cheeses. However, pathogens may be present in "pingo", since MAC are produced from raw milk. Vaccinia virus (VACV), the etiological agent of bovine vaccinia (BV), can be eliminated through milk, including from cows with subclinical infection. Viable VACV particles have already been detected in the "pingo" used in the production of cheeses made from the milk of experimentally infected animals. Another study showed that the consumption of raw milk and MAC are considered risk factors for VACV transmission, since anti-OPXV neutralizing antibodies were detected in people who had no contact with diseased cattle. It is a common fact in the Serra da Canastra region sharing of the "pingo" among properties of the region. Therefore, the "pingo" could be a possible route of VACV spread among farms. Considering "pingo" as a potential source of milk-borne pathogens, including VACV, this study aimed to investigate the presence of VACV in samples of pingo from MAC producing properties of Serra da Canastra, Minas Gerais, Brazil. Eight samples were collected from eight farms from the region, aliquoted and stored at -20°C. The extraction of the viral DNA was done with a commercial kit and, later, the qPCR technique for amplification of the HA gene was performed. The eight samples of "pingo" analyzed showed a negative result for VACV. More "pingo" samples should be evaluated to understand the role of "pingo" as a potential source of VACV spread among farms, including from other MAC producing areas in MG. The microbiological safety of food is extremely important for both industry and the consumer, given that dairy products are important sources of foodborne diseases.

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Palavras-chaves: Cheese, Foodborne, Milk , Vaccinia virus

MULTIPLEX RT-PCR ASSAY FOR SUBTYPING OF SWINE INFLUENZA VIRUS IN BRAZIL

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Resumo

Influenza is an acute respiratory disease of pigs caused by infection with influenza A virus (IAV). The most prevalent IAV subtypes in pigs are H1N1, H1N2 and H3N2. However, genetically distinct viruses circulate in different geographic regions. For this reason, a diagnostic test that is able to identify the viral subtype circulating in swine is important for the monitoring and control of the infection. In addition, such an IAV subtyping assay is not commercially available in Brazil. This study describes the development and validation of a multiplex RT-PCR assay for subtyping of swine IAVs. The assay was performed in two distinct reactions, one was focused on the hemagglutinin (HA) gene (H1 of pandemic origin, H1 of human origin and H3) and the other reaction was focused on the neuraminidase gene (N1 of pandemic origin, N1 of human origin and N2). For the standardization of the assay, reference IAV strains (H1N1, H1N2 and H3N2) were tested against each pair of primers, and also in a 10-fold serial dilutions. Cross-reactivity was not observed in the assay and the limit of detection for the three viral subtypes, in HA and NA reactions, was of 1.9ng/µL of cDNA. The analytical sensitivity of the test was assessed based on 65 IAV isolates, previously characterized by genetic sequencing, resulting in 100% of detection of the correct viral subtype. The assay presented 100% of analytical specificity in the testing of 65 samples considered as negative for IAV, and positive for other viral and bacterial respiratory pathogens. For the diagnostic evaluation, the multiplex RT-PCR was carried out on 77 IAV positive samples collected from pigs between 2010 and 2016. Finally, the RT-PCR assay was able to identify the viral subtype in 74.02% of the samples and the most prevalent subtype was H1N1 (64.9%), followed by H1N2 (29.8%) and H3N2 (5.3%). Furthermore, the test was able to detect mixed viral infections in five samples and reassortant viruses even before performing genomic sequencing. In conclusion, the multiplex RT-PCR assay designed in this study

showed to be a sensitive and specific method for the identification of IAV subtypes in samples collected from pigs. The technique described is cost-effective when compared to other methods such as sequencing, and once implemented in diagnostic laboratories will provide more information on the prevalence of IAV subtypes in swine herds.

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Palavras-chaves: Diagnosis, Influenza, Multiplex RT-PCR, Subtyping, Swine

DIVERSIFICATION OF THE BOVINE PAPILLOMAVIRUS TYPES RELATED TO TEATS WARTS

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Resumo

Papillomaviruses (PVs) are small viruses that comprise a highly diverse group that can produce epithelial proliferative lesions in all amniotes. Bovine papillomavirus (BPV) is recognized as the etiological agent associated with several forms of benign tumors, among them the teat papillomatosis that results in lower profits for the milk industry. Currently, 24 BPV types have been reported, in contrast to more than 200 types of the human papillomavirus (HPVs). The BPVs are assigned in four genera (*Deltapapillomavirus*, *Epsilonpapillomavirus*, *Dyoxipapillomavirus* and *Dyokappapapillomavirus*) whereas the types BPV19 and 21 have not been allocated within any genus. The present study aimed to test 50 cattle teat papillomatosis by histopathology and conventional polymerase chain reaction (PCR) carried out by using the primer pair FAP59 and FAP64 followed by DNA sequencing. Thirty-three out the 50 samples were classified as classical BPV types whereas 17 as putative new types by phylogenetic analysis. These findings add to the expanding genetic diversity of BPV, as also evidencing the possibility of other BPVs has been detected in teats warts besides classical types. Knowledge of the prevalence and of the variety of BPVs is a milestone for the development of appropriate prophylactic and therapeutic measures. Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) supported this work.

Palavras-chaves: Cattle, Papillomavirus, PCR, Sequencing, Teat lesion

IDENTIFICATION OF SWINE INFLUENZA VIRUS SUBTYPES FROM 2012 TO 2015 AND 2017 TO 2018 IN THE SOUTH AND SOUTHEAST OF BRAZIL

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