able to detect viral load of 200 copies/reaction with 100% confidence for HSV2 and 90% confidence for HHV-6. CONCLUSION: The results of validation demonstrated that the kit is a valuable molecular diagnostic tool for virus infections and the implementation of this technique allows the detection of different Herpesviruses and Enteroviruses in CSF samples. This method has been incorporated into the routine of our laboratory allowing the etiological diagnosis and establishment of more effective medical treatment for each patient.

#### **HV190 - CITYPIGEONS AS CARRIERS OF HERPESVIRUS**

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Although they may seem inoffensive, city pigeons may be a public health problem and are of importance to veterinary and epidemiological surveillance. *Columbia livia domestica* is the pigeon specie most widely distributed in urban areas in Brazil. As in most urbanized areas around the world, they have had uncontrolled proliferations and now cohabits with humans in public places, open food courts and many other highly

populated areas. They are proven sources of infection and transmission of various fungal and bacterial diseases but little is known about its potential for the spread of viruses. This study shows the identification of viruses with zoonotic potential in asymptomatic pigeons of the Tietê Ecological Park, São Paulo SP, Brazil, using metagenomic with next generation sequencing analysis and Nested-PCR for validation. This analysis was performed using oropharyngeal and cloacal swabs from 10 *Columbia livia domestica* pigeon specimens collected in 2011. Samples were submitted for pretreatment (filtration and DNase/Proteinase K reaction) and the RNA extraction was conducted through a QIAamp Viral RNA Mini Kit. From an equimolar pool, an RNA library was prepared and was submitted for RNASeq in HiSeq 2500 Sequencing System (Illumina), pairedend (2x 100bp). The genome assembly was made with Metavelvet, and the annotation with UniProt. 83,944,578 reads were obtained, from which 80,67% had Q>30 score. Ten of 39,971 scaffold assemblies matched Human Herpesvirus 6A (HHV6A) (Q69566\_U88). The samples were further investigated by conventional PCR assays for validation using the Van Der Hanter (1996) protocol, which resulted in one positive cloacal swab for Herpesvirus. Herpesviridae is a large family of DNA viruses that cause diseases in animals and in humans. Whilst the initial objective was the RNA virus detection, was also possible the detection of dsDNA viruses as Herpesvirus. The conclusion can be made that metagenomic and NGS analysis followed by PCR for validation is a sensitive, fast and efficient method for virus detection and can be used as a strategy for epidemiological studies. HHV6A has been described to be more neurovirulent, and as such is more frequently found in patients with neuroinflammatory diseases such as multiple sclerosis. Sequencing and phylogenetic analysis are still to be performed. However, these results already indicate that city pigeons may serve as reservoirs for Herpesviruses and may lead to viral transmission to humans.

### **HV191 - DETECTION, GENOTYPING AND VIRAL LOAD QUANTIFICATION OF CHIKUNGUNYA VIRUS IN CANCER PATIENTS**

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Chikungunya virus (CHIKV) is a +(ss)RNA arbovirus that belongs to the Alphavirus genus of Togaviridae family. In 2015, the first autochthonous cases were diagnosed in Rio de Janeiro State, and by April 2016, an outbreak was declared. Little is known about CHIKV infection in cancer patients and whether there was a difference in viral load in the different compartments (serum, plasma and urine) at the time of diagnosis. The aim of this study was to evaluate the performance of a RTqPCR method for diagnosis and viral load quantification, as well as to describe the circulating strain of CHIKV in patients with cancer (immunocompromised) and immunocompetent patients. We herein report on results of 22 CHIKV+ individuals, 14 cancer patients (INCA, Rio de Janeiro), and 8 immunocompetent patients, which were studied 37 days after the appearance of the first symptoms. Sample consisted of serum, plasma and urine; the extraction of viral RNA was carried out using the QIAamp Viral RNA Mini Kit (QIAGEN). Quantitation was performed by RTqPCR with CDC recommended primers and probes (TaqMan), and a standard curve. In cancer patients, 13/14 exhibited a positive result in serum, and 12/14 in plasma. Of note, 9/14 had a positive result in urine, and in one case, CHIKV was detected only in urine. Therefore, in cancer patients, the choice sample is serum, being the urine an important diagnostic complement. A positive correlation was observed between serum and plasma ( $R^2 = 0.886$ ,  $p < 0.001$ ) and between plasma and urine viral loads ( $R^2 = 0.685$ ;  $p = 0.029$ ). Viral loads were higher in serum and plasma vs urine ( $\sim 7 \times 10^4$  times) ( $p < 0.01$ ). No significant differences were found between cancer cases and controls. A segment of the CHIKV E1 glycoprotein gene was sequenced in 11 viral isolates, genotyping and phylogenetic reconstruction analyses allowed to determine a 98.99% similarity of Rio de Janeiro circulating virus with the East / Central / Southern Africa (ECSA) genotype. This is the first report of the CHIKV viral characteristics in the state of Rio de Janeiro, preOlympics games.

### **HV193 - AN APPROACH ON ZIKA VIRUS**

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Introduction: Zika virus is belonging to the genus flavivirus, family Flaviviridae, its genetic material consists of singlestranded ribonucleic acid with a positive sense. It is capable of infecting arthropod *Aedes aegypti* and *Aedes albopictus*, very common in countries with tropical climates, are also associated with the transmission of dengue and chikungunya. The virus can cause symptoms ranging from people, usually include hemorrhagic fever, headache, malaise and other symptoms also related to dengue with duration close to one week. One of the severe conditions of disease include impairment of the central nervous system and microcephaly. The relationship between microcephaly in newborns and contagion virus was observed in mid-2014 in Brazil and confirmed by the Ministry of Health at the end of 2015. Materials and Methods: For diagnostic saliva and blood samples are collected from people in risk areas or showing any symptoms of infection. Reverse transcription is performed by polymerase chain reaction (RTPCR) capable of producing multiple copies of the genetic material. The number of bands is observed in agarose gel and compared with data from recent studies on the virus genome. The amount of antibodies that have the individual is also an important factor for diagnosis. Results: Zika virus was observed and first isolated in 1947 in Africa. About twenty years later it was observed in other regions of Africa and Asia and into Oceania and later in Brazil. Much of the infected zika virus may have the mild form of the disease. However, the virus appears to have acquired the ability to infect humans efficiently since it was discovered. Currently, many of their genes are compatible with the human body, facilitating the transfer of the virus by the vectors to humans. Conclusions: The contamination zika virus is fairly frequent in tropical countries that behave *Aedes* mosquitoes. The main concern include the relationship between infection and syndromes such as microcephaly and GuillainBarré syndrome, but more studies are needed to prove this relationship. In Brazil, more efforts are needed to complete elimination of mosquito breeding sites in addition to clinical tests for rapid diagnosis.

**HV194 - ANALYSIS OF MUTATIONAL PROFILES OF HIV1 NEF IN PATIENTS SAMPLES WITH DIFFERENT HAART RESISTANCE PROFILES**

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Even after 30 years since the beginning of the HIV / AIDS epidemic, the disease still is an important public health issue. The introduction of antiretroviral therapy (HAART) positively affected the course of the disease, increasing the survival of infected individuals. However, in the absence of a cure for the disease, the treatment must be adopted for a long period of time and with the course of treatment, selection of viruses harboring drug resistance mutations is a limiting factor for the treatment continuity. We have previously characterized that the viral protein Nef regulates the activity of the viral Protease, and its absence decreases the sensibility of Protease to currently used Protease inhibitors. Given this, the study of the interactions of HIV1 proteins during its replicative cycle remains important to characterize new specific targets for drug intervention during viral replication. As well as, will provide data on the impact of these mutations occurring in genomic regions outside the target genes for the current therapy. Therefore, this study aims to analyze the nef gene from samples of patients under HAART regimen and presenting different profiles of drug resistance mutations in the RT and PR genes. Analyses of nucleotide sequences from different groups (high number of resistance mutations in RT and PR versus no resistance mutations in these genes), we saw that the synonymous/nonsynonymous mutation rate is higher than 1 in both groups, indicating that Nef is under negative selection. By observing the amino acid sequence from these samples, we found that there was a conservation of functional protein domains. However, we noted the presence of polymorphisms and some specific amino acid modifications that occur specifically in the group presenting high number of resistance mutations in RT and PR. In the region of  $\alpha$  helix we've noticed insertions in 67% of the study samples and in all of this insertions, the occurrence of Proline which can be related to stringencin this region. We also noticed changes in the diacidic domain in samples with mutations in the protease region compared to samples who didn't present mutations in the protease where, for this samples, there was an insertion of another acid

amino acid in this domain. Therefore, it is necessary to test if these changes could alter the protein structure and if they can affect the function of the viral PR or even the sensitivity to the protease inhibitors.

**HV204 - OUTBRED GENETICALLY SELECTED MICE FOR HIGH OR LOW ANTIBODY RESPONSE AS A MODEL TO STUDY THE IMMUNE RESPONSE TO HEPATITIS B ANTIGENS**

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The ability to produce antibody in response to immunization with hepatitis b surface antigen (s antigen) is controlled by autosomal dominantly expressed hla class ii molecules. as a consequence marked variation in antibody production to hepatitis b vaccines was found after immunization with this antigen alone. aiming circumvent the non-responsiveness a new generation vaccines containing epitopes of pre-s1/s2 and s proteins have been developed. since 1998 instituto butantan has been producing the recombinant hepatitis b vaccine containing only the s antigen. in order to study the modification in the immunogenicity promoted by different hepatitis b antigens a new yeast expressing the s protein and other surface components, pre-s1 and pre-s2, was produced and tested in genetically selected murine model. the two mice lines used were obtained by bidirectional selection according to the high (hiii) or low (liii) antibody responsiveness against salmonella flagelar antigens and showed to be useful to investigate the different patterns of immune response to rabies virus and mouse hepatitis virus 3. samples of purified s and pre-s1-s2/s antigens were evaluation by sds-page and by western blot, using anti-s specific serum and pre-s1 and pre-s2 mabs. hiii and liii mice groups received two doses of the s or pre-s1-s2/s antigens adsorbed in aluminum hydroxide by the subcutaneous route with 30 day-interval. individual serum was periodically collected and tested by elisa for specific antibodies titration. in addition, after an intraperitoneal antigen injection spleen cells were isolated and incubated for 3 days at 37oc in 5% co2 with s or pre-s1-s2/s antigens followed by stained with anti-mouse apc-cy7-cd4 and apc-cd8 antibodies and analyzed on facs. the liii mice immunized with s antigen presented higher specific igg

titles at 15 (p=0,02) and 30 (p=0,03) days than the hiii. the antibodies response to pre-s1-s2/s antigens were lower than the response to s antigen in liii (p= 0,04 and 0,01) in all times but equal in the hiii line. initial data indicated that hiii e liii mice have differences in cd4/cd8 response after immunization with s antigen but not with pre-s1-s2/s antigens. these preliminary results showed that both antigens elicited different levels of antibody response and also indicate that hiii and liii mice can be used as a model for studies of hepatitis b antigens response.

#### **HV211 - IS THE HTLV1 INFECTION RELATED TO METABOLIC DISORDERS?**

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HTLV1 causes a persistent and highly dynamic infection and it has associated with neoplastic disorders as well as degenerative inflammatory diseases. So far it is unclear why some people infected with HTLV develop diseases and other not. Early biomarkers have been studied. In order to know epidemiological and clinical laboratory aspects of this infection in the Southern region, HTLV positive individuals attended at Centro de Referência em Prevenção, Assistência e Tratamento CEPART in Itabuna have been monitored. The study was approved by CEP (CAAE 22727114700005526). After signing the TCLE, it was applied a structured questionnaire. Blood and feces were collected with vacuum blood tube and sterile pot, respectively, for hematological, biochemical, immunological and parasitological analyses and co-infections research. Blood samples were collected from all patients (100%) while feces samples only in 44.23%. It was analyzed 55 HTLV 1 infected individuals and 116 noninfected. Individuals infected were mostly women (82.70%), with low income and education levels. Epidemiological data suggest patterns of gender, income and education similar to other regions. Among the infected, 17.30% presented at least one reactivity to other serological marker (mainly total antiHbc), presented a higher number of basophils, and 23 (44.44%) individuals were parasitized. The main parasite was

Endolimax nana. Some metabolic disorders were detected in HTLV infected patients, as increased levels of triglyceride (>150mg/dL) (p=0.047). In addition, total cholesterol levels were higher in women HTLV positive (p=0.02). As the nervous system has a high percentage of lipids in their composition, dyslipidemia can be related to viral pathogenesis, and the higher levels of cholesterol in infected women can be associated to the preponderance of HTLV infection in this group. Evidence for an association between metabolic disorders and HTLV1 infection has been suggested, but this question remains unclear and need to be clarified.

#### **HV216 - MAMMARENAVIRUSES IN WILD RODENTS (CRICETIDAE:SIGMODONTINAE) DETECTED BY METAGENOMIC TOOLS**

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A complex mixture of predisposing factors has created new opportunities in our modern world for the emergence of infectious diseases of animals and humans. This is largely related to globalization and environmental degradation, which increases contacts between animals and humans. Viruses that have their origin in animal world are the most abundant biological entities on the planet. Small mammals such as wild rodents are important reservoirs of viruses. During June 2008 to July 2009 we collected 560 wild sigmodontine rodents in the northeastern region of São Paulo State. In a previous study, we have found mammarenaviruses antibodies in the wild rodents. Thus, we show here a viral metagenomic aiming to detect and characterize viral genomes in sigmodontine from the study region. From all viruses (22%), 13% belonged to Arenaviridae family.

We have found Latino and Oliveros mammarenaviruses in *Akodon montensis*, and Latino, Oliveros and Pinhal mammarenaviruses in *Calomys tener*. This is an ongoing study and we intend to further understand mammarenavirus ecology, the dynamics of infections in nature in order to predict future threats to animal and human health.

#### **HV225 - MOLECULAR DETECTION OF NOROVIRUS AND ADENOVIRUS IN CHILDREN'S DAY CARE IN ANANINDEUAPA**

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Acute gastroenteritis (AGE) is a worldwide impact problem in terms of health being considered a major cause of morbidity and mortality among children under five years. Norovirus (NoV) and enteric adenovirus (AdVE) are important cause of outbreaks of gastroenteritis in children's day care, hospitals, nursing homes, ships, hotel and restaurant settings. This study aimed the detection and molecular characterization of NoV and AdVE in children attending two public children's day care localized in Ananindeua, Para. The study involved fecal samples collected from children less than six years old that attending two children's day care. The NoV detection was realized by enzymatic immunoassay (EIA) and for AdVE by the polymerase chain reaction (PCR). The nucleic acid was extracted by silica method. The NoV genotyping was performed by the analyze of the regions A (JV131/JV12Y and JV12Y/NoroIIR) and B (Mon 431/432/433/434). For AdV, we used the nested PCR with primers Hex 1Deg/Hex 2Deg and Hex 3Deg/Hex 4Deg as described by Allard et al., 2001. The sequencing was performed using the Big Dye Kit® in ABI Prism 3130xL DNA Sequencer (Applied Biosystems, USA). The sequences was aligned by the Bio Edit program (v.7.1.3.0) and compared with others registered in GenBank and Norovirus Genotyping Tool version 1.0. During the study period (August/2014 to April/2015) 135 samples were analyzed, among them

22 came from symptomatic children (presence of three or more diarrhea episodes in 24 hours) and 110 from asymptomatic ones (without diarrhea at least 72 hours before collection). The positivity for NoV and AdV was 13.3% (18/135) and 62.8% (66/105) respectively. The AdV circulated during the period analyzed with fluctuations in the frequencies along the months, for NoV the monthly distribution was more restricted probably due to low number of symptomatic cases. Among the NoV positive samples sequenced, three were genotyped as GII.P4, GII.P7 and GII.P12. For AdV the genotypes detected involved the enteric 40/41 as well as non-enteric genotypes. The results demonstrated that NoV and AdV are circulating among children attending in day care centers. These viruses circulated asymptotically and with wide genetic diversity, during the period analyzed, enlightening a relevant aspect, indicating that this event is frequent. These data are important because they demonstrate the molecular epidemiology of these viruses, which need further investigation.

#### **HV228 - PAN DIAGNOSTICS OF HUMAN VIRAL PATHOGENS THROUGH MULTIPLEX PCR AND MASSIVE PARALLEL SEQUENCING**

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The spectrum of diseases that affect humans is responsible for considerable morbidity and mortality. Most of these diseases are caused by a wide variety of viruses, in the majority causing respiratory and enteric infections. The development of molecular diagnostic methods and new generation sequencing has enabled the rapid and precise detection and identification of these agents. Similarly, the spread of these viruses has been increased with air travel growth along with the large agglomeration of individuals from different countries, especially in major international events. In this scenario, this work aims to develop a universal diagnostic method, or pan diagnostics, for detection and identification of all known human viral pathogens, to be used as an epidemiological surveillance tool and last diagnostic approach. These pan viral diagnostics is part of a major pan diagnostic method that comprises the detection of all know human pathogens, including eukaryotes and

prokaryotes. The method is based on PCR amplification, in a single tube, and parallel massive sequencing of specific sequences for each human viral pathogen present in different types of biological samples such as blood, respiratory secretions, saliva, bronchoalveolar lavage, cerebrospinal fluid, urine and stool. So far, 2034 primers were designed for the detection of 402 viruses, comprising all known human viral pathogens, in addition to the design of synthetic genes to be used as control targets in this diagnostic methodology. To initially establish the methodology a subset of synthetic genes and primers targeting respiratory viruses will be tested. The development of this project will allow the creation of an analytical center of high technology and biosafety to be used in the diagnosis of human pathogens.

#### **HV230 - ADENOVIRUS DETECTION IN PATIENTS UNDERGOING KIDNEY TRANSPLANT IN BELÉM PA**

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Adenovirus (AdV) is nowadays considered to be one of the main causes of gastroenteritis outbreaks and sporadic cases of diarrhea, leading the infected person to both ambulatory and hospital cares, presenting symptoms such as diarrhea, vomiting and fever. It is understood that gastroenteritis is also one of the most common complications observed in individuals undergoing kidney transplant, and its cause is often unknown. Therefore, this study aims to detect AdV in stool samples from kidney recipient patients, with/without diarrhea, hospitalized, treated and followed up at the Ophir Loyola Hospital in BelémPA. As a cohort study, 8 patients were included and followed up each month for a period of one year posttransplant. Fecal samples were collected during their monthly visit to the hospital or when presenting a diarrheic episode. The DNA extraction was performed using the PureLink Viral RNA/DNA Mini Kit, according to manufacturer's instructions. For molecular detection of AdV both PCR and NestedPCR were performed, using the pairs of primers Hex1deg/Hex2deg and NeHex3deg/NeHex4deg in the first and second steps, respectively. Of the 52 stool samples collected from the 8 patients included in the study until

now, 12 (23.1%) showed positive results when using the PCR technique, whereas in the NestedPCR the number of positive samples increased to 35 (67.3%), being 6 from patient coded as PTR001, 6 from PTR004, 5 from PTR005, 6 from PTR008, 4 from PTR009, 4 from PTR032, 2 from PTR054 and 2 from PTR055. Interestingly, some patients had AdV positivity in consecutive months of collection, suggesting a prolonged virus shedding (e.g. PTR005), while others showed positive samples in alternate months, suggesting reinfection (e.g. PTR032). Another interesting data is that from the 35 positive samples, 12 (34.3%) had no gastroenteritis symptoms reported by the patients. The other 23 positive samples came from patients that had at least one symptom reported (14/40%) or had no data available (9/25.7%). In Brazil, there are few studies on the incidence of these viruses in cases of gastroenteritis in patients undergoing kidney transplant. In this research, it was demonstrated the AdV circulation in this group of patients. These data will certainly help in a better understanding of the epidemiology of these viruses in gastroenteritis cases in a post kidney transplant scenario.

#### **HV232 - COINFECTION BETWEEN ZIKV AND DENV DURING DENGUE OUTBREAK IN SÃO JOSÉ DO RIO PRETO, SP**

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Zika virus (ZIKV) is a reemerging flavivirus, first isolated in Uganda in 1947 during the course of a yellow fever virus serosurvey. The first cases of ZIKV infections in Brazil have been documented in Bahia, Rio Grande do Norte and São Paulo. Until April 2016, 39,993 confirmed cases were reported in the country based on clinical-epidemiological or laboratory data. We report two cases of coinfection of ZIKV and different DENV serotypes in a city located in the northwest region of São Paulo State. In 2016 April, two patients were attended at the emergency unit of the reference hospital in São José do Rio Preto presenting acute febrile illness. Dengue was suspected based on clinical-epidemiological data. Blood samples were collected one day after the onset of symptoms

and the patients were screened for DENV by NS1 and/or IgMIgG. PCR/qPCR assays were applied to the RNAs to determine the diagnosis of DENV 14, ZIKV or CHIKV. Both patients were positive to ZIKV, while patient 1 was also positive to DENV 1, and patient 2 to DENV 2. Both were negative to CHIKV. Patient 1 was a 41years old male, without comorbidity, presented with complaints of myalgia, headache, arthralgia, chills, initiated one day prior to examination. No fever or bleeding was reported. The NS1 test was negative and the hemogram was normal. Patient 2 was a 36years old female, without comorbidity, presented with complaints of myalgia and headache, one day prior to examination, with no report of fever, nausea, vomiting, abdominal pain, or bleeding. The blood count showed mild leukopenia. The tourniquet test was positive and dengue NS1 test was negative. An IgM antidengue ELISA was positive. Both patients were discharged after support therapy. São José do Rio Preto is hyperendemic for dengue and is located within the yellow fever transmission region with confirmed cases of ZIKV, SLEV and CHIKV. The city is facing a dengue epidemic by DENV1 and 2, with few detections of DENV4. In previous outbreaks, coinfections among DENV2/3 and DENV3/SLEV were reported. It is believed that coinfections occur only during outbreaks of multiple serotype and where there is a high prevalence of the urban vectors. The sexual transmission may be considered as a possible in the cases of coinfection, together with the natural infection through vector bite. With the recent introduction of ZIKV and CHIKV in the Americas and the resultant cocirculation, situations like this will become more frequent.

**HV247-“TYPIFICATION OF HUMAN PAPILLOMAVIRUS AND PRESENCE OF RISK COFACTORS FOR CERVICAL AND ANAL LESION DEVELOPMENT IN FEMALE SEX WORKERS, DURING 20122013”**

**Valenzuela, A.; Picconi, M.A.; Mongelós, P.; Rodríguez M.I.; Medina, G.; Gimenez, G.; Castro, A.; Cardozo, P.; Castro, W.; Páez, M.; Kasamatsu, E.; Gonzalez, J.; Basiletti, J.; Aguilar, G.; Suarez, Z.; Valdez, R.**

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4. SERVICIO DE VIRUS ONCOGÉNICO, INSTITUTO NACIONAL DE ENFERMEDADES INFECCIOSAS, “DR. MALBRÁN” ADMINISTRACIÓN NACIONAL DE LABORATORIOS E INSTITUTO DE SALUD
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The human papillomavirus high oncogenic risk (HR-HPV) is the major etiological agent of cervical and anal cancer. Female sex workers (FSW) constitute a risk group to be infected with HPV. This descriptive study of transverse cut was aimed to detect HPV types and determine the presence of cofactors of risk associated with the development of lesions in the cervix and anus of 144 FSW period 2012/2013. HPV types, Chlamydia trachomatis (CT) and Herpes simplex virus (HSV) were detected by PCR and genital lesions by cytology. The frequency of HPV and HRHPV detected was 50% and 42.4% in cervical and 56% and 36.1% in anus. 43.1% and 24.3% of women had HPV infection and HRHPV in both locations, in HPV positive women bearing cervical infection more often of anus infection ( $p < 0.0001$ ). HPV 16 was the most common. 1.4% and 8.3% of women had abnormal cervical and anus cytology. It was also noted that HRHPV positive women had a higher frequency of infections with Chlamydia trachomatis ( $p = 0.004$ ), and HSV ( $p = 0.002$ ) in the cervix. These results suggest that HRHPV positive FSW should be controlled at both areas frequently to identify persistent viral infection and prevent the development of genital lesions. They also

suggest the need to strengthen surveillance of infections by Chlamydia trachomatis and HSV in women HRHPV positive, considering that studies have found that they are at higher risk of developing cancer precursor injuries and cancer.

#### **HV263 - DETECTION AND QUANTIFICATION OF EPSTEINBARR VIRUS IN PLASMA FROM PRE AND POST KIDNEY TRANSPLANT PATIENTS**

Brasil Costa, I.; Silva, M.J.M.; Barros, I.C.; Oliveira, B.S.J.; Mendes, W.R.B.; Resque, H.R.; Monteiro, T.A.F.; Viana, C.A.; Silva, A.B.; Migone, S.R.C.

1. INSTITUTO EVANDRO CHAGAS
2. HOSPITAL OPHIR LOYOLA

EpsteinBarr Virus (EBV) latently infects more than 90% of the world population and is effectively controlled by the immune system. However, patients undergoing kidney transplant require immunosuppressive therapy as a measure to prevent graft rejection. The immune depletion, caused by the use of such drugs, enables an increase in the virus reactivation potential. The present study aims to investigate the EBV presence and viral load in plasma samples from patients undergoing kidney transplant at the Ophir Loyola Hospital, reference in kidney transplant in northern Brazil. An analytical and longitudinal study was conducted using 132 plasma samples obtained from 66 kidney transplant patients, being a sample collected immediately before transplant, and another one 30 days after surgery. For viral detection and quantification a qPCR test whit EBV qPCR Alert kit (Nanogen) was used and, as endogenous control, the amplification of the human betaglobin gene was evaluated, previously added in the extraction step. For statistical analysis, it was used the statistical package BioEstat 5.3. Among the pretransplant samples, tested for the presence of the EBV viral genome, 4.5% (3/66) were positive. Only one sample (1.5%) was positive after transplant. It was observed a quantification of 106.32, 46.88 and 32.48 copies/ml of plasma for pretransplant samples PTR33, PTR38 and PTR50, respectively. Post transplant sample (PTR69) showed extremely low quantification (0.05 copies/ml). There was no significant difference when comparing the pre and posttransplant as the results ( $p = 0.625$  McNemar test). The positivity for EBV in the plasma of pre and posttransplant patients was low, which can be explained by the low virus infection/

reactivation, or by a low sensitivity in the EBV research in acellular blood samples. The low quantification observed may indicate that there is a subtle viral load fluctuation that may or may not be detected by the qPCR methodology. Further studies are needed taking into consideration the type of biological sample used, the immune suppression scheme and the increase of the number of patients involved.

#### **HV267 - HIGH RISK HUMAN PAPILLOMAVIRUS DETECTION IN CERVICAL SAMPLES FROM WOMEN OF THE DISTRICT OF ITAUGUÁ, PARAGUAY, 2014-2015. PRELIMINARY RESULTS**

Caballero, S.V.; Kasamatsu, E.; Rodriguez, M.I.; Soilán, A.; Ortega, M.; Mongelós, P.E.; Páez, G.M.; Cristaldo, C.; Castro, A.M.; Basiletti, J.; González, J.; Picconi, M.A.; Hernández, M.; Almonte, M.; Herrero, R.; Mendoza, L.

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2. HOSPITAL DE SAN LORENZO
3. HOSPITAL NACIONAL DE ITAUGUA
4. INSTITUTO NACIONAL DE ENFERMEDADES INFECCIOSAS
5. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

Paraguay has the seventh highest incidence of cervical cancer in Latin America. Human papillomavirus high oncogenic risk (HRHPV) is a necessary factor for cervical cancer. In Paraguay there are not studies on HPV types circulating in the general population. The aim of this crosssectional study was to determine HRHPV types present in cervical samples of women aged 30 to 64 years of the district of Itauguá, Paraguay, period 2014-2015. In total 822 women were surveyed and then they were invited to the health post where cervical samples for convencional cytology and detection of HRHPV by Hybrid Capture 2 were obtained from those women who had agreed to participate. Positive samples for HRHPV were typified by PCR followed by reverse hybridization (PGMYCHUV validated by WHO LabNet HPV). A prevalence of 13,7% of HRHPV (113/822 samples), and of 3,8% of abnormal cytology (31/822 samples) was observed. Furthermore 78 of 113 samples of HRHPV positive women were typified and among these in 75.6% of women (59/78 samples) were observed simple infections and in 24.3% of women (19/78 samples) were observed multiple infections. The most common viral

type was HPV16 followed by HPV 31. This study showed a high prevalence of HRHPV, which partly explains the high incidence of cancer in Paraguay. In addition, these results may be useful as baseline prevaccination data for a future virological surveillance. Finally, this study suggests that HPV testing could be implementing as a primary screening method in Paraguay.

#### **HV268 - DIVERSITY OF HUMAN PAPILLOMAVIRUS IN THE ANAL CANAL OF PARAGUAYAN FEMALE SEX WORKERS, PERIOD 20122014**

Riveros, J.F.; Valenzuela, A.B.; Correa, R.M.; Mongelós, P.E.; Giménez, G.; Rodriguez, M.I.; Medina, G.; Castro, A.M.; Kasamatsu, E.; Páez, G.M.; Basiletti, J.; González, J.; Picconi, M.A.; Mendoza, L.P.

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A great diversity of human papillomavirus (HPV) types is detected in different anatomical locations. HPV infections are associated with the development of anogenital lesions in women. Knowledge about anal HPV infection among women is limited. This crosssectional study analyzed the frequency of cutaneous and mucosal HPV infections in 107 anal canal samples of Paraguayan female sex workers and also described the characteristics that favor HPV infection, period 20122014. Detection and typing of HPV was performed by two generic PCRs combined with their respective reverse hybridization assay (PCRRLB). In total, 63/107 (59%) anal samples were positive for mucosotropic HPV, being HPV 16 the most frequently type detected. Furthermore, positive cases of cutaneous HPV were detected in 31/107 samples (29%), being HPV 9 the most frequently type observed. Furthermore, in 21/107 samples (20%) were observed coinfection between mucosotropic and cutaneotropic HPV. Among the factors predisposing to HPV infection it was observed that 54/107 of women (50%) had 12 sexual partners per week, 36/107 of women (34%) used condoms only with some sexual partners, 54/107 (50%) of women had anal sex, and 46/107 (43%) of women were smokers. In conclusion, this study showed a high frequency of mucosotropic HPV, such as HPV 16 that could cause anal lesions. This data suggest the need for periodic monitoring of this population risk. Finally, the presence of cutaneotropic HPV were detected as simple

infection and as coinfections with mucosotropic HPV, however more studies are needed to understand their role in anal canal.

#### **HV272 - TOXICITY TEST AND ANTIVIRAL ACTIVITY OF THE CHONDRACANTHUS CHAMISSOI EXTRACT AGAINST YELLOW FEVER VIRUS**

Mayta, E.H.; Mamani, E.Z.; Mariano, M.A.; Sevilla, L.D.; Vásquez, K.V.; Quintana, A.B.; Sulca, J.H.; Gonzalez, M.R.; Oliveira, D.B.; Durigon, E.L.

1. UNIVERSITY NATIONAL MAYOR DE SAN MARCOS
2. ICB-II-UNIVERSITY

Yellow fever virus (YFV) affects annually around 200,000 people in tropical regions of South America and Africa. Although an effective vaccine (17D strain) is available, its use is not helpful as it is incomplete in many areas. There is no specific antiviral therapy for a treatment of Yellow fever (YF). Seaweeds full of bioactive compounds which could help to develop antiviral drugs. In this work, we describe in vitro antiviral activity of Chondracanthus chamissoi extracts against (YFV). The C. chamissoi were collect in Perú. The ethanolic extraction phase gametophytic these seaweeds were used for obtaining sulfated carbohydrates and carrageenan were tested for antiviral properties against YFV in cells. The Seaweeds has antiviral properties, and the activity against YFV needs to be tested. The toxicity test was evaluated by inoculating different dilution (101 to 106) of the carbohydrate extracts (625 mg/ml) and the carrageenan (10 mg/ml) which is produced in the gametophyte face and diluted in maintenance media (MM). Then the dilutions were inoculated in a VERO76 cells confluent monolayer using 24well plates, and incubated at 37oC. The morphology of cells was evaluated for 9 days. The plates were stained. For evaluation of antiviral activity against YFV the carbohydrate extracts and carrageenan (diluted 1:10) were mixed (1:1) with YFV (17D vaccine strain), diluted from 104 to 108 for the antiviral activity. After an hour of incubation at 37oC, the mix extract-virus was inoculated in 96 well plates of VERO76 cells and incubated at 37oC for 5 days. Then the plates were stained. Both extracts had not toxicity in the concentrations tested (carbohydrate extracts (31.25 mg/ml) and the carrageenan (0.5 mg/ml)) and they had antiviral activity against YFV (103.5 TCID50/ml). It is necessary to continue the evaluation of the antiviral

activity by using plate assay to know the percent of reduction produced with a specific concentration of the Seaweed extract.

