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ANALYSIS OF COLOSTRAL ANTIBODIES TO HERPESVIRUS 1 AND 5 IN MURRAH BUFFALO CALVES

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ABSTRACT

Familiar agriculture is predominant in the production of milk buffaloes in São Paulo southwest, Brazil. The production of buffaloes' milk is sold almost exclusively to cheese production inside and out São Paulo State. This search was done in a proprerty located in Alambari, São Paulo, specialized in the production of Murrah buffaloes, with 21 animals, born between March and May in the year 2017. Blood was collected at 24 to 48 h from the birth, every 15 days and at the completetion of weaning (91 days of age). The samples were collected in vaccum tubes of 10 mL with coagulum activator, by a puncture of the jugular vein. With the application of the virus neutralization tests, we could see 90.47% reagent animals for BoHV-5 and or BoHV-1 in the first two days of life. The geometric medium titles (GMT) of antibodies reduced significantly from the time 0 (1.4563) to the time 6 (05721) (P=0.0001), showing adequate transference of passive immunity through the ingestion of colostrum by buffalo calves, with the persistence of antibodies until 3 months old. Considering that most buffalo calves were reagents after sucking the colostrum, it is possible to conclude that there is a great occurrence of BoHV-5 and or BoHV-1 once there is a crossed reaction among these and the cattle with no historic of vaccination for the agents.

Keywords: *Bubalus bubalis*, buffaloes, herpesvirus-5, herpesvirus-1, virus disease, colostrum, feeding with colostrum

INTRODUCTION

The immunoglobulins (Ig) in the intestine show efficient activity in the resistance against pathogenic organisms that infect calves orally. The IgG in the intestine is enough to resist to the digestion offering an immunologic response. Studies documented the relative resistance of IgG to the degradation of proteins in the intestine. An important source of IgG in the intestine of newborn calves is the circulating IgG, that is absorbed by the ingestion of colostrum in the first 24 h of the animal life (Quigley, 2003).

Many bacteria and viruses that infect the

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calves are enteric, generally causing intestinal problems and signs of diseases (diarrhea, dehydration). The immunoglobulins in the intestine may help the animal to create an efficient response when they connect to the antigenic linking of the specific pathogen. Thus, the movement of IgG from the circulation to the intestinal lumen would be a way to give immunity as a response to the pathogens that infect the animal by the fecal-oral way (Quigley, 2003).

The BoHV-5 is the agent that causes bovine herpetic - encephalitis, after the primary infection, the virus may stay hidden, without showing clinic signals. However, when animals are exposed to stressful situations, the virus may be reactivated, leading to clinic signs of the disease and the possibility of other animals' infection. It may reach especially young bovines (Rissi *et al*., 2007). Stressing situations like transport, delivery, vaccinations, or cortisone treatment may stimulate the reactivity of the disease (Riet-Correa *et al*., 1996).

The mucosa of the respiratory and genital apparatus are the main routes of infection. The transmission occurs by the intimate contact with these surfaces, but the virus BoHV-5 is also spread by the aerosols and body secretions (Riet-Correa *et al*., 2001).

The common clinic signs of herpetic bovine encephalitis are deep depression, anorexia, nasal and ocular discharge and visible nervous signals by phases of excitation, during which we may observe animals presenting nistagmus, shaking, walking back or in circles, and even falling. Blindness, shaking walks with incoordination of the back members, teeth screaking and laying down, doing movements like driving with pedals may also occur (Riet-Correa *et al*., 1996).

The bovine infection in the rhinotracheitis, also known as IBR is caused by bovid herpesvirus kind I (BoHV-1), which also attacks buffaloes. The animals may show also hiperexcitement, been necessary the differential diagnosis from other diseases that can happen in the central nervous system, such as Hydrophobia, Butolism and Malignant Catarrhal Fever (Typhoid). The virus belongs to the herpesviridae, which infect the animals and stays occult in the healthy organism but may cause an outbreath when the herd present a decreased immunity. The transmission of this disease occurs through direct acrogenous way among the individuals and through the contaminated semen (Leite; Bastianetto, 2009).

IBR is a communicable disease that causes abortion, besides breathing problems, conjunctivitis, metritis and nervous symptoms. Contamination may occur through infected semen used in artificial insemination, infected cows, symptomatic or not. The apparent symptoms of IBR are abortion after the fifth month of pregnancy, pustules (red spots) inside the vulva and the vagina, conjunctivitis, and vaginal flow. The prejudices caused by IBR are the increase in calving interval, leading to smaller number of calf birth and the decrease of the milk production (Ferrera *et al*., 2007).

