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MULTIPLICATION OF BOVINE HERPESVIRUS TYPE 1 (BOHV-1) AND 5 (BOHV-5) IN DIFFERENT CELL LINES

Autores: Jaqueline Peruzzo [1], Anne Caroline Ramos dos Santos [1], Fernando Finoketti [1], André Ferreira Hennigen [1], Ricardo Rohweder [1], Paulo Michel Roehe [1]

Instituição: [1] UFRGS - Universidade Federal do Rio Grande do Sul (Av. Sarmento Leite 500, Farroupilha - Porto Alegre - RS)

Resumo

Bovine herpesviruses types 1 (BoHV-1) and 5 (BoHV-5) share genomic homology equal to 85% or higher, depending on the viral strain. In the present study, a comparison was performed between the permissivity of two cell lines, "Cell Resistant to Infection with BVDV" (CRIB) and Baby Hamster Kidney (BHK-21), to six BoHV strains of different subtypes: BoHV-1.1 (EVI-123), BoHV-1.1 (SV63/06), BoHV-1.1 (56/06), BoHV-1 (SV1613), BoHV-1.2b (PG1779) e BoHV-5 (N569). All viruses were multiplied on both cell lines and titrated in 96-well microplates. Infectious titers were calculated and expressed in 50% cell culture infectious doses per 50 µL ($CCID_{50}$). In BHK-21, the BoHV-1 strains achieved the following titers: $10^{4,25}$ (EVI-123/BoHV-1.1), 10^{3} (SV63/06/BoHV-1.1), 10^{2} (56/06/BoHV-1.1), 10^{1} (SV1613/BoHV-1) and 10^{2} (PG1779/BoHV-1.2 b), while BoHV-5 strain achieved an infectious titer of 10^{7} (N569/BoHV5). With CRIB the titers obtained after viruses' multiplication were: $10^{5,75}$ (EVI-123/BoHV-1.1), $10^{7,25}$ (SV63/06/BoHV-1.1), $10^{7,5}$ (56/06/BoHV-1.1), $10^{7,5}$ (SV1613/BoHV-1), $10^{7,5}$ (PG1779/BoHV-1.2 b) and $10^{5,5}$ (N569/BoHV5). The results of this study show that BoHV-1 strains significantly produced higher titers (≥1 $\log_{10}$) in CRIB, while BoHV-5 strains produced higher titers in BHK-21 (≥1 $\log_{10}$). We conclude that both cells are permissive to viral replication when in contact with these herpesviruses. There is an apparent relation between virus type and cell line that seems to affect the productive performance of the infections. Subsequent studies will be executed to uncover the reasons for these *in vitro* behavior differences. Financial support: CAPES, CNPq, FINEP.

Palavras-chaves: Herpesvirus, Titration, Cell Culture

CHARACTERIZATION OF THE VIROME IN NASAL CAVITY OF NURSERY PIGLETS

Autores	Anne Caroline Ramos dos Santos [1], Márcia Regina Loiko [3], Deisi Maria Pereira [1], Ana Paula Muterle Varela [1], Raíssa Nunes dos Santos [1], André Ferreira Hennigen [1], Fabiana Quos Mayer [2], Paulo Michel Roehe [1]
Instituição	[1] UFRGS - Universidade Federal do Rio Grande do Sul (Rua Sarmento Leite, 500 - 90050-170 Bairro Farroupilha Porto Alegre/RS Brasil), [2] IPVDF - Instituto de Pesquisas Veterinárias Desidério Finamor (Estrada Do Conde, 6000 - Sans Souci, Eldorado do Sul - RS, 92990-000), [3] Feevale - Universidade Feevale (Av. Edgar Hoffmeister, 500, Zona Industrial Norte, Campo Bom, RS - CEP 93700-000)

Resumo

The microbiota of piglets is of particular interest, as it may affect the susceptibility to infections and interfere with growth performance. To date, the characterization of the respiratory virome of piglets in the initial periods of life has been scarcely investigated. In this study, the nasal virome of 4-5 weeks old nursery pigs with or without signs of respiratory disease was examined by high throughput sequencing (HTS). Nasal swabs were collected from 60 piglets (30 healthy and 30 diseased). The samples were pooled and submitted to viral DNA and RNA extractions and sequenced in the MiSeq (Illumina) platform. The reads obtained were filtered with Trimmomatic 0.39, reassembled with Spades meta 3.10.1 and analyzed with Geneious software. The total number of reads sequenced pool varied between 84.580 to 183.976. These were compared to the database of viral sequences at the protein level using K-mer to lowest common ancestor mapping. On average, 11,77% of the reads showed similarity to viral sequences deposited at GenBank database. Among the viral contigs, 64,4% presented homology with sequences of eukaryotic virus genomes and 35,6% with prokaryotic viral genomes. Higher viral diversity was detected in diseased piglets. Most of the genomes in the diseased group displayed similarity with members of the families *Astroviridae*, *Caliciviridae*, *Circoviridae*, *Coronaviridae*, *Herpesviridae*, *Parvoviridae*, *Picobirnaviridae*, and *Reoviridae*. The most abundantly identified viral genomes in diseased group were porcine cytomegalovirus (PCMV, 63.6%), porcine astrovírus (PAsV, 9.6%) and porcine sapovirus (PSaV, 7.2%). Whereas in healthy group, most genomes identified were members of families *Circoviridae*, *Herpesviridae*, *Parvoviridae*, and *Picobirnaviridae*. The most abundant viral genomes in healthy group were PCMV (95.6%) and circular single-strand DNA virus (ssDNA, 3.2%). The results showed higher viral variability in respiratory disease-affected piglets, where PCMV was the predominant viral genome in both groups.

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Palavras-chaves: metagenomic analyses, piglet, sequencing, vírus

CORONAVIRUS DETECTED IN BATS FROM PARK OF INSTITUTO BUTANTAN, SÃO PAULO, BRAZIL.

Autores AMANDA DE OLIVEIRA VIANA [1], Carla Menequim Barbosa [1], Erick Gustavo Dorlass [1], Elizabete Captivo Lourenço [3], Erika Hingst-Zaher [2], Edison Luiz Durigon [1]

Instituição [1] USP - Universidade de São Paulo (Av. Prof. Lineu Prestes, 1374 - Butanta, São Paulo - SP, 05508-900.), [2] IBu - Instituto Butantan (Av. Vital Brasil, nº 1500. Butantã, São Paulo.), [3] UERJ - Universidade do Estado do Rio de Janeiro (Rua São Francisco Xavier, 524, Maracanã, Rio de Janeiro .)

Resumo

Bats are very important to one health, having a particular interest as a host for some viruses. Usually the RNA viruses are more associated with these mammals. Among the RNA viruses found in bats the Coronavirus, cause of Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). Coronavirus are divided in four genus: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus*. *Alpha* and *Betacoronavirus* emerged in bats. We analyzed the presence of Coronavirus in a population of bats in Park of Instituto Butantan, in urban area of São Paulo City. Oral and rectal swab samples were collected from 47 bats, captured between 2017 and 2018, with mist nets in the Butantan Park, in São Paulo city, Brazil. Swabs were placed in cryotubes containing 500µL of VTM and kept in ultra-freezer (-80°C). DNA extraction was *automated* performed with the "MagMAX™ Express" (Applied Biosystems®) using the "*MagMAX™-96 Total Nucleic Acid Isolation Kit" according to the manufacturer's instructions*. For an enhanced sensibility, detection was made using a protocol to avian Coronavirus developed by Poon et al. (2009) and vaccine was used as positive control. Sequencing was performed with Sanger's technique using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and ABI PRISM 3130XL DNA Sequencer (Applied Biosystems). An oral and rectal swab from six individuals of bats showed to be positive for *Alphacoronavirus*. One male and one female of *Artibeus fimbriatus* and a male of *Glossophaga soricina* were detected with an *Alphacaronavirus* not yet identified and found in Costa Rica. Only this species of bats, among seven species of bats was found with this virus. The virus found this work was found to others researches in Costa Rica and São Paulo. Considering the importance of bats, Coronavirus and the location of this study, is very important to screen this population of bats.

Palavras-chaves: Coronavirus, Alphaviridae, Bats, São Paulo

DETECTION OF TESCHOVIRUS A IN DIFFERENT BREEDING STAGES OF A SWINE HERD

Autores MERIANE DEMOLINER [1,2], Ana Karolina Antunes [1,2], Paula Rodrigues de Almeida [1,2], Fernando Rosado Spilki [1,2]

Instituição [1] Feevale - Câmpus II - Universidade Feevale (ERS 239, 2755 - Vila Nova, Novo Hamburgo - RS, 93525-075), [2] Feevale Techpark - Universidade Feevale (Av. Edgar Hoffmeister, 600 - Zona Industrial Norte, Campo Bom - RS, 93700-000)

Resumo

Teschovirus A (TVA) is a non-enveloped RNA virus in the genus *Teschovirus*, family *Picornaviridae*. Some species of TVA frequently circulate in swine populations without causing clinical signs. However, the clinical manifestations may include polioencephalomyelitis, mainly by TVA-1 that is responsible for causing severe encephalomyelitis outbreaks. *Senecavirus A* (SVA) belongs to the same family but in the *Senecavirus* genus. It is an emerging virus, responsible to cause idiopathic vesicular disease in swine. In the beginning of 2019, a swine herd from Santa Catarina, Brazil started to present ulcerative lesions on limbs and nostrils, affecting animals from nursery over 130 days until the termination stage. In this case, the foot-and-mouth disease possibility had been discarded by the responsible veterinarian. The aim of this research was to investigate the circulation of TVA and SVA in this herd. To this, swine were necropsied and samples of skin lesion, liver, lung, kidney, lymph nodes, tonsil, paw, bladder, central nervous system (CNS), serum, cerebrospinal fluid, urine and, feces were collected. Additionally, skin swab, serum saliva and feces were collected from several breeding stages of the herd. Lastly, ration samples from the affected farms were collected too. The samples were previously macerated and added in 1mL Eagle's Minimum Essential Medium, after 1h incubation at 4ºC, the viral RNA extraction was performed and immediately the cDNA was synthesized. To molecular detection, a PCR was performed for SVA genome and, a nested PCR was performed for TVA genome. Both molecular techniques used primers that targeting the 5´-UTR region. Of a total of 94 samples, 31 were positive to TVA. Among the positive samples, 14 were from faecal sample, 14 from skin swab, 3 from saliva, and ration samples were all negative. High prevalence of TVA circulation was observed in swines from nursery to termination of the herd. The cause of the lesions could not be addressed, since skin is often contaminated with feces, and in these cases the animals were excreting TVA in feces. *Picornaviridae* family members are known to cause CNS, upper respiratory, myocardial and skin infections, such as SVA in swine and enterovirus 71 and coxsackie virus A infections in humans. Further sequencing and isolation of the TVA detected here must be performed in order to understand its phylogenetic relationships and role as a contaminant or a causative agent of these lesions.

CAPES, CNPq, FEEVALE

Palavras-chaves: TVA, Picornaviridae , Swine

EVALUATION OF RABIES LYSSAVIRUS REPLICATION AND CELL GROWTH IN DIFFERENT CONCENTRATIONS OF N2A CELL LINE

Autores Tamires Santos de Arruda [1], William de Oliveira FAHL [1], Iana Suly Santos Katz [1], Graciane Maria Medeiros Caporale [1], Francielle Cristina de Freitas Montibeller [1], Keila Iamamoto Nogi [1]

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

Resumo

The laboratory diagnosis of rabies has the direct fluorescent antibody test (DFAT) as the gold standard test, but the WHO recommends performing the viral isolation test in mice or cell culture (VICC). This project aimed to evaluate the viral replication of the VICC test when initial concentrations of $5x10^5$ and $2.5x10^5$ cells/ml were used and to compare cell growth curves when final concentrations of $1.6x10^4$ cells/mL and $1x10^4$ cells/ml were used. A total of 14 samples with minimal viral titer of 10^3 $TCID_{50}$/ml were selected, and two positive controls. For the evaluation of viral replication, samples were inoculated into 96-well plates and observed at time intervals (24, 48, 72 and 96 hours) by adding cells at a concentration of $5x10^5$ cells/ml and $2.5x10^5$ cells/ml. After each interval, DFAT was performed for each plate. For the cell growth curve analysis, cells were added in quadruplicate in 96-well plates with final concentrations of $1.1x10^4$ cells/ml and $1.6x10^4$ cells/ml at the same time intervals (24, 48, 72 and 96 hours), and the procedure was repeated three times. The cells were suspended with culture medium and Trypan blue, and they were counted in a Neubauer chamber. Viral replication was observed in all samples from 24h until 96h, for both initial cell concentrations. However, it was possible to observe the presence of larger foci at the initial concentration of $2.5x10^5$ cells/ml when compared to the concentration $5x10^5$ cells/ml. In the cell growth curve evaluation, at a final concentration of $1.1x10^4$ cells/ml, the following cell growth rate was observed in relation to the time intervals: 24-48h - 219%, 48-72h - 14% and 72-96h - 17%. Already at the final concentration of $1.6x10^4$ cells/ml: 24-48h - 63%, 28-72h - 4%, 72-96h - 38%. Student's t-test showed a difference in cell growth at 24 and 72 ($p=0.0031$), but no difference was observed at 48 and 96 hours. The results suggest that when there are lower concentrations of cells, there is less nutrient consumption and longer preservation of environmental conditions, increasing the phase of cell proliferation, which may favor the formation of larger foci in viral isolation.

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Palavras-chaves: N2A CELL LINE, VIRAL ISOLATION, RABIES LYSSAVIRUS, DIAGNOSIS, CELL CONCENTRATION

FIRST DETECTION OF ANTI-OROPOUCHE NEUTRALIZING ANTIBODIES IN SERA SAMPLES FROM SLOTHS (BRADYPUS VARIEGATUS) FROM ALAGOAS STATE

Autores THIAGO PINA GOES DE ARAUJO [1], DANILO MACHADO DE MELO [1], FLÁVIO MARTINS DOS SANTOS [1], THAYNA MARIA DA SILVA TORRES [1], ANA CARLA EUGÊNIO LIMA [1], MARIA LUISA BESERRA DE FARIA FERREIRA LEITE [1], ANA RACHEL VASCONCELOS DE LIMA [1], ALESSANDRA ABEL BORGES [1]

Instituição [1] ICBS - UFAL - INSTITUTO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE, UNIVERSIDADE FEDERAL DE ALAGOAS (AV. LOURIVAL DE MELO MOTA S/N, CEP: 57072-900, MACEIÓ – AL, BRAZIL)

Resumo

Oropouche virus (OROV) belongs to the *Orthobunyavirus* genus, *Peribunyaviridae* family (order *Bunyavirales*), and is a causative agent of arboviral febrile illness in Brazil. OROV presents two transmission cycles: 1) urban cycle (*Culicoides paraensis* is the primary vector)*;* and 2) sylvatic cycle, in which there is evidence that sloths (*Bradypus tridactylus*), nonhuman primates and wild birds play a role as vertebrate hosts.The potential of OROV to spread to other geographical areas has been recognized in recent years, increasing the possibility of the disease to emerge to non-endemic areas of Brazil. Laboratory diagnosis of Oropouche fever is achieved by viral isolation, as well as by detection of NT, HI, and IgM antibodies. Our aim was to standardize a 50% plaque reduction neutralization test (PRNT50) to use as screening test of sera samples collected from quarantined wild animals, at Instituto Brasileiro do Meio Ambiente (IBAMA) from Maceió city. For this, Vero E6 cell monolayers at 70% confluency were infected with a mix of OROV (50-100 PFU of strain BeAn19991 P/3) plus 2-fold dilutions of the heat-inactivated test sera, incubated at 37ºC for 1 hour covered with carboxymethylcellulose overlay, followed by incubation at 37ºC for 3 days. In this study, we screened 29 serum samples from *Bradypus variegatus* sloths by PRNT50, which 9 (34.61%) presented neutralizing antibodies with titers ranging from 1: 4 to 1: 8. The samples were further tested for viral genome detection by one-step RT-PCR, but all of them were negative. This was the first study that investigated the presence of antibodies against OROV in sloths from Alagoas. Our data evidence the epidemiological importance of the study, since it demonstrates the existence of the contact of these sentinel animals with this virus. Therefore, our finding generates a warning signal for the health surveillance organs for the possibility of epidemic outbreaks caused by OROV.