#### **HV273 - DETERMINATION OF CYTOLOGICAL STATUS OF CERVICAL SAMPLES FROM WOMEN INFECTED WITH HIGH RISK HUMAN PAPILOMAVIRUS**

**Hermosilla, J.; Iwasaki, R.; Mamani, E.; Mayta, E.; Hernández, V.; Arias-Stella, J.**

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2. INSTITUTO DE PATOLOGÍA Y BIOLOGÍA MOLECULAR ARIAS STELLA

The cervical cancer is one of the types of cancer with the highest incidence (third worldwide) and mortality (fourth worldwide). The cervical cancer's development is gradual and it can be diagnosed in preinvasive phases through the Pap test; however, this test shows a high rate of false-negative results. The presence of Human Papillomavirus, especially the High Risk Human Papillomavirus or HRHPV, is strongly related with precancerous cervical lesions and cancer, for that reason, many studies suggest the molecular diagnosis of HRHPV as a complementary test. The aim of this work was to determine the cytological status of cervical samples from women infected with HRHPV. For that, we tested 241 cervical samples (positives for HRHPV) with the Pap test, following the liquid-based cytology method. The results were expressed following The Bethesda System for Reporting Cervical Cytology. 29.5% (n=71) of the samples didn't show neoplastic or dysplastic cells (Negative for the test), while 70.5% of samples showed cellular abnormalities classified in: Atypical Squamous Cells of Undetermined Significance or ASCUS (n = 30), Low Grade Squamous Intraepithelial Lesion or LSIL (n = 126), High Grade Squamous Intraepithelial Lesion or HSIL (n = 14). The obtained results showed that a high rate of women infected with HRHPV didn't show cellular abnormalities. Taking into account that many authors have reported that women infected with HRHPV (either with precancerous lesions or not) show a high rate of malignant progression, it is important to take the molecular diagnosis of HRHPV (cotesting) in order to improve the screening of patients with high risk to develop cancer.

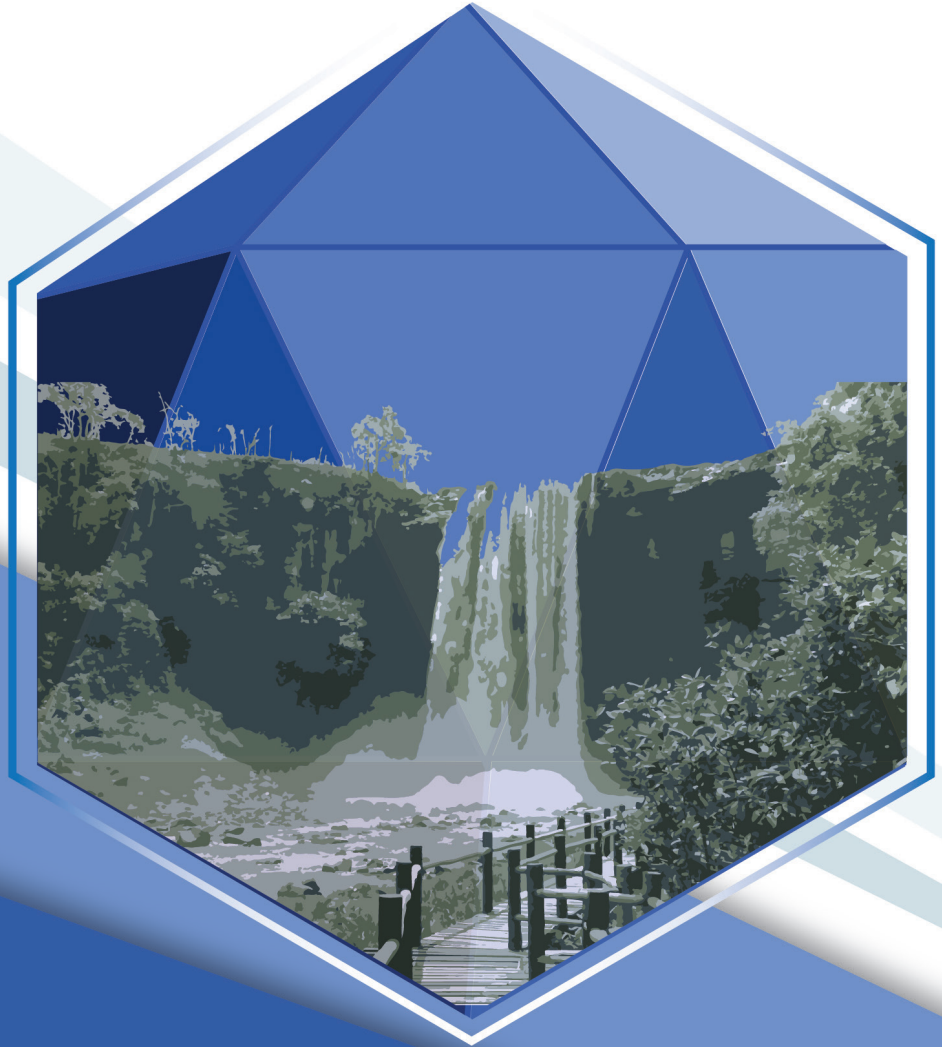
#### **HV275 - PHYLOGENETIC CHARACTERIZATION OF A POSSIBLE NEW HIV-1 RECOMBINANT FORM CIRCULATING IN SOUTHERN BRAZIL**

**Coltro, V.P.; Pinto, A.R.; Graf, T.**

HIV-1 genome is composed by two linear copies of single stranded RNA, combined with enzymes required for replication. One of these enzymes, reverse transcriptase (RT) has the ability to change substrate during transcription, being able to generate a recombinant genome among two or more strains. This feature contributes for the appearance of a wide variety of circulating recombinant forms (CRFs). In southern Brazil epidemic of HIV/AIDS has a distinct character of national scenario. In the states of Rio Grande do Sul (RS) and Santa Catarina (SC) there is a prevalence of subtype C (around 56%) and B (around 22%) that co-circulate on this region approximately for 40 years. Despite this, to date, only one CRF had been described in southern Brazil (CRF31\_BC), reflecting the absence of studies and therefore masking the real scenery of the circulating viral forms at the region of Brazil. Previous studies have found in Joinville/SC a group of three sequences with an uncommon phylogenetic classification. Starting from a BLAST for each of these three sequences, other five were retrieved from public databases, totaling eight sequences isolated from Brazilians HIV positive individuals collected between 1992 and 2013. The recombinant analyses were performed on RDP3 software using the MaxChi, 3seq, Chimaera, BootScanning and RDP methods. Bayesian phylogenetic analyses were performed using a relaxed molecular clock model, GTR+G4+I nucleotide substitution model and Bayesian SkyLine coalescent model as implemented in BEAST software. Recombination analyses showed that eight sequences exhibit the same recombinant form. This recombinant is composed by a central fragment with approximately 373 bp correspondent to the subtype B flanked by two fragments of subtype C. The phylogenetic analyses performed for each fragment indicated that both parental are Brazilian. Besides, fragment corresponding to subtype B grouped with a sequence from RS, showing the south region as the most likely place of emergence. Additionally, phylogenetic analysis showed that this recombination event occurred in Brazil in 1982 (95% HPD: 1979-1987), consistent with the time of co-circulation between subtypes B and C in southern

Brazil. Although the strong evidences of the occurrence of recombination presented in this study, further studies involving the full genome of these samples are necessary in order to determine such a new CRF between HIV-1 subtypes B and C. Financial support: CNPq and FAPESC.

# *IMMUNOBIOLOGICALS IN VIROLOGY - IV*



**IV63 - DEVELOPMENT AND IMMUNOGENICITY OF A NEW INACTIVATE VACCINE FORMULATED WITH A BRAZILIAN VARIANT OF AVIAN INFECTION BRONCHITIS VIRUS (IBV) ENCAPSULATED IN CHITOSAN NANOPARTICLES**

Lopes, P.D.; Okino, C.H.; Fernando, F.S.; Casagrande, V.M.; Pavani, C.; Dalmolin, L.F.; Tamanini, M.L.F.; Montassier, M.F.S.; Lopez, R.F.V.; Montassier, H.J.

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2. EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA
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Infectious Bronchitis (IB) is an acute viral disease caused by IBV. IB is distributed worldwide and in Brazil remains as one of major infectious diseases that affects poultry farms due to the emergence of Brazilian (BR) variant strains and insufficient crossimmunity induced by the commercial vaccines. The objective of this study was to develop and evaluate the protection conferred by an inactivated vaccine formulated with a BR variant strain of IBV encapsulated in chitosan nanoparticles and administered by ocular route (IBVCSN) in SPF chicks. Ionic gelation technique was used for the formulation of nanoparticles. The size and zeta potential of the nanoparticles were measured (ZetaSizer) and the encapsulation efficiency was assessed by measuring protein by the Bradford technique and detection of the viral genomic RNA by RTPCR. The degree of protection induced by the vaccine was compared with other anti-IBV inactivated vaccine incorporated into an oil adjuvant, administered by intramuscular route (IBVOIM). Four groups of chicks were vaccinated with different vaccine protocols, associating or not a prior immunization with attenuated H120 vaccine and four weeks later, the chicks were challenged with the homologous virulent strain. Two other groups were kept as positive control (infected chicks) and negative control (uninfected and unimmunized chicks). At 5 days postinfection, the chicks were euthanized to collect tracheal samples for evaluation of ciliostasis and quantification of viral load by RTqPCR techniques. The best formulation of nanoparticles resulted in 88% encapsulation efficiency,

average size 339 nm and zeta potential of 19.9 mV and encapsulation efficiency of IBV. The chicks vaccinated only with IBVCSN and previously vaccinated with attenuated vaccine followed by a booster vaccination with IBVCSN or IBV OIM showed complete protection to challenge with the homologous strain, as demonstrated by the maintenance the integrity of tracheal ciliary movement and the presence of low viral loads, while the chicks vaccinated only with IBVOIM showed partial protection against homologous challenge. The IBVCSN vaccine alone or combined with live attenuated vaccine conferred to immunized chicks an effective protection against challenge with a BR variant homologous strain. In conclusion, this type of vaccine is a new generation of immunogen with a great potential to induce effective protection at mucosal sites of chickens.

**IV139 - STANDARDIZATION OF THE CONSTRUCTION A RECOMBINANT ANTIBODY LIBRARY ANTI HCV FROM PATIENTS DIFFERENT DEGREES OF WITH FIBROSIS**

Watanabe, T.; Silva, C.N.; Hebel Barboza, F.; Moraes, L.N.; Ferrasi, A.C.; Pardini, L.M.C.; Pardini, M.I.M.C.; Grotto, R.M.T.

UNIVERSIDADE ESTADUAL PAULISTA

The phage display technique for generating recombinant antibodies is an alternative to hybridoma technology. The phage display allows the generation of recombinant antibodies from mRNA obtained of the patients with HCV, liver fibrosis confirmed by biopsy and naive treatment. The standardization of the construction technique of a library for phage display is necessary to ensure the variability of antibodies. To standardize the library were separated two groups of patients with chronic hepatitis C with different degrees of fibrosis according Metavir score: F1/F2 and F3/F4. The mRNA was extracted from whole blood. The reverse transcription performed with random primer and specific primers (for IgG and IgM). The obtained cDNA was purified and PCRs reactions were performed using specific protocol described by Barbas (2001). To amplification the antibody genes using PCRs involve the high and light antibody chain amplification separately. Products of each chain of the antibody (heavy chain: Vh + Ch and light chain: V k + Ck) were joined by overlap PCR. A second reaction was an overlap PCR to join the two strands (VhCh+VkCk)

forming a single product (Fab). The results showed that DNA contamination during RNA extraction is a problem for amplification antibody specific genes. Then the extracted RNA was incubated with DNase improving the amplification process. During amplification phase the techniques limitations were resolved using pure RNA, analyzed by fluorescence method. The purification of the retrotranscription product demonstrated better results during amplification phase. Other differential procedure was the use of the higher specificity and sensibility PCR enzymes. The purification of the PCR products using freeze-squeeze with an in house protocol improved all amplifications reactions. The modifications performed in original protocols showed concentration and pattern of bands consistent with the expected size of the fragment. The product obtained was subjected to cloning and sequencing for confirmation of the antibody library. The standardization of the construction of antibodies libraries enables a good assay reproducibility without lost the variability of antibodies, allowing the creation of a library with great potential for application in the detection and monitoring of the development of fibrosis and the discovery of new antibodies with potential therapeutic.

#### **IV180-DEVELOPMENT OF A LIQUID PHASE BLOCKING ELISA (ENZYMELINKED IMMUNOSORBENT ASSAY) FOR PORCINE CIRCOVIRUS TYPE 2 ANTIBODY DETECTION**

**Araujo Jr., J.P.; Cruz, T.F.; Vaz, M.R.; Portela, L.M.F.**

*UNIVERSIDADE ESTADUAL PAULISTA*

PCV2 (Porcine circovirus type 2) is causative agent of several syndromes named porcine circovirus associated disease (PCVAD). Antibody quantification is important for serological monitoring in swine herds, in association with protection against PCV2, and in serological surveys, in the assessing the effectiveness of vaccines in development. The aim of this work was to standardize a liquid phase blocking ELISA (LFBELISA) for PCV2 antibodies quantification in mice sera, which may be used in research of experimental infection or vaccine development. The optimal concentration of antiPCV2 rabbit IgG used as trapping antibody was defined as 5 µg/mL. The optimum dilution of test serum (1:25), detector antibody (1:800) and antigen (1:2) were established by chessboard titration. It was used as detector antibody

a polyclonal porcine antiPCV2 serum and viral antigen consisted of swine testicle (ST) cell supernatant persistently infected with PCV2. To perform the LFBELISA, ten to nine-week-old female BALB/c mice were injected intramuscularly, with 50 µl of 5 x 10<sup>4.86</sup> DICT50 of commercial vaccine against PCV2. An identical booster immunization was performed 21 days later. The animals were challenged with PCV2 twice at 50 and 52 days after the first vaccine dose, with 100 µl of 5 x 10<sup>5.19</sup> TCID<sub>50</sub> by the intraperitoneal route. Serum samples were collected at 36, 64 and 84 days after immunization and used in LFBELISA test. The microplate was coated with trapping antibody, after incubation, the reaction microplate was blocked. Liquid phase (antigen and serum test) was transferred to the reaction microplate, and detector antibody, peroxidase-conjugated rabbit anti-swine IgG, and H<sub>2</sub>O<sub>2</sub>/TMB were added. The plate was washed after each step, except for the H<sub>2</sub>O<sub>2</sub>/TMB solution. The reaction was stopped (2M HCl) and the results reported as percentage inhibition (PI). A commercial ELISA kit was used for comparison with the LFBELISA, and it was performed according to the manufacturer's instructions, but with some modifications. Both tests were able to detect a high amount of antiPCV2 antibodies in the analysed samples presenting good correlation, showing seroconversion after the challenge with PCV2. Thus, LFBELISA was a method simple to perform, rapid, specific and convenient for the detection of antibodies against PCV2 in studies of experimental infection, and in the development of vaccines against PCV2 in mice due to high analytical specificity.

#### **IV181 - EVALUATION OF SEROCONVERSION INDUCED BY LIVE VACCINE OF NEWCASTLE DISEASE SUBMITTED OF BRAZILIAN OFFICIAL QUALITY CONTROL DURING MARCH/2015 TO MAY/2016**

**Orsi, M.A.; Benites, C.I.; Silva, L.A.; Rodrigues, R.L.; Lima, T.S.; Leal, F.S.; Pereira, G.A.F.; Reischak, D.**

*LABORATÓRIO NACIONAL AGROPECUÁRIO*

Newcastle disease virus (NDV, Paramyxoviridae family, Avulavirus genus) is the agent that causes one of the most important diseases in birds and represents a threat to industrial aviculture, leading to sanitary barriers. Prophylaxis of many avian diseases is based primarily on active immunization through live vaccines. Serological tests may be used to verify the vaccination effectiveness

in field animals and indirectly as vaccine efficacy a in a quality control processes. This test allows to estimate the quantity of antibodies to a specific viral antigen. The commercial avian vaccines in Brazil are submitted to official quality control. In this control, seroconversion is one of the main assays, which checks the production of specific virus antibodies after immunization. This study aimed the evaluation of the seroconversion results obtained by monovalent and combined vaccine submitted to the official quality control during 15 months. For each seroconversion, two vaccine bottles were diluted in PBS and 10 birds were inoculated in BSL-3 isolator (age and method according to manufacturer). After 21 days, the animals were anesthetized and blood collected by cardiac puncture. Specific Pathogen Free birds (SPF) unvaccinated were used as negative control. The sera were analyzed for the detection of antiNDV antibodies by indirect ELISA (commercial kit). 27 batches of monovalent vaccines were tested (6 companies strains: B1, LaSota, VGGA, C2, PHYLMV42, VH and CL/79) and 7 batches of combined vaccines (4 companies strains: Clone 30, B1, LaSota and VH). The monovalent vaccines showed ELISA values ranging from 614 to 7,017, the CL/79 strain showed higher values (3,579 to 7,017). 5 batches resulted negative ELISA test: 4 PHYLMV42 strains and 1 C2 strain. In these samples HI/NDV was performed with LaSota antigen presenting results from 1:20 to 1:456, being considered approved for this technique, which is considered as Gold Standard. The NDV fractions of combined vaccines showed ELISA results ranging from 1,606 to 3,275 therefore approved. The results of monovalent vaccine for PHYLMV42 and C2 strains indicate that it should preferably be submitted to the HI test. As ELISA is used for screening in vaccinated birds, further studies should be conducted to immune response of these specific strains. It is emphasized that all 34 lots tested had minimum values required by Brazilian law.

#### **IV182 - VALIDATION OF THE SEROCONVERSION METHOD FOR DETECTION OF ANTIBODIES INDUCED BY A NEWCASTLE DISEASE VACCINES**

Orsi, M.A.; Fortunato, E.C.; Ashimine, R.; Catharino, A.M.R.; Nascimento, M.L.J.; Zaroni, M.M.H.; Benites, C.I.; Lima, T.S.; Leal, F.S.; Reischak, D.

*LABORATÓRIO NACIONAL AGROPECUÁRIO*

The method of seroconversion (serology) are commonly used in Avian vaccine quality control to evaluate live vaccine, such as Newcastle disease (ND), which is caused by a virus from the family Paramyxoviridae, genus Avulavirus. This method is used to evaluate the production of specific antibodies against the NDV. Currently, validation and verification of analytical methods are usually required by official laboratories from the Brazilian Government as a mean to increase the quality of these tests, besides being criteria checked in international and national auditing. In this way, the objective of this work was the validation of the seroconversion using the ELISA test to detection of antibodies against Newcastle disease virus after immunization with vaccines used in Brazil. The following parameters were evaluated in the data obtained by ELISA: repeatability, analytical sensitivity, analytical specificity, accuracy and Kappa index. Three groups of birds were tested in three independent rounds, using the SPF birds with one dayold which were vaccinated in the eye using antigens of NDVLANAGROSP (origin by National Service Veterinary Laboratory, NSVL). The birds were kept in BSL3, the seroconversion was detected in 21 days p.i. The indirect ELISA (ELISAI) method using the commercial kit of NDV was used for the detection of antiNDV antibodies. In ELISA test, there were differences between positive and negative sera for NDV. In addition, when was compared the results of 27 sera from vaccinated birds and 25 sera from unvaccinated birds, by ELISAI IgG/IgM sera it was showed the accuracy (98%), sensitivity (96%), specificity (100%), repeatability (96,3%). This strong coincidence was measured by Kappa index (96%) with the proportion of false positive+ false negative (4%). This means that the vaccinated birds showed positive results in the ELISA test. Our results indicate that this method were very effective to measure the production of protective antibodies in vaccinate birds for NDV, via a single inoculation.

**IV242 - SEARCHING FOR A MURINE MODEL OF INFECTION BY OROPOUCHE VIRUS TO EVALUATE THE IMMUNE PROTECTION CONFERRED BY ITS NUCLEOCAPSID PROTEIN**

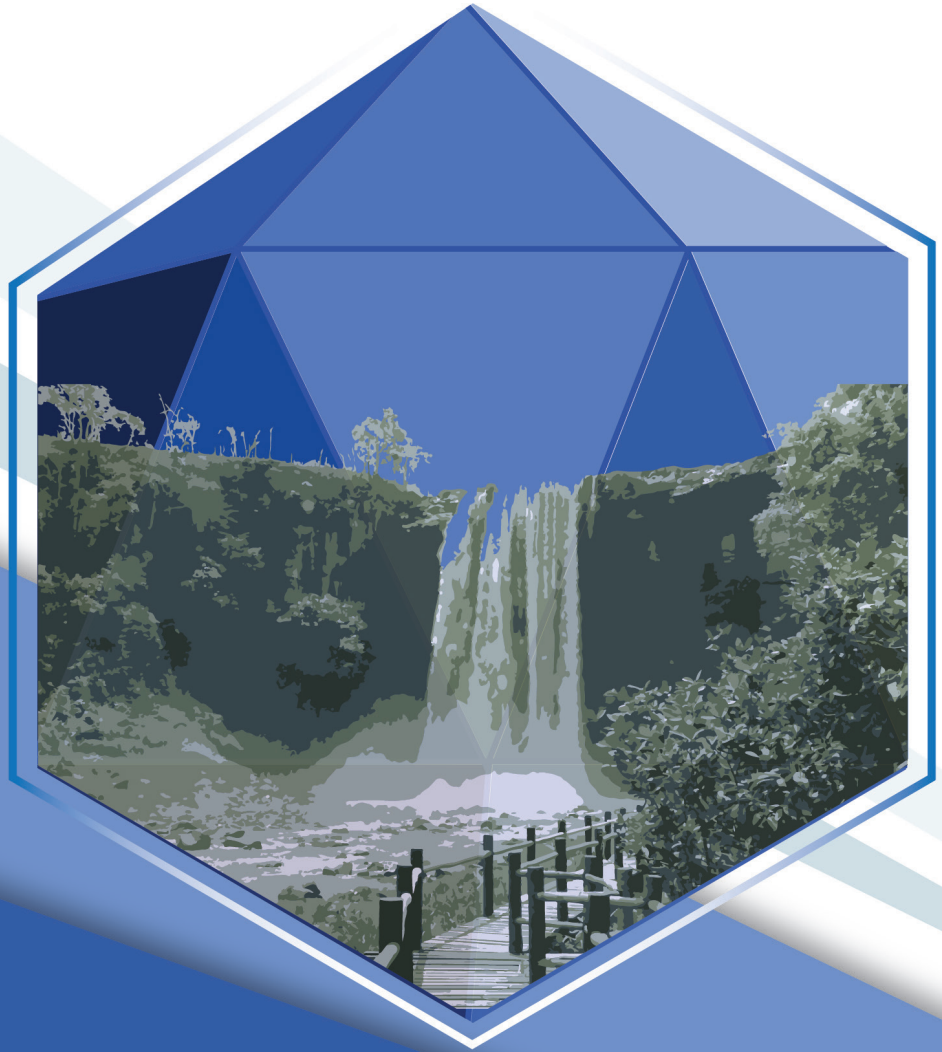
Zapana, P.R.R.M.; Lauretti, F.; Barbosa, N. da S.; de Oliveira, A.S.; de Souza, W.M.; Fumagali, M.J.; Badra, S.J.; Figueiredo, L.T.M.

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The Oropouche virus (OROV) is an arbovirus, which infection usually occurs in the Amazon region causing acute febrile disease outbreaks that occasionally may be associated with meningoencephalitis. It is assumed that it has infected at least 500,000 people in Brazil, making it the second arbovirus in the number of cases, just behind dengue virus. Thus, despite the importance of OROV, there is no vaccine available against it. Therefore, this project proposes the usage of the recombinant nucleocapsid protein of OROV (NrOROV) as immunogen to induce humoral immune response, which may protect mice against infection. The presence of antibodies to NrOROV in the experimental model was evaluated by ELISA, neutralization (PRNT), immunofluorescence, and Western blot. Moreover, the organs of the animals were assessed by RTPCR to amplify the S segment of OROV. In this study, we used the animal model of Swiss mice of 6 weeks old and these were inoculated intracerebrally with 50LD50 of OROV, all of these developed encephalitis (100% mortality). The animals were immunized with NrOROV/Al(OH) at 21, 28, and 35 days of life and challenged with OROV 14 days after the last immunization. Blood samples were collected before and after the challenge, and the organs were collected after it. High titers of IgG antibodies in the animals were observed, being them IgG1 NrOROVspecific, suggesting a humoral immunity mediated by Th2 and also the presence of IgG2a NrOROVspecific, an immune mediated by Th1. Post vaccinated sera (PVS) were evaluated for the presence of antibodies capable of recognizing infected HeLa cells by immunofluorescence. After 24 hours of infection with OROV, it was observed high fluorescence intensity in most of the cells membrane, indicating the presence NrOROV on it. The specificity of the PVS to OROV was confirmed by western blot using purified NrOROV and infected lysed HeLa cells. The neutralizing capacity was determined by PRNT50, showing a 1:20 titer. All vaccinated animals after the challenge were

positive for RTPCR only in the brain, and among them, one also positive in the liver. Vaccination with NrOROV/Al(OH) stimulated humoral and cellular responses with high levels of IgG1 and IG2a. However, when the mice model was intracerebrally challenged, it did not show a protective immunity despite the development of neutralizing antibodies. Lymphocyte proliferation assays and cytokine levels will be measured in the sera of vaccinated animals to evaluate the ce.

# *PLANT AND INVERTEBRATE VIROLOGY - PIV*



**PIV16 - CHARACTERIZATION OF TWO GENOMOVIRUS ASSOCIATED WITH NONCULTIVATED PLANTS IN BRAZIL**

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In recent years, new groups of circular replication-associated protein encoding singlestranded (CRESS) DNA viruses have been described infecting animals (including humans), plants and fungi. One of these groups are the gemycircularviruses, which have been detected in insects, in the faecal matter of various animals, and in fungi infected cassava leaves. It has not yet been unequivocally demonstrated that gemycircularviruses can infect plants, as the virus isolated from cassava is more related to mycoviruses and infectivity tests were not carried out. Here, we describe gemycircularviruses associated with two noncultivated hosts which are important invasive species in field crops in Brazil: *Euphorbia heterophylla* (two samples showing yellow mosaic, leaf curling, and stunting collected in Rio Grande do Sul state) and *Momordica charantia* (samples collected in Minas Gerais state). Viral genomes (aprox. 2,100 nt) were amplified by RCA, cloned and sequenced. Samples of *E. heterophylla* were also infected by *Euphorbia* yellow mosaic virus, but begomoviruses were not detected in *M. charantia*. A Bayesian inferred tree based on full length sequences clustered *Euphorbia heterophylla* associated circular virus (EHAsCV) and *Momordica charantia* associated circular virus (MCasCV) in a well supported clade with *Sclerotinia sclerotiorum* hypovirulence (SsHADV), *Hypericum japonicum* (HJasCV), Cassava (CasCV) and Dragonfly (DfaCV) associated circular viruses, with EHAsCV more closely related to DfaCV and MCasCV to HJasCV. Comparing the genome organization of EHAsCV with that of SsHADV we identified a putative coat protein (298 amino acids, 37% identity with the SsHADV CP), two domains of a putative replication associated protein probably expressed from spliced transcripts (126 aa, 63% identity with the helicase domain, and 205 aa, 64% identity with the catalytic domain SsHADV Rep), and the nanonucleotide TAATATTAT (the genome of MCasCV contains a TAATGTTAT nonanucleotide) within

a potential stemloop sequence. Infectivity tests are being performed in *E. heterophylla*, *M. charantia* and *Nicotiana benthamiana* plants.

**PIV18 - VIRAL DETECTION OF TREE SPECIES FROM A NURSERY IN THE FEDERAL DISTRICT**

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Tree species are of great economic, social and environmental importance. However, these kinds of plants can also be affected by several pathogens, including viruses, in which information is scarce and incipient. This work was conducted to prospect viruses in plants of eight botanical species by serological and biological tests. Samples were collected from symptomatic and asymptomatic plants. Firstly, samples were mechanically inoculated onto eight indicator species. Symptoms were evaluated at 7, 14, 21, 28, 35 and 60 days after inoculation (dai). Moreover, serologic evaluation was performed in the 28th dai, using polyclonal antibodies for detection of Cucumovirus (Cucumber mosaic virus - CMV), Potyvirus (Potato virus Y - PVY; Watermelon mosaic virus - WMV; Pepper yellow mosaic virus - PepYMV; Papaya ringspot virus strain watermelon - PRSVW and Zucchini yellow mosaic virus - ZYMV). According to serological test results, seven out of eight botanical species *curupita* (*Couroupita guianensis*), *copaibeira* (*Guibourtia hymenifolia*), *eucalypt* (*Eucalyptus* spp.), *ipê rosa* (*Handroanthus impetiginosus*), *ipê verde* (*Cyrtanthus antisiphilitica*), *jatobá da mata* (*Hymenaea courbaril*) and *pau santo* (*Kielmeyera coriacea*) were negative for all the antibodies tested. Only *pau Brazil* (*Caesalpinia echinata*) was positive for CMV, WMV, ZYMV, PepYMV and, PVY, while no reaction was observed against PRSVW antibodies. On the other hand, in the host range assay, mosaic symptoms were observed on *Gomphrena globosa* and *Nicotiana tabacum* cv. TNN, when rub inoculated with sap from *ipê verde*, *jatobá da mata* and *eucalypt*. In the serology assay of indicator plants, it was observed that *G. globosa* inoculated with sap of *ipê verde* were positive for ZYMV and WMV. When it was used *pau Brazil* inoculum, it was possible to detect PepYMV and WMV in TNN, while *G. globosa* reacted positively to ZYMV. These results demonstrate the potential of tree

species to serve as reservoirs of viruses belonging to Potyvirus genus.

**PIV23 - GENETIC STRUCTURE AND VARIABILITY OF SIDA MICRANTHA MOSAIC VIRUS (SIMMV) AND TOMATO YELLOW SPOT VIRUS (TOYSV) POPULATIONS INFECTING NONCULTIVATED HOSTS**

**Ferro, C.G.; Xavier, C.A.D.; Silva, J.P.; Pereira, H.M.B.; Godinho, M.T.; Lima, A.T.M.; Lau, D.; Zerbini, F.M.**

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Begomoviruses are responsible for serious diseases in several crops of great economic importance, especially in tropical and subtropical regions. Noncultivated plants act as begomovirus reservoirs, enabling the occurrence of mixed infections where recombination can occur. A high species diversity of begomoviruses in noncultivated plants, especially in *Macroptilium* and *Sida* spp., has been observed in studies conducted in several countries in the Americas. Many of the viral species found in these hosts also infect cultivated plants, reinforcing the importance of characterizing begomovirus populations infecting these noncultivated hosts. Understanding the dynamics and genetic variability of viral populations is important to assist on the prediction and consequent prevention of new virus diseases in cultivated plants. In this work, fulllength begomovirus DNAA and DNAB components of *Sida micrantha* mosaic virus (SiMMV) and Tomato yellow spot virus (ToYSV) were sequenced from samples of the noncultivated hosts *Sida* spp. (Malvaceae) and *Leonurus sibiricus* (Lamiaceae), respectively, collected in the states of Rio Grande do Sul, Paraná and Mato Grosso do Sul between 2009 and 2011. Total DNA was extracted from pressdried samples and fulllength genomes were enriched by rolling circle amplification. Unit length genomes were excised with restriction enzymes, ligated into plasmid vectors and completely sequenced. Pairwise sequence comparisons were performed with SDT v.1.2 using the MUSCLE alignment option. Phylogenetic trees were constructed using Bayesian inference performed with MrBayes v. 3.0b4. Recombination analysis was performed with RDP v.4.5.1 using default settings. We found a high genetic variability for both begomovirus populations, with the SiMMV population showing greater genetic variability for both DNA components compared

to the ToYSV population, and the DNAB being more permissible to variation than the DNAA for both viruses. Phylogenetic analysis and population subdivision tests indicated geographical structuring. A higher number of recombination events were detected in both SiMMV components compared with ToYSV, and for both viruses the DNAB was most prone to recombination than the DNAA. Although recombination partly explains the high genetic variability found for these two viruses, mutational dynamics was the primary factor in the diversification of both viral populations.

**PIV24 - REVEALING THE COMPOSITION OF BEGOMOVIRUS POPULATIONS IN CULTIVATED AND NONCULTIVATED HOSTS WITH NEXTGENERATION SEQUENCING**

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UNIVERSIDADE FEDERAL DE VIÇOSA

Begomoviruses (whiteflytransmitted, singlestranded DNA plant viruses) are among the most damaging pathogens causing epidemics in economically important crops worldwide. Tomatoinfected begomoviruses emerged in Brazil in the mid1990's following the introduction of *Bemisia tabaci* Middle EastAsia Minor 1 (MEAM1, previously known as biotype B). Several lines of evidence indicate that these viruses evolved from indigenous viruses infecting noncultivated hosts. However, tomatoinfected viruses are only rarely found in noncultivated hosts, and viceversa. 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 changes rapidly, with the viruses which are better adapted to the new host predominating. To test this hypothesis, we collected tomato and *Sida* sp. plants growing next to each other, as well as whiteflies, at two locations (Coimbra and Florestal, both in Minas Gerais state, Brazil). Viral infection was confirmed by rolling circle amplification and digestion with MspI. Total DNA from one tomato and one *Sida* sp. sample from each location, and from pools of whiteflies from each location, was sequenced in the Illumina HiSeq 2000 platform. Following a highly stringent set of criteria, reads were

mapped to a data set including all DNAA and DNAB of New World begomoviruses. For each read, the three best hits were recorded and three files were prepared, with reads mapping to (i) Tomato severe rugose virus (ToSRV), (ii) *Sida micrantha* mosaic virus (SiMMV) and (iii) any other begomovirus. The results indicate that >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. These results are consistent with the hypothesis that the composition of viral populations shifts after transfer to a different host.

