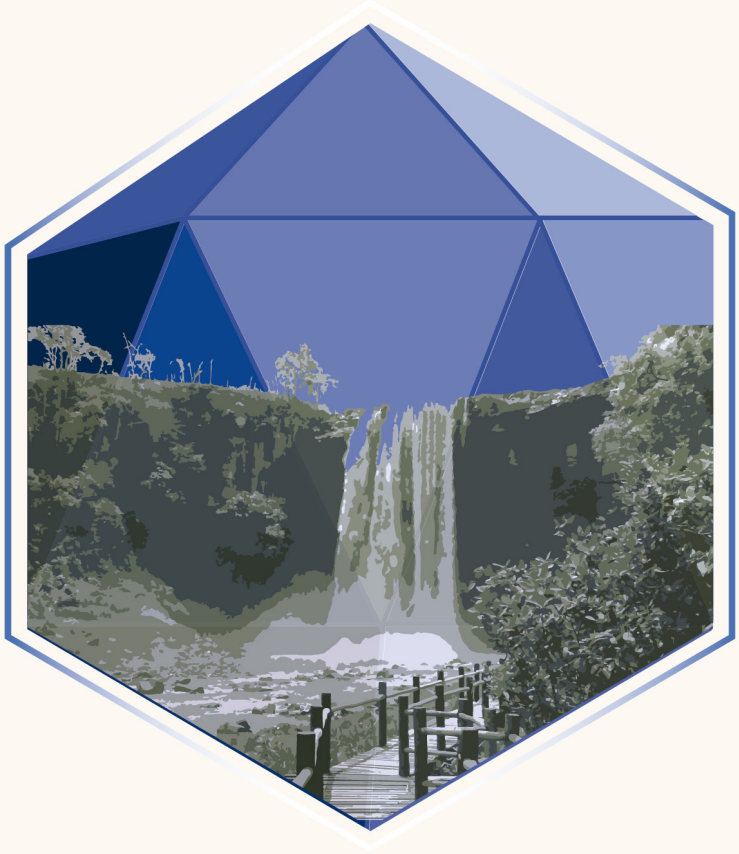
ISSN 519 - 2563

VIRUS Reviews and Research



XXVII Congresso Brasileiro de Virologia

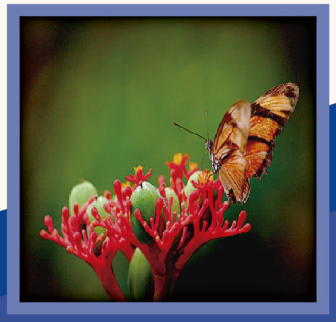
XI Encontro de Virologia do Mercosul

18 a 21 de setembro de 2016

PIRENÓPOLIS • GO

POUSADA DOS PIRENEUS RESORT

sbv.org.br/congresso



Vol 20 (2), August-December 2016

JOURNAL OF THE BRAZILIAN SOCIETY FOR VIROLOGY

Virus Reviews and Research

Journal of the Brazilian Society for Virology

Virus Reviews & Research Vol 20 (2), August-December 2016

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

Editors
Edson Elias da Silva
Fernando Rosado Spilki

Address
Universidade Feevale, Instituto de Ciências da Saúde
Estrada RS-239, 2755 - Prédio Vermelho, sala 205 - Laboratório de Microbiologia Molecular
Bairro Vila Nova - 93352-000 - Novo Hamburgo, RS - Brasil
Phone: (51) 3586-8800
E-mail: F.R.Spilki - fernandors@feevale.br
http://www.vrrjournal.org.br

PBS (1020% w/v) and RNA reverse transcription was conducted using MMLV® (Invitrogen). The positive samples were sequenced in the automated sequencer ABI 3500 (Applied Biosystems®). Electropherogram quality analysis and the consensus sequence were performed using Phred and CAP3 software (http:// asparagin.cenargen.embrapa.br/phph/). Similarity searches were conducted against sequences deposited in GenBank using the BLASTn (http: //www.ncbi. nlm. nih.gov/BLAST/). Phylogenetic trees were generated by neighborjoining method with the Kimura 2parameter model using the Mega 6.0 software. Bootstrap values for phylogenetic trees were determined using 1000 replicates. Of the 89 samples tested, 7 (7.56%) were positive for the ORF1 fragments. The sequences confirmed that all of the samples identified in the present study were classified as genotype 3. Detection of HEV in stool samples of swine feces by RTPCR shows that there may be a direct or indirect human exposure to the agent and suggests that there may be an endemic circulation of HEV in pig farms.

VV260 - ANALYSIS OF SINGLENUCLEOTIDE POLYMORPHISMS IN THE APOBEC3Z3 GENE IN NATURALLY FELINE IMMUNODEFICIENCY VIRUS INFECTED DOMESTIC CATS

Franco, A.C.; Cano, L.; Costa, C.; Duda, N.C.B.; Firpo, R.M.; Nunes, R.; Finoketti, F.; Correa, R.; Roehe, P.; Amorim, F.