Financial support: Ministério da Saúde/CNPq/SESAU-AL/ FAPEAL

Palavras-chaves: Bradypus variegatus, Neutralizing Antibodies, Oropouche virus

SURVEILANCE OF INFLUENZA A VIRUS IN DOMESTIC PIGS IN NORTHEAST BRAZIL

Autores Adalúcia Silva [1], Edmilson Oliveira-Filho [2], Gustavo Lima [1], Antônio Filho [3], Rafael Rosa [1], Christian Reis [1], Abelardo Júnior [4], Laura Gil [1], Alexandre Machado [1], Daniel Perez [6], Lindomar José Pena [1]

Instituição [1] IAM - Fundação Oswaldo Cruz-Fiocruz, Instituto Aggeu Magalhães (Recife), [2] Institute of Virology, Berlin, Germany - Institute of Virology, Berlin, Germany (Germany), [3] UFRPE - Universidade Federal Rural de Pernambuco (Recife), [4] UFV - Universidade Federal de Viçosa (Viçosa), [5] CPqRR - Fundação Oswaldo Cruz-Fiocruz, Centro de Pesquisa René Rachou/I (Belo Horizonte), [6] University of Georgia - University of Georgia (USA)

Resumo

Swine production in Brazil has grown over the last 15 years due to several improvements in the production chain, such as farm technification, genetic improvement and control of the main diseases that hinder production. Influenza A virus (IAV) is a major cause of respiratory disease in pigs, but the epidemiology of swine influenza in Brazil is still poorly understood. The main objective of this work was survey IAV in domestic pigs in the state of Pernambuco, Brazil. To this, we collected nasal swabs and/or lungs samples from 500 pigs in farms and slaughterhouses in different regions of Pernambuco State between 2017 and 2018 and tested them using the OIE qRT-PCR protocol. We found 48 (9.6%) samples positive for IAV. We have also collected blood from 340 animals who and tested them by hemagglutination inhibition test (HI) using A/California/04/2009 (H1N1) and A/Pernambuco/01/2019 (H3N2) viruses. We found 86% positive for H3N2 and 71% positive for H1N1, suggesting that IAV is widely spread in Pernambuco. Thus, we have identified, for the first time, IAV in pigs in this region of the country. These studies provide valuable information for swine producers, health authorities and the scientific community regarding the epidemiology of IAV in pigs in Brazil.

Palavras-chaves: Influenza A, Swine, Epidemiology

MULTIPLEX REAL-TIME PCR VALIDATION FOR DETECTION OF PCV2A AND PCV2B

Autores Victor Hugo da Silva [1], Camila Alves Ferreira [1], Paula Martins Uchoa de Sousa [1], Alessandra Marnie Martins Gomes de Castro [1]

Instituição [1] FMU - Centro Universitário das Faculdades Metropolitanas Unidas (Rua Ministro Nélson Hungria, 541 - Vila Tramontano, São Paulo - SP, 05690-050)

Resumo

Porcine circovirus type 2 (PCV2), discovered initially in 1998, has been associated with several disease manifestations in pigs denominated PCV2 associated disease. Today it is well established that several main PCV2 genotypes circulate in pigs also known as PCV2a, PCV2b and PCV2d worldwide. Real-time or quantitative PCR (qPCR) assays have been used for specific identification of the target sequence by fluorescent probes and so improves the specificity of assays. Another feature of qPCR is the ability to perform multiplex assays by using differences of fluorescent probes in the same reactions. In this report, a multiplex real-time PCR (multiplex qPCR) was validated for simultaneous detection of PCV2a and PCV2b. Briefly, fragments containing specific primer and probe annealing sequences for PCV2a and PCV2b were synthesized, inserted into a plasmid vector and cloned into an E. coli K12 DH10B™ T1R. The plasmid DNA from the transformed bacteria was purified, the concentrations determinate by spectrophotometer and sequenced to verify sequence identity. Serial dilutions were performed and used as stand curve. The reactions were performed in a final volume of 25 µL containing 5.0 µL DNA; 13 µL of TaqMan ™ Universal Master Mix II (Thermo Fischer Scientific, USA), 0.5 µL of each probe at 10 µM (PCV2a VIC-GGG GAC CAA CAA AAT CTC TAT ACC CTT T-MGBNF and PCV2b FAM- CTC AAA CCC CCG CTC TGT GCC C-QSY); 1 µL of 10 each primer at 10 µM (PCV2abF: 5 'GGCGGTGGACATGATGAGA 3' and PCV2abR: 5 'GCAGGGCCAGAATTCAACC 3') and sterile MilliQ water qsp. The amplifications conditions were 50 ºC for 2 minutes, 95 ºC for 10 minutes followed by 40 cycles of 95 ºC for 15 seconds and 60 ºC for 1 minute and run on a QuantiStudio3 (Applied Biosystems, USA). The detection limit of the multiplex qPCR was 25.4 and 25.2 copies of DNA/µL for PCV2a and PCV2b, respectively. The standard curves of multiplex qPCR showed that all parameters analyzed were within the acceptable range, i.e. the efficiency (ε) from 98.9 to 101.9; correlation coefficients (R2) from 0.998 to 0.989 and slope from -3.32 to 3.24. Similar detection limit and standard curves parameters results were obtained from the singular qPCR assays with both viruses separately. The specificity of the multiplex qPCR was performed using 10 clinical samples that were previously sequenced and the assay was able to classify correctly the PCV2a and PCV2b samples.

Financial support: FAPESP 2015/07994-7 and 2017/21592-4.

Palavras-chaves: Diagnosis, Molecular biology, Swine, Virus

EVALUATION OF THE EFFECTS OF AN HVT-INFECTIOUS BURSAL DISEASE VECTOR AND AN IMMUNOCOMPLEXED VACCINE ON THE IMMUNE SYSTEM AND PRODUCTION PARAMETERS OF COMMERCIAL BROILERS

Autores: Tobias Fernandes Filho [1], Melquiades Dvojatzki Junior [1], Lourenço Sausen [1], Leandro Selau [1], Filipe Santos Fernando [1]

Instituição: [1] BI - Boehringer Ingelheim (Rochaverá Corporate Towers. Av. Nações Unidas, 14.171 - Torre Marble – 18º andar - Santo Amaro, São Paulo - SP, 04794-000, Brazil)

Resumo

The infectious bursal disease (IBD) is a widespread infection of broilers caused by an avian *Birnavirus* with higher mortality, condemnations and feed conversion rates leading to production losses in poultry industry. Most of the impact is due to the viral replication in B lymphocytes progenitors in the bursa, resulting in severe immunosuppression not just in humoral but also in cellular immune response. An effective manner to prevent from IBD is through the use of IBDV vaccines at hatchery with replicating attenuated intermediate plus stains of IBDV viruses (Winterfield 2512) complexed with antibodies or with vectored HVT (Fc-126) vaccines expressing IBDV VP2 protein. Although both vaccine technologies are effective in Gumboro disease control the use of a replicating intermediate plus strain of the IBDV virus would mimic the same impact of wild IBDV on bursa infection and consequent impairment of humoral and cellular immune response, in more attenuated way but still demanding energy from the birds to deal with viral replication and its apoptosis and inflammation effects. To understand if immunocomplexed Winterfiled 2512 IBDV vaccines could lead broiler to an immunosuppression condition in the field, broilers vaccinated *in ovo* with W2512 IBDV-antibody complex or HVT-IBD vector vaccines were grown in a large Brazilian broiler company in the state of Paraná. To access the impact of both IBD vaccines in bursa damage and cellular immune response birds in the field had bursa collected to measure histological lymphoid depletion and quantification of total leukocytes, T CD8 and B lymphocytes and heterophil phagocytosis activity through flow cytometry at 21 and 28 days of age. The results brought to evidence the damage in the bursa due to a virus complexed with higher scores for lymphoid depletion and a leucopenia especially for B and T CD8 lymphocytes that increased with age. Heterophil phagocytosis was also affected within the group that have received W2512 immunocomplexed vaccine, with higher phagocytosis indexes. Nevertheless, the feed conversion rate was lower for the group that had been vaccinated with vectored HVT-IBD. In conclusion, adopting a vaccination with a live intermediate plus strain of IBDV in the hatchery for broilers causes damage to the bursa and also to both innate and adaptive immune response which result in a certain degree of immunosuppression, resulting in higher cost of production and higher feed conversion rate.

Palavras-chaves: Infectious bursal disease, Immunossupression, Gumboro disease, Broilers, B lymphocytes

EPIDEMIOLOGIC SURVEY OF EQUINE INFECTIOUS ANEMIA VIRUS IN EQUIDAE FROM NORTHEASTERN BRAZIL

Autores Camila de Sousa Bezerra [1], Denise Batista Nogueira [1], Davidianne de Andrade Morais [1], Clebert José Alves [1], Maria Luana Cristiny Rodrigues Silva [1], Sérgio Santos de Azevedo [1]

Instituição [1] UFCG/CSTR - Universidade Federal de Campina Grande Centro de Saúde e Tecnologia Rural (Avenida Universitária, SN, Santa Cecilia, Patos,PB CEP: 58700-970 Brazil)

Resumo

Equine infectious anemia (EIA) is caused by EIAV (equine infectious anemia virus), a member of the Retroviridae family, genus Lentivirus. This disease causes economic impact, because in Brazil positive animals must be sacrificed, as established in the PNSE of the Ministry of Agriculture, Livestock and Supply. In Brazil, the frequency of infection has already been observed in the states of Rondônia (9.6%), Acre (7.5%), Pará (17%), Minas Gerais (3.1%) and Mato Grosso do Sul (24, 8%). The objective of this work was to determine the epidemiological profile of EIAV in equidae from the states of Paraíba, Pernambuco, Rio Grande do Norte and Ceará, during the rainy (May and June) and dry (October and November) periods of 2017 and 2018. For the serological diagnosis of EIA the agar gel immunodiffusion test (IDGA) was used. During the rainy season of 2017, 22 of the 1,842 (1.2%) animals tested were positive for EIAV (12 from Ceará, eight from Paraíba and two from Rio Grande do Norte states). In the dry season, of the 1,564 animals sampled, 29 (1.9%) were positive in the serology, of which 20 belonged to Ceará, seven to Paraíba and two to Rio Grande do Norte. There was no positive result in the state of Pernambuco in the months corresponding to the study in 2017. In the analysis of data from 2018, it was observed that during the rainy season, 26 of the 1,636 horses were seroreactive (1.6%), with 19 cases resulting from Ceará, four from Paraíba and three from Pernambuco, with no positive animals occurring in the state of Rio Grande do Norte during this period. While in the dry season, 32 out of 1,526 animals were seroreactive to the EIAV, of which 26 were from Ceará, three from Paraíba, one from Rio Grande do Norte and two from Pernambuco. Among the four states represented in the present study, Ceará presented the largest number of positive animals, this being the holder of large herd of equidae, as well as the occurrence of horse events, with the largest contact of infected and susceptible animals. The increase in EIA cases in the dry period compared to the rainy season may be associated with increased vector proliferation, which usually occurs between the end of the rainy season and the beginning of the dry period. The results observed in this study demonstrate the circulation of EIAV in four states of Northeastern Brazil, suggesting a correlation between the dry period and the increased frequency of infection.

Palavras-chaves: Equine infectious anemia , Infection, Horse, Agar gel immunodiffusion test , Caatinga

BAT INFLUENZA A VIRUS H18N11 IN BRAZILIAN BATS ARTIBEUS LITURATUS

Autores: Angélica Cristine de Almeida Campos [1,2], Luiz Gustavo Bentim Góes [1,2], Andrés Moreira-Soto [2], Cristiano Carvalho [3], Guilherme Ambar [4], Anna-Lena Sander [2], Carlo Fischer [2], Adriana Ruckert Rosa [5], Débora Cardoso Oliveira [5], Ana Paula Geraldes Kataoka [5], Wagner André Pedro [3], Luzia Fátima Alves Martorelli [1], Luzia Helena Queiroz [3], Ariovaldo Pereira Cruz-Neto [4], Edison Luiz Durigon [1], Jan Felix Drexler [2]

Instituição [1] USP - Universidade de São Paulo (Av. Prof. Lineu Prestes, 1374 - sala 225 - Depto Microbiologia - ICB II), [2] CHARITÉ - Charité - Universitätsmedizin Berlin (Helmut-Ruska-Haus Charitéplatz 1 10117 Berlin, Germany), [3] UNESP - Universidade Estadual Paulista "Júlio de Mesquita Filho" - Faculdade de Medicina Veterinária - Câmpus de Araçatuba (Rua Clóvis Pestana, 793 - Dona Amélia - Araçatuba, SP/SP - CEP 16050-680), [4] UNESP - Universidade Estadual Paulista "Júlio de Mesquita Filho" - Instituto de Biociências - Câmpus de Rio Claro (Avenida 24 A,1515 - Bela Vista - Rio Claro/SP - CEP 13506-900), [5] CCZ-São Paulo - Centro de Controle de Zoonooses, São Paulo, SP, Brazil (R. Santa Eulália, 86 - Santana, São Paulo - SP, 02031-020)

Resumo

Influenza A viruses (IAV) are major causes of human disease. Until recently, IAV were deemed to be maintained in avian reservoirs. In first studies from 2012 and 2013, the first bat IAV termed H17N10 and H18N11 were discovered in *Sturnira lilium* (little yellow-shouldered bat) and *Artibeus planirostris* (flat-faced fruit-eating bat). So far, only four individual bat specimens yielded IAV genomic sequences during the pivotal investigations. To investigate bat IAV epidemiology, we sampled 533 individual bats representing 27 species and 3 families across 28 sampling sites located in southern/south-western Brazil during 2010-2014. Intestine specimens from all bats were tested using two highly sensitive, broadly reactive nested RT-PCR assays targeting different regions of the IAV PB1 gene. In two samples of *Artibeus lituratus* bat species was found H18N11 Influenza A virus. High concentrations, tested by real-time-PCR, suggested fecal shedding. Genomic characterizations revealed conservation of viral genes across different host species, countries and sampling years, suggesting a conserved cellular receptor and wide-ranging occurrence of bat influenza A viruses.

Palavras-chaves: Influenza A virus, Bats, H18N11, Atlantic Rain Forest

NEWCASTLE DISEASE VIRUS REPLICATION IN DIFFERENT CELL SYSTEMS

Autores Maria Angela Orsi [1], Ana Paula de Morais [1], Matheus C. Martini [1], Laís S. Rizotto [2], Hikary Moriyama [1], Ana Cristina Alves de Almeida [1], Tânia Rosária Pereira Freitas [3], Clarice Weis Arns [1]

Instituição [1] UNICAMP - Department of Genetics, Evolution, Microbiology and Immunology, Institute of Biology, University of Campinas ((Rua Monteiro Lobato, 255, Campinas, São Paulo), [2] USP - Faculdade de Medicina Veterinária and Animal Science, São Paulo University (Piracicaba, São Paulo), [3] LFDA/MG, - Federal Agricultural Defense Laboratory in Minas Gerais (Pedro Leopoldo, Minas Gerais, Brazil.)

Resumo

Repeated studies have been shown that isolates of vaccine-derived Newcastle disease virus (NDV) from different species of wild birds and the number of recovered vaccine-derived virus coincident with the most widely vaccine used in the world (B1 and Lasota). Usually, the NDV has been isolated in chicken embryos (CE) as standard method before cell line adaptation. This present study reports the trials to B1 strain infection establishment in five different cell lines: Chicken embryos related (CER); Baby hamster kidney (BHK-21); Mouse macrophages (J774 A.1); *Aedes Albopictus* larvae (C6/36) and *Eidolon Helvum* kidney (EidNi/41). For each cell line flasks (25cm^2) were seeded with 3X10^{4}cells/mL in culture medium for each cell line and supplemented 10% fetal bovine serum. At 85% cell confluence, were exposed 900 µL of B1 strain ($10^{-8.5}$DIE$_{50}$/mL) inoculated directly on each monolayer's different cells. Four mammalian cells: CER, BHK-21, EidNi/41 and J774 were incubated by 45 min/37^{0}C and for insect cell C6/36 the incubation was for 75 min/28^{0}C. The cell medium was replaced and the flasks incubated at 37^{o}C and for C3/36 at 28^{o}C until the cytopathic effect (CPE) was observed over 70% or 5 days post infection (PI). The cells passages were frozen, thawed, centrifuged and virus supernatant aliquots keep at -80^{0}C. It was repeated for three passages. The virus CPE has been observed at least 24 hours (h) until 120h PI. For J774 cell lines until 72 h PI, BHK-21 and CER until 96 h PI, C6/36 and EidNi/41 until 120 h PI. The comparative kinetics of the NDV proliferation among the cells assays and compare with the standard CE inoculation was performed by quantitate qPCR. Briefly, the RNA was extracted using the QIAamp viral RNA mini kit, Taq Man RNA to CT1step kit (Applied) was used to perform qRT-PCR assays. The fragments of matrix gene (121pb) were amplified and detected using the primer; F (M+4100), R (M-4220) and P (M+4169). The infection at the all cell subcultures have varied. The CT in the first cell passage ranged from 14.00 to 18.38, in the 2nd 15.80 to 25.50, in the 3rd 20.40 to 30.73. From the 2nd to the first passage, the difference ranged from -0.2 to 8.8 Ct. in the 3rd to 2nd passage 0.05-7.82 Ct. The best performance was from the CER, but until 2$_{nd}$ passage was CER, J774 and EidN1/41 cells lines. However, preliminary results showed susceptibility to all five cell lines evaluated against NDV.