#### PIV36 - IDENTIFICATION OF SUSCEPTIBLE HOSTS TO TOMATO CHLOROSIS VIRUS INFECTION IN BRAZIL

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2. EMBRAPA HORTALIÇAS

The tomato (*Solanum lycopersicum*) is one of the most important vegetables grown in Brazil due to the extensive cultivated area and the great socioeconomic importance. However, the tomato crop cultivation is severely hampered by the attack of a wide range of insect pests and diseases, including those of viral etiology. Recently, a crinivirus has emerged as one of the most frequent virus occurring in tomatoes. Tomato chlorosis virus (ToCV) is the only crinivirus reported in Brazil. It was reported in 2008 in the state of São Paulo (Brazil), and soon became widely spread in the major producing states of the country. Little is known about its biological characteristics involving aspects of the host range. Thus, this study aimed to identify cultivated and non cultivated plants that are susceptible to ToCV infection in experimental and natural conditions. A total of 50 plant species was evaluated by inoculations performed with 50 viruliferous adult whiteflies *Bemisia tabaci* biotype B (MEAM1) that had acquired ToCV (isolate ToCVBR) in infected tomato plants. To confirm infection, plants were tested by RTPCR using ToCV specific primers, and back inoculated to tomato plants. Out of the 50 plant species tested, nine were susceptible to ToCV infection, *Gomphrena globosa*, *Chrysanthemum coronarium*, *Datura stramonium*, *Nicotiana benthamiana*, *N. tabacum* cv. TNN, *Nicandra physaloides*, *Physalis angulata*, *P. pubescens* and *Solanum americanum*. Back infection to tomato plants was successful in all plant species, except

for *G. globosa*. To detect ToCV in natural conditions, 120 symptomatic or asymptomatic weed samples were collected close to tomato fields, and tested by RTPCR. The following species were found naturally infected by ToCV: *Amaranthus hybridus*, *N. physaloides* and *S. americanum*. This is the first report of the susceptibility of the ornamental plant *C. coronarium* to ToCV. It was shown that weeds represent potential sources of ToCV virus to tomato crops, particularly the species *A. hybridus*, *S. americanum*, *N. physaloides* and *P. angulata*, plants frequently found in tomato fields. Therefore, a ToCV disease management strategy should include the control of infected weeds close to tomato fields.

#### PIV45 - FIRST REPORT OF AN AMALGAVIRUS IN BRAZIL

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3. EMBRAPA QUARENTENA VEGETAL

Tomato fruits are famous for their antioxidant and anticancer compounds, being extensively used in salads, sauces and sophisticated dishes. Brazil is the eighth largest producer, with approximately four million tons produced in 2013. However, this crop is the target of several pathogens that can limit its production. Viruses cause some of the most important diseases in tomatoes, due to their high incidence, prevalence, and difficulty to control. A virus survey was performed using a nextgeneration sequencingbased analysis in tomato samples collected in two regions, CampinasSP (libraries Ahol, Toca1, Toca2) and BrazlândiaDF (library Braz). Viral particles were semipurified and total RNA was extracted using RNeasy® kit (Qiagen) according to manufacturer's recommendations. Samples were sequenced on an Illumina platform HiSeq2000, at Macrogen Company (Seoul, South Korea). Obtained sequences were trimmed with Trimmomatic 0.35 and contigs were assembled using the Velvet algorithm (word size 29 Kmer). The generated contigs were transferred to the Geneious software 9.0.5 (Biomatters) and submitted to BLAST analysis. Sequences sharing high identity with an amalgavirus were detected in all four libraries. Two contigs of 1.120 nt and 441 nt from Brazlândia and Campinas, respectively, shared 100%

identity with an amalgavirus sequence (accession NC\_011591, E value=0.0). A pair of specific primers targeting the partial overlapping region between coat protein and RNA polymerase genes was synthesized and used for RTPCR detection tests in the original samples, in new randomly fieldcollected samples from Brazlândia-DF, and in tomato seedlings germinated on filter paper. This amalgavirus was detected in the original samples, Ahol, Toca1, Toca2 and Braz, and the resulting 440 bp amplicons shared ca. 95% nucleotide identity with amalgaviruses isolated in the United States, Bangladesh, China and Mexico. The virus was detected in 98% of the samples collected in BrazlândiaDF (total=54) and in seedlings of three out of the five commercial varieties evaluated, with an incidence ranging from four to 36%, suggesting seed transmission ability. These results strongly suggest the presence of an amalgavirus in Brazil, and the final cloning and sequencing tests are currently being carried out before officially notifying its presence to the Ministry of Agriculture.

#### **PIV53 - MOLECULAR DETECTION OF JOHNSONGRASS MOSAIC VIRUS IN FORAGE CROPS IN BRAZIL**

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Considering that pasture grasses are the basis of cattle feeding in Brazil, the study of its phytopathogenic agents is crucial for the development of resistant and more productive grasses cultivars. Plants showing virus-like symptoms have been frequently found in forage species as *Brachiaria* sp. and *Panicum* sp., representing a potential risk for its cultivation. Since there is a lack of information about the occurrence and damages caused by viral infections in forage plants, our aims here were to detect and characterize Johnsongrass mosaic virus (JGMV) in *Panicum* and *Brachiaria* plants collected at the forage germplasm bank of EMBRAPA National Center for Beef Cattle Research. Specific primers were designed from the sequences previously obtained by nextgeneration sequencing and used to amplify the coat protein (CP) and cylindrical inclusion (CI) genes. The amplified PCR products were cloned in pGEM®T Easy vector (Promega), sequenced by Sanger and analyzed

using the software Geneious 9.0. From all plants tested, the JGMV CP gene fragment of 924pb was amplified, producing 39 sequenced clones. The phylogenetic analyses demonstrated that Brazilian's isolates comprise a monophyletic group in which we observed that viral clones derived from the same plant were often dispersed in the phylogenetic tree, suggesting that virus coinfections with distinct JGMV genotypes are common. These results suggest that JGMV is indeed an important pathogen of forage crops, the variation at the sequence of the CP protein could be responsible for the previously described host range variation, cross protection, and virulence among JGMV isolates. In addition to the CP gene, the amplification and sequencing of the CI gene are currently being performed to be used as an additional comparative tool to determine the diversity of JGMV in Brazil.

#### **PIV55 - EVALUATION OF DAMAGE CAUSED BY TOMATO CHLOROSIS VIRUS (TOCV) AND TOMATO SEVERE RUGOSE VIRUS (TORSV) IN SIMPLE AND MIXED INFECTION IN SWEET PEPPER**

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Sweet pepper is cultivated in all regions of Brazil but the Southeast and Midwest States are the largest producers. Tomato severe rugose virus (ToSRVGenus Begomovirus) and Tomato chlorosis virus (ToCVGenus Crinivirus) are of great agricultural importance and can be found infecting this culture. To assess the damage caused by these pathogens in sweet pepper, cultivars MagaliR and RubiaR were inoculated with these viruses by the vector *Bemisia tabaci* MEAM1 cryptic species (biotype B), in single and mixed infections. Adults of *B. tabaci* were maintained during 24 hours in cages containing sweet pepper plants infected by ToCV, ToSRV and ToCV+ToSRV to acquire the viruses. Fifty whiteflies were then transferred to healthy plants placed in cages during 1 week for the inoculation access period. As NestedRTPCR (Primers pairs HS11/HS12 and ToC 5/ToC6) is not efficient to detect ToCV in sweet pepper, the inoculated plants were used as inoculum source for the virus transmission by whiteflies to healthy tomato cv. "Mariana", well known as susceptible

to ToSRV and ToCV, to comprove that the primary inoculation of the sweet pepper plants was succeeded. Total RNA and DNA was extracted from tomato and a PCR (Primers pair PAL1v1978/PAR1c496) and a Nested-PCR, as previously described, were performed to detect ToSRV and ToCV, respectively. The number, weight and diameter of the fruits and plant height were analyzed with ASSISTAT software using ScottKnott test, and  $P = 0.05$  of significance limit was used for the statistical analysis. The fruits diameter and plant height were not directly influenced by ToCV and ToSRV infections, however, the infected plants showed a reduction of 25 % and 14% in the number and weight of the fruits, respectively. The results demonstrate that although the symptoms can be tenuous in sweet pepper fruits, the infected plants had a reduction in the productivity.

#### **PIV56 - POPULATIONAL DYNAMICS OF BEMISIA TABACI MEDITERRANEAN SPECIES (BIOTYPE Q) IN BRAZIL AND VIRUS TRANSMISSION**

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*Bemisia tabaci* (Hemiptera: Aleyrodidae) is a highly polyphagous insect and considered a supervector of viruses that alone transmits over 300 species, representing 5 genera, including DNA and RNA viruses of several shapes. More than three decades after the *B. tabaci* Middle EastAsia Minor 1 species (MEAM1, biotype B) invasion in Brazil, the presence of the *B. tabaci* Mediterranean species (MED, biotype Q) was first reported in Rio Grande do Sul, and recently in São Paulo and Paraná States. In 2015, a first survey in São Paulo State revealed that MED species was present only in commercial ornamentals greenhouses and not in open field neither in vegetable cultures, where MEAM1 prevailed ever since. In 2016, however, a second and more extensive survey performed in São Paulo and Paraná showed that MED has spread through several important vegetable cultures, like tomato, cucumber and sweet pepper, either in greenhouses and open fields located near where MED was detected in ornamental plants. The endosymbiont sets are different as well.

*Arsenophonus*, *Hamiltonella* and *Rickettsia* were detected by PCR and confirmed by sequencing and FISH analysis for MED from São Paulo and Paraná in 2015, differing from MED detected in Rio Grande do Sul that harbored *Hamiltonella* and *Cardinium*, and from the MED collected in 2016, whose sets were composed by *Arsenophonus*, *Hamiltonella*, *Rickettsia* and *Wolbachia*. A pure colony of MED was reared and transmission tests with Tomato severe rugose virus and Tomato chlorosis virus were performed and demonstrated that MED is an efficient vector of these viruses, representing a new concern for Brazilian agriculture.

#### **PIV57 - VIRUS TRANSMISSION BY BRAZILIAN NATIVE AND INVASIVE SPECIES OF BEMISIA TABACI**

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3. INSTITUTO AGRONOMICO DE CAMPINAS

In Brazil, the first major invasion event of *Bemisia tabaci* occurred in the 1990s by ornamentals plants in São Paulo state when Middle East–Asia Minor 1 species (MEAM1, biotype B) was first detected. More than two decades later, the presence of the Mediterranean invasive species (MED, biotype Q) was reported in Rio Grande do Sul, suggesting that these insects were introduced probably from Uruguai. More recently MED was also detected in São Paulo and Paraná States associated to ornamental plants, indicating different invasions. Besides invasive species, there are also two indigenous species, New World 1 (NW1) and New World 2 (NW2), formerly known as biotype A, which have not been completely displaced by MEAM1. The ability of the native species NW2 and the recently introduced MED species to transmit viruses was investigated. Our work provided evidence that the native NW2 species is an especially good vector of Bean golden mosaic virus (BGMV) and Euphorbia yellow mosaic virus (EuYMV) but can also transmit the carlavirus Cowpea mild mottle virus (CpMMV) that infects beans and soybeans, as well as the crinivirus Tomato chlorosis virus and the begomovirus Tomato severe rugose virus (ToSRV). Begomoviruses

associated to weeds were also detected directly from NW2 specimens collected in the field, indicating that NW2 might be important to maintain dispersion of begomovirus that infect weeds in Brazil. Concerning MED species, a pure colony was reared on cotton plants. This population contains Hamiltonella and Richettsia as secondary endosymbionts, with a frequency of 14% and 29%, respectively. These specimens were able to efficiently transmit ToCV and ToSRV+ToCV to tomato plants and CpMMV+BGMV to beans. Other tests are in progress and will help to better clarify the importance of the recently introduced MED in Brazil.

#### **PIV66-UBIQUITIN-RELATED RING E3 OVEREXPRESSION ON NICOTIANA BENTHAMIANA: EFFECT ON TOMATO CHLOROTIC MOTTLE VIRUS INFECTIVITY**

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Ubiquitination is involved in the regulation of many signaling pathways, through the control of cellular proteins degradation. This process involves an enzymatic cascade that tags the substrate by the attachment of ubiquitin molecules with the participation of E1 ubiquitin activating enzyme, E2 ubiquitin conjugation enzyme, and E3 ubiquitin ligase. Several plant viruses show the ability to disturb the ubiquitination pathway by inducing, inhibiting or modifying these enzymes, particularly E3 ligase that confers specificity to the substrate. To study Tomato chlorotic mottle virus (ToCMoV) infection in tomato (*Solanum lycopersicum*) and its resistance mechanism shown by 'LAM 157' line, cDNA libraries from inoculated plants were sequenced by RNAseq and compared with the near isogenic line, the susceptible variety 'Santa Clara'. In silico analysis identified two RING finger proteins related to E3 ligases complex as differentially expressed in inoculated plants. qRT-PCR analysis confirmed the high expression levels of Ring E3 transcripts in 'LAM 157' plants nine days after ToCMoV inoculation. Therefore, to study the effect of Ring E3 on ToCMV infection, transgenic plants of *Nicotiana benthamiana* were evaluated. For that, Ring E3 cDNA was PCR amplified, cloned into pENTR/DTOPO vector, recombined to the binary vector pK2GW7,0 and

transferred to *Agrobacterium tumefaciens* GV3101. Leaf discs of *N. benthamiana* were inoculated and cultured under kanamycin selection. Six plants tested positive for the transgene by PCR. T1 seeds of one of the transformation events (3.2) were germinated in MS medium containing kanamycin (100 mg/ml) and plantlets with well developed roots were transferred to soil. Fourteen transgenic plants were inoculated with ToCMoV by biolistics, using as control, seven wild type (WT) *N. benthamiana* plants. Preliminary results showed that *N. benthamiana* plants overexpressing Ring E3 gene were smaller and showed stronger ToCMoV symptoms than WT plants. Assays to determine virus presence and quantification, as well as the Ring E3 expression in the transgenic plants, are underway. These findings will help to elucidate how Ring E3 overexpression interferes with viral accumulation and its involvement in ToCMoV-susceptibility and resistance mechanisms in plants.

#### **PIV67 - GENE SILENCING IN WHITEFLY (BEMISIA TABACI) BY ORAL ROUTE USING VIRUS INDUCED GENE SILENCING (VIGS)**

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Virus induced gene silencing (VIGS) is widely used in plants to downregulate the expression of a target gene. In a similar approach, VIGS can be adapted for gene silencing in sucking insects and other plant parasites such as nematodes. The whitefly (*Bemisia tabaci*) is an important insect pest causing damage to many crops around the world, including many plants of the Solanaceae and Fabaceae families. Whiteflies are also responsible for transmitting important plant viruses such as begomovirus and crinivirus, which motivates numerous studies aiming alternative strategies for their control. The objective of this work is to test the effectiveness of VIGS for gene silencing in whitefly by the oral route. The target genes are the vATPase subunit A and Ribosomal Protein L9 (RPL9), both essential for the insect and known to cause a high mortality rate in *B. tabaci* when silenced. Total RNA was isolated from whiteflies and cDNA was synthesized.

Fragments of about 200 nucleotides were amplified and cloned in vector pCR8/GW/TOPO® (Invitrogen). After sequencing, the fragments were transferred by LR recombination to the viral vector PotatoVirus X (PVX). This vector was transferred transformation to *Agrobacterium tumefaciens* 'GV3101', and inoculated in plants. PVX empty vector (pGR107) and PVXGFP was used as controls and to analyze suitable host plant for the virus infection. Six plants species were initially tested: *Nicotiana tabacum*, *Datura stramonium*, *Solanum melongena*, *Abelmoschus esculentus*, *Capsicum annum* and *Brassica oleracea*. In *Nicotiana tabacum* cv. TNN and *Datura stramonium* the virus was detected by RT-PCR, 28 days after inoculation, in noninoculated leaves, confirming the systemic infection in the plants. *N. tabacum* cv. TNN and *D. stramonium* are known hosts for the whitefly and were shown to be also good hosts for PVX and therefore, were chosen for further experiments. Studies of vATPase and RPL9 gene silencing in the whitefly using PVX-mediated VIGS are in progress.

#### **PIV82 - THE COMPLETE GENOME SEQUENCE OF MELON YELLOWING ASSOCIATED VIRUS DETERMINED BY NEXT GENERATION SEQUENCING**

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1. UNIVERSIDADE DE BRASÍLIA
2. UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
3. UNIVERSIDADE DE BRASÍLIA
4. EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA, CENTRO NACIONAL DE PESQUISA DE AGROINDÚSTRIA TROPICAL
5. EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA HORTALIÇAS

The Northeast region of Brazil is the major melon producing zone in the country, being responsible for 95% of the total national production. A devastating disease has been reported in melon plants since 1999. It is known as "yellowing of melon plants" (Amarelão do meloeiro), which is associated to a viral agent, Melon yellowing associated virus (MYaV). This virus belongs to genus *Carlavirus* in the family *Betaflexiviridae*, formed by a linear ssRNA (+) genome of ca. 9 kb. The genome contains six ORFs. The complete genome sequence of MYaV is still not available, thus the 'Next Generation

Sequencing' (NGS) strategy was applied to unravel the genome sequence of an isolate of MYaV. Melon samples used in this study were collected from Jaguaribe-Açu agricultural center (CE/RN), which produces ca. 81% of the total national production, and where the virus incidence is frequently high. Plants exhibiting yellowing symptoms were subjected to viral semi-purification according to Cali Moyer (1991) protocol, with modifications. Total RNA was extracted from semi-purified virus preparations with Trizol LS Reagent (Invitrogen), and dried using RNastable (Biomatrica). The ribosomal RNA molecules were removed from the extract and the remaining RNA was sequenced at MacroGen, Inc. (South Korea) by Illumina 2000 HiSeq with 100 bp pairedend. Mapping and assembly of viral quasicomplete genome sequence were done with the Software Geneious 8.1. The genome, lacking its 5' and 3' ends, was approximately 9 kblong, in a typical carlaviral genomic organization with six ORFs. Pairwise nucleotide comparison and phylogenetic analysis confirmed that this virus belongs to the genus *Carlavirus*. Based on the species demarcation criteria of this genus, viruses sharing nucleotide (nt) sequence identity of CP or polymerase genes lower than 72% or amino acid sequence identity lower than 80% are classified as distinct species. The nt sequence of the CP of this study shared 97% identity with the MYaV CP gene available in GenBank and isolated in 2010 (ID: AB510477), and 57.9% with Sweet potato yellow mottle virus (SPYMV), the closest member among the carlaviruses. The nt sequence of the RdRp gene (ORF1) shared the highest identity of 57.2% with SPYMV. In conclusion, the complete genome sequence of MYaV showed a typical carlaviral genomic organization, as well as its genome nt identity with other viruses low enough to be considered as a distinct viral species in the genus *Carlavirus*.

**PIV100 - MOLECULAR CHARACTERIZATION AND COMPLETE GENOME SEQUENCE OF A TOBACCO INFECTING TOMATO BLISTERING MOSAIC VIRUS**

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1. EMBRAPA RECURSOS GENÉTICOS E BIOTECNOLOGIA
2. UNIVERSIDADE DE BRASÍLIA

Tomato blistering mosaic virus (ToBMV) is a monopartite singlestranded positive sense RNA virus member of the Tymovirus genus (family Tymoviridae). This virus has solanaceous plants as natural hosts and was reported causing severe symptoms of leaf mosaic and blistering in tomato plants. In a previous work, a tobaccoinfecting ToBMV was near completely sequenced using Illumina® sequencing technology (GenBank KJ940970). In this study, we determined the 5' and 3' terminal sequences of this virus isolate completing its entire genome. Total RNA was extracted from tobaccoinfected plants using Trizol® reagent. The 3' RACE was done by adding a poly(A) tail to the viral RNA genome and synthesized the firststrand cDNA using Superscript III reverse transcriptase (Invitrogen) and Oligo(dT)50 anchor-primer. RTPCR and nested PCR were conducted using LongAmp Taq DNA polymerase (NEB) and specific forward primers designed based on the contig from the Illumina sequence. To determine the 5' terminal sequence, the firststrand cDNA was synthesized using Superscript III and specific reverse primer. A poly(G) tail was then added to the cDNA. RTPCR and nested PCR were done using LongAmp Taq DNA polymerase (NEB) and specific ToBMVtobacco reverse primers and a poly(G) anchor forward primer. The resulting 3' and 5' terminal fragments were cloned and sequenced. We were able to identify 18 nucleotides at the 5' terminal and five additional nucleotides at the 3' terminal. Primers were designed to amplify the complete ToBMV-tobacco genome and the GibsonAssembly® approach was used to construct a fulllength clone. ToBMVtobacco sequence comprises a total of 6,280 nucleotides and shares 88% identity with the ToBMVtomato isolate SC50 (GenBank KC840043) and 78% identity with the ToBMV Solanum violaefolium isolate SP01 (GenBank KT834406). The ToBMV isolate from tobacco does not display systemic symptoms in tomato plants, neither in cultivated varieties or wild relatives. This characteristic

makes tobaccoinfecting ToBMV an excellent candidate for virusinduced gene silencing (VIGS) vector for tomato functional genomics studies.

**PIV106 - FIRST REPORT OF SIDA MICRANTHA MOSAIC VIRUS IN OXALIS SPP**

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2. UNIVERSIDADE DE BRASILIA

Begomoviruses are singlestranded DNA viruses transmitted by the whitefly Bemisia tabaci. Their genomes are either monopartite or bipartite and known as DNAA and DNAB. Sida micrantha mosaic virus (SiMMV) is a bipartite begomovirus of the new world that has already been described infecting tomato, soybean, common bean, okra, cotton, and Sida spp. Plants in Brazil. In this report, we characterized SiMMV isolates from Oxalis sp. Four Oxalis spp. plants were collected in urban areas of Brasília, Distrito Federal, and Londrina (Paraná State). The collected samples displayed symptoms of yellow mosaic and leaf distortion, typical of begomovirus infection. Total DNA was extracted from the samples using CTAB method, and begomovirus infection was confirmed using the degenerate primers pAL1v1978 e pAR1c496. Total DNA of each sample was submitted to rolling circle amplification (RCA) using Phi29 DNA polymerase for viral DNA enrichment. RCA products were digested with the enzymes ClaI, EcoRV, SacI, EcoRI and HindIII, cloned into pBluescript SK+ and completely sequenced. BLASTN comparison and Species Demarcation Tool (SDT) (v.1.0) analysis were done to identify the virus species infecting the plants. Complete DNAA and DNAB clones were obtained from the Oxalis sp. samples with genomic organization of typical bipartite begomovirus. DNAA clones shared 93% nucleotide identity amongst them and Species Demarcation Tool (SDT) analysis resulted in identities ranging from 82% to 96% with the 19 SiMMV full DNAA isolates in Genebank. These results confirm that Oxalis sp. plants were infected with SiMMV. DNAB clones were 90% identical, and identities ranging from 75% to 93% with all 16 SiMMV DNAB isolates present in Genebank. This is the first report of SiMMV infecting Oxalis sp. which broadens the host range of this begomovirus.

**PIV109 - IDENTIFICATION OF A NEW VITIVIRUS IN ARRACACIA XANTHORRHIZA BY NEXT GENERATION SEQUENCING**

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Vitiviruses belong to the Betaflexiviridae family and possess a positivesense single stranded RNA genome of about 7.37.6 kb. The genomic RNA of vitiviruses has a 5' cap, a 3' poly(A) tail and are known to be organized into five overlapping open reading frames. Viruses belonging to the Vitivirus genus has a restricted host range and are natural transmitted by pseudococcid mealybugs, soft scale insects and aphids, either by mechanical inoculation or by grafting. NextGeneration Sequencing (NGS) technology is a very powerful tool for detecting and discovering novel viral genomic sequences without having prior knowledge. Here we describe a putative new member of the Vitivirus genus that was discovered by NGS from arracacha (*Arracacia xanthorrhiza*) plants. The complete genome sequence was confirmed through Sanger sequencing. Based on the sequence information obtained by our NGS approach, primers were designed to amplify specific overlapping regions of the new virus genome. To this aim, total RNA was extracted from *Arracacia xanthorrhiza* using TRIzol reagent (Invitrogen). RTPCR was performed using the SuperScript™ IV Reverse Transcriptase kit (Thermo Fisher Scientific) and Long Amp Taq DNA polymerase (NEB). In order to determine both genomic termini, 3'RACE and 5' RACE protocols were performed, determining the whole Vitiviral genomic sequence. The amplified fragments were gelpurified with the GE Healthcare Life Sciences kit and send for sequencing (Macrogen, Korea). The genomic organization resembles closely that of grapevine viruses D and A. After amino acid sequence analysis, the putative CP showed 47% to 50% of identity with the coat proteins encoded by grapevine vitiviruses. According to the vitivirus species demarcation criteria, viruses from different species should have less than 80% of identity at amino acid level or less than 72% of nucleotide identity of the coat protein or polymerase gene. Giving these findings the newly discovered virus infecting arracacha is considered a new species of the Vitivirus genus.

**PIV123 - THE HIGHLY DIVERGENT JOHNSONGRASS MOSAIC VIRUS ISOLATE FROM PENNISETUM PURPUREUM REPRESENTS A POTENTIAL THREAT TO CORN CROPS IN BRAZIL**

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1. UNIVERSIDADE DE BRASÍLIA
2. EMBRAPA RECURSOS GENÉTICOS E BIOTECNOLOGIA
3. EMBRAPA GADO DE CORTE
4. EMBRAPA CERRADOS

In the last years, plants showing viruslike symptoms have been observed in the main pasture grass growing areas. Plants of *Pennisetum purpureum* line CNPGL 00211 showing mosaic symptoms on leaves and growth reduction were collected in Minas Gerais State, Brazil. Flexuous elongated potyviruslike particles were observed in the leafdip preparation of diseased plants by electron microscopy. The complete genome sequence (9865 nucleotides) of this highly divergent Johnsongrass mosaic virus isolate (JGMVCNPGL) was determined using Illumina sequencing. The virus JGMVCNPGL was mechanically inoculated into 14 putative host plants to determine its host range and the characterization of symptoms expression. These plants were kept in greenhouse for 30 days and the symptoms monitored during this period. The first symptoms appeared at 13 days after inoculation (dpi) and the infection was confirmed by RTPCR using specific designed primers (JG8352F CAAAGCCCCATACTTGTCGG; JG9413R TTAGCCCCACGGTATGAATG). Only 10 hosts were susceptible to the JGMVCNPGL isolate presenting mainly two types of symptoms: chlorotic veins observed in *Zea mays* 2B587, *Zea mays* 3646H1 and *Millet* ADR500 and mosaic symptoms in *Brachiaria brizantha* cv. Arapoty, *Brachiaria brizantha* cv. Xaraés, *Panicum maximum* cv. Mombaça, *Panicum maximum* cv. Massai, *Panicum maximum* lineage C12, BRS Capileto and *Sorghum bicolor* BRS332. The JGMVCNPGL isolate was unable to infect *Wheat* BRS264, *Hordeum vulgare* L. VCUCPAC, *Crotalaria juncea* and *Glycine max* under the tested conditions. Comparatively, JGMVCNPGL host range was similar to those reported for JGMVN and JGMV MDO. A comparative analysis of the complete genome showed a nucleotide identity of 80% nt (86%

aa) with Johnsongrass mosaic virus Australia. However, the CP identities were slightly above 78% and 82% (nt and aa, respectively), close to the species demarcation values. Crucially, JGMVCNPGL isolate was able to infect maize genotypes, suggesting that this virus represent a potential threat to this important crop. Brazil is the world's third largest maize producer, planting, yearly, over 15.8 million ha, which represents 80 million ton of maize grains productions. The biological implication of this striking difference among JGMV isolates worldwide and its evolutionary history remains to be elucidated

#### **PIV125 - GENOME CHARACTERIZATION OF RHABDOVIRUS DETECTED IN MOSQUITOES TRAPPED AT RIO DE JANEIRO STATE**

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Many arboviruses are responsible for significant human diseases, ranging from mild febrile illness to encephalitis and death. During the last decade the world has been facing the burden of introduction and re introduction of arboviruses such as zika virus, dengue virus, chikungunya virus, japanese encephalitis virus, west nile virus and yellow fever. Most of the characterized virus species related to human diseases belong to 3 families: Togaviridae, Flaviviridae, and Bunyaviridae. However, a significant number of viruses is also distributed among 3 other families: Reoviridae, Rhabdoviridae, and Orthomyxoviridae. Besides the species related to human diseases, a huge diversity of insectonly viruses exist. Very little is known about the diversity, transmission, physiologic effects in host and the impact of insect ecology or even their impact in human populations in this whole scenario. The continuous search for new species is first step to improve this knowledge. The family Rhabdoviridae is very diverse consisting of 6 well

established genders and 130 unsigned viruses widely distributed in nature from vertebrates, invertebrates and plants. The objective of this study was the screening through familyspecific RTPCR of 16.163 mosquitoes collected from 2012 to 2015 in several areas of Rio de Janeiro State. The vectors were separated by genus/species and sex. RNA were extracted and analyzed in the form of a mixture. From all samples two were positive, one collected in 2012 and the other in 2015. The genome sequences obtained for both samples presented homologies with rhabdovirus detected in America.

#### **PIV126 - TEMPLATEBASED MODELING AND MOLECULAR DYNAMICS OF THE TOSPOVIRUS GROUNDNUT RINGSPOT VIRUS NUCLEOPROTEIN: INTRAMOLECULAR INTERACTIONS AND RNA ENCAPSIDATION**

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The understanding of protein folding mechanisms and the advances in the bioinformatics field have provided tools to modeling and predict three dimensional structures of plant virus proteins. The nucleoprotein (NP) crystal structures of related RNA virus families were elucidated and despite having different sizes and distinct NP folding structures, these proteins share common features and architectural principles when forming NPNP multimers and NP-RNA complexes. Due to their genetic relationship, the La Crosse virus (LACV) crystal structure in complex with ssRNA was selected as template for a templatebased modeling approach and molecular dynamics simulations to predict a three dimensional model for the NP of the tospovirus Groundnut ringspot virus (GRSV). The GRSV NP monomer was predicted to possess thirteen helical segments and two small beta-sheets organized in a globular core domain (26223 aa) containing a deep positively charged groove with the two terminal chains forming a Nterminus arm (125 aa) and a Cterminus arm (224258 aa). Both N and Carms extend outwards from the globular core domain and they interact with the globular core domain of neighboring monomers to mediate the multimerization, supporting the "headtotail" model. The RNA is primarily bound at

the central RNA binding groove and the key residues for this interaction are mainly located in this groove. RNA is strongly bent at each NP–NP interface and is largely solvent inaccessible in the tetramer structure. The dimensions of the groove allow accommodation of ssRNA and further analysis showed that the majority of residue-nucleotide interactions occur with the ribose and the phosphate moiety, suggesting a non sequence-specific ssRNA interaction. During the simulation time, the globular core domain did not reveal any loss of secondary structure, increase of radius of gyration or persistent increments on RMSD values, which supports the model quality. The RMSF calculations indicate the N-terminal arm as a very flexible region. Most of the key residues are conserved among all tospoviruses. Copies of the NP form oligomers that interact with the viral RNAs to build ribonucleoprotein complexes (RNPs) that are proposed to be transported via plasmodesmata and are templates for RNA replication and transcription. The proposed model may shed light on the mechanisms of RNP shaping and allow the identification of essential amino acid residues as potential targets for tospovirus control strategies.