The geographic distribution of the infection by BoHV-1 by the proof of neutralization of virus BoHV-5 is not well known, especially due to the crossed serological reactivity (VN). Sporadic events of meningitis-encephalitis caused by BoHV-5 were reported in Australia, the United States, Italy and Hungarian (Maidana *et al*., 2013). The diagnosis of these agents may be performed by indirect methods as virus neutralization, immunofluorescence, and immunoperoxidasis. However, the BoHV-1 and BoHV-5 show 85% of similarity in their sequences of desoxirribonucleic

acid (DNA), which difficult the adequate distinction between these two genotypes, because crossed reactions may occur (Meyer *et al*., 2001).

Data about the transference of colostral passive immunity of antibodies for BoHV-5 and BoHV-1 in buffalo Murrah calves is very scarce, but still very important. These pathogens may negatively affect the commercial production of the buffaloes and bovines, since they are endemic in Brazil and in many other regions in the world.This work had the objective to evaluate the transference of the passive immunity for the BoHV-5 and BoHV-1, to the newborn Murrah calves through colostrum ingestion, seeking to evaluate the conditions to protect against these endemic diseases from 0 to 90 days of age.

MATERIALS AND METHODS

Twenty-one buffalo newborn calves (12 males and 9 females), from buffaloes cows with no historic of vaccination, participated in this study in a joined propriety (bovines and buffaloes). Calves were sampled at 24 to 48 h from the delivery (TO), every 15 days (T1 until T6), and by the day of the completion of wean (at 91 days of age). The samples were collected in vacuum tubes of 10 ml with activation of coagulum by puncture of the jugular vein. The blood was kept at an ambient temperature until coagulation and the separation of the serum. The serum samples were transfer into sterile tubes of 1 ml and freezer stored at -20°C.

The analysis of the blood serum was done in the Laboratory of Bovine Viruses, Center of Research and Development of Animal Health, Biological Institute of São Paulo, São Paulo, Brazil by the method of viral neutralization which were divided in two parts: the selection and designation of antibodies. The qualitative analysis was done to classify the animals in reagents and not reagents to BoHV-5 and/or BoHV-1.

The sera were submitted to a temperature of 56°C for 30 minutes, to inactivate the complement, to a later virus neutralization facing the virus BoHV-5 and BoHV-1. The VNT was conducted in 96-well plate format, on the flat botton. The samples were diluted in series, in the logarithmic base 2, from the dilution 1:2 until 1:1024 using the element Eagle's minimal essential medium (MEM), in duplicates. After that 50 µL of virus BoHV-5 were added in each hole of the plate (2000 TCID 50/mL -50% tissue culture infective doses), and isolated and characterized. After the incubation of 18 to 24 h in a sterilizer at 37°C with 5% of CO_2 in each hole, 100 µL of suspension of Madin-Darby bovine kidney (MDBK) cells, in the concentration of $3x10^5$ cells/mL, were added with 10% of bovine embryo serum (SFB).

The reading was realized through microscope after 4 days of incubation at 37°C and 5% of CO_2 and the infectivity was shown by the visible cellular surface effect in the plates. The title of antibodies was shown as the largest dilution of the serum that inhibited completely the infectivity in both holes of each dilution, being considered reagent the sample that showed title ≥2.

The titles of BoHV-5 and or BoHV-1 were analyzed by Proc Mixed for mixed models, being the time considered as repeated measures. For the analyses, 15 different structures were of tested alteration, being the one that was better adjusted to the statistic model was chosen in the smallest value of the criterion of information Akaike corrected (AICC) (WANG; GOONEWARDENE, 2004). The model included Time as a fixed effect and the animal as random effect. For all variables the comparison was done according to the test of

Turkey adjusted and the level of significance of 5% was adopted.

RESULTS AND DISCUSSIONS

The frequency of buffalo calves presenting antibodies against BoHV-5 and or BoHV-1 after ingestion of colostrum in the first 24 h of life was 90.47% (19/21, 11 males and 8 females), and the titles of antibodies tended to a reduction according collecting time (Figure 1).

In the study of Scheffer (2013), with healthy 339 buffaloes of both sexes, older than 12 months of age, that weren´t vaccinated, 149 animals (44 %) showed antibodies for BoHV-5. This study also showed that the tests of viral neutralization revealed the circulation of herpesvirus in the herd, but with no discimination of the reagents among infections by BoHV-1, BoHV-5 or BuHV-1 due to the crossed reaction.

Zanco *et al*. (2018), tested 92 samples of blood serum of female Murrah and Mediterraneas buffaloes in reproductive age, created in Vale do Ribeira (São Paulo State), for BoHV-1 and BoHV-5 by virus neutralization. These authors also found a high rate of reagent animals (78.3%, 72/92).