Acknowledgment and financial support: CNPq, FAPESP, FUNCAMP AND IB-UNICAMP

Palavras-chaves: Newcastle disease virus, Replication , different cells systems

IN VITRO CHARACTERIZATION OF DIFFERENT GENETIC LINES OF RABIES VIRUS ISOLATED FROM NON-HEMATOPHAGOUS BATS

Autores: Jaíne Gonçalves Garcia [1,2], Camila Mosca Barboza [1,2], Marcélia Emanuele Sad Fernandes [1,2], Raphaela Zamudio [2], Ana Lee Francisco [1,2], Adriana Candido Rodrigues [2], Karin Correa Scheffer Ferreira [2], Helena Beatriz de Carvalho Ruthner Batista [2,1]

Instituição [1] UFABC - Universidade Federal do ABC (Av. dos Estados, 5001 – Bairro Santa Terezinha, Santo André – SP), [2] IP - Instituto Pasteur de São Paulo (Avenida Paulista 393 - Cerqueira César SP)

Resumo

Rabies is one of the oldest known human diseases. Caused by the rabies virus (RABV), this zoonosis has worldwide distribution and still causes the death of approximately 60,000 people per year. Despite advances in research, there are still gaps in knowledge about the maintenance of RABV in nature, especially aspects related to its biology and its mechanisms of adaptation to hosts. In recent years, reports of isolation of RABV in different species of bats have raised Public Health concern. The possibility of these animals developing synanthropic habits, considering that they are reservoirs for RABV, may cause a higher risk of human infections. This study aimed to elucidate adaptive aspects of the maintenance of RABV in different species of non-hematophagous bats. Therefore, 9 samples of RABV isolated from different species of bats were selected. These samples were subjected to genetic characterization of RABV and genetic identification of the species. After selection and characterization of the samples, they were submitted to passage in *Swiss albino* mice and later adapted to *in vitro* growth in Human Embryonic Kidney (HEK 293T) cells. Each serial passage in cells is subjected to Direct Immunofluorescence Test. The following genetic lineages of RABV were identified: genetic lineage from frugivorous bat *Artibeus lituratus* (3 samples), genetic lineages from insectivorous bats from the specie *Eptesicus furinalis* (3 samples) and from the specie *Tadarida brasiliensis* (3 samples). With the genetic identification of species, it was possible to identify that each genetic lineage of RABV was maintained by the respective bat specie, and no spillover cases were verified. So far, RABV samples have been subjected to 6 passages in HEK293T cells, and one of the isolated samples of *A. lituratus* (1233) and one of the isolated samples of *E. furinalis* (964) are already adapted *in vitro*. These are preliminary results that show that the RABV is more adapted to such species, considering the samples used in this study. At the end of the *in vitro* adaptation of the samples, they will be submitted to viral replication kinetics assays. With this work, it will be possible to propose diagnostic and surveillance tools to control rabies specific in non-hematophagous bats.

Palavras-chaves: In vitro characterization, Non-hematophagous bat, Rabies

VIROME OF THE NASAL CAVITY OF SWINE PRIOR TO SLAUGHTER

Autores André Ferreira Hennigen [1], Anne Caroline Ramos dos Santos [1], Márcia Regina Loiko [1], Deisi Maria Pereira [1], Ana Paula Muterle Varela [1], Fabiana Quoos Mayer [2], Paulo Michel Roehe [1]

Instituição [1] UFRGS - Universidade Federal do Rio Grande do Sul (Av. Sarmento Leite, 500 - Farroupilha - Porto Alegre - RS), [2] IPVDF - Instituto de Pesquisas Veterinárias Desidério Finamor (Estrada Do Conde, 6000 - Sans Souci, Eldorado do Sul - RS)

Resumo

High-Throughput Sequencing (HTS) allows the assessment of genetic diversity of microorganisms present in biological samples. Knowledge on the microbiome of swine is important as it may affect the susceptibility to pathogens and interfere in productivity. In this study, we analyzed swine nasal virome using HTS. At 173rd day of age, 15 days before slaughtering, sixty pigs had their nasal secretions collected with sterile swabs at a swine farm with respiratory disease history. The viral DNA was extracted by a standard phenol method and the RNA was extracted with TRIzol-chloroform. The DNA and cDNA libraries were prepared with the Nextera XT kit and HTS performed on an Illumina Miseq sequencer. The reads obtained from sequencing were filtered with Trimmomatic 0.39, assembled with metaSPAdes 3.10.1 and analyzed with Geneious 9.1.8 software. The total number of reads after the trimming process varied between 131,180 to 141,089. These were compared to the viral sequences database at the protein level using K-mer to lowest common ancestor mapping. On average, 18.58% of the reads showed identity to viral sequences deposited at the GenBank database. Among the viral contigs, 93.25% presented similarities with sequences of eukaryotic virus genomes and 6.75% with prokaryotic viral genomes. The virome results show a low diversity of viruses. Sequences with significant identity to porcine picobirnaviruses (PPBV) and circular viruses were detected. Among these, picobirnavirus related sequences were the most abundant. PBV (picobirnaviruses) are commonly found in the gastroenteric tract of different species. However, the lack of data regarding the presence of PBV in the respiratory tract of pigs hinders the identification of a possible pathogenic role in this species. The circular virus related sequences found here display identity to porcine stool-associated circular virus and porcine associated porprismacovirus, both groups of viruses to date not yet associated to diseases. The low diversity of viral families found on the animals' nasal cavity may have been influenced by a number of environmental factors that will be further investigated to understand the relations of this host with its viruses and other microorganisms. Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Pesquisa (CNPq), Financiadora de Estudos e Projetos (FINEP).

Palavras-chaves: Virome, Swine, High-Throughput Sequencing

DETECTION OF NEUROPATHOGENIC GENOTYPIC VARIANT OF EQUINE HERPESVIRUS TYPE 1 (EHV-1) ASSOCIATED WITH ABORTION AND REPRODUCTIVE PROBLEMS IN BRAZIL: DIAGNOSIS AND CASE DESCRIPTION

Autores: Luisa Feliciano de Souza Franklin [1], Marcelo Ferreira Catizane [1], Aila Solimar Gonçalves Silva [1], Ana Carolina Diniz Matos [1], Anna Gabriella Guimarães [1], Grazielle Cossenzo Florentino Gallinari [1], Izabela de Assis Rocha [1], Maria Isabel Maldonado Coelho Guedes [1], Raffaella Bertoni Cavalcanti Teixeira [1], Zélia Inês Portela Lobato [1], Erica Azevedo Costa [1]

Instituição [1] UFMG - Universidade Federal de Minas Gerais (Av. Pres. Antônio Carlos, 6627 - São Luiz, Belo Horizonte - MG, 31270-901)

Resumo

Equine herpesvirus type 1 (EHV-1) is a highly prevalent virus, affecting up to 80% of the world's equine population, causing extensive economic losses in the equine industry. The virus causes respiratory and neurological diseases, neonatal mortality, and outbreak of abortion, usually in the final third of pregnancy. Over the past decade, the incidence of abortion caused by EHV-1 has decreased, possibly due to vaccination practices. In contrast, cases of neurological disease increased significantly over the past 15 years. Different strains vary in their abortogenic potential, as well as in neuropathogenicity. Currently, one of the major markers for neuropathogenic potential of EHV-1 strains is related to a single nucleotide polymorphism of viral DNA polymerase gene (ORF 30). Infections with this marker are associated with a high and prolonged viremia, which may indicate a selective advantage of the neuropathogenic strain, which is refractory to vaccination and is related to abortion cases in some countries. In this study, 35 animals with reproductive problems were analyzed, 30 cases of abortion, 3 cases of neonatal mortality and 2 cases of stillbirths. The animals belonged to 15 properties of four different states of Brazil (Minas Gerais, Rio de Janeiro, Sao Paulo and Mato Grosso do Sul). A PCR reaction assay was performed to detect partial gene that encodes viral ORF 30, which was posteriorly confirmed by sequencing. The DNA of EHV-1 was detected in 7 of 30 abortion cases and the neuropathogenic marker was found in 6 of 7 EHV-1 positive cases by sequencing positive samples. Neuropathogenic EHV-1 was detected in mares vaccinated against EHV-1 and EHV-4 and the vaccination protocol was performed as recommended by the manufacturer. DNA of EHV-4 was detected in only one of the abortion cases analyzed. The results demonstrates the relevance of neuropathogenic strain as the cause of abortion in pregnant mares, even with vaccine control in the brazilian properties. Financial support: CNPq, FAPEMIG, CAPES

Palavras-chaves: herpesviridae, reproductive failure, stillbirth, neonatology

DETECTION OF EQUINE GAMMAHERPESVIRUS 2 IN HORSES

Autores: Sofia Cicolo [2], Angélica Cristine Gois de Almeida Campos [1], Cairo Monteiro de Oliveira [1], Daniele Bruna Leal de Oliveira [1], Edison Luiz Durigon [1], Vanessa Barbosa da Silveira [1], Luiz Gustavo Bentim Góes [1], THAÍS POLTRONIERI DOS SANTOS [2], Carla Bargi Belli [2], Raquel Yvone Arantes Baccarin [2]

Instituição: [1] ICBII-USP - Instituto de Ciências Biomédicas II da Universidade de São Paulo (Av. Prof. Lineu Prestes, 1374 - Butantã, São Paulo - SP, 05508-900), [2] FMVZ-USP - Faculdade de Medicina Veterinaria e Zootecnica da Universidade de São Paulo (Av. Prof. Orlando Marques de Paiva, 87 - Butantã, São Paulo - SP, 05508-010)

Resumo

Introduction: Equine herpesvirus (EHV) comprises a group of viruses that are divided into three subfamilies: Alpha-, Beta- and Gammaherpesvirinae, responsible for affecting different species of horses. Among the EHV species, EHV-2 is an important gammaherpesvirus known to cause infection in equine populations worldwide. This virus is often transmitted horizontally from its mother to newborn foals via the nasopharyngeal pathway or through contact with other foals, which may or may not show clinical signs of disease. EHV-2 is most commonly associated with upper respiratory tract infections of the animal, causing pharyngitis that can progress to more severe forms of disease, occasionally causing death of the animal. Few studies report the circulation of this virus in horses in the Brazil. Objective: The aim of this study is to evaluate the presence of herpesvirus in different equine samples. Methods: Blood, urine, cerebrospinal fluid, brain and swab samples were collected from seventeen symptomatic and asymptomatic horses from the University Hospital of the Faculty of Veterinary Medicine and Zootechnics of the University of São Paulo (HOVET-USP). The analysis of the samples was performed from the automated extraction of total nucleic acids, followed by amplification by PCR and nested assays targeting the Herpesviridae viral polymerase gene. Results: Herpesvirus was detected in two samples (serum and nasal swab) from two horses (11.7% - 2/17). The analyzed fragments were segregated with samples from the Equine Gammaherpesvirus 2 (EHV-2) group. Conclusion: This study shows that despite the few reports and information about this virus in Brazil, it has circulated, causing infections and subsequent death of horses in the country.

Palavras-chaves: EQUINE, HERPESVIRUS, GAMMAHERPESVIRUS 2, DETECTION

AMINO ACID VARIATIONS IN PARTIAL HA SEQUENCES OF H1 SWINE PANDEMIC FLU, FROM 2009 TO 2015

Autores: Ana Luiza Soares Fraiha [1], Ana Carolina Diniz Matos [1], Beatriz Senra Álvares da Silva Santos [2], Maria Isabel Maldonado Guedes [1], Erica Azevedo Costa [1], Alexandre de Magalhães Vieira Machado [2], Zélia Inês Portela Lobato [1]

Instituição [1] UFMG - Universidade Federal de Minas Gerais (Av. Pres. Antônio Carlos, 6627 - São Luiz, Belo Horizonte - MG, 31270-901), [2] Fiocruz Renê Rachou - Fundação Oswaldo Cruz Renê Rachou (Av. Augusto de Lima, 1715 - Barro Preto, Belo Horizonte - MG, 30190-002)

Resumo

Since the 2009 pandemic, Influenza A virus (IAV) has threatened Brazilian swine herds. Although there are different subtypes circulating in the country, 2009 pandemic H1N1 virus (H1N1pdm09) has higher prevalence reports. In 2014, a vaccine against H1N1pdm09 was licensed and is the only one available for swine influenza prevention in Brazil. IAV has a segmented negative-sense genomic RNA contributing for rearrangements and RNA-polymerase lack of proofreading during replication may results in mutations, specially at the glycoproteins haemagglutinin (HA) and neuraminidase (NA) which are the main targets of host immune response. Five antigenic sites were identified at HA: Sa, Sb, Ca1, Ca2, Cb and substitutions of amino acids at these sites, during infection, are associated with antigenic changes. Monitoring the changes is important to understand IAV evolution and selection of vaccine strains. The objective of the study was to compare partial sequences of H1N1pdm09 virus detected in swine during the years 2012-2015 to other Brazilian sequences already deposited at GenBank. Forty-five samples of RNA extracted from clinical samples of swine, positive for H1N1pdm09 by a Nested RT-PCR, were used in the study. Eighteen samples were from 2012/2013 and twenty-seven from 2014/2015. Samples were subjected to a RT-PCR to amplify a 616bp conserved region of HA. The amplicons were sequenced and nucleotide sequence data analyzed. Consensus sequences were aligned with Brazilian H1N1pdm09 sequences detected in swine from 2009-2010 and HA sequences of H1N1pdm09 used in human and Brazilian swine vaccines, previously deposited at GenBank. Amino acid translation was performed and variations among the sequences were analyzed. Twenty-three amplicons were sequenced, 6 from 2012/2013 and 17 from 2014/2015. Amino acid analysis allowed the identification of Sa, Ca2 and Sb antigenic sites. Four partial sequences from 2012/2013 presented amino acid variations at Sa and six from 2014/2015 at Ca2. Punctual variations in other residues could also be observed in samples from 2012-2015 compared to those from 2009/2010 and vaccine strains. The data suggests that H1N1pdm09 virus may have evolved over the years and changes in immunologically important epitopes may have occurred. Surveillance of swine IAV is important to monitoring variation which could help establish updates for vaccine production and also to compare genetic characterization data with human IAV. **Financial support: CNPq, FAPEMIG.**

Palavras-chaves: Brazil, hemagglutinin, sequencing, swine Influenza Virus

FELINE IMMUNODEFICIENCY VIRUS (FIV): OCCURRENCE IN NORTHERN REGION OF CEARÁ, BRAZIL

Autores: Bruno Marques Teixeira [1], Sueli Akemi Taniwaki [2], Ana Kétylla Ponte Prado Rodrigues [1], Meylling Mayara Linhares Magalhães [1], Thiago Luiz Mendes Arcebispo [3], Gissandra Farias Braz [1], Paulo Eduardo Brandão [2], Marcos Bryan Heinemann [2], Marcos Xavier Silva [3], Margaret J. Hosie [4]

Instituição [1] UNINTA - Centro Universitário INTA - UNINTA (R. Antônio Rodrigues Magalhães, 359 - Dom Expedito, Sobral - CE, 62050-100), [2] VPS - FMVZ - USP - Departaments of Preventive Veterinary Medicine and Animal Health, School of Veterinary Medicine and Animal Science, University of São Paulo (Av. Prof. Dr. Orlando Marques de Paiva, 87 Cidade Universitária "Armando Salles de Oliveira" São Paulo – SP CEP 05508-270), [3] DMVP - EV - UFMG - Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais (Av. Antônio Carlos 6627 Caixa Postal 567, Campus Pampulha da UFMG CEP: 31270-901. Belo Horizonte, MG), [4] MRC - CVR - University of Glasgow - Medical Research Council; Centre for Virus Research, University of Glasgow, Glasgow, UK. (464 Bearsden Rd, Bearsden, Glasgow G61 1QH, United Kingdom)

Resumo

The prevalence of feline immunodeficiency virus (FIV) was investigated in domestic cats in northern Ceará, Brazil. Samples from 296 cats were collected and tested using anti-FIV antibody screening, with confirmation of positive results by polymerase chain reaction (PCR). Seventeen cats (5.74%) tested positive for FIV, two female (0.67%) and fifteen males (5.06%). Phylogenetic analysis of *gag* and *pol* gene sequences indicated that the FIV isolates circulating in the study area belonged to subtype B.

Financial Support: Centro Universitário INTA – UNINTA; CNPq; FUNCAP

Palavras-chaves: Feline immunodeficiency virus, Domestic cat, Brazilian Northeast, Ceará

FIRST COMPLETE GENOME CHARACTERIZATION OF A BRAZILIAN BEAK AND FEATHER DISEASE VIRUS ISOLATE

Autores Matheus A. Duarte [1], João M. F. Silva [1], Tatsuya Nagata [1], Clara R. Brito [1], Danilo S. Teixeira [1], Fernando L. Melo [1], Bergmann M. Ribeiro [1], FABRÍCIO SOUZA CAMPOS [2]

Instituição [1] UnB - Universidade de Brasília (Campus Universitário Darcy Ribeiro, Bloco E s/n 1º andar - Asa Norte, DF, 70910-900), [2] UFT - Universidade Federal do Tocantins (Rua Badejós, Chácaras 69/72 s/n Zona Rural, Gurupi - TO)

Resumo

Circoviruses are non-enveloped, single-stranded, circular DNA viruses. They belong to the family *Circoviridae* and have genome size ranging between 1.7-2.3 kb. The group was taxonomically reviewed in 2016. Currently, the family is composed by two genera, *Circovirus* and *Cyclovirus*. *Circoviridade* comprises important animal pathogens, like beak and feather disease virus (BFDV) and porcine circovirus 2 (PCV-2), causes important environmental and economic losses in Psittaciformes species and pig industry, respectively. Genome sequences of these two circoviruses are the most reported in the family. However, no complete genome sequence of a Brazilian BFDV isolate was described yet. Based on this scenario, a high-throughput sequence method was employed to perform this genomic characterization. Feces samples of *Amazona aestiva* were collected from the Veterinary Hospital, University of Brasilia. The sequencing was performed using Illumina HiSeq 2500, 100 paired-end. Reads were trimmed and the contigs *de novo* assembled. Genome-wide pairwise identity is used as species demarcation criteria in *Circoviridade* with 80% identity as threshold and was applied for BFDV contig analysis. The present isolate is phylogenetically closer to the Polish isolate BFDV-U_PL-543_2008 (JX221029.1). Nucleotide identity was 94.9 %. The complete genome sequence found has 1,991 nt in size and is in accordance to other BFDVs. A 270-fold coverage was obtained, and 5339 reads were assembled. The two major ORFs (Rep and CP) were identified. However, differently from other BFDVs isolates, a point mutation was detected. A change of a cytosine (C) to a thymine (T) drives a premature stop codon producing a truncated CP, that is supported by a 345-fold coverage. Another ORF that codes for a hypothetical protein has high identity to C-terminus of CP. The circovirus' conserved nonanucleotide motif (TAGTATTAC) present in *ori* region was also observed. Similar to other circoviruses, the rolling circle replication (RCR) motifs at N-terminus of Rep were identified, motif I (FTLNN), motif II (PHLQG) and motif III (YCSK). Moreover, the superfamily 3 (SF3) helicases motifs, Walker-A (GPPGCGKS), Walker-B (VLDDF) and motif C (IITSN), were detected in Rep. These different features may help to explain the epidemiology of this viral disease in the country, that has great important in the veterinary medicine.

