

type 1, 2 and 5 (BoHV-1, -2, -5), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), vaccinia (VACV) and bluetongue virus (BTV) were individually amplified in cell culture and the supernatant used to immunize rabbits. Only VACV were inactivated and administered with adjuvant. The animals were immunized subcutaneously five times at intervals of 14-21 days, and five days after the last boost were bled. The whole blood were processed for serum separation, aliquot and stored at -20°C until test. No clinical signs were observed after immunizations. From each animal were obtained at least 15 mL of serum. The antiserum was used as primary antibodies in the immunoassays. To determine the concentration of specific antibodies, several dilutions (1:100 to 1:51.200) were tested against homologous viruses and heterologous field isolates. The higher dilution with specific positive reaction and lower background was considered to the working dilution. In general, lower dilutions (< 1:800) presented unspecific reaction in mock-infected cells. Usually, the dilutions used for immunoperoxidase were higher than those used in immunofluorescence and range from 1:1.600 to 1:25.600 for the homologous viruses. No differences were observed when field isolates were tested. In conclusion, all polyclonal antibodies produced were able to react specifically in the immunofluorescence and immunoperoxidase assay, making then an important tool to detection and characterization of several bovine viruses in the routine diagnostic or research. Financial support: CAPES, FAPERGS.

#### VV557 - CONCOMITANT INFECTIONS DUE TO BOVINE LEUKEMIA VIRUS AND MYCOBACTERIUM BOVIS IN DAIRY CATTLE FROM NORTHERN PARANÁ

Bronkhorst, D.E., Alfieri, A.A., Affonso, M.Z., Oliveira, V.H.S., Negri Filho, L.C., Okano, W., Headley, S.A., Araujo Junior, L.D.

*Universidade Norte do Paraná, UNOPAR, Rodovia PR218 Km 1 Medicina Veterinária 86702000 Arapongas - PR*

Enzootic bovine leucosis is caused by the Bovine Leukemia Virus (BLV), Family Retroviridae. This disease produces severe economic losses because of infertility, reduced milk production, culling, and death, and might predispose the affected animal to other diseases. Bovine tuberculosis (BT) is caused by *Mycobacterium bovis*. BT is a chronic disease associated with reduced milking production, carcass condemnation at slaughter, and is an important zoonotic disease. This study evaluated the presence of EBL and *M. bovis* by in a herd of dairy cattle. During May 2013, serum samples were obtained from 16 female, Black and White Holstein cattle located in the city of Jandaia do Sul, PR. All animals were positive for *M.*

*bovis* by the tuberculin skin test, with bacterial isolation via the Stonebrink method, and confirmed by PCR assays targeting specific amplicons of *M. bovis*. Serology was performed by using the Agar Gel Immunodiffusion (AGID) Test to detect anti-BLV antibodies. From the animals evaluated, 37.5% (5/16) were seropositive for anti-BLV antibodies; however most of these (62.5%; 11/16) reactive negatively. In this study, coinfections due to *M. bovis* and BLV were demonstrated in 37.5% of the animals; a similar study done in Pernambuco demonstrated that 12.3% (37/299) of the animals investigated were coinfectd by these infectious agents. Additional studies must be done to determine the effects of BLV in predisposing affected animals to tuberculosis.

#### VV561 - NEUTRALIZING ANTIBODIES FOR VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN HORSES FROM BRAZILIAN PANTANAL

Pauvolid-Corrêa, A., Juliano, R., Velez, J., Schatzmayr, H., Nogueira, R.M.R., Komar, N.

1. *Fundação Oswaldo Cruz, Fiocruz, Av. Brasil 4365, Rio de Janeiro, RJ 21045-900, Brasil*

2. *Empresa Brasileira de Pesquisa Agropecuária Pantanal, Embrapa Pantanal, Rua 21 de Setembro, 1880, Corumbá, MS 79320-900, Brasil*

3. *Centers for Disease Control and Prevention, CDC, 3156 Rampart Road, Fort Collins, CO 80521, Brasil*

The Brazilian Pantanal hosts large concentrations of diverse wildlife species, including migratory birds, and therefore this region is potentially important for arbovirus studies in South America. Neutralizing antibodies for equine encephalitis viruses, including Eastern equine encephalitis virus (EEEV) and Western equine encephalitis virus (WEEV) have been reported in Pantanal equines. To better understand the alphavirus circulation in the region, a serosurvey for Venezuelan equine encephalitis virus (VEEV) was conducted with 760 equines from 15 beef cattle ranches of the Brazilian Pantanal. The sera were titrated by 90% plaque-reduction neutralization test (PRNT90) for VEEV and then the seropositive samples tested for EEEV, WEEV and Mayaro virus. Serum was considered seropositive to VEEV when it reduced at least 90% of the formation of plaques and its neutralizing antibody titre was four-fold greater than what was observed for the other tested alphaviruses. From a total of 760 equines, of which 277 were immunized with bivalent vaccine composed of EEEV and WEEV and 483 were unvaccinated, four (0.5%) had neutralizing reactivity (PRNT90 titre  $\geq$  1:10) for VEEV regardless of vaccine status. Employing the criterion of four-fold greater titre among all alphaviruses tested, one four-year old stallion with history of vaccination was considered seropositive for VEEV with PRNT90 titre