**PIV145 - NGS STRATEGY REVEALED THREE PUTATIVE MEMBERS OF A NEW GENUS IN THE POTYVIRIDAE FAMILY NATURALLY INFECTING STYLOSANTHES**

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1. EMBRAPA GADO DE CORTE
2. UNIVERSIDADE DE BRASÍLIA
3. EMBRAPA CENARGEM

Next generation sequencing (NGS) is quickly emerging as the go-to tool for plant virologists when sequencing whole virus genomes and undertaking plant metagenomic studies for new virus discoveries. Two *Stylosanthes* sp. samples were shipped to sequencing through the use of NGS and three novel potyviruses, preliminarily named as Poty 1, Poty 2 and Poty 3, were discovered naturally infecting *Stylosanthes* plants. These samples were collected in experimental fields of Embrapa Beef Cattle in Mato Grosso do Sul, showing typical leaf mosaic symptoms. To obtain a viral enriched fraction, the leaves were ground in phosphate buffer, filtered and centrifuged through a sucrose cushion. Viral RNA was extracted using RNeasy Mini Kit following the

manufacturer's instructions. The RNA samples were pooled and sequenced at MacroGen INC. (Korea) using Illumina HiSeq 2000 technology. Based on the results from the consensus NGS, primers were designed for whole genome and these viruses were confirmed in the infected samples by RTPCR. The 3' ends were confirmed by using oligodT primers with specific forward to each virus. The 5' ends were confirmed with the techniques of the SMART PCR and RACE. The complete genomes were determined to comprise of 9213 nucleotides for Poty 1, 9197 nucleotides for Poty 2 and 9425 nucleotides for Poty 3 (excluding the polyA tails). The complete virus genomes and CP sequences were compared with sequences available in GenBank. The highest nucleotide identities of 43%, 39% and 56% were determined compared to other potyviruses, respectively. The genomes were deduced to encode a single open reading frame (polyprotein) on the plus strand. Phylogenetic analysis based on the whole genome sequences and coat protein amino acid sequences showed that the new viruses found are most closely related to the Blackberry virus Y (Poty 1 and Poty 2) and the Rose yellow mosaic virus (Poty 3). The biological features of these new potyviruses are currently being investigated.

**PIV151 - HIGH INCIDENCE OF MIXED DNA AND RNA VIRUS INFECTIONS IN COMMON BEAN IN CENTRAL BRAZIL**

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2. EMBRAPA RECURSOS GENÉTICOS E BIOTECNOLOGIA
3. EMBRAPA ARROZ E FEIJÃO

Common bean is one of the most important protein food source consumed worldwide, mainly in Africa, South and Central Americas. In Brazil, there are several diseases affecting bean fields, including viral diseases, such as the Bean golden mosaic virus (BGMV), responsible for losses that can reach 100%. During the winter crop season in 2016 a very high incidence of virus-like symptoms of mosaic, leaf curling and deformation, and plant dwarfing was reported by farmers in central areas of Brazil. Bean plants were collected in commercial farms in Luziânia, Cristalina and experimental plots in Goiânia and Brasília. Total DNA was extracted using

CTAB method. Total RNA was obtained using Trizol® reagent. Samples were tested for the presence of DNA and RNA viruses that are commonly found infecting beans in Brazil. Begomoviruses BGMV, Macroptilium yellow spot virus (MaYSV), and Macroptilium yellow net virus (MaYNV) were detected by PCR using specific primers. Detection of the RNA viruses Cowpea mild mottle virus (CPMMV Carlavirus), Bean rugose mosaic virus (BRMV Comovirus) and a new, yet not fully characterized rhabdovirus (Bean associated rhabdovirus BAR), recently found by our group was performed by RTPCR with specific primers. For the samples tested thus far, a high incidence of mixed infection, usually with three viruses was detected in all sampled areas. BGMV and CPMMV were present in 100% of the plants while BAR had an incidence of 60-100%, varying according to the region where the samples were collected. BRMV was detected in a few samples while MaYSV and MaYNV were not identified in any of the samples. This widespread of mixed infection is causing extensive yield losses in the bean crop and will likely impact on availability and influence market prices.

#### **PIV155 - A PLANT VIRUS COAT PROTEIN AS A CARRIER PROTEIN FOR MEDICAL INTEREST EPITOPES IN BACULOVIRUS/INSECT CELL SYSTEM**

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2. UNIVERSIDADE DE BRASÍLIA

The baculovirus expression vector system (BEVS) has been widely used to produce a large number of recombinant proteins and is becoming one of the most powerful, robust, and cost-effective system for the production of proteins. The success of the system is due to the intrinsic security and the high yields of protein expression. BEVS can be used for the production of virus-like particles (VLPs). The VLPs can be obtained basically by expression of recombinant capsid proteins in a variety of heterologous systems, that promote the self-assembly of proteins into structures similar virus particles. VLPs have antigenicity similar to that of the native virus, but they lack genetic material, thus are not infectious. Tomato blistering mosaic virus (ToBMV) is a plant virus infecting plants from the genus *Solanum* and, in this work, candidate as a carrier protein for medical

interest epitopes. Therefore, we are assessing the assembly of tymoviruslike particles (tVLPs) using BEVS. In this work, the potential use of tVLPs for displaying biopharmacological epitopes with medical interest and one epitope of Chikungunya virus (CHIKV) envelope protein 2 (E2) was selected. CHIKV is a mosquito-borne viral disease that causes headache, fever and severe joint pain. Since 2004, this virus is affecting thousands of people around the world. Importantly, there is no available serological kit for CHIKV detection produced in Brazil. Therefore, we will use the tVLPs displaying the CHIKV E2 epitope to generate diagnostic kits for human serum. For this purpose, we are analyzing the deletion construct of the first 23 a.a. of ToBMV CP whether this deletion will interfere with the correct assembly of the tVLPs. If not, we will replace these 23 a.a. by CHIKV epitope. At first, the deletion mutant gene of ToBMV CP was cloned to pFastBac1 vector. The constructs were sequenced and the DH10Bac strain of *Escherichia coli* that contains BacMed was transformed with selected clones to generate recombinant baculoviruses by prokaryotic transposition and viral DNA transfection into insect cell. The protein expression of this construct is now on evaluation by immunoblotting using specific anti-CP antibody.

#### **PIV164 - A NEW PUTATIVE GEMCIRCULARVIRUS DETECTED IN COMMON BEAN IN BRAZIL**

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1. EMBRAPA RECURSOS GENÉTICOS E BIOTECNOLOGIA
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4. UNIVERSITY OF ARIZONA

Genomoviridae is a recently created family of circular, single-stranded DNA viruses. The family is composed of one genus, Gemycircularvirus with a sole recognized species, *Sclerotinia gemycircularvirus 1*. The representative isolate, *Sclerotinia sclerotiorum* hypovirulence associated DNA virus 1, (SsADV1) was discovered in 2010, infecting the fungus *S. sclerotiorum*. There are more than one hundred SsADV1-like putative viruses described in different hosts and environmental samples, including water from rivers, treated and

untreated sewage, animals, humans and plants. In this study, we report a putative gemycircularvirus (GemyCV) associated to common bean plants. A single 123nt read with similarities with GemyCV sequences was identified in a 454pyrosequencing NGS library prepared from bean DNA, isolated from samples showing virus symptoms collected in Arcoverde, Pernambuco, Brazil. Backto back primers were designed to recover full viral genomes from individual samples using inverse PCR. We were able to recover amplicons of about 2200 pb from three of the bean samples. The fragments were cloned into PCRIITOPOTA vector and sequenced. The sequences were assembled into fullgenomes using Geneious® program. The virus genomes are 2220nt long bearing three ORFs: a putative capsid protein, a Rep and RepA protein, characteristic of GemyCVs. We propose the name Common beanassociated gemycircularvirus (CBaGmV). CBaGmV isolates share 80.480.7% identity with two isolates from Pacific flying fox feces associated gemycircularvirus from Tonga. To further investigate the biology of CBaGmV, infectivity tests are currently in progress.

#### **PIV169 - OCCURRENCE OF BEGOMOVIRUSES ON TOMATOES IN THREE BRAZILIAN STATES**

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1. INSTITUTO FEDERAL GOIANO CAMPUS MORRINHOS
2. EMBRAPA HORTALIÇAS

Begomoviruses (Family Geminiviridae) are the most important viruses infecting tomatoes (*Solanum lycopersicum*) in Brazil. These viruses are transmitted by the whitefly *Bemisia tabaci* MEAM 1 (Middle East Asia Minor 1). Many species are reported infecting tomatoes in Brazil, with prevalence of Tomato severe rugose virus (ToSRV). Based on the geographic distance and distinct cultivation conditions of tomatoes in the country, an irregular distribution of begomovirus species is expected. To test this hypothesis, 313 samples of tomato plants exhibiting typical symptoms of begomovirus infection (such as interveinal chlorosis, mosaic, leaf distortion, and stunting) were collected in Goiás (GO), Paraná (PR) and Rio de Janeiro (RJ) states in 2015. Total DNA was isolated and the presence of

begomovirus was confirmed by PCR using the universal primers PAL1v1978/PAR1c496. A total of 137 samples was PCR positive (27/57 from RJ and 110/216 from GO). Interesting, no sample from PR was PCR positive, but infected by a crinivirus (family Closteroviridae). These 137 infected plants were analyzed using RCA-RFLP with *MspI* restriction enzyme and the pattern of fragments obtained was compared with those known for the main species of begomovirus infecting tomato in Brazil. Representative RCA products were chosen and directly sequenced. It confirmed the presence of three species in single or mixed infection: Tomato mottle leaf curl virus (ToMoLCV), Tomato common mosaic virus (ToCmMV) and ToSRV. In RJ, 15% of the plants were infected by ToCmMV and ToSRV, 41% showed single infection with ToSRV, but the begomovirus present in 44% of the samples is still not determined. In GO, 4.5% of the plants were mixed infected with ToMoLCV and ToSRV, while the remaining (95.5%) showed a single infection with ToSRV. The presence of ToSRV in all these samples was confirmed by PCR with species-specific primers. This preliminary result indicates that even after many years of the last systematic study on the genetic diversity and prevalence of begomovirus species in the country, ToSRV remains the most important species, although the incidence of other species as ToCmMV and ToMoLCV are increasing in some regions in the last years. For a precise taxonomic classification, including the unidentified isolates, clones are being obtained for complete sequencing of each isolate.

#### **PIV172 - IDENTIFICATION OF A NEW POLEROVIRUS INFECTING COTTON IN MATO GROSSO THAT INDUCES APICAL NECROSIS**

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2. TROPICAL MELHORAMENTO E GENÉTICA

Cotton plants from Mato Grosso showing drastic symptoms associated to apical necrosis were seen in distinct cotton crops from Rondonópolis and Serra da Petrolina, Pedra Preta, at Mato Grosso state. Symptoms were observed in adult plants that died after disease manifestation. The middle portion of the diseased plants shows cotton blue diseaselike symptoms, as leaf rolling

and reddening. These symptoms are very similar to the observed in cotton plants infected by CLRDVbreaking resistance virus isolates. However, plants infected with Cotton leafroll dwarf virus (CLRDV – causal agent of cotton blue disease) never present necrosis symptoms. Our group has already studied at least four distinct genotypes of CLRDV and none of these isolates are able to induce apical necrosis. Four samples of plants showing this putative new disease were collect from distinct districts of Mato Grosso state. Total RNA from these samples were extracted and were submitted to CLRDV like polerovirus Nested PCR diagnosis. A fragment corresponding to the capsid region (P3/ORF3) and another corresponding to replicase (P2/ORF2) were amplified, showing that a CLRDVlike virus is present in the four samples. New primers were then used for amplify the silencing suppressor protein (P0/ORF0) in order to analyze that ORF that is the most variable ORF around the CLRDVlike polerovirus as well observed also for other polerovirus genotypes closed related by themselves. The amplified fragments were sent for Sanger sequencing and analyzing the sequences of these three ORFs we expect to identify more deeply this new polerovirus infecting cotton.

**PIV185 - VIRAL BIODIVERSITY IN THE SALIVARY GLAND OF CULICINAE MOSQUITOES CAPTURED IN SYLVATIC AREAS OF CHAPADA DOS GUIMARÃES AND PANTANAL OF MATO GROSSO, BRAZIL**

de Lara Pinto, A.Z.; Carvalho, M.S.; Melo, F.L.; Pinheiro, A.; Serra, O.P.; Bezerra, M.C.F.; Ribeiro, A.L.M.; Dezengrini Shhessarenko, R.

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Arboviruses represent a main public health problem in tropical areas. Mato Grosso presents sylvatic ecosystems harboring a great diversity of vector and host species, intense ecotourism activity in close proximity to populated urban centers, factors contributing to arbovirus emergence. The subfamily Culicinae is the major taxon inside the family Culicidae. Estimates indicate that 371 species classified in this subfamily may circulate in Brazil. This study aimed at identifying the viral diversity in sylvatic culicinae captured in two RAPELD systems present in the National Park of Chapada dos Guimarães (Rio Claro) and in North Pantanal

(Pirizal) in the rainy, intermediate and dry seasons during 20142015. Adult females (n=1.729) captured with Nasci aspirators and CDC light traps were identified alive at a dormant state according to specific dichotomy keys and their salivary gland was dissected. Specimens of 36 species from 8 genera (Aedes, Coquillettidia, Culex, Haemagogus, Mansonia, Psorophora, Uranotaenia and Wyeomyia) comprised 40 pools. Viral RNA was extracted from the minced salivary glands, converted to cDNA, subjected to cDNA secondstrand synthesis and PCR amplification with viral randomic primers (Random KS). Library was prepared with the purified DNA using TruSeq RNA Sample Prep and after sequenced in the Illumina HiSeq 2.500 plataform. Preliminary analysis of the sequences obtained from 25 pools containing species of Aedes, Coquillettidia, Culex, Haemagogus, Mansonia, Psorophora and Wyeomyia using viral RefSeq data available at NCBI generated hits by tblastx with viruses belonging to Flaviviridae, Bunyaviridae, Togaviridae, Reoviridae, Rhabdoviridae and Iridoviridae families, including also some possible plant and insect-specific viruses. Nucleotide identity among the obtained contigs and reference sequences vary among 3060%, suggesting the presence of new viral species within the salivary glands of the mosquitoes included in these pools. Financial Support: CAPES, Rede PróCentro Oeste CNPq.

**PIV189 - VIRAL BIODIVERSITY IN THE SALIVARY GLANDS OF TICKS CAPTURED IN CHAPADA DOS GUIMARÃES AND NORTH PANTANAL, MATO GROSSO, BRAZIL**

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1. UNIVERSIDADE FEDERAL DE BRASÍLIA
2. UNIVERSIDADE FEDERAL DE MATO GROSSO

Ticks are involved in the transmission cycle of several arboviruses worldwide. However, the identification of viruses associated to ticks is scarce in Brazil. The aim of this study is to investigate the presence of viruses in ticks captured in two RAPELD systems present in sylvatic areas of the Chapada dos Guimarães National Park (PNCG) and in North Pantanal (Pirizal), State of Mato Grosso. To achieve that, ticks were captured in the rainy, intermediate and dry season (20142015) in those areas, identified alive according to specific dichotomy keys and

the salivary glands were dissected from adult specimens. 265 specimens (182 adults of *Amblyomma sculptum* and 83 nymphs of *Amblyomma* sp.) were allocated in eight pools. After viral RNA extraction (High Pure Viral RNA kit), reverse transcription (GoScript, Promega), second strand cDNA synthesis (DNA polymerase I large Klenow fragment) and PCR amplification with random primers for viruses (random KS), the DNA product was purified with 20% PEG 8000, quantified (QuantiFluor® ONE dsDNA) and sequenced through Illumina HiSeq 2500 platform after the synthesis of the library (Illumina TruSeq DNA nano). Partial analysis of the sequences obtained from the eight pools indicate the presence of viruses belonging to the families Togaviridae, Flaviviridae, Bunyaviridae, Reoviridae, Arenaviridae, Orthomyxoviridae and Rhabdoviridae and also of some sequences of plant and insect specific viruses. The two pools of nymphs presented hits with RefSeq sequences of Phlebovirus, Nairovirus, Orbivirus, Vesiculovirus and Tospovirus genus. The six pools formed by adult ticks showed mostly hits with sequences of Coltivirus, Asfavirus, Pegivirus, Vesiculovirus and Alphavirus genus. Identity between the contigs and reference sequences were below 70%, which suggests the presence of new viral species in the salivary glands of ticks.

**PIV198 - VIRUSDERIVED GENOMES FROM CULTIVATED VEGETABLES IN DOMINICAN REPUBLIC. A NEW ENVIRONMENT FOR VIRUS EMERGENCE**

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2. MINISTERIO DE AGRICULTURA
3. INSTITUTO DOMINICANO DE INVESTIGACIONES AGROPECUARIAS Y FORESTALES

Dominican Republic (DR) contains large areas for vegetable production which bases a significant portion of this country economy. Chili pepper (*Capsicum frutescens*) and other pepper species, for example, are amongst the top ten commodities exported in quantity and value by DR. Recently, this vegetable production has been threatened by tospovirus infections. While Tomato chlorotic spot virus (TCSV) caused typical tospovirus symptoms in long beans (*Vigna unguiculata*) and chili pepper, Tomato spotted wilt virus (TSWV) was

found in potatoes (*Solanum tuberosum*), tomatoes (*S. lycopersicum*) and sweet pepper (*C. annuum*). Tospovirus species are notorious for inducing substantial losses on vegetable production around the world. The real diversity of plant viruses has been overlooked for a long period of time. Only plants with economical importance and presenting compromising symptoms have mostly been surveyed for identification of disease causative agents. With the accessibility of highthroughput sequencing (NGS) tools, this scenario has changed and viruses not causing apparent disease symptoms have been found in large scales. Here, sequences covering virus-derived genomes were retrieved from RNA sequencing data of symptomatic vegetables collected in DR. RNA genomes of three plant virus genera were obtained apart from the complete genome of TCSV and partial sequences of TSWV. A partial sequence of a Tobacco vein-clearing virus (TVCV) isolate was traced back only in potato plants by RTPCR. A Bell pepper endornavirus (BPEV) isolate, within family Endornaviridae, was found and traced back in sweet pepper. The cryptovirus (family Partitiviridae) Pepper cryptic virus 2 (PCV2) isolate was identified in chili pepper. Finally, a Southern tomato virus (STV) (genus Amalgavirus, family Amalgaviridae) isolate was found infecting tomato. Apart from the viruses above-mentioned, we could assemble the complete genome of a TCSV isolate. The consensus of the three genomic segments were deposited under accession numbers KX463272 (L RNA), KX463273 (M RNA), and KX463274 (S RNA). Whole transcriptome shotgun sequencing of a RNA pool was performed using an Illumina Hi Seq 2000 platform, which ended up in the production of about 53 million reads. The reads were assembled using CLC genomic Workbench. Contigs covering virus-derived genomes were built by BLASTn and BLASTx searches against the virus reference database available in the National Center for Biotechnology Information (NCBI).

**PIV199 - ABSENCE OF BEGOMOVIRUS TRANSMISSION BY SEED IN SIDA SPP**

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The genus Begomovirus (family Geminiviridae) includes a number of plant viruses of economical importance for Brazilian agriculture. Viruses classified in the genus Begomovirus have one or two genomic components and are transmitted in nature by the whitefly *Bemisia tabaci* to dicot plants. Begomoviruses naturally infect several non-cultivated hosts, such as *Sida* spp. and *Macroptilium* spp. These noncultivated hosts may harbor viral populations with a high degree of genetic diversity. Nevertheless, some viral populations seem to be confined to certain species of noncultivated plants. Based on the observation of noncultivated plants newly emerged in the field already showing symptoms of begomovirus infection apparently in the absence of the insect vector, and on recent reports of seed transmission of begomoviruses in sweet potato, bean and tomato, the objective of this study was to analyze the presence of begomoviruses in seeds of *Sida acuta* and *Sida rhombifolia*, as well as the transmission of these viruses by seed. A total of 39 plants of these two species, displaying typical symptoms of infection by begomoviruses, were collected in Viçosa, MG on December 2013 and transferred to a greenhouse. Viral infection was confirmed in 38 of these plants by rolling-circle amplification (RCA) of complete viral genomes. Amplification products were cloned and sequenced, confirming infection of *S. rhombifolia* by *Sida* yellow mosaic virus (SiYMV) and of *S. acuta* by *Sida* yellow leaf curl virus (SiYLCV). Approximately 320,000 seeds were collected from the 38 infected plants. The seeds were surface-sterilized with sodium hypochloride or sulphuric acid, and were ground in groups of 20, 30 or 200 seeds. Total DNA extracted from approximately 80,000 seeds was used for viral detection by RCA, with negative results. Total DNA was also extracted from whole flowers and from flower tissues (sepals, petals, stamens, styles and ovaries) from infected plants and used for viral detection, with positive results in all cases. Seeds from infected plants were treated with sulphuric acid, germinated and 269 plantlets from these seeds were evaluated for the presence of virus by RCA and PCR, with negative results. Together, these results indicate that SiYMV and SiYLCV are capable of infecting

the flower tissues of *Sida rhombifolia* and *Sida acuta*, respectively, however they are not transmitted by seeds in these hosts.

**PIV200 - ANCESTRY STUDY AND FITNESS ANALYZING OF THE VERY CLOSE RELATED TOSPOVIRUSES GROUNDNUT RINGSPOT VIRUS (GRSV) AND TOMATO CHLOROTIC SPOT VIRUS (TCSV)**

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2. UNIVERSIDADE DE BRASÍLIA

Tomato chlorotic spot virus (TCSV) and Groundnut ringspot virus (GRSV) (Tospovirus genus, Bunyaviridae family) are single strand RNA viruses. Their virions are compound by three RNA segments (S RNA, M RNA and L RNA) inside an icosahedral enveloped particle. TCSV and GRSV are closely related phylogenetically and the both were reported infecting tomatoes in 1990's year in Brazil. At the beginning, these viruses were limited to few counties. Nowadays, GRSV incidence has grown and raised it as the main tospovirus species in Brazil. Over the last four years, severe tospovirus infections have spread through Caribbean islands and TCSV became one of the main disease agents in important vegetable's crops. The TCSV has been reported damaging crops fields in the South and Southwest of the United States as well, where GRSV has been stated more than ten years ago. The interaction between GRSV and TCSV was proposed to born the first hybrid tospovirus isolate, which has the S and L GRSV segments and the M TCSV segment (denoted SGRSV MTCSV LGRSV). In attempt to study the competition of GRSV and TCSV in a mixed infection, the fitness of both viruses was measured by qPCR. The M RNA ancestry of GRSV and TCSV was verified to understand the origin of this segment. *Nicotiana benthamiana* plants were infected with the ancient GRSV and TCSV isolates, SA05 and Br03, respectively. After few days, the total RNA was purified and tested with specific primers in the RTPCR. The serological test ELISA was performed to calculate the virus concentration on the *N. benthamiana* leafs. These leafs were used to prepare three different inoculum concentrations, which were rubbed on healthy *N. benthamiana* plants. After few days, the viral titer was measured with specific primers on qPCR. The old

GRSV and TCSV isolates were completely sequenced by Illumina HiSeq 2000. The sequence reads obtained were assembled by CLC Genomic Workbench program and the final contigs were analysed by the GenBank® database. Extensive phylogenetic analysis made by the software PhyML showed that the genetic variability between GRSV and TCSV M RNA is less than in Tomato spotted wilt virus (TSWV) species, the virus type in the genus. The M RNA phylogeny does not separate GRSV and TCSV segments in different groups, signaling that probably they share the same M RNA. As result, the qPCR analysis showed that TCSV was more efficient in replication, even when mixed with a higher amount of GRSV.

#### **PIV208 - A NEW CLOSTEROVIRUS FOUND IN ARRACACIA XANTHORRHIZA BY NEXT GENERATION SEQUENCING**

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1. EMBRAPA HORTALIÇAS
2. UNIVERSIDADE DE BRASÍLIA

Arracacia xanthorrhiza, known as mandioquinhasalsa (MS) in Brazil, is a root vegetable originally from the Andes and belonging to family Apiaceae. It is a vegetatively propagated plant and viral infection symptoms are frequently observed. Next generation sequencing (NGS) has proven to be an efficient tool for viral metagenomic analysis, without the need of previous viral genome knowledge. Here we describe the identification and genome analysis of a novel closterovirus found in A. xanthorrhiza by NGS. RNA from viral enriched preparation after differential centrifugation of plant extracts were sequenced by Illumina HiSeq 2000 platform. Reads were analyzed, assembled, and submitted to blastx analysis against the RefSeq Viral database. The contig of 15.756 bp with coverage of 4464 reads share a high identity to closteroviruses, and similar genomic organization. The genus Closterovirus (family Closteroviridae) comprises species with monopartite positive single strand RNA genome whose size varies from 14.5 to 19.3kb. Based on the sequence information obtained by metagenomic analysis, specific primers that amplify overlapping regions of all genome were designed. Initially, the presence of this new closterovirus was confirmed in 21 MS plants from 47 total plants based on RTPCR. Then, one sample (MS#6)

was selected and used for complete genome sequence through Sanger sequencing. To determine the 5' and 3' terminals the RACE approach was successfully used. The complete genome of this closterovirus encodes 9 potential open reading frames and shows the typical organization of closteroviruses. The putative heat shock protein 70 homolog (HSP70h), RNA dependent RNA polymerase, and coat protein genes showed 3744, 2633, and 1835% amino acid sequence identities with other closteroviruses genome, respectively. A phylogenetic tree based on HSP70h gene showed that Beet yellows virus and Grapevine leafroll associated virus 2 are their closest relative to this virus. In conclusion, this study shows evidence of the presence of a putative new species in genus Closterovirus in Arracacia xanthorrhiza of Brazil. Considering that the sequence similarities of all taxonomically relevant proteins between this new Arracacia virus and recognized closteroviruses are far below the species demarcation threshold proposed by the Closteroviridae Study Group, we propose this virus to be representative of a new species in the genus, for which we propose the name "Arracacha virus 1".

#### **PIV210 - IDENTIFICATION BRUGMANSIA SUAVEOLENS MOTTLE VIRUS IN BRUGMANSIA SP**

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2. EMBRAPA HORTALIÇAS
3. UNIVERSIDADE FEDERAL DO PARANÁ
4. ESALQ

Plants of the genus Brugmansia (Solanaceae) are bushy-like trees that can reach up to 4.6 metres high. In Brazil, plants of this genus are popularly known as 'trombeteira' (trumpet) or 'saia branca' (white skirt). They are used as ornamental plants, because of their beautiful, large and tubular shape flowers. A leaf sample of Brugmansia sp. with mosaic and vein clearing symptoms was collected in 2015, in Curitiba - PR (Parque do Papa, S 25°24'40" W 49°16'13"). There is a report of the infection of the potyvirus Brugmansia suaveolens mottle virus (BsMoV) in a B. suaveolens plant, observed in Campinas SP (isolate BsCampinas). As the symptoms observed in this 'trombeteira' plant was distinct from the one collected in Campinas, it was supposed that a different virus could be causing the viruslike symptom in this plant.

Initially, transmission electron microscopy analysis indicated the presence of long and flexuous particles, and cytoplasmic inclusions, typical of potyvirus. Then, biological characterization was performed by mechanical inoculations in twelve indicator plants and symptoms were recorded. The test plants inoculated with the BsCuritiba isolate showed symptoms similar to those induced by the isolate BsCampinas. The test plants *Nicotiana benthamiana*, *N. tabacum* cv. TNN and *N. rustica* reacted with symptoms of necrotic spots and necrosis in the veins. The virus produced strong necrosis on *Physalis pubescens*, *Datura metel* and *Nicandra physaloides*, resulting in plant death. The molecular identification of the BsCuritiba was initiated by a search of a potyvirus using a potyvirusuniversal primer pair. Total RNA was extracted and subjected to RTPCR using primers that amplify a 1.7kb fragment in the 3' end of the genome. The amplicon was cloned and the insert sequence determined. The sequence share a nucleotide identity of 98% with the BsCampinas isolate (Accession AB551370). It was concluded that *Brugmansia suaveolens* mottle virus is also present in 'trombeteira' plants in Curitiba.

**PIV217 - COMPARATIVE GENOMIC ANALYSIS OF TWO ANTICARSIA GEMMATALIS CLONES OBTAINED FROM A NATURAL ISOLATE FOUND IN THE FIELD**

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UNIVERSIDADE DE BRASILIA

Baculoviruses are pathogenic to insects and have been effective in controlling agricultural and forest insect pests. In Brazil, the baculovirus *Anticarsia gemmatalis* multiple nucleopolyhedrovirus (AgMNPV) has been used as a biological insecticide since the early 80's to control the soybean caterpillar, *Anticarsia gemmatalis*, a major pest of this crop. In this study, we sequenced the entire genome of two clones (Ag01 and Ag16) derived from a natural population of AgMNPV occurring in the field (Ag79). The Ag01 and Ag16 clones were obtained by either using the plaque assay technique (Ag16) or by serial dilution (Ag01) of the Ag79. These isolates were selected for genomic analysis because they showed differences in the virulence pattern in the bioassay. DNA from both clones was extracted and sequenced using the pyrosequencing technique, and the data was analyzed using the Geneious software R6. The entire genome

sequence of the Ag2D (genebank) was used as a reference. The results showed that the major discrepancy between the Ag01 and Ag16 and the genome of reference Ag2D occurred in the pe38 gene. This gene is involved in viral DNA replication, and transactivation of viral early genes transcription. In the Ag2D isolate, the p38 gene presents itself divided in two ORFs while in the Ag01 and Ag16 clones only one ORF was found. Similarly, the he65 gene (unknown function) also have a single ORF arrangement compared to the Ag2D genome, in which the gene is split into two ORFs. Interestingly, in the broa (ORF6) and bro-b (ORF7) genes of the Ag16 clone, the major difference was a deletion of about 700 bps, resulting in a fusion of both genes into a single ORF. This was not observed neither in the Ag2D nor in the Ag01 clone. These clones have polymorphism in genomic regions which have not been described in the literature yet. The comparative genomic analysis is an approach that can bring relevant information on virus diversity and the mechanisms used by the virus to adapt to a new environment.

**PIV218 - DETECTION OF CULEX QUINQUEFASCIATUS NATURALLY INFECTED BY INSECTSPECIFIC VIRUSES IN CUIABÁ, MATO GROSSO, BRAZIL**

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1. UNIVERSIDADE FEDERAL DE MATO GROSSO
2. INSTITUTO EVANDRO CHAGAS

Insectspecific viruses comprise a new group of viruses capable of replicating only in invertebrate cells. *Culex flavivirus* is an insectspecific virus of the *Flavivirus* genus isolated from *Culex* spp in several countries. This study aimed to investigate the frequency of natural infection of adult mosquitoes by viruses in Cuiabá city, Mato Grosso, Brazil. To achieve that, *Culicidae* females (n=4,556) belonging to 14 species sampled in 200 urban census tracts pooled according to collection site, species and gender were subjected to RTPCR for a NS5 region, nucleotide sequencing, viral isolation in C6/36 cells and IonTorrent platform. *Culex Flavivirus* (CxFV) was detected in 16/403 (MIR=4.6) pools of *Cx. quinquefasciatus*, the most abundant species. The nucleotide sequences presented a high similarity with CxFV sequences from México, Uganda and Brazil.

Phylogenetic analysis showed that the Cuiabá isolates are closely related to Africa/Caribbean/Latin American genotype (genotype II) based on envelope protein. This is the first report of CxFV in centralwestern region of Brazil. Studies suggest that *Cx. pipiens* are the main host of genotype I strains and *Cx. quinquefasciatus* of genotype II strains of the virus. One pool of *Cx. quinquefasciatus* females presented also the genome sequence of a Negevirus, similar to species Bustos and Dezidougou viruses (MIR=0,3). Negevirus is a novel group of insect specific viruses composed by at least nine species capable of infecting several hematophagous insects, as mosquitoes and sand flies, presenting a wide geographical distribution. The pathogenicity of CxFV and negevirus for humans and their interference with arbovirus replication in competent vectors is largely unknown, however, recent studies suggest that the insect-specific viruses could alter the vector competence of the mosquitoes for some arboviruses resulting in superinfection exclusion or by alteration of the vector's immune response.

**PIV219 - IN VIVO AND IN VITRO VIRULENCE ANALYSIS OF A BACULOVIRUS ISOLATED FROM CHRYSODEIXIS (=PSEUDOPPLUSIA) INCLUDENS, A SOYBEAN PEST IN THE BRAZILIAN CERRADO**

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1. EMBRAPA RECURSOS GENÉTICOS E BIOTECNOLOGIA
2. EMBRAPA SOJA

Baculovirus are important biological control agents of Lepidoptera. In February 2014, larvae with symptoms of viral infection were observed in populations of *Chrysodeixis* (=Pseudoplusia) *includens* infesting soybean field at BuritisMG (S15o22.2' W46o50.7'). Observations of larval tissue under optical microscope showed the presence of typical virus particles of Nucleopolyhedrovirus (NPV). The virus was identified as *Pseudoplusia includens* single nucleopolyhedrovirus (PsinSNPV) by transmission electron microscopy. The present work was carried out in order to investigate the potential of this viral isolate to control this insect pest. Two bioassays were performed. In the first, larvae infected with virus were macerated and incorporated to the artificial diet and offered to 432 *C. includens* larvae

(third instar). In the second, semipurified virus particles were incorporated to the artificial diet and offered to third instar *C. includens* larvae. The mortality was verified at 10 and 15 d.p.i. and the lethal concentration LC50 and LC99 were calculated using Probit analysis. To select a good candidate for in vitro multiplication of the virus, analysis was performed with six different lepidopteran cell lines: *Bombyx mori* (BM5), *Lymantria dispar* (IPLBLD625Y), two *Trichoplusia ni* (BTITn5B14 and TN368), and two *Spodoptera frugiperda* cells (IPLB-SF21AE and Sf9). The cells were seeded at a density of 1x10<sup>6</sup> per 60mm<sup>2</sup> dish. The virus was obtained from infected larvae hemolymph at 4 d.p.i., treated and allowed to be adsorbed by cells during 1 hour. Then, the cells were kept in TNMFH complete medium and incubated at 27°C. Morphological analysis was monitored by light microscopy during five days, using an Olympus CK2 optical microscope. The best results in the bioassays were obtained with the semipurified virus. The LC50 obtained was 10,918 polyhedra/ ml artificial diet (pol/ml) and the LC99 was 247,710 pol/ml, at 10 d.p.i. Furthermore, at 15 d.p.i. the LC50 was 6,709 pol/ml and LC99 was 146,880 pol/ml. In addition, preliminary analysis of the cell lines incubated with this isolate showed typical cytopathic effects as cell rounding, nuclear hypertrophy and the presence of polyhedra in Tn5B14 cells at 4 d.p.i. The bioassays showed potential to use this virus isolate as a biopesticide. Moreover, the Tn5B14 cell line demonstrated to be prospective for further in vitro studies.