Financial Support: CNPq, FAPDF

Palavras-chaves: circovirus, high-throughput sequence, CP, Rep, ssDNA virus

THE PROPOSED AVIAN CHAPPARVOVIRUS: A NOVEL PARVOVIRUS FOUND IN BRAZILIAN WILD BIRDS' FECES BY A METAGENOMIC APPROACH

Autores: Matheus A. Duarte [1], João M. F. Silva [1], Tatsuya Nagata [1], Clara R. Brito [1], Danilo S. Teixeira [1], Fernando L. Melo [1], Bergmann M. Ribeiro [1], FABRÍCIO SOUZA CAMPOS [4]

Instituição [1] UnB - Universidade de Brasília (Campus Universitário Darcy Ribeiro, Bloco E s/n 1º andar - Asa Norte, DF, 70910-900), [4] UFT - Universidade Federal do Tocantins (Rua Badejós, Chácaras 69/72 s/n Zona Rural, Gurupi - TO)

Resumo

Chapparvovirus is a new virus genus recently proposed that belongs to *Parvoviridae*. This family comprises small non-enveloped ssDNA viruses with non-segmented and linear genome of 4 - 6.3 kb in size, involved in many clinical and subclinical animal infections. Chapparvoviruses exhibit a wide host range, infecting birds, dogs, rodents, bats, Tasmanian devils and swines, and have been identified by metagenomics analyses in feces as well as integrated to mammalian and avian genomes. The Brazilian Cerrado fauna shows very wide diversity and can be a potential viral reservoir. However, the wild animal virome of this biome is unknown. Based on this scenario, a high-throughput sequencing (HTS) constitutes a robust tool for the identification of new virus species in this environment and was applied in the present study. Feces samples of Cerrado birds (*Amazona aestiva* and *Sicalis flaveola*) were collected from the Veterinary Hospital, University of Brasilia. The sequencing was performed using Illumina HiSeq 2500, 100 paired-end. The reads were trimmed and the contigs *de novo* assembled. This new virus showed closer sequence identity to turkey parvovirus TP1-2012/HUN, a Chapparvovirus genus member, with 45.3% NS1 amino acid sequence identity. This protein is used as demarcation criteria for genus and species in *Parvoviridae* family, with 30% identity as threshold to novel genus determination. The genomic sequence found has 4425 nt in size and is in accordance to other chapparvoviruses. The 5'- and 3'-ends showed palindromic sequences that are responsible for the folding of the parvoviruses' terminal hairpins, essential to DNA virus replication. Two main ORFs were identified (NS1 and VP1), occurring a 62 nt overlap between them, that is the biggest one observed in this group or in any parvovirus genus of vertebrate hosts. The parvoviruses' conserved motifs (GPXNTGKS) and (HVH) were found coded in the genome. Similar to other chapparvoviruses, this new virus lacks the phospholipase A2 motif, recognized for playing a role in release of virus particle from endosomes in *Parvoviridae*. The phylogenetic trees of NS1 protein and virus genome sustain with high bootstrap values that this genus is monophyletic and closer to *Muscovy duck parvovirus*. These different features attach importance to study Chapparvovirus and understand your biology.

Financial Support: CNPq, FAPDF

Palavras-chaves: high-throughput sequencing, Cerrado, NS1, VP1, ssDNA virus

WILD ANIMALS AND THEIR IMPORTANCE FOR THE MAINTENANCE OF RABIES IN THE STATE OF SÃO PAULO – DATA COLLECTION

Autores: Ana Lee Aparecida Francisco [1,2], Gilmara de Sousa Silva [2], Marlon Benedito N. Santos [2], Helena Beatriz de C. R. Batista [2,1], Keila Iamamoto [2], Karen M. Asano [2], Enio Mori [2], Willian de O. Fahl [2], Karin C. Scheffer [2]

Instituição: [1] UFABC - Universidade Federal do ABC (Avenida dos Estados, 5001 Santo André), [2] IPSP - Instituto Pasteur de São Paulo (Avenida Paulista, 393)

Resumo

Rabies is an acute and progressive encephalitis caused by a virus that belongs to *Rhabdoviridae* family, *Lyssavirus* genus and *Rabies lyssavirus* (RABV) species. Considering the decrease in rabies cases in dogs and cats and the maintenance of control measures against rabies in herbivores, wild animals have been highlighted as important reservoirs of this disease. In addition, it is worth noting the increased risk to humans due to the synanthropic habits of some wildlife species. The aim of this study was to conduct a retrospective study on rabies positivity in wild animals, referred for diagnosis in the reference laboratory of the State of São Paulo. For this, we used the results obtained by direct immunofluorescence and viral isolation s from different groups of wild animals received from 1996 to 2016, from the State of São Paulo. During this period, the total number of wild animal samples received was 49,310, of which 881 (1.79%) were diagnosed as positive for rabies virus. Evaluating the results by animal group the positivity in the group of chiroptera was 1.80% (865 / 47,937), while the group of wild canids obtained a positivity of 4,8% (7/145) and the group of non-human primates with 1.11% (6/541). The group of cervids, marsupials and procionids obtained a positive sample during the study period. The data obtained in the present study reiterate the importance of wild animals in strategies to limit the spread of rabies, such as coordinated epidemiological surveillance, laboratory diagnostic procedures that allow the integrated study of genetics and ecology, thus providing knowledge of the dynamics of rabies in rabies. wildlife beyond the relevance of epidemiological surveillance, where the strategies adopted for adequate surveillance and control of rabies according to the epidemiological cycle in circulation and the region are evaluated.

Palavras-chaves: Rabies, wild animals, diagnosis, direct immunofluorescence, São Paulo

EQUINE INFECTIOUS ANEMIA (EIA) IN DONKEYS, NORTHEAST, BRAZIL

Autores Bruno Marques Teixeira [1], Viviane Maria Dias Costa [2,1], Andreia Elisa Cursino [2], Ana Paula Moreira Franco Luiz [3], Gissandra Farias Braz [1], Betânia Paiva Drumond [2], Erna Geessien Kroon [2]

Instituição [1] UNINTA - CENTRO UNIVERSITÁRIO INTA (R. Antônio Rodrigues Magalhães, 359 - Dom Expedito, Sobral - CE, 62050-100), [2] ICB/UFMG - Laboratório de Vírus- Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (Av. Pres. Antônio Carlos, 6627), [3] Viriontech do Brasil - Viriontech do Brasil (Avenida José Cândido da Silveira)

Resumo

The population of donkeys (*Equus asinus*) is concentrated in Northeastern Brazil for around 90%. Due to the agricultural modernization, these animals are being abandoned by their owners. As such, it is common to come across many of these animals wandering through the roads with ignored sanitary status, being potential transmitters of infectious agents. Among the infectious agents that can be transmitted by these animals, equine infectious anemia virus (EIAV) can be included. EIAV belongs to the family *Retroviridae* and genus *Lentivirus*. AGID is the official test designated by OIE for the worldwide diagnostic of EIA and is a highly specific test to identify infected animals, however, it has a low diagnostic sensitivity. Therefore, this test has a large number of false negative results, especially in donkeys, a species which is resistant to viral replication and shows a low viral load and late humoral response to the virus when compared to *Equus caballus*. This study aims to examine the serological status of EIAV in the wandering donkeys, using different techniques and also identifies the EIAV molecularly. A total of 124 donkeys were randomly selected in the state of Ceará. For each animal a data sheet with identification, clinical examination and body scoring were recorded. Blood samples were collected for accomplishment of the three diagnostic tests for EIA (AGID, ELISA recombinant protein gp90 and p26) and the detection of the proviral DNA. Immunological tests confirm AIEV in donkeys, however in AGID only one animal was positive (0.81%), compared with 21,8% (27/124) in the rgp90 ELISA and 10,5% (13/124) in the rp26 ELISA. In 8,1% (10/124) of the samples proviral DNA was detected. Thus, in light of the results it can be concluded that donkeys can also be carriers of the EIAV and may be possible sources of infection for horses, mainly in the Brazilian northeast.

Financial Support: Centro Universitário INTA – UNINTA; CNPq; FUNCAP; FAPEMIG.

Palavras-chaves: Equine infectious anemia virus - EIAV, Donkey, Diagnostic tests

SUPPRESSION OF STAPHYLOCOCCUS AUREUS BIOFILM FORMATION BY BACTERIOPHAGE VB_SAUM_UFV4 IN A DYNAMIC AND STATIC SYSTEM

Autores Adriele do Carmo [1], Larissa Araújo [1], Clara Laguardia [1], Isabela Paes [1], Roberto Dias [1], Marcella Vieira [1], Jéssica Silva [1], Vinícius Duarte [1], Sérgio Paula [1]

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

Resumo

Staphylococcus aureus is an opportunistic pathogen that affects humans and animals, being considered the main causative agent of bovine mastitis, one of the main diseases that affect dairy herds, reducing milk production and quality. Because they cause infections in the mammary glands, it is often associated with milk contamination and is responsible for outbreaks of diseases transmitted by the consumption of contaminated milk and dairy products. This microorganism causes great economic losses for the sector, as it is difficult to control, due to the expression of multiple resistance genes and the formation of biofilm. Biofilms are aggregates of microbial cells surrounded by a exopolymers matrix, highly organized and is an important virulence factor for species, because cells present in the biofilm are more resistant to antimicrobial agents, sanitizers and the action of the host immune system. Thus, an alternative method for biofilm biocontrol formed by *S. aureus* is phage therapy. Phages have been used since the early 20th century to treat bacterial infections, and have been shown to decrease biofilm formation due to enzymatic degradation of the layer surrounding and protecting microorganisms. In this work, the potential of bacteriophage vB_SauM_UFV4 was assessed in the preventive action of biofilm formation developed by multidrug-resistant *S. aureus* isolate obtained from milk samples collected from a mastitis casuistic production system. Biofilm formation in the presence of virus (MOI 1.0) was investigated under dynamic conditions through flow cells with polycarbonate coupons (FC BST Biosurface Technologies Corporation© 71) and under static conditions in 96 well flat bottom polystyrene plates, in both experiments occurred at 37 ° C for 72 hours. Bacterial growth was monitored (D.O600) and biomass of biofilm was determined by the microtiter plate-based crystal violet assay, measuring the absorbance of the supernatant obtained obtained after the biofilm discoloration step. In addition, the action of phage on biofilm formation in both systems was assessed by fluorescence microscopy using the dyes 4,6-diamino-2-phenylidol (DAPI), propidium iodide and fluorescein isothiocyanate (FITC). From the data obtained in this work it was possible to observe that vB_SauM_UFV4 interfered negatively in biofilm formation in both analyzed systems, showing to be a promising strategy in biofilm biocontrol formed by *S. aureus* such as pipes, milk cans and milk cooling tanks.

Palavras-chaves: Bacteriophage, Biocontrol, Biofilm

METAGENOMIC DETECTION OF HEPACIVIRUS IN ORAL AND RECTAL MICROBIOME OF OPOSSUMS FROM CAMPINAS METROPOLITAN REGION, STATE OF SÃO PAULO, BRAZIL

Autores: Paulo Vitor Marques Simas [1], Leonardo Cardia Caserta [1,2,3], Gabriela Mansano do Nascimento [2,3], Lok Raj Joshi [2,3], Raphael Mausbach Simão [4], Michael Edward Miller [1], Diego G. Diel [2,3], Clarice Weis Arns [1]

Instituição: [1] UNICAMP - University of Campinas (Laboratory of Animal Virology, Institute of Biology, Campinas/SP, 13083-970, Brazil), [2] SDSU - South Dakota State University (Animal Disease Research and Diagnostic Laboratory, Department of Veterinary and Biomedical Sciences, Brookings, SD 57007), [3] CORNELL - Cornell University (Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Ithaca, NY 14853, USA), [4] USP - University of São Paulo (FZEA, Pirassununga/SP, 13635-900, Brazil)

Resumo

The significant biodiversity found in Brazil is a potential for the emergence of zoonosis. Identifying natural reservoir species and characterizing which traits are associated with pathogens occurrence can be key to control emerging infectious diseases. Marsupials *Didelphis spp.* are widely distributed in the Americas and the species *Didelphis albiventris* and *D. aurita* are common in the most populated areas of Brazil. They adapt to a broad variety of habitats, including great urban centers and secondary forests modified by human action. However, their potential as reservoir species for zoonotic pathogens has not been deeply studied yet. Here we describe the oral and rectal/fecal microbiome (including viruses and bacteria) of 16 healthy opossums (*D. albiventris* and *D. aurita*) captured in three forest fragments in Campinas Metropolitan Region, São Paulo State, Brazil, using high throughput sequencing. Differences between composition and origin of microbiomes were observed. Oral microbiome presented higher bacterial diversity than anal. At species level, we detected sequences from *Campylobacter, Salmonella, Burkholderia, Chlamydia* and *Hepacivirus,* which are microorganisms of high zoonotic potential. Some pathogens species were detected only to one opossum specie or only one sampling location. Extrinsic factors like habitat fragmentation as well intrinsic factors like diet and phylogeny could potentially play a role on the patterns observed. *Hepacivirus* was detected in all samples, suggesting that opossums may be reservoir for this zoonotic virus and should be investigated as possible hosts to other viruses from the *Flaviviridae* family as well. The detection of these pathogens in such broadly distributed animal species warns to the possibility of disease emergence in other species including humans, especially when their habitats overlap.

Palavras-chaves: Didelphis albiventris and D. aurita, Microbiome, Opossum, Reservoir, Zoonotic diseases

CHARACTERIZATION OF ROTAVIRUS POSSESSING A DS-1-LIKE VP3 GENE FROM PIGS IN BRAZIL: EVIDENCE FOR ZOOANTHROPONOTIC TRANSMISSION.

Autores Mayara Neves [1], Alcines Sousa Junior [1], Patrícia Lobo [1], Renato Bandeira [1], Luana da Silva Soares Farias [1], Joana Mascarenhas [1]

Instituição [1] IEC - Instituto Evandro Chagas (Br 316, km 07, s/n)

Resumo

Porcine group A rotavirus (RVA) strains SUI15A and SUI24A were suggested to have genes of human origin and VP3 gene possessing DS-1-like backbone. The aim of the present study was to analyse the genome of two strains (SUI15A and SUI24A) and understand the evolution of a rare human-like M2 genotype in pigs. On partial genomic analysis, strains SUI24A (G3-P[13]-I5-R1-C1-M2-A8-N1-T7-E1-H1) and SUI15A (G3-P[x]-Ix-R1-C1-M2-Ax-Nx-T7-E1-H1) were found to have VP3 gene RVA different from those of typical porcine RVA strains described in Brazil and worldwide. This genotypic constellation was a novel constellation that has not been reported previously in both humans and pigs. Furthermore, on phylogenetic analysis, of VP3 gene of strains appeared to be of human origin. Therefore, suggested to have evidence for human-to-porcine zooanthroponotic transmission.

Financial support: CNPq, FAPESPA.

