

TRANSFER COLOSTRUM OF PASSIVE IMMUNITY  
BY ANTIBODY IN MURRAH CALF BUFFALO

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**ABSTRACT**

The predominant buffalo in southwesten, São Paulo, Brazil is the Murrah. The greatest parts of the farms that explore the buffalo milk production in this region is compound by families and their products are sent to specialized farms. This study was developed in a mixed farm situated in a town called Alambari- São Paulo, specialized in the production of Murrah buffalo milk with 21 animals, born between March and May 2017. Samples of blood were taken between 24 and 48 h from the birth, each 15 days and by the day they stopped sucking completely (in the 91<sup>th</sup> daylife). We collect blood in 10 ml vacuum tubes without anticoagulant to obtain serum with a coagulant substance, by puncture of the jugular vein. Through the buffalo's calves blood serum by virusneutralization, we could find 91.30% of reagent calf buffaloes for the BoHV-1 (bovine alphaherpesvirus type 1, etiologic agent of IBR/IPV (infectious bovine Rhinotracheitis/Vulvovaginitis-IBR/IPV) in the first two days of life. The Geometric Media Titles (GMT) of antibodies for the BoHV-1 reduced a lot by the time of 0 (1.88) to

the time of 6 (0.97) ( $P < 0.0001$ ) showing effective transference of passive immunity after having colostrum for buffalo calves with the persistence until three months old. Considering that most of buffalo calves were reagent for BoHV-1 after drinking colostrums, it was possible to deduce that there is a great occurrence of IBR/IPV and viral activity for this agent, once buffalo calves received colostrum and buffaloes are not vaccinated against IBR / IPV.

Therefore, it can be concluded that the mothers suffered infection and transferred antibodies to the calves.

**Keywords:** *Bubalus bubalis*, buffaloes, BoHV-1, viral disease, colostrum, IBR/IPV

**INTRODUCTION**

The colostrum appears in the mammary glandula before parturition that is the first milk produced after the calving. The colostrum is very important to the newborn calf. It is highly nutritious and some kinds have essencial

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components for survive during the new birth period. The immunoglobulin existent in a high level in the bovine colostrums is the IgG. Through drinking the IgG, it gives systemic immunity to the newborn animal. This is specially important once the placenta of bovines and buffaloes, being sindesmocorial it does not allow the transfer immunoglobulins of the mother's system to the fetus during the pregnancy (Swenson and Reece, 1993).

The calves are completely dependent about drinking colostrums to acquire immunity, called colostral passive immunity, until their own body start producing their own antibodies, called active immunity. The immunoglobulins are responsible for the defense of the organism until the calves start producing their own antibodies two or three weeks after the birth, when exposed to the agents (Salles, 2011).

The calf must suck colostrum by the first time in about 3 h after birth, directly from the mother, and stay with her for at least 12 h after birth, to have the opportunity to suck when they want and more often. It is very important for the calf to suck the colostrum as soon as it can for the quantity of immunoglobulins decrease in the milk while the cow is drawn and the absorption of the immunoglobulins in the intestine decreases after some hours (Salles, 2011).

Bessi *et al.* (2002) identified in their study that the average of serica IgG concentration were  $0.762 \pm 1.13$  mg/mL for Netherlander bovine calves that had not sucked colostrums,  $13.886 \pm 5.52$  mg/mL for calves that had sucked a meal when they were born and  $26.896 \pm 12.45$  mg/mL for animals three days after birth, after two meals of colostrums.

The bovine infective rhinotracheitis, also known as IBR/IPV, is caused by bovine herpesvirus

type 1 (BoHV-1) also attacks buffaloes. The animals can show breathing and neural manifestations, being necessary a differential diagnosis of other illnesses that happen respiratory system and central nervous system. The virus belongs to the Herpesviridae family it is able to infect the animal and stay lactent in healthful animals and become active when the animals are exposed in different conditions, as abrupt variations of temperature, lack of food, conditions that can change immunity of the animal to a low level. Transmission of this illness occur through a direct contact with infected animals and also through the contaminated semen (Leite and Bastianeto, 2009).

That transmissive sickness can causes reproductive problems, as abortion any time of pregnancy pustular and necrotic lesions in the vulva and vagine, conjunctivitis and vaginal flowing. Beside it can cause breathing alterations, conjunctivitis, metritis and neural problems. The prejudices caused by IBR/IPV are the increasing of the interval between the parturition ou calving, for the smaller number of calves birth and the decrease of milk production (EMBRAPA, 2007).

Fusco *et al.* (2015) related in Italy that samples collected from new born buffaloes and those that had suffered euthanasia were positive for BoHV-1 and negative for all other investigated pathogenesis (Bovine Diarrhea virus, bovine Coronavirus, virus of Schmallemborg, virus of the frontier sickness, type Chlamydomphila, *Coxiella burnetii*, *Leptospira* spp., *Neospora caninum*, *Toxoplasma gondii*, Herpesvirus-2).

Studies indicate that the BoHV-1 shows the same level of pathogens, including death, in the newborn buffaloes, because the samples of aborted fetal tissue and the cervical rubbings were positive for the virus. The results suggest that BoHV-1 may be responsible for abortions observed in buffaloes

(Fusco *et al.*, 2015).

The infected buffaloes by BoHV-1 may therefore, become a carrier of BoHV-1 and a potential source of infection for the animals. Any systemic plan of control or eradication of the IBR to be applied in these farms should involve not only bovine cattle, but also buffaloes. For this reason, some authors suggest that the sanitary measures applied to cattle should be extended to buffaloes (Fusco *et al.*, 2015).