**PIV220 - COMPARISON OF THE INFECTIVITY OF TWO SPODOPTERA FRUGIPERDA CELL LINES TO SFMNPV**

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1. EMBRAPA RECURSOS GENÉTICOS E BIOTECNOLOGIA
2. UNIVERSIDADE PAULISTA
3. EMBRAPA MILHO E SORGO

The fall armyworm, *Spodoptera frugiperda*, is a severe pest in South America causing damage to different crops, especially in maize. The *Spodoptera frugiperda* Multiple Nucleopolyhedrovirus (SfMNPV), a baculovirus highly pathogenic to this pest, has been largely used as a biocontrol agent. So far the baculovirus production has been done by multiplication of the virus in its insect

host in despite of difficulties as intense cuticle lyses and cannibalism behavior. Therefore, optimization of baculovirus in vitro production is essential as an alternative technology. In the present work, the SfMNPV production in IPLBSF21AE and Sf9 cell lines were compared. The polyhedra yield was determined as well as the kinetics of viral protein synthesis. In addition, larval mortality was determined by virulence assays with 3th to 4th instar larvae. Cells seeded at a density of  $2 \times 10^6$  in a T25 flasks were incubated with the SfMNPV I19 isolate for 1h adsorption time and kept in TNMFH complete medium at 27°C. At 5 dpi the cells were collected by centrifugation at 3000rpm for 5 min. The cell pellet was disrupted by treatment with 1% SDS, for 1h, at 27°C. Polyhedra was placed in a Neubauer chamber and counted under an optical microscope. For kinetics of the protein synthesis both cell lines were seeded at a density of  $1 \times 10^6$  per 60mm<sup>2</sup> dish and incubated with the virus. After that, pulse labelling was carried out by addition of 50 uCi of [<sup>35</sup>S] methionine per dish at 0h, 24h, 48h and 72h pi. Analyses of the labelled proteins was done by SDS-PAGE followed by autoradiography. Comparison of the two cell lines infected with the virus showed that polyhedra production was similar in both cells ranging from 200400 polyhedra/cell. As expected, the kinetics of radiolabelled proteins showed that the cell protein synthesis was shut off while an intense band of approx. 30 kD (polyhedrin) was synthesized in SF21 and Sf9 cells. Assays with *Spodoptera frugiperda* larvae showed that the virus produced in cell culture was pathogenic to its host. The present data indicates that cell culture is a viable system for baculovirus in vitro production and reinforces the need to optimize strategies for large production in bioreactors.

**PIV221 - INFLUENCE OF THE AGE OF TOMATO TRANSPLANTS ON THE RATE OF INFECTION AND SYMPTOM SEVERITY CAUSED BY THE BEGOMOVIRUS TOMATO SEVERE RUGOSE VIRUS**

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1. UNIVERSIDADE DE BRASÍLIA
2. EMBRAPA HORTALIÇAS

High incidence of viral diseases is reported in tomato plants, particularly those caused by begomoviruses (Fam. Geminiviridae, gen. Begomovirus) in Brazil.

These viruses are transmitted by the whitefly *Bemisia tabaci*. Tomato severe rugose virus (ToSRV) is the most important begomovirus reported in the country. The control of these viruses is difficult, and usually rely on the use of resistant cultivars and the chemical control of insect vectors. There is a lack of information on the cultural practices that could be implemented to reduce the incidence of the begomovirus in the field. The objective of this study was to evaluate the influence of transplants age of tomatoes on the begomovirus infection rate and symptom expression. The susceptible cultivar H9553 was used with whitefly inoculation. Transplants of 20, 30, 40, 50 and 60 days after seeding (DAS) were distributed in three blocks (completely randomized) with 10 plants for each treatment. Inoculation was performed by *B. tabaci* biotype B viruliferous to ToSRV. Negative controls consisted of plants inoculated with aviruliferous whiteflies and plants without the presence of whiteflies. The evaluation was performed visually and by detection tests based on PCR and hybridization. There was a clear difference in the rate of infection and severity of the symptoms observed in plants inoculated at distinct ages. The treatment with plants of 20 DAS had the highest incidence of infection (50%), followed by plants with 30, 50, 60 and 40 DAS. Plants inoculated 40 DAS showed the lowest rate with 20% of infection. For symptom severity, plants inoculated at 40 DAS showed the least severe symptoms, followed by plants inoculated at 60 DAS. The results suggest that the age of the transplants influence the incidence rate and severity of a disease caused by a begomovirus. Further studies are in progress to confirm this result.

**PIV223 - IMMUNOSTAINING OF ROOT TISSUE OF NICOTIANA BENTHAMIANA INFECTED WITH PEPPER RINGSPOOT VIRUS**

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1. UNIVERSIDADE DE BRASÍLIA
2. CENTRO NACIONAL DE PESQUISA EM HORTALIÇAS

The genus Tobravirus is formed by three species: Tobacco rattle virus (TRV, type species), Pea early browning virus (PEBV) and Pepper ringspot virus (PepRSV). The genome of a tobavirus is formed by two segments of single stranded RNA molecules. Because of their ability

to be transmitted by nematodes of genera *Trichodorus* and *Paratrichodorus* they are thought to be present in high titers in the root tissues. The objective of this study was to immunolocalize the PepRSV capsid protein (CP) in the root tissue of *Nicotiana benthamiana*. Plants of *N. benthamiana* were inoculated with the CAM isolate of PepRSV. After 14 days, the roots were collected, fixed (3% paraformaldehyde, 0.1% glutaraldehyde), treated with the polyclonal antibody against the CP of PepRSV, produced in the Laboratory of Virology of the 'Centro Nacional de Pesquisa em Hortaliças', and later treated with antirabbit conjugated with alkaline phosphatase (AP). Chromogenic substrates, 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium chloride (NBT), were used for immunodetecting AP activity. BCIP is hydrolyzed by AP and intermediates undergo dimerization with the help of NBT. At the end of the reaction an insoluble darkblue precipitate is formed consisting of NBT-diformazan and 5,5'-dibromo-4,4'-dichloro indigo. The immunostained tissue was analyzed with the Leica TCS/SP5 confocal laserscanning microscope (Leica Microsystems, Wetzlar, Germany). Strong chromogenic signals were observed in the phloem cells of the root tissue in PepRSV infected *N. benthamiana* plants suggesting that the virus efficiently translocate to root tissues. No positive signal was observed in noninoculated plants of *N. benthamiana* (negative control). We concluded that the CAM isolate of PepRSV translocates via vascular system (phloem) to the root tissue, similar to the other two tobnaviruses, TRV and PEBV.

#### **PIV226 - A NOVEL CYTHORHABDOVIRUS IN ARRACACHA (*ARRACACIA XANTHORRHIZA*)**

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Arracacha (*Arracacia xanthorrhiza*) is one of the most important cultivated Andean roots, belongs to the family Apiaceae, which includes carrot, celery and parsley. It is vegetatively propagated, and therefore it accumulates high amounts of degenerative pathogens such as viruses. The viral metagenome sequencing brings many possibilities of identifying unknown

viruses, overcoming previously technical barriers. Therefore, through next generation sequencing (NGS) and metagenomics analysis a novel plant virus related to Rhabdoviridae family was found infecting arracacha plants. Here we describe the molecular characterization of this putative new rhabdovirus genome. To this extent, based on NGS sequence information, primers were designed to amplify the full viral genome containing overlapping regions. Initially, the presence of this new putative plant rhabdovirus was confirmed by RTPCR in 36 arracacha plants out of 47 analyzed. One plant was selected and total RNA extracted aiming to amplify five overlapping regions of the genome. All amplified fragments were sequenced by Sanger sequencing. The RACE technology was used to determine both 5' and 3' terminals. The genomic organization resembles those of plant rhabdoviruses. Six open reading frames (ORFs) were identified in the antigenomic orientation of the negativesense, singlestranded viral RNA, in the following order 3'NP4bM GL5'. Amino acid sequence analysis of the putative nucleoprotein (N) showed 941% identity with N proteins encoded by other plant rhabdovirus genomes. Phylogenetic analysis of the N and polymerase (L) amino acid sequence indicated that this arracacha-infecting rhabdovirus is related to viruses belonging to *Cytorhabdovirus* genus, and are closely related to Alfalfa dwarf virus. According to Rhabdoviridae Taxonomy Group (ICTV), genus classification based on sequence diversity has thus far correlated 100% with classification by intracellular virus maturation. Giving these findings the novel virus found infecting arracacha should be considered as a new species of the *Cytorhabdovirus* genus.

#### **PIV231 - DISCOVERY OF A NOVEL DICISTROVIRUS ISOLATE IN TOMATO LEAF SAMPLES**

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The family Dicistroviridae is composed of viruses that infect invertebrates, including insects such as honeybees and hemipterans. Therefore, these viruses might provide practical applications for controlling agricultural arthropod pests. Our group is currently making efforts

to identify whiteflyinfecting viruses that can be used as biological control agents in integrated pest management programmes. The surveys are carried out directly in whiteflies and also in whiteflyinfested plants. Here, we report the identification of a dicistrovirus using a next generation sequencing (NGS) approach. Tomato leaves were collected in São Paulo state in 2013 and kept at 80 °C until processing. After semipurification of virus particles by differential centrifugation, total RNA was extracted and subjected to NGS sequencing at Macrogen, Inc. Reads were trimmed with an automatic Phred score on Trimomatic before contigs were assembled using the Velvet algorithm (91kmer) and analysed for their shared identities with other viruses in a RefSeq database using MegaBLAST on Geneious software. Forty contigs ranging from 190 to 1,228 nt shared high percentage identity (>80%) with the dicistrovirus Aphid lethal paralysis virus (ALPV). These contigs were used as reference for sequence extension using the Geneious mapper. By using this strategy, a 9,936 ntlong sequence was generated from 278,669 reads. The sequence presented 86% overall identity with ALPV (acc. JQ320375, 97% query cover, Evalue=0.0) and two major ORFs, the first ranging from nt 592 to 6699 and coding for a putative protein similar to ALPV nonstructural polyprotein (92% identity, Evalue=0.0) and the second ranging from nt 6896 to 9301, coding for an ALPVlike capsid protein (92% identity, Evalue=0.0). As this virus presumably infects insects, pathogenicity tests were initiated in insect cell lines. The extract containing semipurified particles used for NGS sequencing was filtered and inoculated into lepidopteran cell line UFLAg. Cytopathic effects such as vacuolization and cell rounding were evident following ten days postinoculation. Furthermore, reinoculation of the cell media into healthy cells consistently produced cytopathic effects. These results demonstrate the feasibility of using NGS methods for discovery of new virus isolates.

### PIV233 - SEQUENCE VARIABILITY AND EVOLUTION OF TOMATO CHLOROSIS VIRUS IN BRAZIL

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1. INSTITUTO FEDERAL GOIANO
2. EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA
3. INSTITUTO FEDERAL GOIANO

Tomato chlorosis virus (ToCV, genus Crinivirus, family Closteroviridae) is a whiteflytransmitted crinivirus with a bipartite RNA genome (RNA1 and RNA2). The RNA1 encodes proteins involved in replication of viral RNA and suppression of gene silencing, while RNA2 encodes proteins likely involved in viral movement and encapsidation. In the last years, this virus is emerging as a serious threat to tomato crops in Brazil. During 2015, surveys were done on tomato (*Solanum lycopersicum*) virus diseases in three states of Brazil: Goiás (GO), Paraná (PR) and Rio de Janeiro (RJ). Samples exhibiting interveinal chlorosis on the basal leaves, typical symptoms of ToCV, were collected. Total RNA was extracted from 64 samples and the presence of ToCV infection confirmed by reverse transcriptionPCR (RT-PCR) using the specific primers, which amplifies a fragment of approximately 463 pb. A total of 55 samples were PCR positive (14/14 from GO, 23/23 from RJ and 18/27 from PR). In order to study the variability and evolution of ToCV species infecting tomatoes in Brazil, 10 isolates from each Brazilian state were selected. Primers were designed to amplify three genomic regions coding three genes, p22 (RNA1), HSP70h and CP (RNA2), which amplify fragments of 720, 936 and 917 pb, respectively. PCR products were cloned and sequenced. Preliminary results based on p22 protein from a population of PR state indicate nucleotide diversity ranged between 0.1 and 0.3% and interpopulation between 0.1 and 3.2%. Virus evolution predictions based on synonymous/nonsynonymous rates indicate that this population is under positive selection suggesting adaptation to a new ecological niche. Interesting, in phylogenetic analyses, these isolates grouped in a regular manner suggesting a geographicalbased evolution pattern. However, only one location has been completely analyzed, data from other regions (RJ e GO) will be useful to determine the true diversity of Brazilian isolates.

**PIV235 - DETECTION OF LEISHMANIAVIRUS IN LEISHMANIA PARASITES FROM AMAZON REGION OF BRAZIL**

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3. INSTITUTO EVANDRO CHAGAS/CENTRO DE INNOVAÇÕES TECNOLÓGICAS

The Leishmanivirus (LRV) genus is part of Totiviridae family and includes endogenous doublestranded RNA virus of Leishmania sp., where a virus-host relationship is unclear. Thus, this study aimed to investigate the presence of LRV at Leishmania sp. strains isolated in the Amazon region. The detection of Leishmanivirus was conducted in 40 Leishmania strains belonging to the collection of the Leishmaniasis Laboratory at the Evandro Chagas Institute (Pará State/ Brazil). The first step was precipitation of the total doublestranded RNA of Leishmania sp. strains, followed by sequencing using Ion Torrent PGM™ platform and mapping the generated readings against the reference genome (Leishmania RNA virus 1 and Leishmania RNA virus 2) available at GenBank (NCBI). For positive samples, genome was assembled by De novo approach. Among readings generated, only eight samples had nucleotide sequences related to the virus, comprising the following species of protozoa: Leishmania (V.) shawi, Leishmania (V.) guyanensis, Leishmania (V.) Lindenberg, Leishmania (V.) lansonii and Leishmania (V.) braziliensis. All viral readings were related to Leishmania RNA virus 1. Thus, it is confirmed that the occurrence of this species is related to protozoa strains of the "New World". The occurrence of Leishmanivirus was also detected in Leishmania strains (V.) guyanensis isolated from Bradypus sp. and this finding was not further reported. Furthermore, genome assembly analysis revealed possibility of the presence of more than one viral strain within the same host. Thus, the use of new generation sequencing becomes extremely useful for detection and characterization of viral strains, such as Leishmanivirus.

**PIV237 - AN SYBR GREENBASED REALTIME RTPCR ASSAY TO DISTINGUISH GROUNDNUT RINGSPOT VIRUS AND TOMATO CHLOROTIC SPOT VIRUS**

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Groundnut ringspot virus (GRSV) and Tomato chlorotic spot virus (TCSV) belong to the genus Tospovirus (Bunyaviridae family) and have a similar host range, causing high economic losses in several vegetable crops in Brazil. Their genomes consist of three segmented RNAs: L, M and S, in which they share biological and serological properties, making it difficult to accurately distinguish these viruses in single or mixed infections. In this study, we developed a highly sensitive and rapid detection method for GRSV and TCSV by Sybr Green based Real Time RTPCR. Firstly, specific primers were designed to amplify a fragment of approximately 150bp in the same region of the segments L and S of each virus based on alignment of complete genome. An isolate of GRSV (Tom#47) and TCSV (Tom#59) from tomato plants were mechanically inoculated separately in healthy Datura stramonium and Nicotiana rustica plants, and after the appearance of symptoms, leaves were collected for total RNA extraction using TRIzol reagent (Invitrogen), following the manufacturer's protocol. cDNA synthesis was performed using MMLV reverse transcriptase (Invitrogen) with random primers. Real Time PCR was performed using Power Up SYBR Green Master Mix (ThermoFisher) following manufacturer's instructions and the RotorGene equipment (Qiagen). Melting temperature analysis generated a segment-specific amplicon at 79,2°C, 76,2°C, 75,8°C and 78,2°C for GRSVL, GRSVS, TCSVL and TCSVS, respectively. The analysis indicated no primer dimers in the assay. No crossreaction was observed with GRSV, TCSV and also to Tomato spotted wilt virus. Therefore, the SYBR green-based real time RTPCR assay could provide a rapid, sensitive, specific and reliable alternative approach with lower costs for high throughput screening of suspected tospovirus-infected samples with the most prevalent species in Brazil. In addition, the developed method can be used to monitor virus epidemiology, to study virus resistance and virus synergism under field conditions.

**PIV239 - IDENTIFICATION OF A POTATOINFECTING BEGOMOVIRUS IN THE CENTRAL REGION OF BRAZIL**

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1. EMBRAPA RECURSOS GENÉTICOS
2. EMBRAPA HORTALIÇAS

Potato is one of the most important vegetable crops in Brazil. Production is concentrated mainly in three regions, South, Southeast, and CentralWest. Cristalina county, located in the state of Goiás, is considered as one of the major region for potato production in the country. In 2012, the production of potato reached 300,000 tons (10% of national share) in 6,000 hectares planted in Cristalina. Monitoring potato plants exhibiting begomoviruslike symptoms has been performed in potatogrowing areas since 2011. The incidence of symptoms in potato fields was generally low during 20132014 growing seasons. The objective of this work was to identify begomovirus species infecting potato plants in the central region of Brazil. Leaf samples were collected from 20 plants showing yellow mosaic and leaf deformation symptoms in potatogrowing fields, 50 to 60 days after planting, at Cristalina in 2014. Total DNA was extracted from samples and tested by polymerase chain reaction (PCR) using degenerate begomovirusgroup specific primers, which amplify a DNA fragment of ca. 1.1 kbp of the A component. Ten begomoviruspositive samples were selected to Rolling Circle Amplification (RCA) using Phi 29 DNA polymerase. RCA products were digested with restriction enzymes and Hind III was selected for cloning the genome. RCA digested products were electrophoresed on 1.2% agarose gel and a DNA fragment of ca. 2.6 kb was gelpurified, and cloned into pBluescript vector. Out of the 20 samples, 15 were positive by PCR, showing a ca. 1.1 kb DNA amplicon. Initial sequences of 750 bp, from DNAA of all ten selected samples, share from 94% to 98% nucleotide identity with Tomato severe rugose virus (ToSRV), a pathogen that primarily infects tomato. It is concluded that ToSRV is likely to be the predominant begomovirus in potatoes in the Cristalina region.

**PIV240 - VIRAL BIODIVERSITY IN PLEBOTOMINAE CAPTURED IN PANTANAL AND CHAPADA DOS GUIMARÃES OF MATO GROSSO, BRAZIL**

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3. UNIVERSIDADE FEDERAL DE BRASILIA

Arboviruses transmitted by phlebotominae present considerable medical importance, since many were isolated from these arthropods and humans in the Brazilian Amazon. This study was conducted to investigate the viral biodiversity in phlebotominae captured with CDC traps in two sylvatic areas of Cerrado of Mato Grosso containing RAPELD systems: the Chapada dos Guimarães National Park (PNCG, Rio Claro) and North Pantanal (Pirizal) during the rainy, intermediate and dry seasons of 20142015. After identification with a specific dichotomy key, 105 specimens (90 belonging to genus *Lutzomyia* [*L. witmani*, *L. evandroi*, *L. carmelina* and *L. sherloki wilsoni*], and 15 to genus *Brumptomyia*) were allocated 11 pools. These pools were minced and, subjected to viral RNA extraction, reverse transcription, synthesis of the second strand of cDNA and PCR amplification with viral randomic primers (random-KS). The purified and quantitated DNA product was sequenced by illumina HiSeq 2500 platform. Partial analysis of contigs obtained from five pools of *Lutzomyia* sp. revealed hits with sequences of vesiculovirus, pegivirus, flavivirus, phlebovirus, nairovirus and tospovirus. Since the whole arthropod was used in the experiment, is possible that some of these sequences originated from their gastrointestinal contents or either from their exterior parts in contact with plants and animals.

**PIV241 - PRODUCTION OF INFECTIOUS CLONES OF WEEDINFECTING BEGOMOVIRUSES FOR PATHOGENICITY STUDIES**

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*EMBRAPA HORTALIÇAS*

The genus Begomovirus has the highest number of known viral species. Some of them are responsible for large agricultural losses such as Tomato severe rugose virus (ToSRV) in Brazil. Euphorbia heterophylla and Sida spp. are weeds frequently found in tomato production fields and act as hosts of ToSRV and other begomovirus species. The aim of this work was to obtain infectious clones of weedinfesting begomoviruses, Euphorbia yellow mosaic virus (EuYMV) and Sida micrantha mosaic virus (SiMMV), for studies of characterization, interaction between viruses and pathogenicity in tomato plants. EuYMV and SiMMV isolates were obtained from *E. heterophylla* and *S. santaremnensis* plants naturally infected in the field. Total DNA was extracted and the begomovirus species identified by PCR. Circular DNA was amplified by the rolling circle amplification (RCA) technique followed by digestion with restriction endonucleases and dimeric molecules (4.8 to 5.4 Kb) were recovered after electrophoresis. The inserts were cloned in the pCAMBIA0380 vector. Two clones were obtained for each viral component. The EuYMV DNA-A clones shared 97 to 99% to EuYMV DNAA accession JF56676; the EuYMV DNAB clones 98% to EuYMV DNAB accession JF756678; the SiMMV DNAA clones 99 to 100% to SiMMV DNAA accession JX415194; and SiMMV DNAB clones 96% to SiMMV DNAB AJ557452. The recombinant plasmids were transformed into *Agrobacterium tumefaciens* and inoculated in test plants. Inoculated plants are under analysis. These clones will be useful for studies on pathogenicity and virus-virus interaction in weeds and tomato plants.

**PIV248 - RECOMBINATION EVENTS IN FULL GENOME SEQUENCES OF APPLE STEM GROOVING VIRUS**

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Apple stem grooving virus (ASGV), type species of the genus Capillovirus, is disseminated worldwide. No vector is known and the virus is transmitted only by grafting, usually causing a latent infection in most commercial cultivars of apple trees. However, infected scions grafted onto sensitive material display reduction of yield, loss of fruit quality and tree decline. Currently twenty three complete nucleotide sequences of this virus species are available in the GenBank. Here we report recombination events along the complete genome sequences of ASGV using two Brazilian isolates (M2193 and M220) and sequences available in the GenBank database. Confidence values for the recombination events were evaluated by seven programs built in the RDP4 software package: RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan and 3Seq. Events detected by RDP with a pvalue under  $1 \times 10^6$  were considered significant in this study. In a full-length genome analysis, six potential recombinants, not previously reported, were detected. Four recombinants apple isolates, NC\_001749 (Japan), JQ308181 (China), KJ579253 (China) and M2193 (Brazil) showed as major parents, apple isolate M220 (Brazil), lily isolate D16681 (Japan), apple isolate M2193 (Brazil) and lily isolate AB004063 (Japan), respectively. JQ308181, M2193 and KJ579253 shared the same minor parent, M220. NC001749 showed as minor parent, JQ308181. One recombinant pear isolate (JN701424 from China) showed citrus isolate LC143387 (Japan) as major parent and apple isolate KF434636 (China) as minor while the recombinant LC143387 was originated from JN701424 (majorparent) and pear isolate AY596172 (Korea). Among recombinants shown we have observed recombination between isolates from different countries and different hosts species. Recombinant events were likely a result from exchange of propagation materials and vegetative propagation and could play a role in virus evolution, emergence and epidemiology. It likely contributes to virus survival, adaptability to environmental factors and new hosts, increase in virulence and genetic diversity.

**PIV249 - PEPPER RINGSPOT VIRUS ISOLATE CHARACTERIZATION AND PRODUCTION OF A POLYCLONAL ANTIBODY**

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2. CENTRO NACIONAL DE PESQUISA EM HORTALIÇAS

The genus Tobravirus is formed by three species: Tobacco rattle virus (TRV, type species), Pea early browning virus (PEBV) and Pepper ringspot virus (PepRSV). PepRSV is the only tobnavirus that occurs in Brazil and has been detected in the field of tomato, pepper and artichoke crops. The objective of this study was to produce a polyclonal antiserum against PepRSV to be used in diagnosis. The CAM isolate of PepRSV was propagated in *Nicotiana benthamiana*, and the particles were purified according to the protocol of Nixon & Harrison (1959). Purified particles were used for three immunizations in rabbits at intervals of three weeks after emulsification with Freund's complete (1st) and incomplete (2nd, 3rd) adjuvant. Bleeding started one week after the last immunization. The purified virus preparation observed in transmission electron microscopy confirmed the presence of tubular and rigid particles with two different lengths. This preparation contained a protein of ca. 30 kDa observed in a polyacrylamide gel. This protein reacted positively with the produced polyclonal antibody in a Western blotting test. Then, indicator plants were inoculated with PepRSV and tested by DotELISA. The virus caused local lesions in *Chenopodium quinoa*, *C. amaranticolor*, *C. murale*, *Nicotiana physaloides*, but no systemic infections were observed in these plants. The systemic necrotic lesions were observed in *Gomphrena globosa*, *Nicotiana rustica*, *N. tabacum* cv. Samsun and cv. TNN. The chlorotic rings and line patterns were observed in *Capsicum annuum* cv. Casca Dura Ikeda, *Solanum lycopersicum* cv. Santa Clara. *N. benthamiana* showed crumpling symptom. In general, the symptoms in *S. lycopersicum* and *C. annuum* and *N. benthamiana* plants were disappearing with time. Serological tests confirmed the specificity of the antibody with the positive reactions observed only in inoculated plants. ELISA tests were done every week up to six weeks post inoculation. The virus could be detected in the indicator plants with systemic infection in all time points, although symptom was not clear, suggesting that the virus remained present

in the plant after symptom disappearing. Only the local symptomatic leaves of *Chenopodium* spp and *N. physaloides*, but not upper leaves, became positive in serological detection. No signal was observed in healthy plants. It is concluded that the polyclonal antibody reacted specifically with PepRSV and can be used for a rapid serological detection of the virus.

**PIV250 - STRANDSPECIFIC REALTIME RTPCR ASSAYS FOR QUANTIFICATION OF GENOMIC AND ANTIGENOMIC RNAs OF TOMATO SPOTTED WILT VIRUS**

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Tomato spotted wilt virus (genus *Tospovirus*; family *Bunyaviridae*) (TSWV), infects over 1000 plant species and causes significant economic damage to many agronomic and horticultural crops. Their genome are three singlestranded, negativesense RNAs that are individually encapsidated, they differ in size and are called large (L), middle (M) and small (S). For viruses having a singlestranded, negativesense RNA genome, standard qRT-PCR assays do not distinguish between the genomic (negativesense genome) and antigenomic RNA (positivesense). Thus, these methods are unable to determine viral genome copy numbers. To better understand TSWV replication and transcription cycle, quantitative detection methods for distinguishing TSWV genomic RNA (gRNA) and antigenomic RNA (agRNA) in TSWV-infected plants are indispensable. Therefore, in this study, a strand-specific realtime RT-PCR method was developed to quantify independently the two types of TSWV viral RNA (gRNA and agRNA) of the three segments. This method is based on reverse transcription using tagged primers added of a 'tag' sequence at the 5' end. Realtime PCR using the 'tag' portion as the forward primer and a segment-specific reverse primer ensured the specificity for quantifying both types of RNA. Validation of this strategy has been performed with synthetic RNA transcripts obtained from full-length TSWV segment clones by T7 RNA polymerase and demonstrated that assays could discriminate the correct RNA strand with greater than 1000-fold fidelity. After validation, this method will be applied to evaluate the gRNA and agRNA levels of L, M and S segments in tomato plants infected with TSWV at different times

postinfection. In conclusion, a realtime RTPCR for absolute quantitation of specific viral RNA fragments in TSWVinfected plants was developed for the first time. The development of this assay will be helpful for further studies on the pathogenesis and control strategies of TSWV, understanding the tospovirus virus life cycle, including transcription and replication.

#### **PIV252 - VIROME OF SWEET POTATO GENOTYPES**

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The sweet potato (*Ipomoea batatas* L.) is a vegetable of great global importance and is currently the sixth most consumed food crop in the world. In Brazil, the sweet potato is grown in all regions with the main importance to the Northeast where it is greatly appreciated by population. The sweet potato production can be affected by the action of various pathogens such as viruses that reduce the productivity due to viral accumulation caused by vegetative propagation, the main method of propagation of the culture. Thus, the aim of this study was to apply metagenomic analysis by Illumina sequencing to verify the viral diversity in sweet potato samples from different regions of Brazil. For this, 100 samples of sweet potato were used to a virus enrichment process by differential centrifugation where each plant was weighed to 1g to form a composite sample. After this, RNA extraction was performed using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions and send to Illumina HiSeq sequencing. The reads obtained were assembled using the CLC Genomics Workbench 8.5 software, generating 2.698 contigs. The contigs were analyzed using the BLASTX program (GenBank) against viral RefSeq database. The genome analysis was performed using Geneious 9.0 program, and as a result, four viral sequences related to Potyvirus genus (Potyviridae), one sequences related to Carlavirus genus (Betaflexiviridae) and one to Crinivirus genus (Closteroviridae) were found. Thus for the future studies, used specific primers, for each viral species, will be designed and synthesized for detection of virus in each plant separately. Financial support: CNPq.

#### **PIV264 - FIRST REPORT OF TOMATO SEVERE RUGOSE VIRUS IN COMMON BEANS IN BRAZIL**

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In Brazil, diseases caused by begomoviruses may lead to severe losses in common beans and tomatoes. In beans, the disease is primarily caused by Bean golden mosaic virus and in tomatoes, Tomato severe rugose virus (ToSRV) is currently the predominant begomovirus found in the country. During epidemiological studies conducted in 2013-2015 in tomato production areas, 479 bean plants cv. Carioca were collected from around tomato fields located in five municipalities in the central part of Brazil. DNA was extracted and used in a PCR test with the degenerate begomovirus primers PAR1c496/PAL1v1978 and a total of 204 samples were positive. The 204 DNA extracts of these begomoviruspositive samples were used in another PCR with ToSRVspecific primers, and 14 samples, all from symptomless plants, were positive. Then, direct sequencing of the RCA products of the 14 ToSRVpositive bean samples was performed with the begomovirus degenerate primer PARc496, which generated sequence of the 5' end of the capsid protein and the intergenic region. The sequences shared 96.98% identity with the sequence of ToSRV (accession FJ824808), confirming that these bean plants were infected with ToSRV. One of these samples was selected and the fulllength DNAA component was cloned and sequenced (GenBank accession number KX458238). The sequence of this clone was 99% identical identity with a ToSRV isolate from tomato (accession JX415196). To confirm the susceptibility of common beans to ToSRV, bean plants (cv. Topcrop) were agroinoculated with infectious ToSRV DNAA and DNAB dimeric clones ToSRV1164 of a tomatoinfecting isolate of ToSRV. Controls were bean plants agroinoculated with the BGMV DNAA and DNAB clones BGMVBRCAM and an empty pCAMBIA vector, and tomatoes (cv. Glamour) agroinoculated with ToSRV. By twenty one days after agroinoculation, common beans agroinoculated with ToSRV did not show obvious symptoms, but all plants (18/18, results of three independent experiments) were infected based on detection of the virus by PCR. Common

bean and tomato plants agroinoculated with BGMV and ToSRV, respectively, developed typical symptoms. The bean plants agroinoculated with empty pCAMBIA remained healthy and were negative for begomovirus infection. These results indicate that ToSRV induces a symptomless infection in common beans, and suggest that infected beans could serve as reservoirs of ToSRV for the tomato crop.

