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Avian metapneumovirus (AMPV) is responsible for major economic losses in domestic poultry throughout the world. The most severe clinical effects of infection are seen in turkeys, but there are significant economic losses in commercial chickens where it has also been implicated in swollen head syndrome. AMPV is a member of the family *Paramyxoviridae*, subfamily *Pneumovirinae* and genus *Metapneumovirus*. AMPV have been classified into four subgroups, A thought to D. AMPV subgroups A and B were first detected in 1978 in South Africa and where subsequently found in Europe, Israel Asia and America. AMPV subgroup C was first detected in the USA in 1996, subgroup D was first detected in France. Serological evidence indicated that AMPV is widespread in broilers, broiler breeder and layer flocks from different regions of Brazil, and the virus was first isolated in 1995. The aim of this work was the cloning of the glycoproteins G and F from a Brazilian isolate AMPV (antigenic subgroup A) in a prokaryotic system to proceed the sequencing of larger genomic fragments. We would like to examine, there are mutations on the G an F protein gene. The Brazilian strain AMPV-669-BR, was propagated in CER cells (Chicken related embryo), following routine protocols, and titrated by visualization of syncytia formation. The RNA was extracted using a High Pure Viral Nucleic extraction kit™ (Roche). cDNA was synthesized using a High capacity cDNA kit (Applied Biosystems™). The cloning of PCR product G and F genes were performed by using the plasmid vector pCR®8/GW/TOPO® TA Cloning® (Invitrogen Ltd., California, USA), procedures were performed according to the manufacturer's instructions. The confirmation the identity and orientation inserts, the PCR products and pTOPOG and pTOPOF were sequenced using specific primers G and F genes and primers M13 vector, were sequenced three times each, both in forward and reverse directions using ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems™). Obtained nucleotide sequences were compared with homolog sequences deposited at *GenBank* using the program NCBI BLAST N (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The similarity sequences have demonstrated the higher degree homology to G protein gene and to F protein gene, thus confirming the identity of the target genomic inserts. The clones generated here may be used in further studies regarding the biological effects of those proteins both *in vitro* and *in vivo*.

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408 - ELISA RGP90 AS AN ALTERNATIVE METHODOLOGY TO DIAGNOSE EQUINE INFECTIOUS ANEMIA IN SOUTHERN PANTANAL, BRAZIL

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As the official diagnostic test for equine infectious anemia (EIA), Brazilian governmental policy for prevention and control of EIA determines to be used the agar gel immunodiffusion (AGID) test with p26 core protein as antigen. The World Organisation for Animal Health (OIE) states that AGID and enzyme-linked immunosorbent assays (ELISAs) are both accurate and reliable tests, classifying the later as alternative tests. Internationally, there are four ELISAs available, three using p26, and one p26 and gp45, the viral transmembrane antigen. Brazilian researchers developed an indirect ELISA with a recombinant glycoprotein of the viral surface, the rgp90. Antibodies against the gp90 are usually detected before the antibodies against p26, and when the viral replication levels are extremely low. The aim of this preliminary work was to compare the performance of the ELISA rgp90 with the officially accepted AGID p26, in samples of equidae from Pantanal. Serum samples of 173 working horses and 24 working mules from 11 farms of the Pantanal sub-regions of Paraguai, Paiaguás and Nhecolândia were submitted to both tests. Nine (5.2%) equine and one (4.2%) mule samples were in the undetermined range of ELISA rgp90, testing all ten positive in the AGID. Prevalence rates in equines were 58.4% (n = 101) in both tests, though 2.9% (n = 5) were positive by AGID and negative by ELISA rgp90, while other 2.9% (n = 5) were negative in the first and positive in the second assay. In mules, prevalence rates were 45.8% (n = 11) by AGID and 37.5% (n = 9) by ELISA rgp90. For equines and mules, respectively, the copositivity rates were 95.0% and 81.8%, conegativity rates were 92.1% and 100.0% and agreement rates were 93.9% and 91.3%. Samples positive by ELISA rgp 90 and negative by AGID could reflect recent infection. Since the used rgp 90 is based in a sequence from the prototype strain of the EIA virus, one reasonable explanation for samples negative by ELISA rgp 90 and positive by AGID would be a mutation of the *env* gene in the local strains. The obtained results, and its sensitivity and facility to process several samples in a short period of time, indicate that the ELISA rgp 90 is a good alternative methodology to be employed in the routine screening of herds tested

once by AGID and that intend to keep the status of controlled for EIA.
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409 - OROPOUCHE VIRUS: SEROLOGIC EVIDENCE OF RECENT CIRCULATION IN ALTAMIRA, PARÁ

