

segments of double-stranded RNA (dsRNA). Objective (s): Standardize a methodology of quantitative Real Time PCR (qPCR) to assessing the presence or absence of rotavirus A-H in fecal samples collected from different breeding herds of buffaloes diarrheal occurred of Marajó Island and Marabá district, Pará state, Brazil. Material and Methods: Stool samples were obtained from samples Virology Section, at Evandro Chagas Institute collected from different breeding from State of Pará. Stool samples were suspended in buffer Tris/Calcium, followed by nucleic acid extraction and after were subjected to qPCR, to detect the rotavirus groups. Results: Forty seven positive samples (51.64%) were detected belongs to the groups A, B and C of RV. Of these, were observed amplifications in 48.35% (44/91) to RV of A group; 2.19% (2/91) to RV of C group; and 1.09% (1/91) to RV of B group. Furthermore, were observed concomitant amplifications for the groups A and B in 1.09% (1/91), suggesting the existence of mixed infection. Conclusion: The recent literature shows the existence of RV of several groups (A, B and C) in domestic mammals, bovines and buffaloes. The present study corroborates with the data existent on literature and show a necessity of a further research of the diarrheal cases occurred different breeding to determine the frequency and distribution of RV of several groups between the existent herds' on the region. Financial Support: Fundação Amazônia Paraense de Amparo à Pesquisa (FAPESPA); Instituto Evandro Chagas (IEC); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

#### **VV301 - CONTINUOUS CIRCULATION OF HUMAN-ORIGIN SEASONAL INFLUENZA A VIRUSES IN SWINE IN BRAZIL**

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In the last years, following the introduction of pandemic H1N1 (H1N1pdm) influenza A virus (FLUAV) in swine in 2009, genetically diverse FLUAVs of subtypes H1N1, H1N2 and H3N2 have been detected in swine in Brazil. The eight gene sequence analysis of 16 FLUAVs isolated from pigs between 2009 and 2012 revealed the

circulation of H1N1pdm and reassortant H1N2 and H3N2 viruses containing HA and NA genes of human-origin seasonal influenza virus and the internal gene segments derived from H1N1pdm. Passive monitoring of FLUAV in pigs continued from 2013 to 2015 and resulted in the isolation of thirty-six viruses from nasal swab and lung tissue samples collected from clinically affected pigs from farms in the southern region of Brazil. Sequencing of HA and NA gene segments were performed by ABI 3130xl and the phylogenetic relationships of each analyzed gene were inferred with the Neighbor Joining method in MEGA 6.0. The sequence analysis revealed seven H1N2, four H1N1pdm and five H3N2 FLUAVs. Eighteen viruses had only the NA gene segment identified, resulting in additional eleven N1 and seven N2 sequences. On the H1 phylogeny, the seven H1N2 viruses isolated in pigs in 2013 and 2014 were grouped with other Brazilian H1N2 virus detected previously. The Brazilian H1N2 viruses were separated into two subclades supported by a 100% bootstrap. On the H3 phylogeny, the five H3N2 viruses isolated in 2014 and 2015 were grouped with other four Brazilian H3N2 viruses detected in pigs in 2011 with a 99% bootstrap support. The analysis of the NA gene segment of H1N2 and H3N2 viruses revealed the formation of two subclades, one clade with a 97% bootstrap support comprising two H1N2 viruses detected in pigs and in wild boars in 2011 and a second clade with 81% bootstrap support comprising H1N2 and H3N2 viruses detected in pigs from 2011 to 2015. Within the second N2 clade, the H1N2 viruses clustered separately from the H3N2 viruses (99% and 100% bootstrap support, respectively). The H1N2 and H3N2 FLUAVs detected in pigs in Brazil are distinct from other human-origin H1N2 and H3N2 viruses detected in swine in other countries. These findings show the very dynamic epidemiology of influenza virus in pigs and highlight the importance of human-to-swine transmission in the generation of influenza virus diversity in swine in Brazil. Financial Support: EMBRAPA (02.11.01.006.00).