#### **PIV266 - REACTION OF LETTUCE GENOTYPES TO LETTUCE MOSAIC VIRUS AND CHARACTERIZATION OF THE EIF4E ALLELE**

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Lettuce mosaic virus is one of the major virus occurring on lettuce (*Lactuca sativa* L.). LMVMost are able to overcome the resistance of the recessive genes mo11 and mo12 found in lettuce and so far as we know there are no resistant or tolerant varieties for these isolates. These recessive genes encode the eukaryotic translation factor eIF4E, also related to the recessive potyvirus resistance in other plant species. In this work lettuce genotypes belonging to a collection of FCA / UNESP-Botucatu were evaluated for reaction to isolate AF199 (LMVMost) and the presence of possible variations in the eIF4E gene were evaluated. Varieties Calona and Salinas88, previously reported with the tolerant genes mo11 and mo12, respectively, were used as control for the eIF4E sequence pattern. The sequence for the others genotypes analyzed was highly conserved and typical for eIF4E0 (mol0), related to the susceptible genotype. Interestingly, some of these genotypes showed delay in symptoms appearing and attenuated symptoms for LMV AF199. These phenotypes could not be correlated with variability's in the eIF4E sequence, indicating that other regions of the lettuce genome could be implicating in the symptomatology reaction to LMV isolates.

#### **PIV269 - FIRST REPORT OF TOMATO SEVERE RUGOSE VIRUS IN SOYBEAN IN BRAZIL**

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Three begomoviruses have now been reported in soybean plants in Brazil, occurring in a low incidence. However, soybeans have the potential to act as inoculum sources for crops such as beans and tomatoes. In addition, because soybean is frequently cultivated in close proximity to beans and tomato in Brazil, whiteflies can transmit begomoviruses among these crops. The begomovirus Tomato severe rugose virus (ToSRV) is the predominant species in tomatoes and can cause yield losses. During epidemiological studies on begomoviruses infecting tomato plants, 295 soybean plants were randomly collected around tomato fields. DNA was extracted and PCR with degenerate begomovirus primers was performed, and 17 positive samples were obtained. The DNA from these positive samples was used in another PCR with ToSRV-specific primers, and four samples were positive. Rolling circle amplification (RCA) and RFLP analysis with *Msp* I enzyme were performed with these ToSRV positive samples. The RFLP patterns of the soybean samples were identical to the ToSRV-infected tomato sample. Two of these samples were selected and the DNAA component was cloned and sequenced. These sequences were 9899% identical to ToSRV (JX415196.1). To confirm the susceptibility of soybeans to ToSRV, soybean plants were inoculated by biolistics using an RCA preparation from one of the soybean samples positive to ToSRV by PCR. By 21 days after inoculation, soybeans inoculated with the RCA from a ToSRV positive soybean did not show any obvious symptoms, but all plants (12/12) were infected based on detection of the virus by PCR. Soybean plants inoculated with RCA product of BGMV-infected soybeans, as positive controls, developed mosaic and mottling symptoms, whereas those inoculated with the RCA product from a healthy plant were not infected. A transmission experiment with whiteflies (*Bemisia tabaci* biotype B) was performed. Soybean infected with ToSRV was used as an acquisition host (48 h acquisition) and these whiteflies were provided a 48 h inoculation access period (IAP) on healthy soybeans. The soybean plants given the 48 h IAP

with whiteflies having acquired the virus from ToSRV-infected soybeans did not develop symptoms, but six of ten plants (results from two independent experiments) were infected based on PCR detection. These results indicate that ToSRV induces a symptomless infection in soybean, and suggest that it may serve as a reservoir of ToSRV for the tomato crop.

#### **PIV270 - CONSTRUCTION OF A NEW EXPRESSION VECTOR BASED ON THE BACULOVIRUS ANTICARSIA GEMMATALIS MULTIPLE NUCLEOPOLYHEDROVIRUS (AGMNPV)**

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Baculoviruses are insectinfecting viruses used as expression vectors of heterologous proteins. The most common method for the construction of recombinant baculovirus is the BactoBac System (Invitrogen). This strategy is based on the sitespecific transposition of an expression cassette (KLM, that contains a kanamycin resistant gene, a LacZbased complementation gene, and a MiniF replication origin) into a modified baculovirus genome (bacmid) maintained in *E. coli*. This work aimed for the construction of a similar expression vector system using the baculovirus genomes of *Anticarsia gemmatalis* multiple nucleopolyhedrovirus (AgMNPV). Several strategies were performed including homologous recombination of KLM into insect cells and direct cloning. For the first strategy, supernatant from a cotransfection experiments having the recombinant virus likely recombined with the KLM cassette were used in 96well end point dilution assays to isolate the recombinant vAgKLM. After six passages, viral DNA was purified PCRchecked using specific oligonucleotides that resulted positive for the construction. Restriction enzyme digestion (HindIII) was also performed resulting in the expected digestion profile, when compared with AgMNPV wildtype. When this viral DNA was transformed by electroporation into *E. coli* cells, no colony forming unit was observed. In the direct cloning strategy, the ligation was transformed by electroporation into *E. coli*

DH10B cells. Colonies were selected by PCR to check for the presence of the KLM cassette. Restriction enzyme digestion (HindIII) was also performed resulting in not expected digestion profile, when compared with AgMNPV. We hypothesized that the virus might be able to express cytotoxic products after bacterial transformation, killing the *E. coli* host. On the other hand, virus lacking this cytotoxic portion would be positively selected during bacterial transformation which explains the second result. Therefore, both restriction enzyme digestion and complete genome sequencing of possible recombinant viruses are needed in order to reevaluate the integrity of the genomes and to confirm for the recombinant viruses.

#### **PIV271 - POTATOINFECTING VIRUSES IN BRAZIL: A SURVEY 20102015**

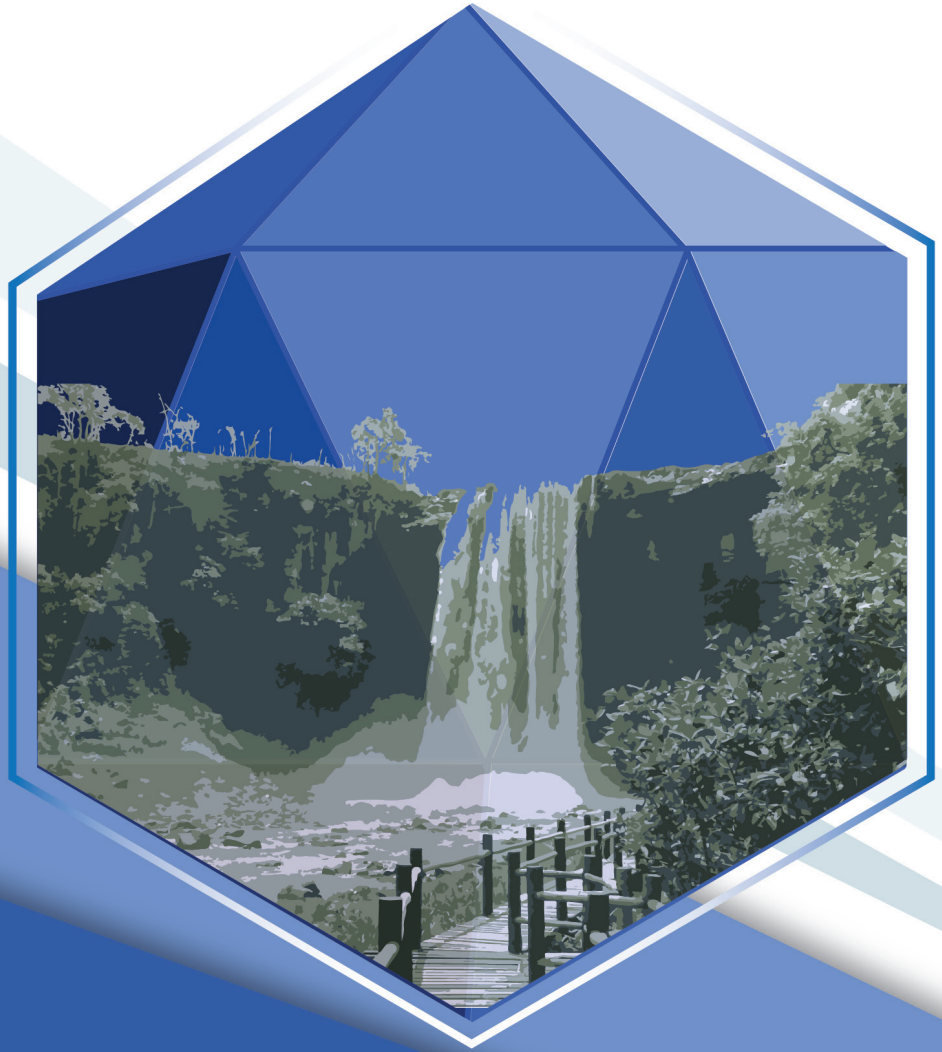
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2. FACULDADE ANHANGUERA

Potato is affected by many viral diseases that can cause severe losses, resulting in low yields and tubers of poor quality. Potato virus Y (PVY; genus Potyvirus; family: Potyviridae) and Potato leafroll virus (PLRV; genus Polorovirus; family Luteoviridae) were the main viral agents infecting potato in Brazil, while Potato virus X (PVX; genus Potexvirus; family Alphaflexiviridae), and Potato virus S (PVS; genus Carlavirus; family Betaflexviridae) were less frequently detected. In the last years, whiteflytransmitted viruses (e.g. begomoviruses and crinivirus) have been detected causing diseases in potato in producing areas around the country, especially after the introduction of *Bemisia tabaci* biotype B. The objective of this work was to perform a survey of viruses infecting potato in the central region of Brazil during 20102015. A total of 200 samples showing mosaic, mottling, interveinal chlorosis and stunting were collected from 10 potato-producing fields (Cristalina and LuziâniaGO; UnaíMG). Whitefly and aphid populations were present on fields sampled. Extracts from leaf samples were tested for PVY, PLRV, PVS and, PVX by DAS-ELISA using polyclonal antibodies. Total DNA was tested for begomovirus infection, by PCR, using degenerate genus-specific primers that amplify a fragment of 1.1 kbp of DNAA component. Total RNA was tested for crinivirus by RTPCR using a pair of specific primers (e. g. Tomato chlorosis virus – ToCV). Eighty samples positive for PVY

by serology were investigated by host reaction after rub-inoculation on *Nicotiana tabacum* cv. TNN plants, and 3-primer RT-PCR tests to determine PVY strains (e.g. PVY<sub>o</sub>; PVYN; PVYNTN), by amplifying fragments of P1 protein, coat protein, and nuclear inclusion body and 3'UTR. Serological and molecular test results indicated the presence of all the viruses infecting potatoes singly or in combination. More than 58% of the samples were virus-infected. PVY was the most frequently found (40%), followed by crinivirus (ToCV; 14%), indicating that PVY is still the most important and, ToCV incidence is increasing. PVS and PVX were encountered in 10% and 3%, respectively. Geminivirus occurred in 10% of the plants. PVY strains induced vein clearing and chlorotic pearl spots (PVY<sub>o</sub>) and vein necrosis on leaves of TNN plants (PVYN; PVYNTN). PVYNTN was the most frequent PVY strain (54%). These data reaffirm the importance as well as the diversity of viruses infecting potato crop in central region of Brazil.

# ***VETERINARY VIROLOGY - VV***



**VV1 - GAMMACORONAVIRUS AND DELTACORONAVIRUS DETECTED IN WILD BIRDS FROM SOUTH AND SOUTHEAST BRAZIL**

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3. UNIVERSIDADE FEDERAL DE MATO GROSSO
4. UNIVERSIDADE DO VALE DO RIO DOS SINOS
5. INSTITUTO CHICO MENDES DE CONSERVAÇÃO DA BIODIVERSIDADE
6. CENTRO NACIONAL DE PESQUISA E CONSERVAÇÃO DE AVES SILVESTRES
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Coronaviruses (CoVs) have a unique replication mechanism, resulting in a high frequency of recombination and high mutations rates, which may allow them to adapt to new hosts and ecological niches. Even though Brazil has 18% of the bird species diversity and the largest number of endangered species in the world, studies about the presence of CoVs in wild birds are scarce. This study performed a retrospective analysis of the presence of CoVs in 754 orotracheal/cloacal swab samples from wild birds collected in different regions of Brazil between 2004 and 2015. Viral screening was performed using conventional RTPCR and nested-PCR. Positive samples were characterized by partial sequencing of the RNAdependent RNA polymerase (RdRp) gene and phylogenetic analysis was performed to investigate the association between virus epidemiology, bird migration routes, and the proximity of urban and poultry regions. Six samples were positive for CoVs by RTPCR, three of which were gammacoronaviruses and three were deltacoronaviruses. This study showed the presence of avian gamma and deltacoronaviruses circulating in different regions of the country, close to urban areas and poultry regions, indicating that wild birds may transport CoVs to different migratory sites and represent a risk to poultry farms and public health in Brazil.

**VV4 - NATURAL INFECTION WITH BOVINE PAPILLOMAVIRUS TYPE 2 IS NOT SUFFICIENT TO CAUSE ENZOOTIC HEMATURIA IN CATTLE**

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Papillomaviruses are DNA doublestrand viruses that infect squamous epithelium of the skin and mucosa in various mammalian species. Bovine papillomatosis is a disease caused by Bovine Papillomavirus (BPV). The virus is described as epitheliotropic, but studies have shown the viral presence in body fluids such as blood and semen. The BPV2 is associated with the development of bladder cancer and enzootic hematuria (EH) in animals fed with fern shoots. One experimental infection study showed that the BPV2 alone can lead to the development of characteristic clinical signs of EH, suggesting that the natural infection itself could lead to the development of the disease. This study aimed to assess whether animals naturally infected with BPV2 but that do not feed on fern shoots can develop EH. In order to do this, it was isolated in the Agronomic Institute of Pernambuco (IPA) urine and blood samples of two groups consisting of 15 animals each. The first group was composed of infected animals with BPV2 and the second with animals not affected by BPV2. These animals underwent periodic clinical examinations by veterinarians. The DNA extraction was performed from samples collected using the PhenolChloroform extraction kit, the DNA was quantified and then was performed Polymerase Chain Reaction (PCR) of FAP 59/64 (Fw 5' TAA CWG TIG GIC AYC CWT ATT3' and Rev 5' CCW ATA TCW VHC CAT ITC ICC ATC3) for general detection of Papillomaviruses and then was evaluated for the presence of BPV2 using specific primers. The results show the detection of Papillomavirus in all studied samples, while BPV2 is shown in more than 90% of the total samples. However, no signal of EH was observed. The results obtained so far suggest that the natural infection with BPV2 is not sufficient for the development of EH. More studies are needed to better understand the disease, to know what is the real role of the virus in the development of bladder cancer and hematuria, how does the interaction between the virus and the fern shoots occur, and if the presence of the virus in the blood and urine plays a role in the disease development.

**VV5 - PRESENCE OF MIXED INFECTION OF DIFFERENT TYPES OF BOVINE PAPILLOMAVIRUS IN BOVINE PERIPHERAL BLOOD IN CATTLE AFFECTED BY PAPILLOMATOSIS**

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*UNIVERSIDADE FEDERAL DE PERNAMBUCO*

The bovine papillomatosis is a disease that affects cattle causing warts along its epithelium that has as etiological agent the Bovine Papillomavirus (BPV). Papillomaviruses are circular doublestranded DNA viruses, it has icosahedral symmetry, nonenveloped, that infect the squamous epithelium of the skin and mucous causing generally asymptomatic infections and various benign lesions that may progress to malignant lesions in various mammalian species. Currently there are 14 types of BPV described in the literature, with the type 14 described in 2015. In Brazil, the disease has been reported in several areas causing serious economic losses to cattle keepers, some even give up this activity. This study aimed to evaluate the presence of Bovine Papillomavirus types 1 to 13 in bovine blood samples. The 36 blood samples were obtained from the Agronomic Institute of Pernambuco IPA, Experimental Station of Itambé. The quality of the extracted DNA was evaluated by primers that attach to the bovine genome (beta-globin). The viral DNA detection was performed by PCR (Polymerase Chain Reaction) using specific primers for the BPV types 1 to 13. The presence of at least one type of viral DNA was found in 100% of the 36 samples, and the mixed infection was detected in 97.2% (35 samples). It was possible to identify up to 5 viral types in the same sample, being 43% of the samples positive for two types of BPV, 40% for three types, 14.2% for four types and 2.8% for five types. The BPV13 was not identified in any of the studied samples. Briefly, the BPV12 (97,2%), BPV11 (70%) and BPV2 (58,3%) types were the most frequent in the blood samples, and then the BPV6 with 22,2%. The BPVs that presented lower frequency were the BPV3 with 5,6% and BPV10 with 2,8%. The BPVs types 1, 4, 5, 8 and 13 were not detected in the samples studied. The results presented here show the wide dissemination of the types of BPVs in the evaluated herd of cattle. Furthermore, it reinforces that the presence of mixed infection for the virus approached has become common in molecular analysis performed by PCR. These

data support other studies that show that BPV, although characterized as epitheliotropic, can be found in other tissues, as in this case, the blood tissue.

**VV22 - PHYLOGENETIC ANALYSIS OF PORCINE GROUP A ROTAVIRUS WITH ZOONOTIC POTENTIAL IN BELÉM, BRAZIL**

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Rotavirus A (RVA) is member of the Reoviridae family, Rotavirus genus and they are a common cause of severe gastroenteritis in humans and animals. RVA genome comprises 11 segments of doublestranded RNA, encoding six structural viral proteins (VP1-4, VP6 and VP7) and six nonstructural proteins (NSP1-5/6). The segmented RVA genome facilitates reassortment between strains, happening both intra and intergenogroup reassortment that allowing the transmission zoonotic. This study aims to molecular characterization RVA in porcine samples in Belém, Brazil. From April 2008 to May 2009, 17 samples porcine RVA positives from five commercial pig farms located in midsized metropolitan middle region of Belém, Pará. Viral genome was extracted and subjected to ReverseTranscription Polymerase Chain Reaction (RT-PCR) targeting VP7, VP1, NSP4 and NSP5 genes. Subsequently, these samples were sequenced and subjected to phylogenetic analysis. All porcine samples were subjected to partial sequencing and the phylogenetic analysis of VP7, VP1, NSP4 and NSP5 genes demonstrated that samples possessed G3/G5, R1, E1 and H1 genotype, respectively. Phylogenetic analysis all genes analysed, demonstrated a high nucleotide identity with strains porcine and human origins detected in Brazil, Argentina e Paraguay. This study indicates that porcine samples have different evolutionary origins, with transmission interspecies from different regions of Brazil and zoonotic transmission within Brazil and between neighboring countries (Argentina and Paraguay) that import porcine. In conclusion, these data are important for epidemiological surveillance mainly because Brazil is an exporter of porcines that can transmit viral strains that infect humans or emergence of new recombinant strains with zoonotic potential.

**VV27 - EVIDENCE FOR A NOVEL AVIAN PARAMYXOVIRUS (APMV14) DETECTED IN MIGRATORY BIRD FROM LAGOA DO PEIXE, RS**

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2. UNIVERSIDADE DO VALE DO RIO DOS SINOS
3. ST JUDE HOSPITAL

Avian paramyxovirus (APMV) belongs to the genus Avulavirus in the Paramyxovirinae subfamily of the family Paramyxoviridae. Members of family Paramyxoviridae are characterized by pleomorphic enveloped particles that contain a singlestranded, negative sense RNA genome. APMV is classified into twelve distinct serotypes (APMV1 to 12) by ICTV Virus Taxonomy 2016. Only recently, in 2016, three APMVs isolated from the rockhopper penguin in the Falkland Islands, the common snipe in France, the Eurasian wigeon in Italy were considered new serotypes of APMV, APMV10, 11 and 12, respectively. An unclassified avulavirus isolated from the wild geese in Japan have been proposed to be serotype APMV13. Here, we propose a new serotype APMV14 based in genetic finds considering that many authors have demonstrated that phylogenetic analysis are suitable to separate the groups in accordance with the serological tests. Since 2005 the Laboratory of Virology of Institute of Biomedical Science USP has a surveillance programme aimed at detecting the presence of avian influenza and Newcastle disease viruses in wild birds and assessing the risk of such viruses spreading to poultry. As part of this programme, cloacal swabs were taken from a *Calidris fuscicollis* (Charadriiforme) captured in April 2012 in the Lagoa do Peixe, RS from which an APMV virus was isolated. The partial sequence (2000 bp) of the L gene of this isolate (RS1177) were aligned with representative viruses of the Paramyxoviridae family including all unclassified Avulavirus available in GenBank. For the construction of the phylogenetic trees, the evolutionary history was inferred using the maximumlikelihood method and the substitution model chosen from a model test done using MEGA 5. Isolate RS1177 had 67.4 % identity to the closest APMV8 and second 67.3 % identity to the APMV2. These are comparable to, or even lower than, the identities seen between the closest groups APMV12

and APMV13 (67.4 %), APMV2 and APMV10 (67.4 %), and APMV1 and APMV9 (66.5 %). The higher divergence between APMVs was 87.4% (APMV4 and APMV 12), the higher divergence of isolate RS1177 was 79.3% with APMV4. We consider that the large genetically distance showed by isolate RS1177 in the present study, indicate that it is sufficiently different from the other APMVs to be considered the prototype strain of a new APMV group, APMV14, with the full name APMV 14/*calidris\_fuscicollis*/Brazil/RS1177/2012.

**VV29 - COMPETENCE IN ANIMAL RABIES DIAGNOSIS**

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In the field of Preventive Veterinary Medicine, it is essential that diagnostic and research laboratories attend to the quite restricted biological competence and safety standards required for differential diagnosis of encefalopathies. Targeting the global livestock business and aiming to conduct diagnostic research about encephalopathies in mammals within internationally recognized competence concepts, the Virology Area of "Centro Estadual de Pesquisa em Sanidade Animal Geraldo Manhães Carneiro" of PESAGRORIO developed technical competence for systemic acting and quality requirements for rabies diagnosis, resulting in accreditation recognition. We developed the quality of diagnostic research of diseases that affect the central nervous system of animals, with primary focus on rabies, and were able to monitor the health of herds in the State of Rio de Janeiro. The activities resulted in the development of a Competence System, called "Sistema de Competencia da Área de Virologia do CEPGM", SCAV, culminating in accreditation by CGCRE INMETRO in the diagnosis of rabies, under the number of CRL 1007. We attended the requirements of ISO / IEC 17025 and the proposed by Ordinance 116, of August 28, 2008, including the conduction of the complete protocol of all processes and tests carried out in the involved laboratories. To suit the conditions of management and techniques for rabies virus diagnosis in mammals, within the 17025 and MAPA requirements, we prepared 01 Manual (MV),

13 management procedures (PGV) and 08 technical procedures (PTV), 02 Preparation Instructions (IPV), 13 Work Instructions (ITV), 06 Remark books (LOV), 53 Forms (FV), 49 Development Models (MDV) and 08 Informatives (IV). It is concluded that the development of SCAV, with its Manual and several technical and management procedures, will support the adaptation to situations arising from the international imposition of sanitary barriers, in order to keep the constant threat of virus severity in animals, and its spread, under control in the country, as well as those that can be introduced at any time, and automatically implies in the design and implementation of appropriate and effective preventive measures by the government and the productive sector.

**VV31 - DETECTION OF ROTAVIRUS A IN DOMESTIC ANIMALS IN AREAS OF ANTHROPOGENIC ALTERATIONS FROM MESOREGIONS METROPOLITAN OF BELEM AND NORTHEAST OF PARÁ STATE**

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Viral gastroenteritis is a condition infectious subject to certain factors of transmission and maintenance of the agents in animal population and the environment. Studies have shown that domesticated animals of several species have been affected by rotavirus infections (RV), symptomatic or asymptotically. The RV offers a wide range of hosts and are classified into nine groups or species designated from A to I, whereas the group A rotavirus (RVA) are widely distributed, especially in young animals. The RV belong to the Reoviridae family, genus Rotavirus, and their segmented genome consist of 11 segments of double stranded RNA (dsRNA). Fecal specimens were collected from asymptomatic domestic animals from areas of anthropogenic changes located in Belém metropolitan mesoregions and Northeast Pará State. Were selected 120 fecal specimens from different animal species with different ages and races belonging to the Santa Bárbara and Viseu cities. The specimens were subsequently subjected to immunochromatography, ELISA, polyacrylamide Gel Electrophoresis and one-step (RTPCR) to gene VP6. All samples studied were negative for polyacrylamide gel electrophoresis, ELISA and Immunochromatographic test. However, of the 120

samples 7.5% (9/120) were positive by RVA for one-step (RTPCR), distributed 5% (6/120) in Santa Barbara and 2.5% (3/120) in Viseu, being six isolated from canine, two swines and one feline. The positive samples were sequenced and after phylogenetic analysis, they were grouped in to genotype I2 of human origin. The results show the circulate of RVA in animals belonging to the areas analyzed and pointed out the requirement for further investigation of domestic animals as RVA reservoirs and zoonotic transmission.

**VV32 - EVALUATION OF ROTAVIRUS A, B, C AND H BY RTPCR MULTIPLEX IN WILD ANIMAL FROM MESOREGIONS OF BELÉM AND NORTHEAST OF PARÁ STATE, BRAZIL**

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Acute gastroenteritis is an important cause of morbidity and mortality both in humans and in animals. Infection by rotavirus (RV) have shown zoonotic character, because its diversity and possibility of rearrangements, affecting a wide variety of species. The RV are classified into nine groups or species designed from A to I, whereas the Group A RV (RVA) infections are widely distributed, especially in young animals. The RV belong to the family Reoviridae, genus Rotavirus, have segmented genome with 11 double RNA segments (dsRNA). The present study aims to evaluate the circulation of rotavirus A, B, C and H in wild mammals, flying mammals and nonflying mammals in the areas of environmental changes in the Metropolitan mesoregions of Belém and Northeast of Pará, Brazil. Faecal samples were collected from flying mammals (bats n=50) and non-flying mammals (rodents n=50) from Santa Bárbara and Viseu cities, Pará, in the period from September/2014 to December/2015. The samples were screened by Immunochromatographic test, RIDAQUICKR Rotavirus. Bats Faecal specimens were extracted in NB3 laboratory using Guanidine isothiocyanate, and submitted to Polymerase Chain Reaction preceded by Reverse Transcription Multiplex (RTPCR Multiplex) for RVA, RVB, RVC and RVH, and positive and negative controls. The amplicons were subjected to electrophoresis in agarose gel 1.5%, and photodocumented. The Chi-

square test, BioEstat 5 Program, with  $\alpha = 0.05$  was applied to compare the positivity among the population studied (bats and rodents). All samples were negative by Immunochromatographic test, although has been observed 4% (4/100) of positive samples for two populations investigated for RVA to Multiplex RT-PCR. When analyzed separately were confirmed in 8% (4/50) of positivity in bats, and no positive samples in rodents population. The positivity significance among populations studied was 0.041 to 0.05. Sequencing demonstrated one sample genotype G1 (99%) and one I2 (99%) closely related to human samples. The results are pioneers and recorded for the first time the occurrence of RVA in Chiroptera in the Amazon region. Further studies to determine the RVA circulation are needed to evaluate the potential of these animals as reservoirs and zoonotic transmission of rotavirus.

**VV33 - GENOME SEQUENCING AND INFECTION IN ANIMAL PRIMARY NERVOUS CELL CULTURE OF BEAN 58058 VIRUS ISOLATED FROM ORYZOMIS RODENTES IN NORTHERN BRAZIL (PARÁ)**

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The poxviruses are enveloped viruses with mean diameter of 200nm and the replication occurs in the cytoplasm. The genome is a doublestranded DNA which in size from 130300kb. In northern Brazil few information is available on BeAn 58058 virus, Cotialike virus, thus we propose to study the genome it using the GS FLX 454 (Roche, Life Science) and Ion Torrent platforms; in parallel an experimental infection in primary neuronal cells of suckling Swiss mice was performed and transmission electron microscopy was used to examine sites of replication of strain BeAn 58058 onto infected Vero and primary cells. For this, we sequenced the nearly complete genome, and infected primary animal culture and Vero cells with a clarified 1:10 brain homogenate infected with BeAn 58058 virus onto the monolayer. The negative staining and ultrathin sections were examined in a transmission electron microscopy (Zeiss EM 900). The virus particles were precipitated using the

polyethylene glycol (PEG) centrifugation as previously described and the supernatant treated with DNase and RNase (Ambion) for host contaminant debris removing. Treated samples were further used for RNA extraction and for fulllength genome sequencing. The genome was obtained employing a de novo hybrid assembly strategy using GS FLX 454 and Ion reads simultaneously in the software Mira 4.0. Visual inspection was performed using the software Genious v.6.1.4. The cytopathic effect was detected 4 days post infection and characterized by membrane fusion and formation of syncytia and vacuoles. Negative staining of supernatants of cultures showed coated viral particles with diameter of approximately 200nm. Ultrathin sections showed enveloped virus particles in the cytoplasm with different stages of maturation. The total genome recovered was 162,700nt in length with a mean coverage of 297x fold. This is the first report of the complete genome sequence for Be An 58058 virus strain isolated in northern Brazil.

**VV39 - SENCAVIRUS A IN SUCKLING PIGS FROM SWINE HERDS, SANTA CATARINA, BRAZIL**

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Senecavirus A (SVA), also known as Seneca Valley Virus (SVV) has been detected in swine, and is characterized by vesicular skin lesions, which are sometimes accompanied by systemic disease. This includes digestive, respiratory, reproductive and nervous system disorders. Clinical signs resemble Footandmouth disease, swine vesicular disease and vesicular stomatitis. SVA is the only member of the genus Senecavirus within the family Picornaviridae. SVA is a singlestranded, positivesense, nonenveloped RNA virus with a genome size of approximately 7.2 Kb. In May of 2016, a pig herd located in Santa Catarina State, Southern Brazil, presented diarrhea, vesicle in the skin and lesions in hoof and snout of suckling piglets, 20 days after the arrival of male and three female adult pigs. The clinical evolution was of one week approximately, and samples from snout, vesicles, livers and hoof were submitted for viral detection. After RNA extraction and cDNA synthesis, polymerase chain reaction was performed with primers targeting the genomic 5'

untranslated region (UTR), namely SenecaV5UTRF 5'TTAGTAAGGGAACCGAGAGG3' and SenecaV5UTRR 5'CTGTAGCTCGCTATGCTAGG3'. Amplicons from positive samples were purified and submitted to sequencing for further genotype characterization. Twelve out of 14 samples analyzed were positive for SV genotype A (SVA) virus. Detection of RNA SAV in suckling pigs allowed us to conclude that this virus may be responsible for this outbreak. Due to the epidemiological investigation conducted, it seems that the adult pigs served as case controls for the introduction of SVA in the farm, thus reinforcing the need of stricter biosafety measures. Many outbreaks have been reported in Southern region of Brazil, and SVA might be included as a differential diagnosis for swine vesicular diseases.

#### **VV40 - ZONOTIC HEPATITIS E IN BRAZIL: A NEGLECTED DISEASE?**

**Rigueira, L.L.; Vilanova, L.F.L.S.; Rigueira, L.L.; Perecmanis, S.**

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Hepatitis E is a zoonotic disease that circulates all over the world. In addition to affecting humans, the Hepatitis E virus (HEV) is also found in pigs, wild boar and other animals. The zoonotic transmission mainly occurs through the direct exposure, by drinking contaminated water (fecal-oral route) or by the consumption of raw or undercooked meat. In Brazil, Hepatitis E is a notifiable disease but it is not routinely investigated, even in cases of unexplained elevated liver enzymes or acute hepatitis. Furthermore, only a few laboratories perform diagnostic tests for the virus. It is known that humans and swine share genotypes 3 of HEV, which circulate in many countries such as Brazil. It is also known that humans with occupational exposure to pigs are at increased risk of HEV zoonotic transmission. Both human and swine HEV antibodies have already been detected in Brazil. Human cases have been detected in all five regions of the country. Swine HEV seems to also be spread all over the country. However, despite all the evidence demonstrating this, the lack of a national epidemiological study about the disease hinders the awareness of its real situation in the country. Even Brazil's Ministry of Health recognizes that many human cases may not have been registered in the country, therefore generating high underreporting. In addition, the HEV circulation in the national swine

herd as well as in other animal species discloses the epidemiological complexity of the disease. The results of all HEV studies conducted in Brazil so far should serve as a warning to the local public health system, but they also reveal that the disease needs to be better investigated.

