

Until now, there is no disease assigned to these emerging viruses in swine and wild boar. The aim of this study was to detect parvoviruses and anelloviruses in lung of captive wild boar. Sixty lung samples were collected in a slaughterhouse in the Rio Grande do Sul State, in 2011 from wild boars that had pulmonary consolidation lesions from two herds (A and B). DNA extraction was performed using a kit based on silica. PCR to detect PPV1, TTSuV1 and TTSuV2 were performed according to described previously, for PPV2, PPV4 and PHoV were standardized in our laboratory. PCR results showed (33%, 20/60) positive samples for PPV1, (26.6%, 16/60) for PPV2, (61.6%, 37/60) for PHoV, (45%, 27/60) for PPV4, (53.3%, 32/60) for TTSuV1 and (51.6%, 31/60) for TTSuV2. In herd A, PHoV was highly detected, with (87%, 27/31), followed by TTSuV2 with (54.8%, 17/31), PPV4 with (51.6%, 16/31), TTSuV1 with (38.7%, 12/31), PPV2 with (29%, 9/31) and PPV1 was not detected. In herd B, TTSuV1 and PPV1 showed the same number of positive samples (68.9%, 20/29) followed by TTSuV2 with (48.3%, 14/29), PPV4 with (37.9%, 11/29), PHoV with (34.5%, 10/29) and PPV2 with (24.1%, 7/29). PHoV was the main virus detected in lung samples, as similarly described previously in Europe. This is the first report of these emerging parvovirus in captive wild boars in Brazil; but, their pathogenicity remains unknown. Financial Support: CNPq and Fapergs

VV1053 - DEVELOPMENT OF REVERSE GENETICS SYSTEMS FOR INFECTIOUS BURSAL DISEASE VIRUS BY YEAST-BASED HOMOLOGOUS RE-COMBINATION

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Infectious Bursal Disease Virus (IBDV) is a pathogen causing immunosuppressive disease in young chickens. In Brazil, the IBDV economic importance is observed in the death of chickens with immunosuppression and acute infection. Viral reverse genetic system is the virus generation/recovery from cloned viral cDNA or in vitro transcribed viral RNA transfection methods. Advances in the replication understanding and vaccines/viral vectors development for IBDV have been achieved by means of viral reverse genetic. Reverse genetic for IBDV has undergone changes over time. The first reverse genetic system for IBDV was possible by transfection into Vero cells with in vitro transcribed viral RNA. Improved techniques were followed by transfection of viral cDNA cloned downstream of the polymerase II promoter. However, all plasmid strategies used to generate particles of IBDV involves multiple rounds of amplification and need of in vitro ligation and restriction sites. Thus,

the aim of this work was to build the world's first reverse genetic system for IBDV by yeast-based homologous recombination in *Saccharomyces cerevisiae*; a more efficient, robust and simple process than cloning by in vitro ligation. wt-BrIBDV was isolated in Brazil and had its genome amplified and cloned in pJG-CMV-HDR vector by yeast-based homologous recombination. The clones were transfected into chicken embryo fibroblasts and the recovery virus (IC-BrIBDV) showed genetic stability and similar phenotype to wild-type virus (wt-BrIBDV), confirming the utility and the practicality of the employed methodology. The genetic stability and phenotype were observed by nucleotide sequence, focus size/morphology and replication kinetics, respectively. The IBDV reverse genetic construction by yeast-based homologous recombination, the first system for IBDV made by this technology and the first developed in Brazil, provides tools to IBDV understanding and create the basis for vaccines and viral vectors development. Financial Support: CNPq, Fiocruz

VV1058 - CONSTRUCTION OF YELLOW FEVER VIRUS 17D CHIMERIC EXPRESSING THE STRUCTURAL PROTEINS OF SAINT LOUIS ENCEPHALITIS, ILHEUS AND ROCIO VIRUSES FOR USE IN DIFFERENTIAL DIAGNOSIS

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Arboviruses, caused by flaviviruses, are involved in outbreaks, which are a major public health problem, such as Dengue (Brazil) and West Nile virus (WNV) (North America). In Brazil, the WNV was recently detected in horses, conducting to current need for WNV, Ilheus virus (ILHV), St. Louis Encephalitis virus (SLEV) and Rocio virus (ROCV) differential diagnosis, therefore present diagnosis methods resulting in cross-reactions between them. Seroprevalence studies for flaviviruses using ELISA often result in cross-reactions, especially with two or more members of the family Flaviviridae coexist in the same environment. The PRNT serological test is the gold standard for flaviviruses assays due to high sensitivity, specificity and low cross-reactivity, but requires the use of live viruses. Some flaviviruses, such as WNV, ILHV, ROCV and SLEV, must be handled in biosafety level 3 laboratories (NB3). An important tool for bypassing the NB3 need is the construction of chimeric viruses. These chimeras are highly attenuated and manipulated in NB2. The aim of this study was to construct chimeras using the infectious clone (pBSC-YFV-17D) from Yellow fever virus strain (17D) attenuated as vector, replacing its structural proteins (prM/E) by ILHV, ROCV and SLEV prM/E proteins. The ILHV, ROCV and SLEV coding regions were commercially synthesized. The chimerical plasmids were constructed by yeast-based homologous recombination. The RNAs in vitro transcribed from the plasmids were transfected into BHK-21 cells and incubated by 48, 72 and 96 hours.