Palavras-chaves: Group A rotavirus, Zooanthroponotic transmission, Swine

GENETIC DIVERSITY OF CANINE MORBILLIVIRUS GENOTYPE CIRCULATING IN THE WEST-CENTRAL REGION, BRAZIL

Autores Vivaldo Gomes da Costa [1], Marielena Vogel Saivish [2], Rebeca Francielle de Lima Silva [2], Roger Luis Rodrigues [2], Marcos Lázaro Moreli [2], Ricardo Henrique Kruger [1]

Instituição [1] UnB - Universidade de Brasília (Asa Norte, Brasília-Distrito Federal, 70910-900), [2] UFG - Universidade Federal de Goiás (Br364, Km 195, Setor Industrial, Jataí-Goiás, 75801-615)

Resumo

Canine morbillivirus (previously known as canine distemper virus (CDV)) is an important viral agent that causes seve and highly contagious diseases in domestic dogs (*Canis familiaris*). CDV is enveloped with single-stranded, negati sense and nonsegmented RNA genetic material, belonging to the genus *Morbillivirus* (family *Paramyxoviridae*). Th virus has a large genetic diversity that divides it into several genotypes. Consequently, the hemagglutinin (H) ger has become the most suitable target to investigate the CDV variability. In view of there is little data that analyze th genetic variability, the objective of this study was to perform a molecular characterization of the H gene from CD in the clinical samples from dogs, which had biological samples collected between the years 2017 and 2018 in th municipality of Jataí-Goiás (Ethics Committee on the Use of animals-UFG: 054/17). The molecular characterizatio was performed for all CDV RNA positive samples by nested RT-PCR (detection of the CDV nucleoprotein gene). sequence, for the obtainment of an amplicon from the H gene, it was done an RT-PCR test with specific primer generating a product with 1189 bp. PCR product was confirmed by DNA sequencing and Neighbor Joinir phylogenetic inferences was performed by Mega 7.0 software. The results showed that only three (3/30) whole bloo samples from dogs with distemper had amplicons for the H gene. The H gene phylogeny showed characterization the lineage in the genotype of South America-I/Europe, with greater similarity to the isolated strain of Urugua (KM280689.1). Thus, a phylogenetic proximity between our isolates and other isolates from Latin America ar Europe was observed. To our knowledge, this is the first study in the region about CDV molecular research and w contribute to molecular surveillance and trace the epidemiological profile of CDV in the study region.

Financial Support: FAPDF, CNPq and CAPES.

Palavras-chaves: Canine distemper virus, Canine morbillivirus, Paramixoviridae, Domestic dogs, Morbillivirus

CHARACTERIZATION OF THE HEMAGGLUTININ PROTEIN GENE OF CANINE MORBILLIVIRUS FROM NATURALLY INFECTED DOGS IN THE STATE OF MATO GROSSO

Autores Mayara Lima Kavasaki [1], Daniel Moura de Aguiar [1], Michele Lunardi [2], Luiz Donizete Campeiro Junior [1]

Instituição [1] UFMT - Universidade Federal de Mato Grosso (Av. Fernando Corrêa da Costa, nº 2367 - Bairro Boa Esperança. Cuiabá - MT - 78060-900), [2] UNIC - Universidade de Cuiabá (Av. Manoel José de Arruda, 3100 - Jardim Europa, Cuiabá - MT, 78065-700)

Resumo

Canine morbillivirus (CDV) causes one of the major infectious diseases of high morbidity and mortality in dogs and wildlife, called canine distemper (CD), belonging to the family *Paramyxoviridae* and genus *Morbilivirus*. The viral genome contains transcriptional units that encodes eight proteins that include nucleoprotein (N) and Hemagglutinin (H). The hemagglutinin (H) is expressed in the viral envelope and has the highest genetic variability among CDV and has been used to characterize phylogenetically into nine strains named America I, America II, Asia I, Asia II, Europe Wildlife, Arctic, South Africa, South America I / Europe and South America II. In the present study, hemagglutinin (H) protein gene was characterized in CDV from naturally infected dogs in the state of Mato Grosso. Central nervous tissue from 6 dogs were collected post mortem between August 2018 and April 2019 and submitted to RT-PCR to detect the nucleocapsid gene (N) of CDV. From the positives, the H gene were amplified, and the nucleotide sequence was aligned by the MUSCLE program and a phylogenetic tree was inferred by the Neighbor-Joining method. Sequenced samples were classified into MT1, MT2, MT3, MT4, MT5 and MT6. Samples MT1 to MT4 were from the municipality of Poconé-MT and MT5, MT6 from Cuiabá-MT. Phylogenetic analysis revealed that the samples positioned in two distinct groups: samples MT1, MT3, MT5 and MT6 were genetically related to isolates from Brazil, Italy and Spain, and the samples MT2 and MT4 were grouped in clade composed by strains from South America, Europe, South Africa and classic vaccine strains (Convac, CDV3, Snyder Hill, and Onderstepoort). All sequences were classified within the South America I / Europe genotype. Financial support: Ministry of Education of Brazil (MEC), Federal Agency for the Support and Improvement of Higher Education (CAPES) and National Council for Scientific and Technological Development (CNPQ).

Palavras-chaves: Canine distemper, Nucleoprotein, Molecular Detection, RT-PCR, Phylogenetic analysis

CHARACTERIZATION OF BRAZILIAN GENETIC LINEAGES OF THE RABIES VIRUS COMPATIBLE WITH SAMPLES ISOLATED IN DOMESTIC AND WILD CANIDS AND VAMPIRE BATS IN RT-QPCR ASSAY

Autores: Maria Eduarda Rodrigues Chierato [1], Débora Fernanda Pedrozo Pavani [2], Alex Domingos Reis [2], Yasmin Machado de Freitas [2], Karin Corrêa Scheffer [2], Helena Beatriz de Carvalho Ruthner Batista [2], Karen Miyuki Asano [2], Willian de Oliveira Fahl [2], Keila Iamamoto [2], Enio Mori [1,2]

Instituição: [1] FMVZ-USP - Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (Av. Prof. Dr. Orlando Marques de Paiva, 87 - Butantã, São Paulo - SP, 05508-900), [2] IP-SP - Instituto Pasteur de São Paulo (Av. Paulista, 393 - Cerqueira César, São Paulo - SP, 01311-000)

Resumo

Rabies is a progressive acute viral encephalitis, caused by Rabies virus (RABV), which belongs to the family *Rhabdoviridae*, genus *Lyssavirus*. The disease is characterized by a central nervous system (CNS) disorder, that presented a high lethality (almost 100%). Because of the RABV adaptation to the determinate animal hosts (reservoirs) over the time, different genomes were formed and are classified as variants (AgV) and/or distinct genetic lineages. In Brazil, the main reservoirs are domestic dogs (AgV1 and AgV2), vampire bats *Desmodus rotundus* (AgV3) and non-hematophagous frugivorous bats *Artibeus lituratus* (AgV3). The aim of this study was to develop a RT-qPCR assay for the detection and genetic lineages characterization of the different variants of the RABV for diagnosis in the Pasteur Institute of Sao Paulo. Samples from cattle CNS (n=40) and domestic and wild canids CNS (n=16), compatible to AgV3 and to AgV2, respectively, were used in order to analyze the specific character of the probes designed to AgV3 and to AgV2 for the RT-qPCR assay. The cattle samples were obtained between the years 2015 and 2016 from the Southeast Brazil and the domestic and wild canids samples were obtained between the years 2007 and 2018 from the North and Northeastern Brazil. Total RNA was extracted from all 56 CNS samples by the guanidinium thiocyanate (TRIzol reagent) method and the RT-qPCR assay was performed using primers and probes to amplify the N gene. The results showed that the probes are specific and sensitive for the respective genetic lineages. Furthermore, the probe for AgV3 did not amplify the wild canids samples, and the probe for AgV2 did not amplify the cattle samples. The results also suggest that the different genetic lineages may be analyzed in a real time RT-PCR assay without the need of sequencing. Besides, this assay may discriminate the main variants of RABV in Brazil and once introduced into the laboratory routine can help implementing surveillance measures and rabies control.

Financial Support: CNPq (404065/2016-3); FAPESP (15/17807-0); CAPES (Finance Code 001).

Palavras-chaves: Genetic lineages, Rabies, RNA, RT-qPCR

A CONTEMPORARY BRAZILIAN SENECAVIRUS A ISOLATE: IN VITRO CHARACTERIZATION - PARTIAL RESULTS -

Autores Manuela Muller [1], Sergio Abreu Machado [2], Diego Gustavo Diel [3], Mathias Martins [1]

Instituição [1] UNOESC - Universidade do Oeste de Santa Catarina (Laboratório de Virologia, Rodovia Rovilho Bortoluzzi, SC480, Km 3.5, Bloco C, Xanxere, SC, Brazil. 89820-000), [2] UNOESC - Universidade do Oeste de Santa Catarina (Laboratório de Biologia Molecular, Bloco C, Xanxere, SC, Brazil. 89820-000), [3] SDSU - South Dakota State University (Animal Disease Research and Diagnostic Laboratory, Department of Veterinary and Biomedical Sciences, Brookings, SD, United States. 57007)

Resumo

Senecavirus A (SVA) belongs to the *Picronaviridae* family and was firstly described in 2002 in United States as a cell culture contaminant and non-pathogenic for animals. However, SVA became a problem between the end of 2014 and early 2015 when it was detected in piglets presenting vesicular disease, diarrhea and death in Brazil. Although the virus remained endemic in the country since then, field veterinarians have reported an increase in the number of cases and a change in the clinical form of the disease in 2019. The virus is apparently more virulent than the one first detected in 2014, especially in finishing pigs. The disease caused by SVA has generated disturbance to the swine production chain in Brazil since it is on the list of diseases that can be confused with foot-and-mouth disease. In this study, a contemporary Brazilian Senecavirus A was isolated. The sample was collected using swab from vesicle in finishing swine with 160 to 170 days old, kept in a farm with 6000 pigs in Minas Gerais State, Brazil. The morbidity was about 20 percent and mortality rate less than 3 percent. The diagnosis was confirmed by PCR using primers that determine amplification of an internal region of the 3D gene of the SVA genome. Simultaneously, the sample was inoculated in cell culture for viral detection. The virus isolation was performed in baby hamster kidney cells (BHK-21). In the first passage of the material under cultivation, a cytopathogenic effect compatible with SVA replication was observed (cell rounding and detachment). The identity of virus was confirmed using two additional technics: nucleotide sequencing of PCR amplicons and indirect immunofluorescence assay (IFA) using monoclonal antibody (mAb). Both tests confirmed that it was an SVA. The next step of the research is to obtain the complete genome of the isolated virus and compare with the SVA that circulated in Brazil in 2014.

Palavras-chaves: emerging infectious disease, idiopathic vesicular disease, picornavirus, Seneca Valley virus, swine

PHYLOGENY OF CIRCULATING STRAINS OF CAPRINE ARTHRITIS ENCEPHALITIS VIRUS FROM GOATS OF THE SÃO PAULO STATE, BRAZIL

Autores: Ingrid Bortolin Affonso Lux Hoppe [1], Igor Renan Honorato Gatto [1], Andressa de Souza-Pollo [1], Andréa Souza Ramos de Medeiros [1], Kayo José Garcia de Almeida Castilho Neto [1], Samir Issa Samara [1], Adolorata Aparecida Bianco Carvalho [1]

Instituição [1] FCAV/Unesp - Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (Via de Acesso Prof. Paulo Donato Castellane, s/nº, CEP:14.884-900, Jaboticabal, São Paulo, Brazil)

Resumo

Caprine arthritis encephalitis virus (CAEV) and Maedi-Visna virus (MVV), also known as Small Ruminant Lentiviruses (SRLVs), cause slow and persistent inflammatory diseases in goat and sheep, respectively, leading to important productive and economic losses worldwide. For a long time, these infections have been considered species-specific, although, several reports showed that natural cross-species infections may occur. Therefore, monitoring the genetic diversity of SRLVs in sheep and goat herds is useful mainly to improve the diagnostic tools used in the control or eradication programs. The present study aimed to characterize genetically SRLVs strains obtained from naturally infected dairy goat herds of the municipality of Jaboticabal, São Paulo State, Brazil. Thus, blood samples of three CAEV-positive goats (121, 171 and 191) detected by agar gel immunodiffusion test were submitted to nested PCR to amplify part of the *gag* gene of the SRLVs and perform the sequencing. In addition, a nested PCR-positive goat sample (V23.2), obtained from another herd of the same municipality, was included in the study. None of the animals have presented clinical signs of SRLVs infection. In the phylogenetic tree, the sequences of this study were grouped in CAEV group type B , subtype B1. Sequences of the goats 121, 171 and 191 were grouped in the same cluster, separately from the sequence V23.2, that showed to be genetically distant from the other sequences obtained in this study. These results indicate that the occurrence of SRLVs strains belonging to subtype B1 remains predominant in Brazil, as reported in previous studies. CAEV is a major disease of goat production and the continued study of its molecular epidemiology is especially important owing to its constant change, related to animal movement, cross-species transmission and the rapid evolutionary rate.

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Palavras-chaves: gag gene, phylogeny, Small Ruminant Lentiviruses, SRLVs

GENETIC CHARACTERIZATION OF AVIAN POXVIRUS IN SOUTHERN BRAZIL

Autores: Leonardo Clasen Ribeiro [1], Francielle Liz Monteiro [1], Matheus Iuri Fruhauf [1], Renata Nobre da Fonseca [1], Gilberto D'Avila Vargas [1], Marcelo de Lima [1], Geferson Fischer [1], Silvia de Oliveira Hübner [1]

Instituição [1] UFPel - Universidade Federal de Pelotas (Campus Universitário Capão do Leão, Prédio 1, Faculdade de Veterinária, Laboratório de Virologia e Imunologia)

Resumo

Avian poxvirus (APV) is an enveloped double-stranded DNA virus which affects a wide variety of domestic and wild birds worldwide. APVs belong to the subfamily *Chordopoxvirinae* and to the family *Poxviridae*. In the genus *Avipoxvirus* there are ten recognized species, being the nomenclature given in accordance with the species in which they were first described. However, new molecular characterization studies corroborate the idea that the same strain may affect different bird species. Thus, the objective of this study was to characterize genetically APVs detected in different bird species in southern Brazil. The samples were received by the pathology department and sent for confirmatory diagnosis at the Laboratory. DNA was extracted from frozen tissue of the lesions and submitted to the PCR, targeting the polymerase and P4b gene. PCR product was purified, and amplicons were sequenced bidirectionally. Results were analyzed to obtain a consensus sequence from each duplicate. Phylogenetic analysis was conducted by the MEGA 6.0, using the *neighbor-joining method*, and the evolutionary distances were computed using the *Kimura 2-parameter model.* Ten samples were analyzed: a sample of domestic fowl (*Gallus gallus domesticus*) grouped in clade A1, and five samples, one of hawk (*Mivalgo chimango*), one of canary (*Serinus canaria*) and three of dove (*Columba livia*), grouped in clade A2. Two canary samples were grouped in clade B1 and one in clade B2. Curiously, the samples of canary and hawk grouped in clade A, which represented by *Fowlpox virus*. These samples demonstrated 90.7 to 100% identity between then, demonstrating that different bird species can be infected by very similar viruses. Studies conducted in Brazil are generally based on clinical diagnosis and histopathology. Thus, the constant monitoring of the evolution of APVs is important an eventual outbreak of the disease and the evolutionary biology of these viruses.

Financial Support: CAPES.

Palavras-chaves: APV, phylogenetic analysis, P4b gene, polymerase gene.

TEMPORAL AND SPACE CHARACTERIZATION OF RABIES IN LIVESTOCK IN THE STATE OF MATO GROSSO BETWEEN 2009 AND 2018

Autores: Emmanuelle Rosa Mutzenberg [1,2], Áquila José Gonçalves Delfino [1], Ernani Machado de Lima [2], Selma Maria Nassarden [2], Rísia Lopes Negreiros [2], Anderson Castro Soares de Oliveira [1], Daniel Moura de Aguiar [1]

Instituição: [1] UFMT - Universidade Federal de Mato Grosso (Avenida Fernando Corrêa da Costa, 2367, Cuiabá-MT), [2] INDEA - Instituto de Defesa Agropecuária do Estado de Mato Grosso (Avenida Jornalista Arquimedes Pereira Lima, 1000, Cuiabá-MT)

Resumo

Rabies is a zoonosis caused by *Lyssavirus*, causing acute fatal encephalomyelitis in farm animals and causing serious damage to livestock. The disease is endemic in the state of Mato Grosso (MT). The present work carried out an epidemiological analysis of rabies outbreaks in MT registered by the state animal health defense between 2009 and 2018.The following data were extracted from the Disease Investigation Forms: number of outbreaks, cases, animal species, positivity rate, and number of notified municipalities. The data of focus, mean, standard deviation and coefficient of variation were evaluated by the *Rcmd*r package of the R software. Maps with focus concentration / km² were constructed using the *QGIS* software. The ARIMA (1,1,1) statistical model to determine the historical series of outbreaks was built by the *TSA* package of the R software. Of the total, 589 cases were diagnosed in 538 outbreaks with an average of 53.8 outbreaks per year and a coefficient of variation of 0.39. It was found a higher occurrence in cattle (89%) and horses (10%), with the highest positivity rate, 35.8% and 33.7%, respectively. Direct immunofluorescence testing diagnosed 89.3% of cases. Ninety-five of 141 municipalities (67.3%) recorded outbreaks that shifted over the years, concentrating mainly on the western (Amazon biome), central and eastern (Cerrado biome) regions of MT. Outbreaks increased from May to July, but without seasonality patterns (p>0.01), with 1% significance level. There was a decreasing trend of outbreaks (p

Financial Support: National Council for Scientific and Technological Development (CNPQ)

Palavras-chaves: Lyssavirus, epidemiological survey, rural cycle, Brazil, animal health defense

IN VITRO SUSCEPTIBILITY OF BOVINE CELLS TO SMALL RUMINANTS LENTIVIRUS

Autores: Maria Aurea Azevedo Nogueira [1], Marcelo Tigre Moura [1], Giselle Ramos da Silva [1], Davi Santos Rodrigues [1], José Wilton Pinheiro Junior [1], Rita de Cássia Carvalho Maia [1], Sergio Alves do Nascimento [1]

Instituição [1] UFRPE - Universidade Federal Rural de Pernambuco (Rua Dom Manoel de Medeiros, Dois Irmãos, Recife. PE.)