Martinez (2017), analyzing 51 female buffaloes of Murrah and Mediterranean races of a dairy cattle from Vale do Ribeira in São Paulo State, collected 692 samples, and 80.4% (556 animals) were reagent to BoHV-1 and 82% (568 animals) were reagent to the virus BoHV-5.

Bharti *et al*. (2015) in his study with Murrah buffalo calves suggest that the levels of immunoglobulins increased after colostrum intake, but with individual variation on immunoglobulins levels by the age of 24 h. No effect of the abrupt weaning on humoral immunity of buffalo calves was identified, as well as the adoption of different age weaning programs in the immunologic development of the buffalo calves.

The dynamic of antibodies to BoHV-1 and or BoHV-5 measured by the method of virus neutralization indicated that there was a transference of antibodies through the colostrum ingestion. However, titles declined over time, from birth until 0-3 months old, alerting to the loss of protection against infection. Consequently, it is necessary the adoption of preventive steps to avoid infections, as the disease is endemic in cattle. There was no clinic signs observed for BoHV-1 and or for BoHV-5 in the experimental group, or depression in animals performance. However, the decline in the titles of antibodies against BoHV-1 and or BoHV-5 shows that there was no active occurrence of infection in these animals during the studied period.

CONCLUSION

Antibodies anti-BoHV-5 and or anti BoHV-1 were present in newborn buffalo's calves after the ingestion of colostrum from naturally infected mothers (not vaccinated), suggesting adequate passive transfer and a high prevalence of these viruses in this herd. Protection was adequate at least until the three months of age, the period when the mother antibodies fall, and the animals become vulnerable to the infection of these viruses.

Most of the the studied buffalo calves, regardless of sex, reached significant tittles of mother antibodies anti-BoHV-5 and or anti-BoHV-1 through the natural suckling and the ingestion of colostrum in the two first days of life.

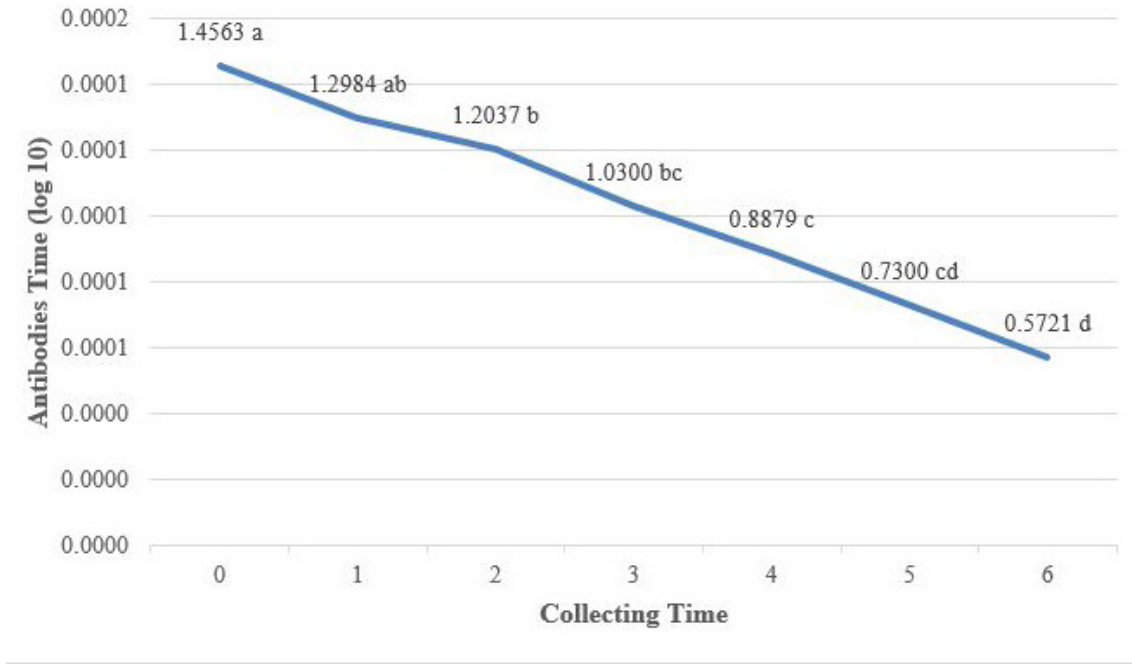

Figure 1. Titles of antibodies (log 10) present in the serum of buffalo calves reagent to the virus BoHV-5 and or BoHV-1.

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