#### **VV47 - DETECTION OF BLUETONGUE VIRUS ANTIBODIES IN CATTLE OF PORTO NACIONAL CITY, TOCANTINS, BRAZIL**

**Negri Filho, L.C.; Silva, L.C.; Marcasso, R.A.; Nogueira, A.H.C.; Okuda, L.H.; Stefano, E.; Pereira, C.E.S.; Veronez, J.V.; Vieira, M.V.; Rodrigues, S.M.C.; Furlan, D.; Pereira, G.R.; Gomes, M.G.T.; Koetz Junior, C.; Pituco, E.M.; Okano, W.**

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2. *UNIVERSIDADE NORTE DO PARANÁ*
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The Bluetongue is an infectious disease that affects all ruminants. It is caused by Bluetongue Virus (BTV), with 27 serotypes distributed worldwide. This virus is primarily transmitted by vectors of the *Culicoides* genus. BTV occurs endemically in temperate and tropical climate zones that provide suitable conditions for high proliferation of *Culicoides*. In countries where Bluetongue is endemic there may be restrictions on international trade in animals. In cattle the infection is usually unapparent and it is detected by the presence of antibodies produced around 10 days after infection. Cattle are considered to be an incubator of the disease due to the long viremia duration. Different studies in Brazil showed high prevalence of BTV in cattle and sheep and they are identified as serotypes 3, 4, 12, 14 and 18. However, there are no data on the BTV in the state of Tocantins. Therefore, the aim of this work was to check the occurrence of antibodies antiBTV in bovines of the Tocantins State. For this study blood serum and semen were collected from 25 Braford cattle, of which were 14 females and 11 males without clinical manifestation of the disease. All animals were older than two years of age and were raised in the municipality of Porto Nacional, state of Tocantins, Brazil. Samples were collected in October of 2015. The analyzes were performed at the UNOPAR Veterinary Medical Diagnostic Center in Arapongas, state of Paraná, following the standard protocol for the Bluetongue Virus Antibody Test Kit,

VMRD Inc. It was performed a screening of samples by AGID technique, which detects antibodies of all BTV serotypes. After that, the samples were sent to the Bovine Virus Laboratory, at the Biological Institute of São Paulo. Serum samples were subjected to virus neutralization tests against BTV4 serotype, where the titers were determined by Reed and Muench method and expressed in log<sub>10</sub>. Samples with titers greater than 0.3 log<sub>10</sub> of virus neutralization were considered reagents. Semen samples were subjected to RTPCR for BTV. In AGID, 76% (19/25) of serum samples were positive, whereas in virus neutralization 100% (25/25) of the samples were positive for BTV4, with titers ranging from 1.0 to 2.2 in logarithmic basis. No semen samples were positive for RTPCR. The high percentage of animals with antibodies shows that in the region studied the BTV4 is endemic, which is justified by the tropical climate favorable to the proliferation of vectors.

#### **VV48 - DETERMINATION OF ANTIBODY TO BOVINE VIRAL DIARRHEA IN CATTLE UNIMMUNIZED AT PORTO NACIONAL COUNTY, TOCANTINS, BRAZIL**

Negri Filho, L.C.; Silva, L.C.; Marcasso, R.A.; Nogueira, A.H.C.; Okuda, L.H.; Stefano, E.; Pereira, C.E.S.; Veronez, J.V.; Vieira, M.V.; Rodrigues, S.M.C.; Furlan, D.; Pereira, G.R.; Gomes, M.G.T.; Koetz Junior, C.; Pituco, E.M.; Okano, W.

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The virus of Bovine viral diarrhea (BVDv) is a RNA virus from Flaviviridae family. The virus has a worldwide distribution, with cytopathic and noncytopathic biotypes. The bovine viral diarrhea is a disease with severe or unapparent clinical signs and it is responsible for high mortality rates and reproductive problems. The seropositivity for this agent in a herd indicates the presence of carriers of the virus that could potentially serve as a source of infection for susceptible animals. The presence of persistently infected (PI) animals is the main form of transmission and maintenance of the virus in a herd. Hence, the prophylaxis and control of BVDv infection consist essentially on the identification and removal of PI animals out of the properties. The BVDv diagnosis is performed through the detection of

the virus or by the demonstration of specific antibodies. Antibodies against BVDv can be detected on serum within 21 days after the infection and remain detectable for at least three years. However, the antibody titers resulting from an old infection will decline with time. The aim of this work was to check the occurrence of antibodies antiBVDv in bovines of the Tocantins State. For this study blood serum and semen were collected from 25 Braford cattle, of which were 14 females and 11 males without vaccination for BVDv. All animals were in reproductive age and were raised in the municipality of Porto Nacional, state of Tocantins, Brazil. Samples were collected in October of 2015. All analyzes were performed at the Biological Institute of São Paulo. Serum samples were subjected to virus neutralization(VN) tests, were titers were determined by Reed and Muench and expressed in log<sub>10</sub>. Samples with titer greater than 1.0 log<sub>10</sub> of VN were considered reagents. Semen samples were subjected to Polymerase Chain Reaction(PCR) for BVDv. The presence of antibodies to BVDv was detected in 100%(25/25) of the serum samples, with titers ranging from 1.0 log<sub>10</sub> and 3.1 log<sub>10</sub>. The results demonstrate that in this property there are animals with high and low antibodies titers, which represents that were newly infected and others that are already declining titration. Through the PCR, 100% of the analyzed semen was negative for BVDv. Since none of these animals have been vaccinated against BVDv and all showed the presence of antibodies, the results indicate that there has been exposure to the virus, and that studies should be conducted on the property to identify PI animals.

#### **59 - ANTIBODY RESPONSE EVALUATION OF DOGS VACCINATED AGAINST CANINE DISTEMPER ASSOCIATED WITH ACUPUNCTURE STIMULATION**

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Acupuncture is one of the oldest methods in Traditional Chinese Medicine consisting in the use of needles for cutaneous stimulation in determined points called acupoints, in order to prevent or treat diseases. It has been shown that they can immuno modulate the defenses, increasing population and activity of many immune

cells. Canine distemper virus (CDV) causes one of the most important infectious diseases of dogs worldwide, a highly contagious illness that leads to neurologic disorders and death. The most susceptible dogs are those unvaccinated, but it is necessary to revaccinate the animals in order to maintain the level of protection. The present study was carried out at the Veterinary Hospital from the Department of Veterinary Medicine, where 18 dogs from various ages were randomly distributed in 5 groups: GI – 1mL of the CDV vaccine (3 animals); GII – 1mL of the vaccine and were stimulated with acupuncture (2 animals); GIII – 1mL of NaCl and were stimulated with acupuncture (4 animals); GIV – 0,2mL of vaccine and were stimulated with acupuncture (4 animals); GV – 0,2mL of vaccine (5 animals). All doses were applied subcutaneously in the right hypochondria, and the acupuncture stimulation were performed in the day (D0) and twelve days (D12) later, using the acupoints IG4(Hegu), VG14(Dahzui) e E36(Zusanli), known for immunological stimulation. The parameters evaluated were hematologic profile and antibody titer antiCDV using the immunochromatographic method to detect IgG, performed on D0 and D12. The results obtained showed that acupuncture stimulation increased the specific antibody titer of 90% of the animals in all experimental groups that received the acupuncture stimulation (9/10). The only animal that received the acupuncture stimulation and showed no increase in antibody production was a 10yearold from Group IV, most likely due to age-related immunosuppression. Interestingly, animals from the negative control group (GIII) with initial low levels of antiCDV antibodies had an increase in specific antibody production due to acupuncture stimulation alone (4/4). The non-parametric MannWhitney U test showed that the results are significantly different from D0 to D12 ( $p < 0.5$ ). These results corroborate many literature findings concerning acupuncture stimulation of immune responses. This work showed that acupuncture immune stimulation may become an interesting partner in increasing vaccine protection against Canine Distemper in dogs.

#### **VV68 - EPIDEMIOLOGICAL AND CLINICOPATHOLOGICAL FEATURES OF CANINE PARVOVIRUS 2C INFECTION IN DOGS FROM RIO GRANDE DO SUL STATE, BRAZIL**

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Canine parvovirus type 2c (CPV2c) emerged in Europe in the early 2000's and rapidly spread out worldwide. Clinical and molecular data demonstrated the widespread distribution of CPV2c among Brazilian dogs. However, detailed clinical and pathological descriptions of cases are still scarce. This research describes the epidemiological and clinicopathological features of 23 cases of CPV2c associated disease in dogs from Rio Grande do Sul state (2014-2016). Fecal samples or intestine segments of 23 dogs with clinical and/or pathological signs compatible with parvovirus were obtained from veterinary clinics and pathology laboratories. The samples were submitted to virus isolation and/or to a PCR for a 583bp of the VP2 gene of the CPV2 genome, following by nucleotide sequencing of the amplicons. Virus isolation and PCR detection were achieved in all samples. Nucleotide sequencing of the amplicons revealed a high amino acid identity among them and with CPV2c standard sequences (99.4 to 100%). Most importantly, all sequences harbored the mutation at amino acid residue 426 in VP2 sequence (asparagine to glutamic acid), which is a signature of CPV 2c. Most affected dogs presented typical clinicopathological signs of parvovirus such as diarrhea, vomiting, hyperemia and hemorrhage of the serous membrane of the small intestine. Also a diffuse segmental granulation, atrophy of the mucosa, necrosis and fusion of crypts, villous atrophy, squamous metaplasia and epithelial syncytia in crypts was observed in most of the cases. Nonetheless, some affected animals presented clinical, pathological and/or epidemiological features divergent from the classical cases. These differences included a wide variation in the color of diarrheic feces. The colour ranged from yellowish, light brownish, greenish, orange brown and brownish (13/23); the number of adult (3/23) and vaccinated affected dogs (11/23); the wide extension of the lesions in small intestine (10/20) and extraintestinal lesions, such as pulmonary edema and convulsion (9/20). These findings confirm the importance of CPV-

2c infection among Brazilian dogs and reinforce the need for its inclusion in the list of differential diagnosis of many diseases, especially for its potential atypical clinicopathological presentations.

#### **VV69 - DETECTION AND IDENTIFICATION OF PESTIVIRUSES IN COMMERCIAL FETAL BOVINE SERUM**

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Bovine viral diarrhoea virus (BVDV) belongs to the family Flaviviridae and genus Pestivirus. Based on genetic and antigenic relationships, field BVDV isolates are classified into two species, BVDV1 and BVDV2. Additionally, a new pestivirus species, named HoBilike, was identified in commercial batches of fetal bovine serum (FBS) from Brazil and, subsequently, isolated from FBS and clinical cases of BVD in several countries. The objective of this study was to detect and identify pestiviruses contaminating commercial FBS produced in Brazil between 2005 and 2015. Seventy six batches of FBS were submitted to RNA extraction, cDNA synthesis and PCR amplification using panpestivirus primers for 5'UTR, followed by nucleotide sequencing and phylogenetic analysis. In addition, FBS were submitted to virus isolation in MDBK cells and virus neutralizing (VN) assays against BVDV1 and BVDV2. Forty two FBS samples (55.3%) were PCR positive for pestiviruses, being 37 (88.1%) BVDV1 (BVDV1a: 25; b: 8 and d: 4). Seven samples contained BVDV2 (16.7%) and 4 HoBilike (9.5%). From the positive samples, six (14.3%) contained more than one genotype (4 had BVDV1 and BVDV2; one had BVDV1 and one had BVDV2 and HoBilike). The nucleotide identity among the BVDV1a ranged from 90.8 to 98.7%; BVDV1b, 93.9 to 96.7%; BVDV1d, 96.2 to 97.6%. The identity among HoBilike viruses varied from 96.6 to 98.7%. In VN assay, 12 samples had antibodies to BVDV1 with titers of 5 to 40; and 12 samples had antibodies to BVDV2, with titers ranging of 5 to 160. These results showed a high level of FBS contamination with pestivirus RNA and call attention for continuous and systematic monitoring to avoid contamination of biological products.

#### **VV70 - NEUTRALIZING ANTIBODIES TO BOVINE ENTEROVIRUS IN CATTLE, HERD FROM DO RIO GRANDE DO SUL, BRAZIL**

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Bovine enterovirus (BEV) a singlestrand RNA and nonenveloped virus, belonging to Enterovirus genus from Picornaviridae family is endemic in cattle herds worldwide and the infection is normally subclinical. Clinical signs may be sporadically reported being associated to gastroenteritis, respiratory disorders and infertility. BEV is detected in high load in bovine feces and may be transmitted to susceptible bovine by contaminated water. Fecaloral route is the main transmission mechanism and BEV particles can be viable for long periods under environmental conditions. The goal of the present study was to survey the presence of BEV neutralizing antibodies in cattle herds from Rio Grande do Sul State, Brazil. Sera came from 49 different municipalities from central, north and northwest of RS, from female beef and milk herds with reproductive losses. Samples were kindly provided by Setor de Virologia from Universidade Federal de Santa Maria. One hundred three n=180 serum samples were submitted to virus neutralization (VN) assay. Sera were diluted from 1:5 to 1:640 and assayed against 100 - 200 TCID<sub>50</sub>/mL of a prototype BEV2 virus strain. Nearly all samples testes showed anti BEV antibodies: 98.3% (177/180). The antibody titers of ranged from 1:10 in 3.8% (7/180) to 1:80 in 23.3% (42/180). These results showed BEV is circulating among southern Brazilian cattle herds.

#### **VV73 MULTIPLEX RTPCR EVALUATION OF ROTAVIRUS A, D, F AND G FROM BIRDS ON PARÁ STATE, BRAZIL**

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Studies demonstrate different birds' species have been affected by Rotaviruses (RV), symptomatic or asymptomatic, specially by RVA and RVC, which are important zoonotic agents, in addition they represent an economy impact, due these animals decreased productivity. The RV offers a wide range of hosts and are classified into nine groups or species designated

from A to I, although in birds the infections have been characterizing by groups A, D, F and G and widely distributed. The RV belong to the Reoviridae family, genus Rotavirus have segmented genome with 11 double-stranded RNA (dsRNA). This study aimed to detect RV by multiplex RTPCR in fecal birds specimens collected from different locations from State of Pará. Nine two fecal specimens have been selected randomly, of different bird's species, from the samples animals Bank been of Rotaviruses Laboratory of the Instituto Evandro Chagas IEC. There specimens have subjected to Polyacrylamide Gel Electrophoresis and Multiplex (RTPCR). All samples from this study were negative by Polyacrylamide gel Electrophoresis, however showed positivity in 10.9% (10/92) by Multiplex (RTPCR), which 6.9% (2/29) represented the Group A, 13.8% (4/29) Group D, 10.3% (3/29) Group F and 3.4% (1/29) to the Group G. Subsequently all positive samples have sequenced to characterization of groups. The results presented record simultaneously detection about RVA, RVD, RVF and RVG from fecal birds specimens in the State of Pará, and show to the multiplex RTPCR efficiency as diagnostic test, noticing the largest necessity investigation of these animals like RV reservoirs and possible chance of zoonotic transmission.

#### **VV76 - IMMUNOGENICITY OF PARAPOXVIRUS OVIS RECOMBINANTS EXPRESSING THE RABIES VIRUS GLYCOPROTEIN AS A CANDIDATE VECTORIAL VACCINE FOR CATTLE**

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The parapoxvirus orf virus (orfv) has several unique properties that make the virus an excellent candidate for vaccine delivery in livestock. To assess the suitability of orfv as a multi-species vaccine delivery vector, we constructed orfv recombinants expressing the rabies virus (rabv) glycoprotein g (gg). expression of gg by recombinants orfv-rabv-gg-1 and orfv-rabv-gg-2 was confirmed in vitro and their replication properties were assessed in bovine cell cultures. then, the immunogenicity of orfv-rabv-gg-1 and orfv-rabv-gg-2 recombinants was assessed in heifers. thirty animals were allocated in two groups (15 heifers each) and immunized intramuscularly with three doses

(days 0, 30 and 390) of orfv-rabv-gg-1 or orfv-rabv-gg-2 containing  $10^{7.9}$  tcid<sub>50</sub>/dose. serum was collected at days 0 (vaccination day), 30 (second dose day), 60 (30 post-revaccination), 180, 390 (third dose day) and 420 (30 days post-revaccination after one year of the first dose - immunization) and tested for rabv neutralizing antibodies by rapid fluorescent focus inhibition test (rffit). all immunized animals developed vn antibodies to rabv 30 post-vaccination. heifers immunized with orfv-rabv-gg-1 developed titers of 80 to 1280 (gmt = 211) and heifers immunized with orfv-rabv-gg-2 developed titers of 320 to 2560 (gmt = 735). the antibody titers at 180 and 360 after vaccination demonstrated the duration of humoral response. after a booster vaccination after at day 390, animals of group orfv-rabv-gg-1 developed titers of 40 to 2560 (gmt = 177) and heifers immunized with orfv-rabv-gg-2 developed titers of 80 to 1280 (gmt = 422). we are currently investigating the impact of vaccination with the recombinants in cellular responses to rabv glycoprotein. these results demonstrate that orfv-rabv-gg-1 and orfv-rabv-gg-2 may be a new option for rabies vaccine cattle and suitability of orfv as a vaccine delivery vector for this species.

#### **VV78 - MOLECULAR CHARACTERIZATION OF GLYCOPROTEIN G OF RABIES VIRUS FROM CATTLE IN THE CENTRAL RIO GRANDE DO SUL STATE, BRAZIL**

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Rabies is a worldwide, generally fatal, zoonosis of mammals, caused by the rhabdovirus rabies virus (RabV). Rabies is endemic in Southern Brazil, especially in Rio Grande do Sul (RS), Brazil where thousands of bovine cases have been reported every year. Molecular and epidemiological investigations of RabV infection have been performed, mainly to identify the virus variants involved in the disease and their geographic distribution. In the present study we performed a molecular characterization of the glycoprotein G (gG) of 78 RabV identified in clinical specimens obtained from bovine in RS between 2012 and 2016. Glycoprotein G is an important RabV protein involved in virulence and its interactions with the immune system. The gG gene was amplified by RT PCR; nucleotide and amino acid

sequences were analyzed. To date, total nucleotide/amino acid sequences were obtained of 22 RabV obtained from cattle from central RS. The amino acid analyzed showed the high conservation of gG from all samples (97.6 to 100% of amino acid identity), mainly in all six antigenic sites (I, IIa and IIb, III, IV, G5 and G1). For phylogeny, all samples clustered together with herbivorous and vampire bat RabV obtained from Genbank and apart of dog, cat, wild animals and human RabV sequences. Three sublineages were detected, clustering two samples from Pinhal Grande county (sublineage 1), identified in 2012 and 2016; three samples of Ivorá, Pinhal Grande and Jaguari counties obtained in 2015 and 2016 (sublineage 2); and two samples obtained from São Pedro do Sul county (sublineage 3) in 2014, indicating divergent virus circulating in central region of RS. Some amino acid mutations were identified in gG sequences, and two sublineages were determined by some mutations: lineage 2, at amino acid position 375 (aspartic acid to asparagine); and lineage 3, at amino acid position 376 (glycine to arginine). The results showed the high conservation of gG among the analyzed samples, mainly in the antigenic sites. On the other hand, our data demonstrate that different RabV variants are circulating among herbivorous of central RS.

#### **VV81 - GENETIC CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF SENECAVIRUS A CIRCULATING IN THE US AND IN BRAZIL**

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Senecavirus A (SVA) has been associated with sporadic outbreaks of vesicular disease in pigs in the US since the late 1980's. Recently, however, an increased number of reports have described the association of SVA with vesicular disease and neonatal mortality in swine. Notably, the number of SVA cases have jumped from two in 2014 to over 100 in 2015, which represents a

significant increase in the incidence of infection. Since November 2014, SVA has also been frequently reported in swine in Brazil. However, the factors that contributed for the emergence of SVA remain unknown. The overall goal of our study was to characterize contemporary SVA isolates to determine the genetic diversity of the strains circulating in the US and Brazil. The complete genome sequences of seventeen SVA isolates obtained in the US and four SVA isolates obtained in Brazil were compared to other SVA sequences available on GenBank. Sequence comparisons revealed that the US contemporary isolates characterized here share 9193% of nucleotide (nt) identity with the prototype US SVA strain SVV001 and an isolate obtained in Canada in 2007 (SVA11559103), 9899% nt identity with other contemporary isolates recently obtained in the US, 9597% nt identity with contemporary Brazilian isolates and 9496% nt identity with a recent Chinese isolate (CH12015). Comparison of the amino acid (aa) sequences of SVA polyprotein (2181 aa) revealed that the US contemporary isolates here share 9799% aa identity with other SVA strains. Comparisons based on a 541 nt region of the VP1 gene revealed a similar genetic heterogeneity between these isolates. A greater genetic divergence (8688% nt identity), however, was observed when the contemporary SVA isolates were compare to historical US isolates obtained prior to 2002. Sequence comparisons between the isolates obtained here and other contemporary or historical strains available on GenBank revealed a high degree of sequence homology between contemporary isolates. Additionally, both US and Brazilian SVA isolates share a high degree of homology with a recent SVA strain obtained in China. Phylogenetic analysis using complete genome sequences of contemporary SVA isolates and a limited number of historical sequences suggest a constant evolution of SVA. Results here provide important information on the genetic diversity of contemporary SVA isolates that have been recently associated with outbreaks of vesicular disease in swine.

**VV101 - NEUTRALIZING ANTIBODIES TO BOVINE ADENOVIRUS TYPE 3 IN CATTLE, RIO GRANDE DO SUL, BRAZIL**

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Adenovirus infections are mostly characterized by ubiquitous nature in many host species, thus being expected that seroprevalence to bovine adenovirus (BAV3) may be high in cattle herds worldwide. However, there is no information regarding the presence of BAV3 specific antibodies in Brazilian cattle. BAV3 is associated with respiratory disorders, conjunctivitis, and pneumonia, and also with enteritis, lymphadenopathy and polyarthritis disease, comprising the so called "weak calf syndrome". Transmission mainly occurs by the fecal-oral route, but can also occur by aerolized droplets. The objective of the present study was to survey the presence of neutralizing antibodies to BAV3 in cattle herds from Rio Grande do Sul State, Brazil. One hundred and seven (n=107) serum samples were analyzed by Virus Neutralization (VN) assay. Serum samples were provided by Setor de Virologia da Universidade Federal de Santa Maria and came from central, north and northwest region of State, both from beef and milk cattle herd and from female gender. The serum samples were diluted from 1:2 until 1:256 in microplates assayed against 100 – 200 TCID<sub>50</sub>/mL of a prototype BAV3 strain. Nearly all samples, 91% (97/107) were positive for antiBAV antibodies. One percent of serum of these animals had 1:4 of titers; however, 60% of serum samples showed titers > 1:256. From these results, we can conclude that indeed BAV3 is circulating among southern Brazilian cattle herds and further attention have to be taken for the presence of typical clinical signs in calves.

**VV110 - ANTIBLUETONGUE ANTIBODIES IN DAIRY CATTLE FROM THE STATE OF PERNAMBUCO, BRAZIL**

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Bluetongue is a noncontagious infectious disease, caused by an Orbivirus from the Reoviridae family, called Bluetongue virus (BTV). This BTV transmission is carried out by hematophagous vector from the *Culicoides* sp. genera, being all domestic and wild ruminants susceptible to BTV infection, especially sheep. Bluetongue is a disease with compulsory notification to the World Organization for Animal Health. Confirmed cases of this disease results in great economic impacts due to the direct and indirect production losses, as well as sanitary restriction measures by importer countries. The lack of data of this infection in the Northeast region of Brazil in the literature prompted the investigation on the present work. Therefore, the aim of this study was to investigate the presence of antiBTV antibodies in the dairy cattle of the state of Pernambuco, Brazil. The mesoregion Ipanema Valley, in the state of Pernambuco comprises six counties (Águas Belas, Buíque, Itaíba, Pedra, Tupanatinga and Venturosa) and its main economic activity is dairy production. During October of 2015 and February of 2016, 358 samples were collected from female dairy cattle in reproductive age, from 18 randomly selected farms. The sampling was designed to determine the occurrence of positive properties and seropositive animals per county. Subsequently, the samples were processed using the Immune diffusion agar gel technique to detect the presence of antibodies. The results showed the presence of antiBTV antibodies in 23.2% (83 / 358) animals. Interestingly, all properties (100%) had at least one positive animal among their herds, with frequency varying between 1.7 and 84.6%. The detection of infected animals is important to establish prophylactic measures in order to reduce the exposure of uninfected members of the herd. The results obtained in this initial work showed circulation of BTV in the mesoregion studied and that prevention and control measures need to be implemented to reduce the dissemination of the agent. Moreover, it indicates

that new studies are necessary in order to identify the circulating serotypes in this region.

#### **VV115 - DETECTION OF AVIAN METAPNEUMOVIRUS IN CAPTIVE ANATIDAE BIRDS**

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Wild birds from Anseriforme order, specially Anatidae family, are considered reservoirs of many pathogens that threats poultry industry. Among these pathogens are the avian metapneumovirus (aMPV), one of the members of the genus Metapneumovirus, the family Paramyxoviridae. The aim of this study was detect the presence of aMPV in captive birds samples. To do so, RNA purification was performed and a conventional RTPCR targeting the N gene with expected fragment of 115bp and a real time RTPCR (RRTPCR) targeting the G gene using primers and probes specific for detection of subtypes A and B were carried out. All assays were validated using aMPV vaccines (subtype A and B) as controls, RTPCR and RRTPCR specificity was assayed by using a vaccinal Newcastle disease virus (strain La Sota). Regarding samples 329 oropharyngeal (OP) and cloacal swabs from captive birds of Anatidae family collected in 4 different locations from São Paulo state, following the guidelines and with the permission of the responsible agency (CEUAUSP and SISBIO), were tested. Samples combining up 5 swabs types in one vial from birds of same sex, specie and cage. In total, 157 samples were tested individually or pooled, comprising 78 OP and 79 cloacal swabs from 3 ducks, 2 swans, 2 drakes and 1 goose species. Additional samples were also tested, those samples were pooled mixing OP and cloacal swabs, 2 each, totaling 172 samples from 2 drakes and 1 duck species. aMPV viruses were successfully detected by all assays; whereas NDV was not detected by those tests. Of the 329 tested samples, 29 (8.81%) were detected by conventional RTPCR, corresponding to 20

(68,96%) samples from *Aix galericulata*, 8 (27.59%) of *Dendrocygna viduata*, and 1 (3.45%) sample from *Aix sponsa*. None of tested samples was detected by specific RRTPCR assays for subtypes A and B. The data shows that there is a circulation of a aMPV between captive birds that belong to the Anatidae family, corroborating previous studies that also found aMPV subtypes A, and B in *Dendrocygma viduata*, *Anas bahamensis*, *Neochen jubata* samples, and subtype C in *Cairina moschata*, *Anas discors*, *Branta canadensis* and wild goose samples. Samples will be molecularly characterized by DNA sequencing to confirm the viral circulation and aMPV subtype that has been found.

#### **VV122 - PREVALENCE OF HEPATITIS E VIRUS (HEV) ANTIBODIES IN DOMESTIC SWINE IN PERNAMBUCO**

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PERNAMBUCO

Hepatitis E is a zoonotic emerging disease worldwide distributed. The causative agent, Hepatitis E virus (HEV) is present in domestic pigs, wild boar, deer and rabbit, while other member of the Hepeviridae family are present in camel, moose, rat, ferret, bats, chicken and cutthroat trout. In Brazil, few reports have shown the presence of HEV in humans, swine and in the environment samples. However, due to the lack of reported human cases it still disputable whether Hepatitis E is a public health issue or a neglected disease in the country. For instance, the presence of HEV the Northeast regions has not been yet reported in animals. In addition, environmental, social and economic differences might play a role in the Hepatitis E transmission chain, concerning the disease severity and even genotype difference. In this study we investigate the HEV seroprevalence in domestic pigs from the state of Pernambuco, northeast Brazil. A total of 229 swine sera samples were collected from 16 farms using intensive and semiintensive pig production systems. The presence of antiHEV IgG antibodies was investigate using the PrioCHECK HEV antibody ELISA kit (Thermo Fisher), specific for porcine (sensitivity of 90.96% and specificity of 94.04%). In addition, an epidemiological study was performed in order to examine the risk factor

associated with the presence of HEV. 82.10% (188) of the sera tested were positive and 17.90% (41) were negatives. All farms were positive with the prevalence rate ranging from 40 to 100%. The results shown very high positive rates among the pig farms in Pernambuco. The presence of HEV in domestic swine poses a great risk of infection to the human population. On another hand, as most of the animals were in poor sanitary conditions, it cannot be discarded the possibility that pigs are getting infected from human excrements (e.g. contact with sewage), which would suggest that the HEV might be disseminated through both human and swine population in the state. We are currently identifying the risk factors associated with the HEV infection/exposition. Future studies should be carried out in order to investigate the presence of HEV in humans and other animal species as well as to find the genotype and subtype involved.

#### **VV124 - COINFECTIONS WITH INFECTIOUS BROCHITIS VIRUS AND NEWCASTLE DISEASE VIRUS IN SAMPLES FROM CAPTIVE BIRDS**

**Ferreira, H.L.; Simão, R.M.; Rizotto, L.S.; Benassi, J.C.; Scagion, G.P.; Barnabé, A.C.S.; Caserta, L.C.; Arns, C.W.; Ferreira, H.L.**

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2. UNIVERSIDADE DE CAMPINAS

Wild birds can be reservoir of many pathogens, including infectious bronchitis virus (IBV) (family Coronaviridae) and Newcastle disease virus (NDV) (family Paramyxoviridae). Coinfections with one or more viruses including NDV, IBV and/or avian influenza virus were observed but they are rare. The aim of this study was investigate the presence of coinfection by IBV and NDV in wild birds. One hundred and forty-eight samples (cloacal and oropharyngeal OP swabs) pooled in up to 5 samples from captive birds allocated in the same cage were tested. Samples were collected in Pirassununga city, Sao Paulo state according to the authorities' guidelines. Viral RNA purification using the QIAmp viral RNA kit (Qiagen) and realtime RT-PCR (RRTPCR) reactions targeting UTR gene of IBV and M gene of NDV, previously described, were carried out. Samples detected by both tests were amplified in chicken embryo eggs to attempt virus isolation and DNA sequencing was done using harvested allantoid liquid. Conventional RTPCR reactions targeting F and S1 genes

of NDV and IBV, respectively, were performed for DNA sequencing. One positive sample was selected for a deep sequencing using Nextera XT kit (Illumina). 18 samples (14 OP and 4 cloacal swabs) and 56 samples (42 OP and 14 cloacal swabs) of tested samples were detected by RRTPCR specific for NDV and IBV, respectively. Among those, eighteen samples (14 OP and 4 cloacal swabs) from *Aix sponsa* and *Aix galericulata* birds of Anatidae family, were detected for both viruses. A sample from *Aix galericulata* was selected for deep sequencing. Phylogenetic analysis from OP swab of *Aix galericulata* based on 527 nt of F gene showed 93% of identity with genotype II of NDV vaccinal strains. Sequencing of S1 gene and analysis of obtained reads from deep sequencing are ongoing to characterize the detected viruses. Our results indicate a high prevalence of IBV and NDV among tested samples, mainly in OP swabs, in agreement with previous studies, since inhibitory substances present in fecal samples may reduce or block RNA amplification. Coinfections with IBV and NDV in ducks detected are in agreement with a previous study. Future studies should be done to elucidate how those interactions can affect viral fitness.

#### **VV141 - POOLED BATS' SAMPLES SHOWED PRESENCE OF CORONAVIRUS IN THREE DIFFERENT SPECIES**

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2. UNIVERSIDADE DE SOROCABA
3. UNIVERSIDADE FEDERAL DO PAMPA
4. BOSQUE DOS JEQUITIBÁS

The Coronaviridae family has been associated with many zoonotic diseases in humans. To date, many bat species, the only truly flying mammals, have been identified as asymptomatic reservoirs for Coronaviruses. The aim of this study was to identify Coronaviruses circulating in different bat species in the cities of Campinas and Rio Claro in the Sao Paulo State - Brazil, in areas of different degrees of environmental conservation (native forests; older, later and most recently reforested areas and urban native forest). Between 2011 and 2015, 429 samples were collected, from which 256 oral and 173 anal swabs

from the Phyllostomidae (8 species), Molossidae (5 species) and Vespertilionidae (1 specie) bat families. To perform a rapid and most effective screening, the samples were organized into pools according to origin of the swab (oral or anal) and bat specie. Thirtythree pools of 140µL each, composed by 20µL of seven samples, were submitted for RNA extraction through a QIAamp Viral RNA Mini Kit. Another twenty pools were obtained from RNA of other samples and were composed by 1µL of each sample (10 samples per pool). All pools were submitted to reverse transcription with High-Capacity cDNA Archive Kit, according manufacturing intructions, and NestedPCR reactions for RdRp gene of panCoronavirus according with the protocol of Chu et al. (2011). The positive pools were subbmited to new analysis in which were conducted new reactions of individually samples. Twentyeight (28) anal swabs from three (3) bat species (*Anoura caudifer*, *Carollia perspicillata* and *Sturnira lilium*) and ten (10) anal swabs from the Vespertiolionidae family (unclassified species) were positive for Coronaviruses. Seven (7) oral swabs (*Carollia perspicillata*) had a positive result. From the positive samples, ten (10) were collected in the Jequitibás Woods, an urban park in the city of Campinas, and all other were from two newly reforested areas surrounding the city of Rio Claro. The PCR products were purified and submitted for sequencing (results unavailable). These primary results indicate that different Brazilian bat species living in close proximity with both human and domestic animal populations may serve as reservoirs for Coronaviruses and demonstrate the importance of new studies to understand the relationship between bat viruses associated with both human and nonhuman emerging diseases.