Resumo

The susceptibility of a primary bovine cell line to viral agents of small ruminants, which do not cause natural infection in this species, was tested. Thus, ear biopsy samples from male bovine with no defined breed, and approximately two years old, were obtained from slaughterhouses. After tissue processing and fragmentation, 1-2mm explants were plated on 60mm petri dishes containing 3mL Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% Fetal Bovine Serum (FBS). Afterwards, weekly passages were performed, where the cultures were trypsinized and subcultivated in 25cm^2 flasks, containing DMEM with Amphotericin B, Streptomycin and Penicillin, and incubated at 37°C. During cell culture 10% FBS was used and for viral inoculation 2% FBS was preferred. The cells obtained (CFBov) were inoculated with Caprine Artrite-Encephalites virus (CAEV-Co) and Maedi Visna virus (MVV) (K1514), and karyotyping was performed for chromosome analysis. The CFBov cells were cultivated continually for 18 months and 48 passages. The monolayers did not show any morphological modifications or reduction in multiplication rate. The CFBov primary cells from bovine ear samples showed susceptibility during *in vitro* infection with both CAEV and Maedi-Visna viruses. The chromosomic analysis of twelve intact cells, showed 2n=60 with all acrocentric autosomes and a submetacentric X, thus confirming that the cells have shape, size and number characteristic of bovine species. In addition to the position of the centromere, the size and bands were evaluated and confirmed, which are the light and dark regions along the chromosome. Each band is distributed differently and specific for each species, leading to an effective analysis. This study corroborates the importance of cell culture techniques as a key player in a veterinary virology laboratory. Due to their growth characteristics, the cells have behaved as a continuous lineage, which will be confirmed with the continuation of the passages. Finally, it is possible to state that the CFBov cells have demonstrated *in vitro* susceptibility to small ruminant virus, and further studies on the pathogenesis of the etiological agent in this species are necessary.

Palavras-chaves: Cell culture, Virus susceptibility, Chromosomic analysis

DETECTION OF HOBI-LIKE PESTIVIRUS IN AN OUTBREAK OF RESPIRATORY DISEASE IN CALVES OF SÃO PAULO STATE, BRAZIL

Autores Ingrid Bortolin Affonso Lux Hoppe [1], Andressa de Souza-Pollo [1], Adolorata Aparecida Bianco Carvalho [1]

Instituição [1] FCAV/Unesp - Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (Via de Acesso Prof. Paulo Donato Castellane, s/nº, CEP:14.884-900, Jaboticabal, São Paulo, Brasil)

Resumo

HoBi-like is an emerging pestivirus of the family *Flaviviridae* detected in cattle herds and biological products, such as fetal bovine serum, in many parts of the world. The virus is associated with a variety of clinical manifestations resembling the infections by bovine viral diarrhea virus (BVDV), such as reproductive and respiratory disorders, persistent infection and mucosal disease. Clinical signs caused by Hobi-like pestivirus can also be confused with those caused by other viruses, making the diagnosis difficult. This study reports the detection of HoBi-like pestivirus in an outbreak of respiratory disease in calves of a dairy cattle herd in São Paulo State, Southeastern Brazil. For that, serum samples and nasal swabs were collected from 44 calves up to one year old, presenting or not clinical signs of respiratory disease. RT-PCR was performed to detect pestiviruses (BVDV-1, BVDV-2 and HoBi-like), bovine respiratory syncytial virus (BRSV) and bovine parainfluenza-3 (BPIV-3); and, PCR was utilized to detect the bovine herpesvirus-1 (BoHV-1) DNA. Both serum samples and nasal swabs of two animals aging 0-3 months and two older calves (6-12 months) were positive for pestiviruses. Sequencing results of the amplified 5'UTR and E2 regions of the nasal swabs identified the HoBi-like pestivirus. The phylogenetic tree of the concatenated sequences of the 5'UTR and E2 regions showed a close genetic similarity among the sequences obtained in this study which were grouped in a same cluster, nonetheless, the sequences were separated in different subgroups according to the age of the calves, evidencing the genetic particularities between the sequences obtained from younger and older calves. Only one Brazilian sequence of HoBi-like, from the State of Mato Grosso do Sul, showed genetic relation with the sequences obtained in this study, but presenting high genetic distance from them (0.033), and the other Brazilian sequences showed to be even more genetically distant (≥0.047). This is the first detection of HoBi-like pestivirus in nasal secretions of calves in an outbreak of respiratory disease in Brazil, and the increasing detection of this virus at field indicates the necessity of a differential diagnosis to enable the implementation of appropriate measures of control and prophylaxis.

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Palavras-chaves: calves, nasal swab, pestiviruses, phylogeny

OCCURRENCE OF GENOGROUP I PICOBIRNAVIRUS IN SHEEP FLOCKS FROM PARANA.

Autores: CAROLINE DO NASCIMENTO FERREIRA [1], DANIELA LORENCENA [1], PATRICK KAISER KNABBEN [1], JOICE APARECIDA DE ANDRADE [1], PETRONIO PINHEIRO PORTO [2], FRANCIELLE GIBSON DA SILVA ZACARIAS [2], LIZA OGAWA [2], ELISABETE TAKIUCHI [1]

Instituição: [1] UFPR - UNIVERSIDADE FEDERAL DO PARANÁ (SETOR PALOTINA - Rua Pioneiro, 2153. Jardim Dallas. CEP 85950-000. Palotina - PR.), [2] UENP - UNIVERSIDADE ESTADUAL DO NORTE DO PARANA (RODOVIA BR-369 KM 54, VILA MARIA, CEP 86360-000, BANDEIRANTES, PR, BRAZIL)

Resumo

Picobirnaviruses (PBV) are non-enveloped virus, with a bisegmented double stranded RNA genome. PBV are classified into genogroups I and II due to the high genetic variability of the segment 2 that encodes its RdRp. PBV is considered an opportunistic pathogen and its role as a causative agent of diarrhea is uncertain. Although PBV have already been detected in a wide range of susceptible hosts, data pertaining to its presence in small ruminants are still limited. In order to assess the occurrence of PBV in ovine host, stool samples from 203 animals were collected between 2017 and 2018 in four sheep flocks located in the municipality of Bandeirantes, Parana State. Sampling was performed from animals with (n=68) or without (n=135) signs of diarrhea. The PBV diagnosis was carried out by silver-stained 7.5% polyacrylamide gel electrophoresis (SS-PAGE) and RT-PCR using the primers PicoB25 (5´TGGTGTGGATGTTTC 3´) and PicoB43 (5´A(GA)TG(CT)TGGTCGAACT T 3´) that amplify a 201 bp fragment of the RdRp gene of GI PBV. The PBV was detected (positive results by SS-PAGE and/ or RT-PCR) in all four herds surveyed, with frequencies of 24.3% (18/74); 30% (3/10); 30.6% (11/36) and 50.6% (42/83). PBV was found in 29.4% (20/68) of the diarrheic and 40% (54/135) of the non-diarrheic samples. Out of the total samples, PBV was detected in 4.93% (10/203) and 34% (69/203) by ss-PAGE and RT-PCR, respectively. From the 10 SS-PAGE positive fecal samples, five were not successfully amplified using GI specific primers, suggesting the presence of PCR inhibitors in the samples or these samples may still belong to the GII PBV. However, since we have not tested the GII specific primer set it is not possible to assign to these PBV this classification. The low sensitivity of the SS-PAGE technique could be considered as a limiting factor for PBV diagnosis. However, the positive results obtained in this assay suggest intense viral replication with faecal excretion of PBV in high titers by these animals. Besides, all positive samples by SS-PAGE were non-diarrheic that reinforces the hypothesis that PBV may not be the primary etiological agent in diarrhea episodes. Although much remains to be understood about the epidemiology of PBV, this study confirms the GI PBV is widely distributed in sheep flocks from Parana State.

Palavras-chaves: Picobirnavirus, sheep, genogroup I, PAGE, RT-PCR

DETECTION OF PICOBIRNAVIRUS IN FECAL SAMPLES FROM PIGS AND PIG FARM WORKERS IN THE WESTERN REGION OF PARANÁ.

Autores DANIELA LORENCENA [1], CAROLINE DO NASCIMENTO FERREIRA [1], THIAGO HENRIQUE BELLE [1], LEONARDO ERENO TADIELO [1], JANAINA LUSTOSA DE MELLO [1], ELISABETE TAKIUCHI [1]

Instituição [1] UFPR - UNIVERSIDADE FEDERAL DO PARANÁ (SETOR PALOTINA - Rua Pioneiro, 2153. Jardim Dallas. CEP 85950-000. Palotina - PR.)

Resumo

Picobirnavirus (PBV) constitute a group of emerging non-enveloped virus with a bisegmented double-stranded RNA (dsRNA) genome. PBV has been detected in a wide range of host species, including terrestrial and marine mammals, reptiles, and birds. Some studies of PBV has been suggested the possibility of inter-species transmission, highlighting the zoonotic potential of PBV in pigs and humans. However, most of the studies have compared PBV sequences obtained from the swine host and from humans without any epidemiological relation between them. This work aimed to investigate the PBV excretion in fecal samples from pigs and their contacts pig farm workers. In order to investigate the PBV infection in swine and human in close contact with these animals, stool samples from 133 pigs and nine pig farm workers were collected in 2018 in three commercial farms located in the western region of Parana State. Fecal samples were submitted to nucleic acid extraction by the combination of phenol chloroform-isoamyl alcohol and silica/guanidine isothiocyanate. The PBV diagnosis was carried by silver-stained polyacrylamide gel electrophoresis (SS-PAGE) for visualization of the dsRNA genome. PBV was detected in all three pig farms surveyed, with frequencies of 24.4% (11/45); 41.3% (19/46); 45.2% (19/42) in pigs and of 0% (0/2); 100% (3/3); 50% (2/4) in their respective pig farm workers. Considering the low sensitivity of the SS-PAGE, the detection of PBV dsRNA using this technique demonstrates the elimination of high titers of viruses in both host species. This report constitutes a preliminary investigation; future studies will include analysis of all samples by RT-PCR and sequencing of the amplified products to assess the identity of the sequences obtained from swine and humans in the same space/time locations. Due to the close relationship between pig farms workers and pigs, new epidemiological studies should be conducted to evaluate the pathogenesis of PBV and to elucidate the role of these viruses in populations exposed to infection.

Palavras-chaves: picobirnavirus, pigs, humans, PAGE

CRITICAL ANALYSIS OF FACTORS THAT MAY INFLUENCE THE DOMESTIC HERBIVORE RABIES DIAGNOSIS

Autores: Vitória Bueno Vilela Silveira [1], Maria Eduarda Rodrigues Chierato [2], Debóra Fernanda Pedrozo Pavani [1], Samira Maria Achkar [1], Helena Beatriz de Carvalho Ruthner Batista [1], Karen Miyuki Asano [1], Keila Iamamoto [1], Willian de Oliveira Fahl [1], Enio Mori [1,2]

Instituição [1] IP-SP - Instituto Pasteur de São Paulo (Av. Paulista, 393 - Cerqueira César, São Paulo - SP, 01311-000), [2] FMVZ-USP - Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (Av. Prof. Dr. Orlando Marques de Paiva, 87 - Butantã, São Paulo - SP)

Resumo

Direct Fluorescent Antibody (DFA) test is the golden standard for rabies diagnosis because it is fast and has high sensitivity and specificity. However, since rabies is almost 100% fatal and the DFA may present low sensitivity in some specific cases, a confirmatory test is recommended. It has been postulated that the bovine species has near 100% of sensitivity in the DFA test, while equine sensitivity may be lower. For this reason, the Pasteur Institute of Sao Paulo uses the RT-PCR as a second test for equines. This study aimed to make a survey of the DFA results from February 2017 to July 2019, and compare the different techniques used for the domestic herbivore rabies diagnosis such as DFA, RT-PCR, and virus isolation (VI) in N2A cells. During this period, the Pasteur Institute tested 457 bovine central nervous system (CNS) samples and 382 equine CNS samples for rabies. Spite of 211 bovines were DFA negative, 8 of them were positive in the RT-PCR. At the same, 248 equines were DFA negative; however, 25 were positive in the RT-PCR. Therefore, 3.8% of bovines and 10% of equines were false negative in the DFA test. Moreover, 3 bovines and 3 equines were positive in the VI test. These results highlight that bovines and equines are indicating that some factors may influence DFA sensitivity. 100% of the false negative bovines (n=8) showed a fast disease progression (2 days), and 50% of them were euthanized. Therefore, the samples could have a low viral load, which may hamper the visualization of the inclusion corpuscle. Due to the lack of data, the same information cannot be applied to equines. Also, the sampling used for the DFA may not have been infected since the virus does not infect the CNS uniformly, which is why a transversal section of the brain stem could be required as recommended in these cases by WHO. On the other hand, especially for bovines, the VI test proved to be a satisfactory technique as a backup of the DFA test. A false negative diagnosis may carry tragic effects such as the death of humans due to the lack of post exposure prophylaxis. Nonetheless, additional studies are required in order to have a better understanding of the causes that may influence the rabies diagnosis and also, how the processing of CNS samples must be performed.

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Palavras-chaves: Cattle, DFA, Equine, Rabies, RT-PCR

MOLECULAR MODELING AND STRUCTURAL ANALYSIS OF THE NS5B POLYMERASE OF NOVEL HEPACIVIRUS AND PEGIVIRUSES INFECTING HORSES

Autores Pedro Pereira Lira Furtado de Albuquerque [2,1], Lucianna Helene Silva dos Santos [5,6,7,8], Deborah Antunes [3,4,1], Ernesto Raul Caffarena [3,4,1], Andreza Soriano Figueiredo [2,1]

Instituição [1] LADTV, IOC - Laboratório de Desenvolvimento Tecnológico em Virologia, Instituto Oswaldo Cruz (Avenida Brasil, 4.365, Pavilhão Hélio e Peggy Pereira - Manguinhos, RJ, Brasil), [2] PROCC, IOC - Grupo de Biofísica Computacional e Modelagem Molecular, Programa de Computação Científica, Instituto Oswaldo Cruz (Avenida Brasil, 4.365, Manguinhos, RJ, Brasil), [3] LMMPF, UFMG - Laboratório de Modelagem Molecular e Planejamento de Fármacos, Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (Av. Antônio Carlos, 6627, Pampulha - Belo Horizonte - MG)

Resumo

Defining the three-dimensional (3D) structure of a viral protein has many biological, evolutionary, therapeutic and prophylactic perspectives. The Non-structural 5B (NS5B) protein of the *Flaviviridae* family is known for its conservation, and as an important drug target. Containing the RNA-dependent-RNA-polymerase (RdRp) domain, the NS5B is responsible for viral replication, though its conformation for the equine hepacivirus (EHCV), equine pegivirus (EPgV) and Theiler's disease associated virus (TDAV), novel equine viral infections from the *Hepacivirus* and *Pegivirus* genus, have not yet been elucidated. Thus, this work aimed to build the first *in silico* 3D model of the EHCV, EPgV and TDAV NS5B protein. EHCV subtypes 1 and 2, obtained in previous epidemiological investigations in Rio de Janeiro, and reference sequences from the EPgV and TDAV were submitted to comparative modeling using hepatitis C virus (HCV) crystallographic structures as templates: PDB 3HHK and 4WTG in open (elongation phase) and closed (chemically active phase) conformation. The following amino acid (aa) numbers are based on HCV reference isolate H77. Primary structure analysis revealed conservation of the catalytic motif A (DxxxD 220-225) and motif C (GDD 318-320). Essential aa for the RdRp activity were present such as G283, T287, N291 in motif B and K153, R158 and L/I160 in motif F, responsible for sugar selection and interaction with the incoming nucleotide, respectively. Some aa, which studies with mutation-induced analysis showed decreased or abolished HCV polymerase activity, were partially conserved in motif B, D and E. Secondary and tertiary analysis revealed conserved folding structures in both open and closed conformation among these flavivirus family members (high TM-score of 0.99 and low RMSD values,

thumb domain, characteristic of the HCV enzyme. In conclusion, primary, secondary and tertiary structure analysis argue toward a similar RdRp mechanism of action for the EHCV, EPgV and TDAV, while the variability may suggest different polymerization rate or efficiency, comparatively to HCV polymerase, although further research is needed.

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Plano de Objetivos e Metas – POM, Instituto Oswaldo Cruz, Fiocruz.