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Feline immunodeficiency virus (fiv) is a widely distributed retrovirus that infects domestic cats (felis catus) and other members of the felidae. fiv infections are counteracted by different immune mechanisms, including the restriction factors, which are proteins that have the ability to hamper retroviruses' replication and are part of the conserved mechanisms of anti viral immunity of mammals. the apobec3 or a3 proteins are the most studied class of restriction factors. such proteins are cytidine deaminases that generate hypermutations in provirus dna during reverse transcription, thus causing hypermutations in the viral genome, hampering virus replication. the feline genome contains four a3h genes named a3z2aa3z2c and a3z3. in addition to these, a fifth transcript, designated a3z2z3, is expressed by readthrough alternative splicing, and its product was also shown to restrict feline retroviruses. in other mammals, a3h singlenucleotide polymorphisms (snps) was shown to alter the stability and cellular localization of the encoded protein, thus influencing its subcellular localization and reducing its anti viral effect. thus, it might be possible that a3h variants would confer different degrees of susceptibility to fiv infections in cats. however, little is known on the genetic variability of a3 genes in domestic cats. the aim of this study was the investigation of the variability of a3h in naturally fiv infected cats. dna obtained from whole blood of 27 fiv positive cats were used as template to amplify a region of a3h that was previously shown to display polymorphisms in the cat population. following the sequencing of the amplified region, the samples were classified in the haplotypes i, ii, iii and iv. twenty one samples showed the a65s snp, of these 18 were heterozygous and 3 homozygous for this polymorphism. this snp was previously associated with susceptibility to the infection. our results indicate that, as previously shown in other mammals, there is variability in a3h genes among the population of domestic cats, which could contribute to susceptibility of domestic cats to retroviral infections; however, these results should be confirmed by more extensive analysis and in vitro experiments.

VV261 - COMPLETE GENOME SEQUENCE OF AN EQUINE INFECTIOUS ANEMIA VIRUS FROM BRAZIL

Araujo Jr., J.P.; Malossi, C.D.; Fioratti, E.G.; Lima, M.F.N.T.; Aguiar, D.M.; Ullmann, L.S.

1. *EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA*
2. *UNIVERSIDADE FEDERAL DO MATO GROSSO*
3. *UNIVERSIDADE ESTADUAL PAULISTA*

Equine infectious anemia virus (EIAV) is a persistent lentivirus that causes equine infectious anemia (EIA). All of the complete genomic sequences published from field virus are from North America, Asian and Europe, and only proviral genomic sequences are available. In Brazil, EIAV is endemic in Pantanal region and euthanasia is not mandatory in these areas. Only the gag sequence is currently available from the Brazilian virus. This study aimed to sequence EIAV's genomic RNA for the first time in naturally infected horses. Plasma of an infected horse from Mato Grosso State was collected.

Total RNA was extracted and used to prepare the dsDNA library with the kit Strand Specific RNA Library Prep (Agilent Technologies). The library was quantified by Illumina Library Quantification kit (Kapa Biosystems) and sequenced with the NextSeq System (Illumina Inc.). Geneious R6 was used to analyze the sequences, using the map to reference tool with complete EIAV genome from isolate DV103(accession no. HM141910). Then, primers were designed to cover the gaps of the consensus sequence, and these PCR products were sequenced by Sanger protocol in a 3500 platform (Applied Biosystems). A new alignment with all the sequences (12 sequences obtained by Sanger and 9,185,813 reads obtained by Illumina) generated a consensus sequence of 7,591 bp in length, presenting 94% of coverage. This isolate has just 82% of nucleotide sequence identity with the main field strains, like EIAV Liaoning (AF327878), Wyoming (AF033820), and Ireland isolates (JX480631JX480634). Furthermore, phylogenetic studies using EIAV sequence against known viral strains of EIAV strongly suggests this isolate comprise a separate monophyletic group. With these results it is possible to better characterize the virus circulating in Brazil and to cope with the challenges of the EIAV diagnosis in Brazil.

VV262 - DETECTION OF THE CURRENT CIRCULATING EQUINE INFECTIOUS ANEMIA VIRUS IN BRAZIL BY QUANTITATIVE PCR

Araujo Jr., J.P.; Malossi, C.D.; Lima, M.F.N.T.; Aguiar, D.M.; Ullmann, L.S.;

1. *UNIVERSIDADE ESTADUAL PAULISTA*
2. *EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA*
3. *UNIVERSIDADE FEDERAL DO MATO GROSSO*

Equine infectious anemia virus (EIAV) is a lentivirus that causes equine infectious anaemia (EIA), a persistent viral infection. The recommended diagnosis method is either agar gel immunodiffusion (AGID) or enzyme linked immunosorbent assay (ELISA). The PCR described by OIE to detect EIAV does not amplify the virus currently circulating in Brazil due to the higher genetic variation. A modification in the NestedPCR protocol to detect an Asian EIAV is used in some Brazilian labs, but some serologically positive samples test negative in the SemiNestedPCR modified. In order to find a more sensitive and more specific molecular technique to detect the viral Brazilian strain, we designed a quantitative PCR based on EIAV sequences from Mato Grosso Brazil obtained in our lab to detect the current circulating virus. The designed qPCR primers amplify a 71 bp product from the 5' LTR region. GoTaq® qPCR Master Mix (Promega) was used in the reaction plus 5 pmol of each primer, 4 uL of gDNA sample (about 100 ng) and nuclease free water to 20 uL. The thermocycling program set up in a 7500 Fast qPCR (Applied Biosystems) was 1 cycle at 95ºC for 10 min, 40 cycles at 95ºC for 15 sec and 60 ºC for 1 min, followed by the standart melting curve. Eighty eight equine sera samples were tested in AGID or ELISA and their gDNA extracted from whole blood was tested with the SemiNestedPCR and qPCR. Equine GAPDH was used in the samples as a control gene and all amplified. Thirty horses were positive in AGID or ELISA, and although 16 were positive both in the SemiNestedPCR and in the qPCR, not the same samples amplified. This result shows that the reactions have different specificity. A test performed with a serially diluted positive sample indicates the same sensibility in both reactions. A qPCR using cDNA of some positive samples had a good amplification too, and a next step is use this reaction for a viral quantification. With these results we concluded that the two reactions can be used in a set to a better detection of the virus circulating in Brazil and maybe to help to classify animals in viremia.