#### **VV154 - COMPARISON OF COMMERCIAL TESTS FOR THE DETECTION OF FELINE IMMUNODEFICIENCY VIRUS AND FELINE LEUKEMIA VIRUS INFECTION**

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Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (FeLV) are members of the Retroviridae Family, and they are responsible for the most important

diseases of the cats. The symptoms are mostly related to immunodeficiency and secondary infections, but tumours and hematological abnormalities are also common. All cats should be tested for these viruses, as the most important way to prevent the spread of infection is the detection of cases. Many rapid immunoassay tests are available for the diagnosis of infection in veterinary practice. Two tests are commercially available in Brazil: a lateral flow enzymelinked immunosorbent assay (ELISA) kit and a lateral flow immunochromatography (LFI) kit. Both tests are rapid immunoassays for simultaneous detection of antibodies against FIV proteins and FeLV antigen in serum, plasma, or whole blood. The LFI assay was recently introduced in Brazil. The aim of this study was to compare LFI kit with lateral flow ELISA kit. One hundred and twenty six plasma or serum samples from healthy and diseased cats were tested using both tests. Real time Polymerase Chain Reaction (qPCR) was chosen for verification of samples with discordant results between the kits. The results showed good agreement between the tests. However, three plasma samples with weak positive result for FIV in the LFI kit were negative in the ELISA kit. These samples were also negative in qPCR. Only one sample showed discordant results for FeLV. In this case, the result was positive in the LFI kit using serum sample and negative in the ELISA kit. When the test was performed using the plasma sample, the result was negative in both tests. This sample was negative in qPCR. The LFI assay had a good performance when compared to the ELISA assay. However, plasma samples generated better accurate results and should be the matrix of choice. Finally, although agreement between the two assays was good, confirmation of positive rapid tests results by qPCR is recommended.

#### **VV163 - METAGENOMIC ANALYSIS FOR CORONAVIRUS DETECTION IN BATS ANAL AND TRACHEAL SWAB SAMPLES**

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Coronaviruses (CoVs) constitute a large viral family associated with respiratory infections. Many CoVs described in the last decade were identified from bat species, which demonstrates a strong association. Also, these animals can play an important role in virus dispersion. Contrasting the big diversity of bats in

our region, there is a small number of research being conducted on CoVs biodiversity in bats from Brazil, and no research at all on Amazon bats. In order to detect quickly all CoVs types from a sample, a PCR technique was established using a primer designed by aligning a polymerase region that is conserved in the whole viral family. The amplification of a 251 base pair fragment is expected. The pancoronavirus PCR is a useful tool to screen positive samples and to detect all coronaviruses types. Objectives: To identify coronaviruses in *Carollia perspicillata* individuals captured in the city of Canaã dos Carajás, state of Pará. Materials and methods: We used anal and tracheal swab samples from 32 individuals totaling 64 samples. RNA was extracted from the 64 swab samples but only 12 of those samples, chosen randomly, continued to PCR using the pancoronavirus primer. 10 out of 12 samples followed for sequencing. Results: 100% positivity for PCR tested samples. The PCR amplification characterized the viral presence in the samples, but sequencing results weren't satisfying as they presented low quality values and further analyses were impossible. Conclusion: The present study was the first to detect coronaviruses in the *Carollia perspicillata* individuals from the State of Pará, although it wasn't possible to make the identification of the viral species and to make the phylogenetic analysis since the obtained sequences showed quality below the expected.

#### VV167 - SURVEILLANCE OF ROTAVIRUS IN WILD NEOTROPICAL PRIMATES

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Introduction: Acute diarrhea is one of the most causes of morbidity and mortality among human and animals, including non human primates (NHP). However, few studies have investigated the occurrence of viral agents that could cause acute gastroenteritis in free living and threatened wildlife, as the goldenheadedlion-tamarins (*Leontopithecus chrysomelas*). The goal of this study was investigate the occurrence of rotavirus (RV) in the Southern Bahia Atlantic Forest, Brazil, using

as sentinel the species *L. chrysomelas*. Material and Methods: Fecal samples were collected individually from groups of primates living in four Atlantic Forest fragments. The animals were captured by tomahawk traps, anesthetized for clinical evaluation and most of fecal samples were collected straight from the rectum. The faeces were stored at 20°C and shipped in dry ice to the Rotavirus Lab at Evandro Chagas Institute, Brazil. Following the Public Health Service, screening tests were performed by immunochromatography specific to RVA inside of the Biosecurity Standards Level 3 Lab. RNA viral extraction was proceeded using a mix of fecal suspensions prepared in TrisCa++ 0,01M pH 7,2. A Polyacrylamide Gel Electrophoresis (PAGE) to determine the electropherotypes of RVA and RVC was performed. And a quantitative real time Polymerase Chain Reaction (qPCR) were made looking for the VP6 gene amplification. Results: All the fifty samples were negative for RV. Despite of the negative results, this study was able to perform a molecular methodology to investigate RVA and RVC in wild primates. Conclusion: This research indicates the absence of RV circulation in these groups of *L. chrysomelas*. However, future studies should be conducted to improve the understanding of the RV epidemiology into the wild adding habitats and other environmental variables that could be affect the virus infection in NHP populations.

#### 178 ZIKA VIRUS IN PERIDOMESTIC NEOTROPICAL PRIMATES FROM AN EPIDEMIC REGION IN BRAZIL

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Zika virus (ZIKV) is a viral disease recently associated with microcephaly and other central nervous system - CNS symptomatology. It was identified in Rhesus monkeys in Uganda and later in humans (Zika fever). In 2015 ZIKV was notified in Northeast Brazil related to CNS alterations and rapid spread. Considering that the virus can infect African monkeys, the aim of this study was to research ZIKV in neotropical primates. Here we show a ZIKV detection in neotropical primates captured in Ceara State, Northeast region and an epidemic area

for ZIKV. The animals were captured from July/2015 to March/2016, during the epidemic period and in areas from Ceara State with reports of microcephaly related to ZIKV. Samples of sera and oral swabs from 25 white-tufted marmosets (*Callithrix jacchus*) and 17 capuchin monkeys (*Sapajus libidinosus*) were tested for ZIKV using real time RTPCR assay as previously described. Seven (7/41) samples were positive and the sequencing showed high similarity with the ZIKV isolates circulating in the Northeast region of Brazil. All positive primates were captured in several regions of the State, including coastal (1 capuchin monkey), semiarid – “caatinga” biome (2 marmosets and 1 capuchin monkey) and a mountain range with rainforest remnants (2 marmosets – a female and its cub and 1 capuchin monkey), marmosets were free ranging and the capuchins were pets. All animals were apparently healthy at the moment of their capture, during the procedures and when released. The samples were collected during the viral epidemics in Ceara State, from animals living in proximity to humans and as these mammals were constantly exposed to *A. aegypti*, it is likely that the ZIKV detected in the primates was of human origin. There is no current information regarding the susceptibility of neotropical primates to ZIKV, however, the yellow fever virus (YFV), another vectorborne flavivirus, has an established sylvatic cycle in Africa and in the America. This is the first report on ZIKV detection among neotropical primates, further information is required to determine if the infection was incidental, as a result of the intense virus circulation and ZIKV will not establish a sylvatic cycle; or if there is the possibility that primates could act as reservoirs, with ZIKV “jumping” from humans to neotropical primates, similar to the sylvatic cycle of YFV described in the New World.

#### **VV186 - HEMORRHAGIC DISEASE IN BROCKET DEER (MAZAMA NANA) CAUSED BY DIFFERENT BLUETONGUE VIRUS SEROTYPES, BRAZIL**

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2. ITAIPU BINACIONAL

Brazil has a great diversity of deer species with eight species currently recognized. Deer breeding in Brazil, for conservation or commercial purposes, has been poorly developed due to difficulties in maintenance of these animals in captivity. Despite the reproductive success, the mortality rate is still high, due to different diseases. Adenovirus Hemorrhagic Disease (AHD), Epizootic Hemorrhagic Disease (EHD) and Bluetongue (BT) are the diseases generally associated with hemorrhagic disease in deer. AHD is a contagious disease caused by Adenovirus hemorrhagic disease virus (AHDV), not yet recognized as a new species by ICTV. Bluetongue virus (BTV) and Epizootic hemorrhagic disease virus (EHDV) are arboviruses transmitted by *Culicoides* sp midges and their distribution corresponds to the vector distribution, which is widespread in tropical and subtropical zones. Bela Vista Biological Sanctuary (CASIB) is a protected area in Itaipu Binational, located in the border of Brazil and Paraguay. There, reproduction of *Mazama nana* (brocket deer) happens continuously. However, outbreaks of hemorrhagic disease have led to death of a considerable number of animals along the years, compromising conservation efforts. In 2015, blood samples of the herd were collected for a serologic survey for BTV. From 32 deer, only one (3.12%) was seropositive, revealing high susceptibility of the animals to BTV infection. From March to June of 2015, four *M. nana* died with clinical signs and macroscopic lesions compatible with hemorrhagic disease. In April 2016, another three *M. nana* died with the same clinical signs. Fragments of tissues were collected and submitted for molecular diagnosis and virus isolation. Real time RT PCR results combined with differential diagnosis for AHDV and EHDV and virus isolation revealed that BTV was the etiological agent of the outbreaks. BTV was identified and isolated in all samples. Different BTV serotypes were identified in three samples from 2015 (BTV3, BTV14, BTV18) and

another two samples from 2016 (BTV19, BTV22). The identification of occurring serotypes is essential for disease epidemiological studies. This is the first report of BTV3, BTV14, BTV18, BTV19 and BTV22 detection in Brazil, and the first confirmed case of BTV isolation affecting wild ungulates in South America. Molecular and serological studies integrated with vectors distribution should be conducted in order to implement programs for conservation of endangered deer species.

#### **VV187 - DETECTION OF VACCINIA VIRUS IN BUFFALO HERDS IN MARANHÃO STATE, BRAZIL**

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2. UNIVERSIDADE DE SÃO PAULO

The production and consumption of buffalo milk and meat has experienced great expansion in Brazil in the last years. The Brazilian herd is estimated in 1.15 million buffaloes, with herds present in all Brazilian regions. Among the pathogens that infect buffaloes, Buffalopox virus (BPXV), considered a Vaccinia virus (VACV) variant, has been circulating among buffaloes, cows and humans in Asia, mainly in India, since the 1960's. The disease caused by BPXV, which has economic and public health impacts, is characterized by exanthematic lesions, similarly as it is observed in cases of bovine vaccinia (BV), caused by VACV, in Brazil. So far, no VACV outbreaks in buffaloes have been reported in Brazil. However, antibodies antiOrthopoxvirus (OPXV) have been detected in asymptomatic buffalo herds in Minas Gerais and Pará (Marajó Island) states, in the Southeast and North Brazilian regions, respectively. Furthermore, VACV DNA genome was detected in serum samples from asymptomatic buffaloes from Marajó Island. Therefore, it is important to study VACV circulation in buffalo's herds from other Brazilian regions. Total blood and serum samples were collected from 33 asymptomatic buffaloes belonging to herds from Maranhão state, Northeast region, which has the fourth largest buffalo herd in Brazil. Serum samples were submitted to immunoperoxidase cell monolayer cell assay (IPMA) and plaque reduction neutralization test (PRNT) to detect total and neutralizing antibodies antiOPXV, respectively. Conventional PCR for the VACV A56R gene

amplification was performed in blood samples. Total and neutralizing antibodies were detected in 17 and seven samples, respectively. Nine samples were positive for A56R gene. Seven samples were sequenced, and they showed 100% similarity. These results confirmed that buffaloes in Brazil are susceptible to VACV infection, corroborating with previous studies. Furthermore, more evidence was shown about VACV circulation among buffalo herds in different Brazilian regions. So far, buffaloes did not showed clinical signs compatible with VACV infection in Brazil, which could suggest that they might act as possible VACV reservoirs. It is well known that VACV is spread among cattle herds in the country, and these findings suggests that VACV may be prevalent in buffalo's herds as well. However, more studies are necessary to determine the importance of buffaloes in the epidemiology of VACV infection in Brazil.

#### **VV188 - METAVIROME OF DOMESTIC PIGEON IN THE STATE OF SÃO PAULO, BRAZIL**

Caserta, L.C.; Simas, P.V.M.; Barnabé, A.C.S.; Nascimento, G.M.; Beck, R.M.; Miller, M.E.; Moraes, A.P.; Lima Neto, D.F.; Felipe, P.A.N.; Arns, C.W.

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2. UNIVERSIDADE ESTADUAL DE CAMPINAS
3. UNIVERSIDADE DE SÃO PAULO  
DEPARTAMENTO DE PROTEÇÃO E BEM ESTAR  
ANIMAL PREFEITURA DE CAMPINAS

The domestic pigeon (*Columba livia domestica*) is one of the main sinantropic birds in Brazil. The gathering of this specie represents a public health problem specially due to the carriage of pathogenic agents. The present study was aimed at identifying viral agents in domestic pigeons by the use of metagenomics. Twenty samples were collected in the Tietê Ecological Park, São Paulo SP, Brazil. Samples were grouped in 2 pools: 10 oropharyngeal swabs and 10 cloacal swabs. A pretreatment was carried out with DNase and Proteinase K, followed by RNA extraction. Libraries were prepared and sent for sequencing in the HiSeq 2500 Sequencing System - Illumina platform at the Central Laboratory of High Performance Technologies LaCTAD (Unicamp). A pairedend 2x100pb run in 1 lane yielded 83.944.578 reads, among which 80,67% presented a quality index  $\geq$  Q30. Similarity analyses were performed by Metavir using a viral genome database. Between analyzed contigs, 91% grouped with dsDNA viruses, with no

RNA stage. Caudovirales are represented by 45% of the sequences in this group whereas the families Mimiviridae and Phycodnaviridae accounted for 17% and 14%, respectively. Within the Caudovirales group the Myoviridae family grouped 55% of the sequences, distributed among several phages, being the most representative the Haemophilus phage Aaphi23 and the Bacillus phage 0305phi836. There was a significant detection of representatives of the Caudovirales order with over 4000 scaffolds clustered, the family Myoviridae had 2200 scaffolds associated followed by the family Siphoviridae and Podoviridae (1800 and 500 scaffolds respectively). Within the Myoviridae family it was noteworthy the subfamily Tenevirinae with 500 associations, more than all the other subfamilies combined but less than sequences unclassified but associated with Myoviridae. The Siphoviridae family was subdivided in the genus Lambdaliikevirus (54 scaffolds), Barnyadilikevirus (24 scaffolds) and Andromedalikevirus (22 scaffolds). A similarity was also found with viruses important for human health, such as HHV6 (9 scaffolds) and RSV (2 scaffolds). Regarding this findings it is known that bacteria serve as natural hosts for some of these viruses, which may reflect the presence of such hosts in the samples. This study provides a description of the basal virome of domestic pigeons, prior to the study of symptomatic birds's virome.

#### VV196 - CITY BATS AS CARRIERS OF RETROVIRUS

do Nascimento, G.M.; Simas, P.V.M.; Barnabé, A.C.S.; Caserta, L.C.; Martini, M.C.; Durães Carvalho, R.; Felipe, P.A.N.; Ferreira Neto, D.L.; Beck, R.M.; Jacomassa, F.A.F.; Moraes, A.P.; Barbosa, C.M.; Miller, M.E.; Oliveira, D.B.L.; Durigon, E.L.; Arns, C.W.

1. UNIVERSITY OF CAMPINAS
2. UNIVERSITY OF SOROCABA
3. FEDERAL UNIVERSITY OF PAMPAS
4. OSWALDO CRUZ FOUNDATION
5. JEQUITIBÁS WOODS
6. UNIVERSITY OF SAO PAULO

Bats have been receiving growing attention for serving as reservoirs for many emerging infectious diseases. A high number of zoonotic viruses in humans and animals have been associated to this reservoir. The intensification of anthropogenic pressure on wildlife habitats facilitates the interspecific transmission of pathogens between bats and humans and domestic animals. For this study,

10 anal swabs and 10 oral swabs were collected from 10 bats belonging to a *Tadarida brasiliensis* colony in the Jequitibas Woods, Campinas, São Paulo, Brazil. Samples were sent for sequencing in the HiSeq 2500 Sequencing System – Illumina platform at the Central Laboratory of High Performance Technologies LaCTAD (Unicamp). A paired-end 2x100bp run in 1 lane yielded 345.409.110 reads, among which 76,47% presented a quality index  $\geq$  Q30. For the assembly of contigs and annotation, MetaVelvet and Metavir 2 were used. Nearly 4% of sequences presented similarity with retroviruses belonging to the subfamily Orthoretrovirinae. Viruses that cause immunosuppression in birds and simians were detected within this family, like viruses of the family of reticuloendotheliosis (6181 scaffolds), including Avian spleen necrosis virus (2564 scaffolds), and Simian retrovirus. In addition, retroviruses that cause tumours in murines, like murine osteosarcoma virus and mouse mammary tumor virus were detected. Therefore, it is possible to conclude that the metagenomic approach using next generation sequencing represents a useful methodology in epidemiological studies and a powerful tool in researches involving the zoonotic potential of bats, not only as reservoirs for retroviruses but also for other important diseases in human and veterinary health.

#### VV245 - CORONAVIRUS IN A THRAUPIDAE BIRD LIVING IN THE URBAN AREA OF CAMPINAS

do Nascimento, G.M.; Beck, R.M.; Caserta, L.C.; Barnabé, A.C.S.; Miller, M.E.; Moraes, A.P.; Simas, P.V.M.; Martini, M.C.; Durães Carvalho, R.; Felipe, P.A.N.; Ferreira Neto, D.L.; Jacomassa, F.A.F.; Barbosa, C.M.; Oliveira, D.B.L.; Durigon, E.L.; Arns, C.W.

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2. FEDERAL UNIVERSITY OF PAMPAS
3. OSWALDO CRUZ FOUNDATION
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6. UNIVERSITY OF CAMPINAS

The recent advances in molecular sequencing techniques are leading to the identification and discovery of many novel viruses in avian species. Between January, 2012 and September, 2014, at least 73 viruses belonging to more than 17 families were identified in birds and published in indexed journals. Among RNA viruses, those belonging to Coronaviridae family were the most

numerous and gammacoronavirus the main genus detected in birds. The main representative within this genus is the infectious bronchitis virus (IBV), responsible for large economical losses in the poultry industry. In the last years, CoVs were isolated from many avian orders, including Passeriformes. It leads to the idea that wild birds are essential as reservoirs and carriers of CoVs. In May, 2016, twenty three birds belonging to the *Tersina viridis* specie, Thraupidae family, were found dead in the urban area of Campinas, State of São Paulo, Brazil. The postmortem examination found that the cause of death was traumatism due to a frontal collision against a window, meaning that the sampling was most likely composed by healthy wild birds. In order to investigate the presence of viruses in these birds, tracheal and cloacal swabs were collected. After the RNA extraction and cDNA synthesis, a nested PCR was carried out targeting the RdRp gene of coronaviruses, using genus-wide universal primers. Samples from 8 different birds were considered suspect after the electrophoresis gel analysis. Five samples were chosen between these suspect and after DNA purification, they were sequenced to verify the presence of the virus. One sample retrieved a 158 bp sequence showing similarity with human, canine, bat and ferret coronavirus. Probably due to the short range and quality of the sequence, it was not possible to perform a more precise phylogenetic analysis of this virus. For this reason, other samples will be sequenced as a next step, to provide a better characterization about what CoVs this specie and order of birds is able to carry.

#### **VV251 - SEROPREVALENCE STUDY OF FELINE CORONAVIRUS (FCOV) INFECTION IN DOMICILED DOMESTICS CATS FROM BOTUCATU CITY, SÃO PAULO, BRAZIL**

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The feline Coronavirus (FCoV) is responsible for causing one of the most important infectious diseases that affect domestic and wild cats, the feline infectious peritonitis (FIP), which is an immunemediated, systemic, progressive and fatal disease. The FCoV is highly contagious and the infection is common in populations of domestic cats worldwide. This study aimed to determine the seroprevalence of FCoV infection in domiciled domestic cats from Botucatu city, São Paulo,

Brazil. Whole blood samples were collected (0.5 to 1.0 ml) from 151 cats, centrifuged, and obtained sera were tested by ImmunoComb® FCoV kit (FIP)® (Biogal Galed Labs. Abs. Ltd.) for detection FCoV antiIgG antibodies following manufacturer's recommendations. A seroprevalence of 65% were observed in 98 of 151 tested sera. It is estimated a FCoV seropositivity of 80% to 90% for animals that live in populated environments such as shelters and catteries, and 25% to 40% for pet cats living in the home environment. The present study found that the FCoV infection is widespread in the population of domiciled domestic cats from Botucatu.

#### **VV257 - DETECTION OF HEPATITIS E VIRUS IN SAMPLES OF SWINE FECES FROM THE STATE OF SAO PAULO BY RTPCR**

**Cortez, A.; Meteorima, C.S.; Sousa, A.O.; Miuagi, S.A.T.; Castro, A.M.M.G.; Brandão, P.E.; Pinto, M.A.; Heinemann, M.B.; Megid, J.**

1. CURSO DE MEDICINA VETERINÁRIA UNIVERSIDADE DE SANTO AMARO
2. FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA UNIVERSIDADE DE SÃO PAULO
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Hepatitis E virus (HEV) is a member of the genus Orthohepevirus in the Hepeviridae family. HEV is a nonenveloped, singlestranded, positivesense RNA virus containing about 7.2 kb in its genome that contains 3 open reading frames (ORFs) ORF1, ORF2 and ORF3 encoding respectively a nonstructural protein, the capsid protein, and a small protein involved in virus egress. HEV genotypes 3 and 4 have been isolated from both humans and animals such as deer, wild boars and pigs and are recognized as zoonotic pathogens. In Brazil, HEV has also been detected in pigs from Rio de Janeiro, Paraná, Pará, Mato Grosso and Sao Paulo states. In addition to swine, HEV infections have been detected in other domestic and wild animal species and the first report of a human autochthonous case was documented in 2010 in Rio de Janeiro. With the aim to evaluate the occurrence of pigs with HEV infection in Campinas Region of the São Paulo State, 89 stool samples were collected between 2008 and 2009 and screened by nested RTPCR using primers targeting ORF1. The viral RNA was extracted with Trizol® according to the manufacturer's instructions, from faecal suspensions in

PBS (1020% w/v) and RNA reverse transcription was conducted using MMLV® (Invitrogen). The positive samples were sequenced in the automated sequencer ABI 3500 (Applied Biosystems®). Electropherogram quality analysis and the consensus sequence were performed using Phred and CAP3 software (<http://asparagin.cenargen.embrapa.br/phph/>). Similarity searches were conducted against sequences deposited in GenBank using the BLASTn (<http://www.ncbi.nlm.nih.gov/BLAST/>). Phylogenetic trees were generated by neighborjoining method with the Kimura 2parameter model using the Mega 6.0 software. Bootstrap values for phylogenetic trees were determined using 1000 replicates. Of the 89 samples tested, 7 (7.56%) were positive for the ORF1 fragments. The sequences confirmed that all of the samples identified in the present study were classified as genotype 3. Detection of HEV in stool samples of swine feces by RTPCR shows that there may be a direct or indirect human exposure to the agent and suggests that there may be an endemic circulation of HEV in pig farms.

#### **VV260 - ANALYSIS OF SINGLENUCLEOTIDE POLYMORPHISMS IN THE APOBEC3Z3 GENE IN NATURALLY FELINE IMMUNODEFICIENCY VIRUS INFECTED DOMESTIC CATS**

**Franco, A.C.; Cano, L.; Costa, C.; Duda, N.C.B.; Firpo, R.M.; Nunes, R.; Finoketti, F.; Correa, R.; Roehe, P.; Amorim, F.**

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Feline immunodeficiency virus (fiv) is a widely distributed retrovirus that infects domestic cats (*felis catus*) and other members of the felidae. fiv infections are counteracted by different immune mechanisms, including the restriction factors, which are proteins that have the ability to hamper retroviruses' replication and are part of the conserved mechanisms of anti viral immunity of mammals. the apobec3 or a3 proteins are the most studied class of restriction factors. such proteins are cytidine deaminases that generate hypermutations in provirus dna during reverse transcription, thus causing hypermutations in the viral genome, hampering virus replication. the feline genome contains four a3h genes named a3z2aa3z2c and a3z3. in addition to these, a fifth transcript, designated a3z2z3, is expressed

by readthrough alternative splicing, and its product was also shown to restrict feline retroviruses. in other mammals, a3h singlenucleotide polymorphisms (snps) was shown to alter the stability and cellular localization of the encoded protein, thus influencing its subcellular localization and reducing its anti viral effect. thus, it might be possible that a3h variants would confer different degrees of susceptibility to fiv infections in cats. however, little is known on the genetic variability of a3 genes in domestic cats. the aim of this study was the investigation of the variability of a3h in naturally fiv infected cats. dna obtained from whole blood of 27 fiv positive cats were used as template to amplify a region of a3h that was previously shown to display polymorphisms in the cat population. following the sequencing of the amplified region, the samples were classified in the haplotypes i, ii, iii and iv. twenty one samples showed the a65s snp, of these 18 were heterozygous and 3 homozygous for this polymorphism. this snp was previously associated with susceptibility to the infection. our results indicate that, as previously shown in other mammals, there is variability in a3h genes among the population of domestic cats, which could contribute to susceptibility of domestic cats to retroviral infections; however, these results should be confirmed by more extensive analysis and in vitro experiments.

#### **VV261 - COMPLETE GENOME SEQUENCE OF AN EQUINE INFECTIOUS ANEMIA VIRUS FROM BRAZIL**

**Araujo Jr., J.P.; Malossi, C.D.; Fioratti, E.G.; Lima, M.F.N.T.; Aguiar, D.M.; Ullmann, L.S.**

1. EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA
2. UNIVERSIDADE FEDERAL DO MATO GROSSO
3. UNIVERSIDADE ESTADUAL PAULISTA

Equine infectious anemia virus (EIAV) is a persistent lentivirus that causes equine infectious anemia (EIA). All of the complete genomic sequences published from field virus are from North America, Asian and Europe, and only proviral genomic sequences are available. In Brazil, EIAV is endemic in Pantanal region and euthanasia is not mandatory in these areas. Only the gag sequence is currently available from the Brazilian virus. This study aimed to sequence EIAV's genomic RNA for the first time in naturally infected horses. Plasma of an infected horse from Mato Grosso State was collected.

Total RNA was extracted and used to prepare the dsDNA library with the kit Strand Specific RNA Library Prep (Agilent Technologies). The library was quantified by Illumina Library Quantification kit (Kapa Biosystems) and sequenced with the NextSeq System (Illumina Inc.). Geneious R6 was used to analyze the sequences, using the map to reference tool with complete EIAV genome from isolate DV103 (accession no. HM141910). Then, primers were designed to cover the gaps of the consensus sequence, and these PCR products were sequenced by Sanger protocol in a 3500 platform (Applied Biosystems). A new alignment with all the sequences (12 sequences obtained by Sanger and 9,185,813 reads obtained by Illumina) generated a consensus sequence of 7,591 bp in length, presenting 94% of coverage. This isolate has just 82% of nucleotide sequence identity with the main field strains, like EIAV Liaoning (AF327878), Wyoming (AF033820), and Ireland isolates (JX480631/JX480634). Furthermore, phylogenetic studies using EIAV sequence against known viral strains of EIAV strongly suggests this isolate comprise a separate monophyletic group. With these results it is possible to better characterize the virus circulating in Brazil and to cope with the challenges of the EIAV diagnosis in Brazil.

#### **VV262 - DETECTION OF THE CURRENT CIRCULATING EQUINE INFECTIOUS ANEMIA VIRUS IN BRAZIL BY QUANTITATIVE PCR**

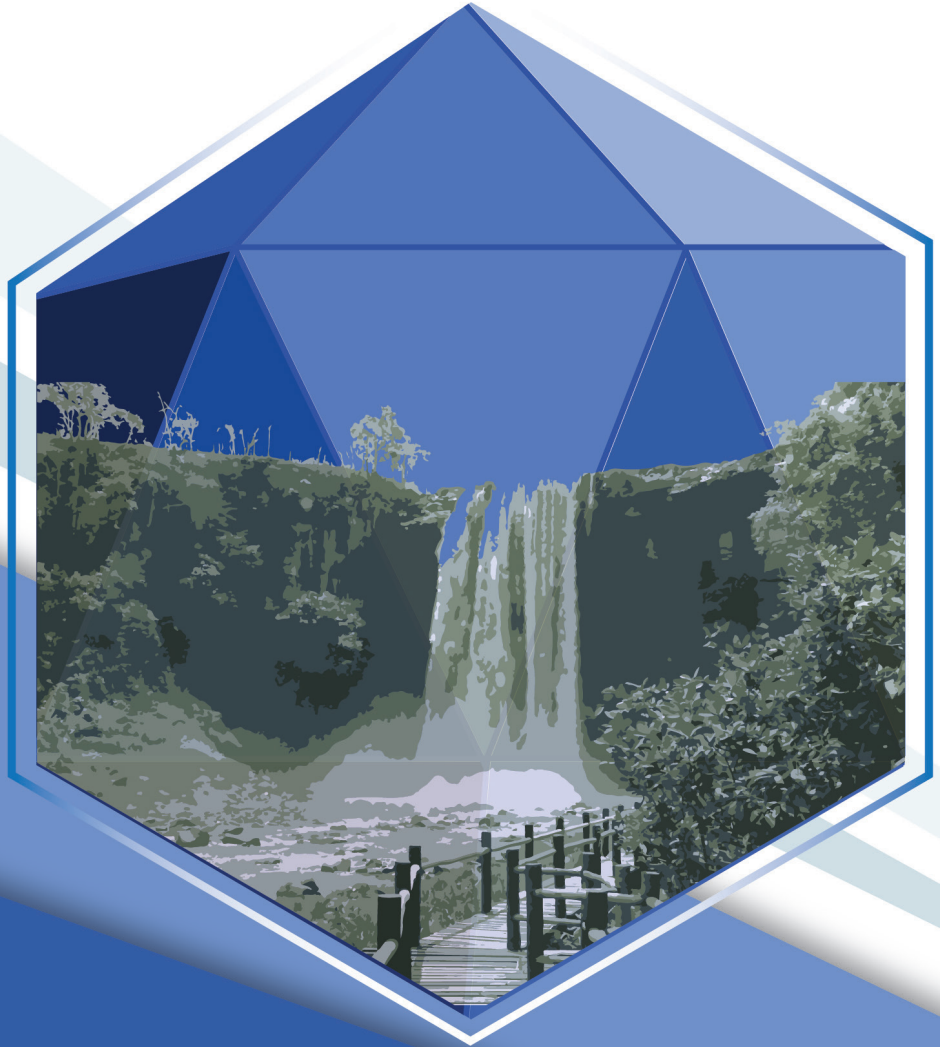
**Araujo Jr., J.P.; Malossi, C.D.; Lima, M.F.N.T.; Aguiar, D.M.; Ullmann, L.S.;**

1. UNIVERSIDADE ESTADUAL PAULISTA
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3. UNIVERSIDADE FEDERAL DO MATO GROSSO

Equine infectious anemia virus (EIAV) is a lentivirus that causes equine infectious anaemia (EIA), a persistent viral infection. The recommended diagnosis method is either agar gel immunodiffusion (AGID) or enzyme linked immunosorbent assay (ELISA). The PCR described by OIE to detect EIAV does not amplify the virus currently circulating in Brazil due to the higher genetic variation. A modification in the NestedPCR protocol to detect an Asian EIAV is used in some Brazilian labs, but some serologically positive samples test negative in the SemiNestedPCR modified. In order to find a more sensitive and more specific molecular technique to detect the viral

Brazilian strain, we designed a quantitative PCR based on EIAV sequences from Mato Grosso Brazil obtained in our lab to detect the current circulating virus. The designed qPCR primers amplify a 71 bp product from the 5' LTR region. GoTaq® qPCR Master Mix (Promega) was used in the reaction plus 5 pmol of each primer, 4 uL of gDNA sample (about 100 ng) and nuclease free water to 20 uL. The thermocycling program set up in a 7500 Fast qPCR (Applied Biosystems) was 1 cycle at 95°C for 10 min, 40 cycles at 95°C for 15 sec and 60 °C for 1 min, followed by the standart melting curve. Eighty eight equine sera samples were tested in AGID or ELISA and their gDNA extracted from whole blood was tested with the SemiNestedPCR and qPCR. Equine GAPDH was used in the samples as a control gene and all amplified. Thirty horses were positive in AGID or ELISA, and although 16 were positive both in the SemiNestedPCR and in the qPCR, not the same samples amplified. This result shows that the reactions have different specificity. A test performed with a serially diluted positive sample indicates the same sensibility in both reactions. A qPCR using cDNA of some positive samples had a good amplification too, and a next step is use this reaction for a viral quantification. With these results we concluded that the two reactions can be used in a set to a better detection of the virus circulating in Brazil and maybe to help to classify animals in viremia.

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