Palavras-chaves: Hepacivirus equino, Pegivirus equino, Theilers Disease Associated Virus, RNA polimerase dependente de RNA, Análise estrutural

DIAGNOSTIC ACCURACY OF LENTZ BODY INCLUSIONS TEST FOR CANINE MORBILLIVIRUS DETECTION

Autores Vivaldo Gomes da Costa [1], Priscila Gomes de Oliveira [2], Marielena Vogel Saivish [2], Andréia Vitor Couto do Amaral [2], Marcos Lázaro Moreli [2], Ricardo Henrique Kruger [1]

Instituição [1] UnB - Universidade de Brasília (Asa Norte, Brasília-Distrito Federal, 70910-900), [2] UFG - Universidade Federal de Goiás (Br364, Km 195, Setor Industrial, Jataí-Goiás, 75801-615)

Resumo

Rapid detection tests are widely used in veterinary clinical laboratories. In this context, the observation of Lentz bodies in erythrocytes and leukocytes is considered definitive diagnosis of distemper; however, its absence does not rule out the possible existence of the distemper. This disease is caused by canine distemper virus (CDV, currently termed canine morbillivirus, family *Paramyxoviridae*); it has worldwide distribution, affects mainly puppies dogs and is responsible for respiratory, gastrointestinal and neurological complications. Therefore, in view of the significant impact of CDV infections on the health of domestic dogs, the aim of this study was to evaluate the accuracy of CDV diagnosis by Lentz bodies in blood samples from dogs clinically suspected of distemper in comparison to gold standard (nested RT-PCR). The present study was approved by the ethics committee on the use of animals of the UFG (N° 054/17). Samples from dogs (n=40) were collected between 2017 and 2018 in the Veterinary Hospital of the UFG, municipality of Jataí, located in the Goiás state, Brazil. CDV RNA was extracted with QIAamp viral RNA® and subsequently nested-RT-PCR were performed for the purpose of detection of the CDV nucleoprotein gene. The Lentz corpuscle survey consisted of blood smear on a glass slide for microscopy, with subsequent staining by Panotic® kit. Analysis of the slides was performed under a 100X objective optical microscope in immersion oil. Sensitivity of the Lentz bodies test was 77.7% (90% CI: 54-100), while the specificity was 74.2% (90% CI: 61-87). The proportion of agreement between the tests by Kappa index had a value of k= 42%. In the Kappa index scale this value is considered of regular agreement. Among the possible causes of false-negative number of Lentz bodies test are the high sensitivity/specificity of nested RT-PCR. While, false-positive number probably occurred due to the formation of artifacts in the staining of slides. These preliminary results demonstrated the highest possibility of occurrence of false positives in Lentz body inclusions research; however future studies must be conducted to better evaluate these hypothese.

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Palavras-chaves: Canine distemper virus, Canine morbillivirus, Paramixoviridae, Domestic dogs, Morbillivirus

PCV2a AND PCV2b DETECTION IN DOGS FROM A NON-VACCINATED PCV2 POSITIVE PIGS' HERD

Autores Camila Alves Ferreira [1], Victor Hugo da Silva [1], Paula Martins Uchoa de Sousa [1], Alessandra Marnie Martins Gomes de Castro [1]

Instituição [1] FMU - Faculdades Metropolitanas Unidas (Rua Ministro Nelson Hungria, 541 - São Paulo)

Resumo

Porcine circovirus type 2 (PCV2), discovered initially in 1998, has been associated with several disease manifestations in pigs denominated PCV2 associated disease. Recently, many researchers have revealed PCV2 could infect many other mammals like mice, calves, minks, dogs and goats. PCV2 has been currently classified (PCV2a - PCV2f), of which PCV2a, PCV2b and PCV2d are predominant. The current study aims to investigate PCV2a and PCV2b in feces samples of dogs from non-vaccinated PCV2 positive pigs' herd. The study was conducted in a PCV2-positive pig herd that has never used a PCV2-vaccine protocol. The herd had four dogs that always had access to installation and had contact with food, feces, oral fluids and mummified from the pigs' herd. A total of 15 fecal swabs were collected from four (C1 to C4) dogs that have access to the pig installations in four different moments (M1 to M4). The swabs were stored in 2 mL microtubes containing 1 mL sterile saline solution. All the samples were storage at -20 °C into DNA extraction that was performed using QIAamp DNA mini kit to quantify the genomic DNA copy numbers of PCV2 by SYBR green quantitative real-time PCR (qPCR). The reactions were performed in a final volume of 25 µL containing 5.0 µL DNA; 13 µL of TaqMan ™ Universal Master Mix II (Thermo Fischer Scientific, USA), 0.5 µL of each probe at 10 µM (PCV2a VIC-GGG GAC CAA CAA AAT CTC TAT ACC CTT T-MGBNF and PCV2b FAM- CTC AAA CCC CCG CTC TGT GCC C-QSY); 1 µL of 10 each primer at 10 µM (PCV2abF: 5 'GGCGGTGGACATGATGAGA 3' and PCV2abR: 5 'GCAGGGCCAGAATTCAACC 3') and sterile MilliQ water qsp. The amplifications conditions were 50°C for 2 minutes, 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute and run on a QuantiStudio3 (Applied Biosystems, USA). PCV2 cycle threshold values were converted into copy number per swabs using standard curve data. Samples with no signal by a cycle-threshold (CT) of 40 were considered negative (number of DNA copies < of the analytical sensitivity). A total of 3/12 samples were PCV2 positive, being two for PCV2a from the same dog (C1) in two different (M1 and M4) and one for PCV2b from dog C3 in one moment (M4). The viral load for PCV2 was of 30 copies of DNA/swabs and for PCV2b ranged from 41 to 51 copies of DNA/swabs. These findings suggested the possibility of PCV2a and PCV2b cross-species transmission in herds with high virus circulation.

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Palavras-chaves: Dogs, feces, Pig's herd, Porcine Circovirus, type 2

IMPROVING INFECTIOUS BRONCHITIS VIRUS ANTIGEN PROCEDURES FOR SEROLOGICAL ASSAYS

Autores MARIA ANGELA ORSI [1], ANA CRISTINA ALVES DE ALMEIDA [1], HIKARY MORIYAMA [1], ANA PAULA MORAES [1], LAÍS SANTOS RIZOTTO , PAULO VITOR MARQUES SIMAS [2], TÂNIA ROSÁRIA PEREIRA FREITAS [3], CLARICE WEIS ARNS [1]

Instituição [1] UNICAMP - DEPARTAMENTO DE GENÉTICA, EVOLUÇÃO, MICROBIOLOGIA E IMUNOLOGIA, INSTITUTO DE BIOLOGIA,UNIVERSIDADE ESTADUAL DE CAMPINAS (RUA MONTEIRO LOBATO, INSTITUTO DE BIOLOGIA, BLOCO E, CIDADE UNIVERSITÁRIA " ZEFERINO VAZ', BARÃO GERALDO, CAMPINAS-SPRArua rur), [2] EMBRAPA - EMBRAPA- SÃO CARLOS (SÃO CARLOS), [3] LFDA/MG - FEDERAL AGRICULTURAL DEFENSE LABORATORY (PEDRO LEOPOLDO- MINAS GERAIS)

Resumo

Infectious bronchitis virus (IBV) causes a highly contagious acute avian disease caused by a coronavirus of the family *coronaviridae*. Several serological methods are applied to determine the level of protection of vaccines and infections. The Hemagglutination Inhibition (HI) test should be applied to IBV differentiation (or subtype).

This study describes an improvement in the procedure for obtaining IBV antigens for the HI test. The Massachusetts-41 (M41) IBV strain was inoculated at 10^4 DIE_{50} into 11-day-old SPF chicken eggs incubated at 37°C. After 72h observing a typical lesion of IBV, allantoic-amniotic fluid (AAF) was collected, clarified at low-speed centrifugation at 1,100 X g for 30 minutes, titrated, filtered (0.22-µm) and kept at -80 ° C. The AAF was then performed to ultracentrifugation at 77,000X g for 2 hours. The pellet was submitted to two distinct treatments. Primarily, pellets with a 60% concentration of antigen was treated with 10 mL phosphate buffer [0.15 M pH 7.2], after than treated with 3 mL of two different solutions, Solution-A (0.01 M Tris HCL buffer - pH 6,4) and solution-B (NaCl, KCl, CaCl2, MgSO4). The second treatment (40% of concentrated virus) was performed to Solution-A, then added to Solution-A and B. Phospholipase C type 1 was added to the both treatments and reached the final concentration of 2.3 and 2.6 units/mL for each treatment. Then, those treatments were incubated for 37^{o}C for 45 minutes, fractionated and kept at -28^{o}C. The antigens prepared above were tested against homologous IBV-Mass-41, avian metapneumovirus, bursal Infectious virus, Newcastle diseases virus sera and human Ig. Our preliminary results showed that the Hemagglutination (HA) titer for each treatment reached 1: 2,048 and 1: 512, respectively. That means, the second treatment showed no advantage over the first treatment. The sensitivity of both antigens was 72.72%. In these assays, the Hemagglutination Inhibition (HI) test was performed without cross reactivity. The next step will be to evaluate the antigen produced in this study with variant IB-virus sera.

Financial support: CNPq, FAPESP, FUNCAMP AND IB-UNICAMP

Palavras-chaves: INFECTIOUS BRONCHITIS VIRUS, ANTIGEN , SEROLOGICAL ASSAYS

BIOCHEMICAL AND HEMATOLOGICAL PROFILE OF PREGNANT RHESUS MONKEYS (MACACA MULATTA) EXPERIMENTALLY INFECTED WITH ZIKA VIRUS AND TREATED WITH SOFOSBUVIR – A DESCRIPTIVE ANALYSIS

Autores Fernanda de Oliveira Bottino [1,2], Noemi Rovaris Gardinali [2], Juliana Gil Melgaço [2], Tatiana Kugelmeier [3], Gisela Trindade Freitas [4], Jaqueline Mendes de Oliveira [2], Marcelo Alves Pinto [2]

Instituição [1] EPSJV/ Fiocruz - Escola Politécnica de Saúde Joaquim Venâncio (Rio de Janeiro, Brasil), [2] IOC/ Fiocruz - Instituto Oswaldo Cruz (Rio de Janeiro, Brasil), [3] ICTB/ Fiocruz - Instituto de Ciência e Tecnologia em Biomodelos (Rio de Janeiro, Brasil), [4] Fiocruz - Bio-manguinhos (Rio de Janeiro, Brasil)

Resumo

Zika virus (ZIKV) infection is highly relevant for public health since a strong association between the occurrence of infection in pregnant women and congenital malformation. However, little is known about hematological and biochemical changes during ZIKV infection in pregnancy. Non-human primates may mimic both ZIKV infection and fetal neuropathogenesis. Our study aimed to perform a descriptive analysis of the biochemical and hematological profile of *Macaca mulatta* experimentally infected with ZIKV and treated with antiviral sofosbuvir (SOF). Four pregnant rhesus monkeys were inoculated subcutaneously with a ZIKV suspension containing 10^7 plaque-forming unit /mL and treated subcutaneously with SOF at 5 mg/kg/day for 15 days after 2nd day post-infection (dpi). Samples were collected from baseline (day 0) and 2, 4, 8 and 12 dpi, followed by biweekly samples until the end of pregnancy. Viral RNA isolation was confirmed by RT-qPCR. Biochemical and hematological analyses were performed by the commercial laboratory Laborlife Clinical Analyzes (Rio de Janeiro), including: aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), c-reactive protein (PCR), total cholesterol (TC), glucose (GLU), leukogram, erythogram and platelet count. Elevation in CK values in the acute phase of infection (2 dpi) were observed in two of four animals (AB28 e AA14). AST, ALT, TC and GLU values remained within the expected normal range for the species, with animals AB18 and AB28 showing, respectively, isolated increases of TC (2 and 8 dpi) and GLU (84 dpi). Anemia, characterized by hematocrit, red blood cell count and hemoglobin dosage decreased, was observed in all animals since the beginning of the experiment. Leukopenia and lymphopenia were also observed in all four animals, both of which were more pronounced during the acute phase. After this phase ends, eosinophilia was observed in three of four animals (AD18, AB28 and AB18). All animals had mild neutrophilia and monocytosis at baseline (day 0), showing no major changes during the experiment. These results suggest a cause-effect relationship between ZIKV infection during pregnancy and changes in biochemical and hematological parameters, such as CK, leukocytes, lymphocytes and eosinophils counts.

Palavras-chaves: Zika virus, Macaca mulatta, Sofosbuvir, Hematology, Biochemistry

IMMUNOGENICITY OF AN INACTIVATED VACCINE AGAINST BOVINE ALPHAHERPESVIRUS TYPE 5, ASSOCIATED WITH THE THERMOLABILE ENTEROTOXIN OF ESCHERICHIA COLI

Autores Cristina Mendes Peter [1], Matheus Iuri Fruhauf [1], Nadálin Yandra Botton [1], Lariane da Silva Barcelos [1], Rodrigo Bozembecker de Almeida [1], Marcelo de Lima [1], Silvia de Oliveira Hübner [1], Geferson Fischer [1]

Instituição [1] UFPel - Universidade Federal de Pelotas (Campus Universitário Capão do Leão, Prédio 1, Faculdade de Veterinária, Laboratório de Virologia e Imunologia)

Resumo

The mucosal immune system represents the initial barrier against several pathogens that use these surfaces as a gateway to the body, such as bovine alphaherpesviruses (BoHV), which use the mucous membranes, mainly nasal and genital, as the starting point of replication. The secretory immunoglobulin A (IgA) plays a fundamental role in the humoral immunity of mucosal surfaces through viral neutralization, an essential mechanism in the defense of the genital mucosa against genitally transmitted pathogens. Due to the great importance of mucosal pathways in BoHV transmission, interest in the development of vaccines that provide mucosal immunity against BoHV becomes evident. In the present study, experimental vaccines containing inactivated BoHV-5 associated with the subunit B recombinant of *Escherichia coli* thermolabile enterotoxin (rLTB) were made for intravaginal application. Thirty bovine females were divided into five groups: Placebo (PL: ova consisting of gelatin + E-MEM), Antigen (AG: gelatin + BoHV-5 + E-MEM), Adjuvant rLTB (rLTB100: gelatin + 100 µg / dose of rLTB + E-MEM), rLTB50 (rLTB50: gelatin + BoHV-5 + 50 µg rLTB + E-MEM) and rLTB100 (rLTB100: gelatin + BoHV-5 + 100 µg rLTB). Two doses of each vaccine were applied at 21-day intervals. The humoral (IgA and IgG) local (vaginal mucus and nasal swab) and systemic (serum) induced response in the inoculated animals was measured by indirect ELISA. Analysis of variance (ANOVA) was used to compare antibody levels in the ELISA test. As a result, the rLTB-containing vaccine at both concentrations was shown to increase IgA and IgG levels in the vaginal and nasal mucosa, and in animal serum (p

Financial Support: CAPES.

Palavras-chaves: Mucosal Immunity, BoHV-5, Cattle, IgA, IgG.

MOLECULAR INVESTIGATION OF THE PRESENCE OF FELINE PARAMYXOVIRUS RNA IN KIDNEYS OF DOMESTIC CATS FROM CUIABÁ, MATO GROSSO

Autores Káryta Maria de Lima Bezerra Bertti [1], Gabriela Molinari Darold [1], Glaycenyra Cecília Pinheiro da Silva [1], Jordan Gregorio Lucca Cardoso [1], Michele Lunardi [1]

Instituição [1] UNIC - Universidade de Cuiabá (Avenida Manoel José de Arruda, 3100 - Bairro Jardim Europa - Cuiabá, Mato Grosso)

Resumo

Paramyxoviruses are enveloped, single-stranded, negative RNA viruses, which have been previously identified in a wide variety of vertebrate hosts. Feline morbillivirus (FeMV) is an emerging member of *Morbillivirus* that was first discovered in Hong Kong in 2012. Recently, its circulation was also demonstrated within cat populations from Japan, Italy, Germany, USA, Turkey, and Brazil. Upon discovery, the presence of FeMV[ML1] was associated with histopathologically confirmed tubulointerstitial nephritis (TIN) and chronic kidney disease (CKD) in cats, however the possible role of the virus as a triggering event of a disease with major systemic changes such as CKD is still uncertain. Moreover, data on the correlation between CKD and FeMV infection are insufficient and have not yet been investigated in Brazil. Thus, the aim of this study was to investigate through RT-PCR the presence of feline paramyxovirus infection in renal tissues of domestic cats, which died due to several causes, in Cuiabá, Mato Grosso state. Kidney samples of 62 domestic cats that died in veterinary clinics and at the Veterinary Hospital of the University of Cuiabá, were evaluated for presence of FeMV RNA by reverse transcription followed by semi-nested PCR assay, in order to amplify a partial fragment of the paramyxoviral L gene. Only five out of the 62 evaluated cats (8,0%) had the presence of the viral RNA in renal tissue. Data of this study showed an overall prevalence of FeMV similar to previously reported frequencies observed in other geographic regions, which ranged from 7 to 44%. This result may be due to sampling from cats that died from various causes, unlike other studies using samples from cats with some nephropathy or lower urinary tract diseases. Nevertheless, the pathogenicity of FeMV is not clear yet due to paucity of isolation of viral strains from diverse geographical regions and the chronic nature of involved diseases, thus requiring further studies to better evaluate the pathogeny of FeMV and establish if this virus is associated with other diseases. Our results confirm the circulation of the FeMV within domestic cats and this is the first study to show the presence of FeMV infection in kidneys of cats in Brazil.