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The Oropouche virus (OROV; *Bunyaviridae*, *Orthobunyavirus*) is a very important arbovirus that causes a public health threat in tropical and subtropical areas of Central and South America where more than a half million persons have been infected. It causes in human an acute febrile illness (Oropouche Fever) accompanied mainly by headache, myalgia, photophobia, retrobulbar pain, and other systemic manifestations. Some days after the end of the fever episode, it is common the resurgence of the symptoms, including fever, however in general with lower intensity. Rarely can appear asseptic meningitis. In the Brazilian Amazon Region, the OROV is being detected since 1960 years, especially in Pará State. This study aimed to investigate the OROV circulation in Altamira, Pará, by the detection of IgM antibodies by immunoenzimatic assay (ELISA-IgM) and detection of total antibodies by Hemagglutination Inhibition test (HI). Serum Samples of 44 patients, collected between December/2008 until May/2009, were processed by the ELISA-IgM for OROV, and 29 (65.9%) were positives. Of the 44 samples, 27 were processed by the HI and in 16 (59.3%) patients, with IgM antibodies for OROV, presented HI antibodies too. The detection of antibodies IgM in Altamira, located in the southeast region of Pará state, revealed OROV's recent circulation in that municipality. The prevalence of HI antibodies for OROV is common in Pará's population, where the wide dispersion and circulation of the OROV is currently associated with cases or outbreak reported in whole state.
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410 - ESTABLISHMENT OF BOVINE CELLS EXPRESSING THE V PROTEIN OF SIMIAN VIRUS 5

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Cells have evolved many diverse mechanisms of innate and acquired antiviral responses to combat virus infection. One of the most important innate antiviral response mechanisms is the production and secretion of interferon (IFN) and the subsequent paracrine activation of signaling pathways via IFN receptors. Simian Virus 5 (SV5) encodes V protein which blocks IFN signaling pathway by targeting the signal transducer and activator of transcription (STAT 1) for proteasome degradation. The goal of this work was to produce bovine cells blocked in the IFN signaling in order to have a tool to study virus-host interactions on the level of innate immune response. To this goal, the V gene of SV5 was inserted in the genome of the bovine cells Madin-Darby Bovine Kidney (MDBK) and cells resistant to infection with BVDV (CRIB). In order to isolate cell clones stably expressing the protein of interest, we used a vector that allows the expression of the transgene and the gene for Geneticin® (GIBCO) resistance from a single transcript (pEF.IRES.neo.SV5 V). Twenty hours before transfections, both cell lines were seeded in 12 well plates at a density of 1.0×10^6 cells/ml. The transfections were performed with 0.3 µg of the plasmid DNA (pEF.IRES.neo.SV5 V) and 4 µl of LipofectAMINE™ 2000 (GIBCO) according to the manufacturer's instructions. To select transfected cells expressing the protein of interest, an antibiotic selection was used (500 ìg of Geneticin/ml) and resistant colonies were isolated. To confirm the presence of the gene in transfected cells, a polymerase chain reaction (PCR) with primers that target the V gene was designed and optimized to detect 10^4 molecules of DNA from 50 ng of total DNA. We obtained 59 colonies (42 CRIB cells and 17 were MDBK cells). In a preliminary test 17 random colonies of either CRIB or MDBK were analyzed by PCR. All the samples yielded the expected fragment size (241 bp) with different intensities, which indicates that different colonies may express the V at different levels. All the CRIB transfected colonies showed a stronger signal than MDBK. With basis on these results, the interferon blockage in these cells will be evaluated in the future after infection with bovine viruses
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411 - DETECTION AND IDENTIFICATION OF AVIAN INFECTIOUS BRONCHITIS VIRUS AND AVIAN METAPNEUMOVIRUS WITH FIELD SAMPLES USING POLYMERASE CHAIN REACTION

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