(7/233), 2.22% (3/135), 0% (0/32), 11.76 % (2/17), 77.17% (71/92) respectively and the titles ranging from 10 to 1280. According to the expected, most of the herds had antibodies for both viruses, indicating that buffaloes were exposed to them. In herds A, B and F paired samples were collect and in B, buffaloes seroconverted to BoHV-1 during the period of study, indicating viral activity. Probably the intensification of production and the increase of animal density, increased the risk transmission and maintenance of these viruses in herds, which points to need for more studies to assess the impact of these diseases in this specie.

VV1283 - DEVELOPMENT OF A MICROPLATE IMMUNOCAPTURE (IC) RT-PCR ASSAYS FOR THE DETECTION OF AVIAN INFLUENZA VIRUS (AIV) AND INFECTIOUS BRONCHITIS VIRUS (IBV)

Borzi, M.M., Piza, V.T., Montassier, M.F.S., Mora, A.G., Okino, C.H., Reischak, D., Mendonça, A., Montassier, H.J.

1. **Universidade Estadual Paulista - Campus de Jaboticabal, UNESP, Via de Acesso Prof.Paulo Donato Castellane s/n 14884-900 - Jaboticabal, SP**
2. **Centro Nacional de Pesquisa de Suínos e Aves, CNPSA - EMBRAPA, Rodovia BR 153, Km 110-Concórdia, SC**
3. **Laboratório Nacional Agropecuário, LANAGRO, Rua Raul Ferrari - Jardim Santa Marcelina - Campinas, SP E-mail: mmborzi@gmail.com**

Rapid, sensitive and specific methods are necessary to confirm the diagnosis of infectious diseases caused by avian respiratory RNA viruses, specially AIV and IBV. Reverse transcription followed by polymerase chain reaction (RT-PCR) are now commonly used for the molecular diagnosis of AIV and IBV in tissue or fluid samples collected from birds suspected of infection by AIV or IBV. The detection of these viruses can be achieved by virus isolation on embryonated chicken eggs, or antigen detection by immuno-histochemical techniques, or molecular methods, such as RT-PCR. Aiming to reduce the time and the number of steps in the molecular diagnostic procedure of these viruses, AIV-IC-RT-PCR and IBV-IC-RT-PCR were developed in this study, considering the virus capture by specific polyclonal antibody preparations from chickens hyperimmunized with AIV or IBV, followed by genomic amplification by RT-PCR. Allantoic fluid samples from embrionated SPF chicken eggs experimentally infected with AIV or IBV, were analysed here. The overall results showed that AIV- or IBV-IC-RT-PCR methods were efficient in detecting the presence of these viruses in infected allantoic or in seeded tracheal and cloacal swabs, with an analytical sensitivity of 0,1 hemagglutinating units (UHA) for AIV, and 102.8 EID50 for IBV. The analytical specificity of the IC-RT-PCR methods were also assessed, by testing other avian viral pathogens, such as avian metapneumovirus, Newcastle disease virus, Gumboro disease virus, and none of these heterologous viruses were detected by AIV- or IBV-IC-RT-PCR, including IBV by the first and AIV by the latter. Thus, the IC-RT-PCR techniques, developed here, have a great potential to be applied in the direct detection of AIV and IBV, as a useful alternative for the rapid and

specific diagnosis of these viruses, because these assays combine the immuno-capture step in a microplate, in which the RT reaction were carried out, with the high analytical sensitivity of PCR.

VV1285 - COMPARISON OF SEMI--NESTED PCR AND QUANTITATIVE PCR ASSAYS FOR THE DETECTION OF CANINE HERPESVIRUS TYPE 1

Kurissio, J.K., Taniwaki, S.A., Cruz, T.F., Araújo Jr., J.P.

Instituto de Biociências de Botucatu - Unesp Botucatu, IBB-UNESP, Distrito de Rubião Jr. S/N. Botucatu-SP - CEP 18618-970 E-mail: jkkurissio@yahoo.com.br

The canine herpesvirus type 1 (CHV-1) is a viral agent involved in respiratory and reproductive problems. However, the diagnosis is not always achieved due to the lack of laboratory tools which allow its spread in canine population. Thus, this paper aims to compare the analytical sensitivity of quantitative PCR (qPCR) and semi-nested PCR (SN-PCR) to detect the CHV-1. For this purpose, kidney DNA sample from a neonate with clinical signs compatible with the infection by CHV-1 was used as positive control to both assays. The specificity of primers was shown by amplicon sequencing, which showed identity to the target region of the thymidine kinase gene of the CHV-1. For comparative test of analytical sensitivity between the two techniques ten-fold dilutions of positive controls in a negative matrix was carried out from pure to 10-7 for qPCR and up to 10-10 for SN-PCR. The qPCR primers were the same of the internal primers of SN-PCR and 2.5% DMSO was added in both reactions. The qPCR detected amplification up to 10-6 dilution. In the standard curve, the reaction efficiency of qPCR was 91.58% with R2 of 0.99. A single melting peak, ranging from 73.4 to 74.9°C, was obtained indicating the specificity of the primers. In the SN-PCR, PCR reaction showed amplification up to 10-4 dilution, with product size about 345 bp. In semi-nested reaction positivity was visualized up to 10-7 with expected amplicon of 168 bp. It was not observed any amplification in negative samples. Both techniques had high sensitivity and are useful for the detection of the CHV-1, what could decrease the risk of infected dogs to spread the infection. The qPCR is the most reliable assay because provides faster and readly results besides its lower risk of contamination.

VV1286 - COMPARISON OF PROPORTION OF GENOTYPES OF NEWCASTLE DISEASE VIRUS VACCINES RECEIVED AND TESTED BY LANAGRO-SP IN THE PERIOD 2000-2005 AND 2008-2010

Orsi, M.A., Camillo, S.C.A., Zaroni, M.M.H., Reischak, D., Fortunato, E.C., Domingues, C.S., Ribeiro, S.A.M., Ramazzoti, A., Almeida, F.S., Sobreiro, K.L.

LABORATORIO NACIONAL AGROPECUÁRIO, LANAGRO-SP, RUA RAUL FERRARI SN, CAMPINAS- SP, BRASIL E-mail: giulia96@yahoo.com

The ND's control in broiler chickens, in Brazil, is focused on the surveillance and active immunization with live lentogenic vaccines, the La Sota, B1, Clone 30, C2 strains belonging to the genotype II (GII), VG-GA and Ulster to the genotype I (GI). Field isolates in different geographic regions are