Financial Support: University of Cuiabá (UNIC)

Palavras-chaves: Brazil, Chronic kidney disease, Feline morbillivirus, RT-PCR

THE FIRST REPORT OF CANINE MORBILLIVIRUS INFECTION IN GIANT ANTEATER (MYRMECOPHAGA TRIDACTYLA) IN BRAZIL

Autores: Melissa Belizário [1], Mayara Lima Kavasaki [1], Adriane Jorge Mendonça [1], Marisol Alves de Barros [1], Thais Oliveira Morgado [1], Carolina Fontana [1], Daniel Moura de Aguiar [1], Mateus de Assis Bianchini [1], Aneliza de Oliveira Souza [1], Amanda Raiza Gonçalves Lima Oliveira Santos [1], Edson Moleta Colodel [1], Lucas Avelino Dandolini Pavelegini [1]

Instituição [1] UFMT - Universidade Federal de Mato Grosso (Av. Fernando Corrêa da Costa, nº 2367 - Bairro Boa Esperança. Cuiabá - MT - 78060-900), [2] UNIC - Universidade de Cuiabá (Av. Manoel José de Arruda, 3100 - Jardim Europa, Cuiabá - MT, 78065-700)

Resumo

Canine Distemper (CD) is a multisystemic and contagious disease caused by Canine Morbillivirus (CDV), an enveloped RNA virus that replicates in epithelial, nervous and lymphoid tissues; it is released in urine, feces, saliva, oral and nasal secretions and its major infection route is from respiratory system. Beside dogs, the disease and natural hosts of CDV include species of wild terrestrial carnivores. The present study reports the natural infection of *Myrmecophaga tridactyla*, a threatened species in Brazil named the Giant anteater, that was maintained hospitalized due to health problems in consequence of mistreatment. During a hematological exam, the presence of Lentz corpuscles were visualized in leukocytes. Posterior analysis of CDV rapid test combined with RT-PCR of N and H genes sequencing and pathological findings confirmed infection by CDV. The consensus sequence generated from the *N* gene amplicon was deposited in the GenBank under the accession number MK552116. Phylogenetic tree of H gene was inferred by the Neighbor-Joining method and our sequence fell into a clade composed of other genotypes classified as South America isolates. The sequence of the *H* gene generated in this study was deposited in GenBank under accession number MN208239. Macroscopy alterations were skin hyperkeratosis and evidence of lobular septa in lung. Histopathologic findings were eosinophilic intracytoplasmic and intranuclear inclusion corpuscles in urinary bladder, kidney, lung, stomach, duodenum and jejunum tissues' cells. Both macroscopy and microscopy alterations observed during necropsy were related to CD disease. Lentz corpuscles usually appears in the early stage of CD with the firsts evidence of infection which is associated with prostration, anorexia, diarrhea, nasal and ocular secretion that started after hospitalization. The results confirmed the first report of CM in a threatened species occurring in the American continent, *Myrmecophaga tridactyla*. This is the second report of CDV infection in the order Pilosa and family *Myrmecophagidae* in the Midwestern region of Brazil. Financial support: Ministry of Education of Brazil (MEC), Federal Agency for the Support and Improvement of Higher Education (CAPES) and National Council for Scientific and Technological Development (CNPQ).

Palavras-chaves: Canine distemper, Infection, wild life, sequencing, RT-PCR

PCR SURVEY OF BOVINE ALPHAHERPESVIRUS 1 DNA IN SEMEN FROM BULLS FROM MATO GROSSO STATE

Autores Raphael Campos Quinteiro [1], Sandro Ribeiro da Costa [1], Gabriela Molinari Darold [1], Glaucenyra Cecília Pinheiro da Silva [1], Jordan Gregorio Lucca Cardoso [1], Michele Lunardi [1]

Instituição [1] UNIC - Universidade de Cuiabá (Rua Manoel José de Arruda, 3100, Jardim Europa, Cuiabá - MT. CEP: 78065-900)

Resumo

Bovine alphaherpesvirus 1 is regarded as one of main viral pathogens associated with negative impacts on reproduction of both beef and dairy cattle. Clinical consequences of this viral infection can occur after acute infection as well as when viral recrudescence takes place after a period of viral latency. Infected bulls usually excrete virus in the semen specially when experiencing the genital disease known as infectious pustular balanosposthitis. In infected cows, endometritis, infertility, abortions, and occasional birth of stillborn or weak calves may be seen. In order to study the frequency of excretion of bovine alphaherpesvirus 1 in semen from bulls from beef and dairy herds of Mato Grosso state, 99 animals aging ³ 24 months from eight different cattle herds, without history of reproduction failure and specific vaccination, belonging to eight municipalities, located in six out of seven macroregions of the Mato Grosso state, had an aliquot of fresh semen evaluated for the presence of viral DNA by using PCR. Total DNA was extracted with QIAamp DNA mini kit (Qiagen), following manufacturer's instructions. To amplify a partial fragment of glycoprotein C gene of bovine alphaherpesvirus 1, a PCR assay employing Platinum *Taq* DNA polymerase (Invitrogen) and the primer pair B1/Bcon was carried out. Despite the amplification of a PCR product with the expected molecular size in some semen samples, through direct sequencing of obtained amplicons the purified DNA was shown to be a result of inespecific amplification. In this investigation, excretion of bovine alphaherpesvirus 1 through semen was not observed in the bulls evaluated. Since we collected only one aliquot of semen for each bull included in this study, it is important to highlight that it is not possible to exclude presence of infection in its latent form. Once bovine alphaherpesvirus 1 can be spread by breeding infected bulls through semen, knowing the frequency of viral excretion in this body fluid of bulls from Mato Grosso is of importance so measures of prevention and control for this specific viral pathogen can be implemented to avoid or limit reproductive losses in breeding female cows.

Financial Support: University of Cuiabá (UNIC)

Palavras-chaves: brazil, cattle, glycoprotein C, PCR, reproduction

CLINICAL AND LIVER HISTOLOGICAL FINDINGS OF A CHRONIC EQUINE HEPACIVIRUS (HEPACIVIRUS A, EQHV) INFECTED HORSE

Autores: Caroline Roberta Soares Salgado [1], Fernanda Nascimento de Godoi [6], Tatianne Leme Oliveira Santos Godoi [7], Bruno Gonçalves de Souza [4], Cassia Maria Molinaro Coelho [4], Cristiane Divan Baldani [5], Anna Paula Balesdent Barreira [3], Leonardo Rodrigues de Lima [3], Renato Sergio Marchevsky [2], Fernando Queiroz de Almeida [3], Marcelo Alves Pinto [1], Andreza Soriano Figueiredo [1]

Instituição: [1] LADTV - Laboratório de Desenvolvimento Tecnológico em Virologia,Instituto Oswaldo Cruz (Av. Brasil, 4365- Manguinhos, Rio de Janeiro-RJ), [2] LANEU - Laboratório de Neurovirulência, Biomanguinhos/Fiocruz (Av. Brasil, 4365- Manguinhos, Rio de Janeiro-RJ), [3] UFRRJ - Instituto de Veterinária,Universidade Federal Rural do Rio de Janeiro (Rodovia BR465, Km 07, s/n Zona Rual-Seropédica, RJ), [4] UFRRJ - Hospital Veterinário, Universidade Fedral Rural do Rio de Janeiro (Rodovia BR465, Km 07, s/n Zona Rual-Seropédica, RJ), [5] UFRRJ - Laboratório de Patologia Clínica, Universidade Fedral Rural do Rio de Janeiro (Rodovia BR465, Km 07, s/n Zona Rual-Seropédica, RJ), [6] UFRRJ - Instituto de Zootecnia, Universidade Federal Rural do Rio de Janeiro (Rodovia BR465, Km 07, s/n Zona Rual, Seropédica), [7] UFRRJ - Sede Administrativa da Fazenda Universitária, Universidade Federal Rural do Rio de Janeiro (Rodovia BR465, Km 07, s/n Zona Rual, Seropédica)

Resumo

The equine hepacivirus (EqHV, *hepacivirus A*) is the closest genetic relative to hepatitis C virus (HCV) and also shares pathogenic similarities. Infection with both hepatotropic viruses may evolve to clearance or chronicity, but unlike HCV, a few equines become chronic infected. The clinical and pathological consequences of the chronic EqHV infection are not well established with limited data available from experimental infection studies. The elucidation of the equine infection characteristics is important to help veterinary clinicians facing this new pathogen and is an interesting comparative model of hepatitis C. The aim of this study was to evaluate the clinical and histological aspects of an EqHV chronically infected horse by analyzing viral load, serum biochemical alterations and the presence of liver damage. A male 10-years-old gelding horse from Rio de Janeiro state, positive for EqHV RNA since 2014 was subjected to blood collection and ultrasound-guided liver biopsy. Blood count, serum levels of liver associated proteins, serum viral load, Hematoxylin and eosin stain (H&E) performen on ultrasound-guided liver biopsies and liver ultrasound images were obtained in June 2018. Liver associated proteins were slightly above reference values in serum: AST 370 IU/L (<366 IU/L), GGT14 IU/L (<13.4 IU/L), total bilirubin 2.8 mg/dL (<2.0 mg/dL), conjugated bilirubin=0.46 mg/dL (<0.4 mg/dL) and unconjugated bilirubin2.34 mg/dL (<2.0 mg/dL). Serum viral load was ≤8.0E+3 copies/mL. Ultrasound examination revealed the liver to be isoechoic to the spleen, which may suggest a diffuse hyperechogenicity of the liver. This can be associated to chronic hepatic changes, but ultrasound-guided biopsy gives more sensitive and specific information in diffuse lesions. H&E staining of the liver tissues showed the presence of focal discrete lymphocyte infiltrates, areas of anucleated hepatocytes, ballooning and fibrosis. There were no alterations in hematological parameters., The results demonstrate a chronic course of EqHV infection of at least 4 years presenting low viral load with mild clinical and histological alterations.

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Palavras-chaves: Equine Hepacivirus, Chronic infection, Histopathology, Clinical aspects

FIRST DESCRIPTION OF THEILER'S DISEASE ASSOCIATED VIRUS (TDAV) IN BRAZIL

Autores Maria Vitória dos Santos de Moraes [1], Caroline Cordeiro Soares [2], Flávia Lowen Levy Chalhoub [3], Ana Maria Bispo de Filippis [3], Debora Regina Lopes dos Santos [4], Fernando Queiroz de Almeida [4], Tatianne Leme Oliveira Santos Godoi [5], Aline Moreira de Souza [6], Tatiana Rozental Burdman [7], Elba Regina Sampaio de Lemos [7], Jenner Karlisson Pimenta dos Reis [8], Oswaldo Gonçalves Cruz [9], Marcelo Alves Pinto [1], Andreza Soriano Figueiredo [1]

Instituição [1] LADTV - Laboratório de Desenvolvimento Tecnológico em Virologia, Instituto Oswaldo Cruz, Fiocruz (Avenida Brasil, 4365 - Manguinhos - Rio de Janeiro - Brasil), [2] LVM - Laboratório de Virologia Molecular, Instituto Oswaldo Cruz, Fiocruz (Avenida Brasil, 4365 - Manguinhos - Rio de Janeiro - Brasil), [3] LF - Laboratório de Flavivírus, Instituto Oswaldo Cruz, Fiocruz (Avenida Brasil, 4365 - Manguinhos - Rio de Janeiro - Brasil), [4] UFRRJ - Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro (Rodovia BR 465, Km 07, s/n Zona Rural, Seropédica - Rio de Janeiro - Brasil), [5] UFRRJ - Coordenação de Produção Integrada ao Ensino, Pesquisa e Extensão, Reitoria, Universidade Federal Rural do Rio de Janeiro (Rodovia BR 465, Km 07, s/n Zona Rural, Seropédica - Rio de Janeiro - Brasil), [6] UFF - Laboratório de Pesquisa Clínica e Diagnóstico Molecular Professor Marcílio Dias do Nascimento, Departamento de Patologia e Clínica Veterinária, Faculdade de Veterinária, Universidade Federal Fluminens (Av. Alm. Ary Parreiras, 507 - Icaraí, Niterói - Rio de Janeiro - Brasil), [7] LHR - Laboratório de Hantaviroses e Rickettsioses, Instituto Oswaldo Cruz, Fiocruz (Avenida Brasil, 4365 - Manguinhos - Rio de Janeiro - Brasil), [8] UFMG - Laboratório de Retroviroses, Escola de Veterinária, Universidade Federal de Minas Gerais (Av. Pres. Antônio Carlos, 6627 - Pampulha, Belo Horizonte - Minas Gerais - Brasil), [9] PROCC - Programa de Computação Científica, Fiocruz (Avenida Brasil, 4365 - Manguinhos - Rio de Janeiro - Brasil)

Resumo

Theiler's disease associated virus (Pegivirus D, TDAV) is a newly described member of the Pegivirus genus (Flaviviridae family) infecting horses. TDAV was described as the causative agent of an acute hepatitis outbreak (also known as Theiler's disease) in the USA, when 4 horses presented elevated hepatic enzymes with icterus, lethargy, hyporexia and photodermatitis, while another 4 horses presented elevated hepatic enzymes without clinical manifestations. Since then, TDAV has been detected as a contaminant of equine-derived serum, but not in the horse population. The aims of this study were to investigate the presence of TDAV in Brazil, to evaluate possible risk factors, presence of liver damage and genetic variability of virus. Study population comprised 500 horses from Rio de Janeiro, Mato Grosso do Sul, Minas Gerais and Espírito Santo states. Information such as age, sex, breed, activity, geographical location and management system were recorded for epidemiological analysis. TDAV was detected by real time RT-PCR using DNA intercalating SYBR Green with primers directed to 5'NC region. RT nested]PCR and semi]nested PCR directed to NS3 region were performed for phylogenetic analysis. For clinical biochemistry analyses, serum was tested in automated analyzer to determine AST, GGT and GLDH levels in either RNA positive or negative horses. To find possible risk factors associated with TDAV infection, univariate and multivariate logistic regression analyses were performed. Prevalence was 1.6% (8/500), detected only in Rio de Janeiro state, in 5 of 6 mesoregions (Metropolitan, Central, North, Northwest and Coastal Lowlands, South being the exception). The age ranged from 3 months to 12 years, but the majority were Sequencing and phylogenetic analysis were performed together with all TDAV nucleotide sequences available in the GenBank. The nucleotide genetic distance within the Brazilian isolates was 7.9%, while within Brazilian isolates and the commercial serum isolates was 11.1%. Tree topology demonstrated the formation of two strongly supported

clades. None of the horses had serum biochemical alterations. In this study we demonstrated for the first time the detection of TDAV RNA-positive horses outside the USA without any clinical signs. Infection was not statistically associated with any of the analyzed variables. TDAV has similar circulating isolates worldwide.

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Palavras-chaves: Theiler's disease associated virus, Prevalence, Risk factors, Phylogenetic analysis

IDENTIFICATION OF CLADE E AVIPOXVIRUS IN BRAZIL

Autores Leonardo Clasen Ribeiro [1], Francielle Liz Monteiro [1], Domitila Brzoskowski Chagas [1], Tamires Ellen Tomio [1], Marcelo de Lima [1], Geferson Fischer [1], Gilberto D'Ávila Vargas [1], Silvia de Oliveira Hübner [1]

Instituição [1] UFPel - Universidade Federal de Pelotas (Campus Universitário Capão do Leão, Prédio 1, Faculdade de Veterinária, Laboratório de Virologia e Imunologia.)

Resumo

Avipoxviruses (APVs) cause fowlpox (FP) disease which results in significant economic losses in domestic poultry due decreased egg production, reduced growth, and increased mortality. APVs are enveloped viruses with a double-stranded DNA genome, belonging to the family *Poxviridae*. Three main clades (A to C) are differentiated into APVs, represented by Fowlpox virus (Clade A), Canarypox virus (Clade B) and Psitacinepox virus (Clade C). Two additional clades (D and E) were also proposed. The constant monitoring of the evolution of APVs with the genetic characterization of new isolates is an important approach to study an eventual outbreak of the disease and the evolutionary biology of these viruses. Thus, the objective of this study is to report the identification of clade E *Avipoxvirus* in Brazil. The sample was obtained from a periocular tumor-like skin lesion found in a young domestic fowl *(Gallus gallus domesticus).* The lesion was nodular, round shaped, crusty and blackened. The bird was kept in a backyard system with thirty animals of the same species, without any vaccination protocol or zootechnical and sanitary control. DNA was extracted from frozen tissue and submitted to the polymerase chain reaction (PCR), targeting the polymerase gene and P4b. Phylogenetic analysis was conducted by the MEGA 6.0, using the *neighbor-joining* method, and the evolutionary distances were computed using the *Kimura 2-parameter* model. The *Avipoxvirus* DNA was detected in the tissue sample collected from a domestic fowl. Phylogenetic analysis revealed that the amplified segment of the P4b and polymerase gene clustered in clade E. The sequence's similarity with APV isolated in Hungary in 2011, and Mozambique in 2016, was 99.2%. The late detection and description of this clade in the country could be, at least in part, due to the recent and scarce molecular characterization studies of APVs in Brazil. Furthermore, APVs are known to infect different species of wild birds, many of which are migratory. Thus, the introduction of new viruses through migratory wild birds is also a possibility. However, these hypothetical scenarios must be confirmed by further studies. Finally, epidemiologic monitoring of APV in domestic and wild-living birds is necessary for a better understanding of many aspects related to the occurrence, host range and genetic diversity of APVs in Brazil.

Financial Support: CAPES.

Palavras-chaves: APV, domestic fowl, fowlpox, P4b gene, polymerase gene