Hubner *et al.* (1996) studying Holland cows that had not been vaccinated and their calves, using the neutralization tests, evidenced the presence of antibodies against BoHV-1 in the calves serum, after they had sucked colostrums, until they were 180 days old. Due to a low prevalence of antibodies among the studied cows (7.3%), just 11 calves were found with a positive presence of antibodies after sucking colostrums. From 11 female calves 6 were born from cows with evidence of latent infection of BoHV-1. Hubner *et al.* (1996) related that there was a gradual fall of colostral anti-BoHV-1 until 180 days from the birth when the calves did not react when tested by virus neutralization test.

Fernandes *et al.* (2016) observed in their study a serum positivity for BoHV-1 in female buffaloes in the state of Paraíba, northeast Brazil and suggested that for the control and prevention some steps should be adopted, as a use of diagnostic tests before buying animals and the use of vaccines to avoid the introduction of infected animals to the cattles and consequent dissemination of infection, decreasing economic lost.

Medeiros (2014) in his search relates that buffaloes are hosts BoHV-1, and they can be disseminator species of this agent for cows and buffaloes. In this study, the presence neutralizants antibodies for herpesvirus in this species indicates a natural exposition to the infection, once the

animals had not received vaccine against BoHV-1.

It is very important the development of knowledge to evaluate the transfer of the passive immunity from the female to the calves, specially infectious agents, that negative impact in buffalo farming. Due to a lack of data the transfer of colostral passive immunity of antibodies anti-BoHV-1 was evaluated in Murrah buffalo calves, being this infection endemic in Brazil and in various regions around the world in both cow cattle and buffalo cattle.

The present work has the objective to evaluate the transfer of passive colostral immunity of antibodies anti-BoHV-1 to newborn Murrah buffaloes calves, and the condition to protect from this endemic disease from 0 to 90 days old.

## MATERIALS AND METHODS

Blood samples were collected from 21 buffalo calves, born from females which had not received vaccine, in a mixed property (bovines and bubalines), being 12 males and 9 females, from 24 to 48 h from birth, each 15 days and in the day they stopped sucking completely (91<sup>th</sup> day of life). The samples were collected in vacuum tubes of 10 ml with coagulum activator, by puncture of jugular vein. The blood remained for some hours in the room temperature until the coagulation and the separation of the serum happens. The sample of the serum was transferred to transferred aseptically to a sterile labeled vial of 10 ml and stored in a -20°C freezer.

The analysis of the serum blood were realized the analysis of the samples was carried out through the neutralization virus test at the Laboratório de Vírus de Bovideos, Biological Institute in São Paulo, using the standards and

procedures defined by the World Organization for Animal Health (OIE, 2016) through the virus neutralization test (VN) was performed to determine antibodies titers (OIE 2016).

The serum was submitted to temperature of 56°C for 30 minutes, to inactivate the complement of the complement, for a later realization of a virus neutralization against the virus BoHV-1. 96-Well polystyrene cell culture plates. The samples were solved in series in the logarithmic base 2, from the dilution 1:2 until 1:1024 using agent MEM. Each dilution was done twice. After, they added in each hole of the plate 50 µl of the virus BoHV-1 (2000 TCID<sub>50</sub>/ml- 50% doses of tissue culture infective) The virus strain used in the virus neutralization test was the Los Angeles (ATCC-VR 188; USA). MDBK cell line (ATCC CCL-22) was used for virus propagation. After incubation of 18 to 24 h in 37°C with 5% of CO<sub>2</sub> were added 100 µl in each hole in suspension of cells MDBK in the concentration of 3x10<sup>5</sup> cells/ml supplemented with 10% of bovine fetal serum (BFS).

The reading was done in an inverted microscope, after 4 days of incubation in 37°C and 5% of CO<sub>2</sub>, and the infectivity was indicated by the cytopathic effect visible in cell monolayer. The title of antibodies was expressed as a bigger dilution of the serum that inhibited completely the infectivity in both holes of each dilution, being considerate reagent the sample that presented title  $\geq 2$ .

The data were analyzed were analysed using the Proc Mixed Models, being the time used as a measure repeated. For the analysis, 15 different structures of tested of “covariance” were tested bring the one which was the best adapted to the criterion of information Akaike corrected (AICC) (WANG) (Goonewardene, 2004). The model includes the Time as a fixed effect and the animal as aleatory effect. For all variables, the comparison

among the treatment were done according to the adjusted Turkey test, the same way for all the tests done, level 5% will be adopted.

## RESULTS AND DISCUSSIONS

Analysing IBR/IPV, the frequency of calves buffaloes of antibodies against BoHV-1 after sucking colostrums in the first 24 h after birth were 90.48% (19/21 animals), being 11 males and 8 females, and the titles of antibodies decreased according to the Table 1 and Figure 1.

The experimental infections with BuHV-1 and BoHV-1 in their natural hosts induce to intense infections with characteristics of pathogenicity similar to other herpesvirus a these data increase the knowledge about pathogenesis of BuHV-1, that was not studied too much, particularly about experimental infections in cattle. It would be interesting to know if this event happens in mixed farms (cattle and buffaloes). This information will allow the rational sanitary measures that may allow decrease of the viral crossing risk and barriers crossings among species, with consequent generation of genetic variants with unknown virulence in mixed calves (Maidana *et al.*, 2016).

Bharti *et al.* (2015) in their study with Murrah buffaloes calves, suggest that the levels of immunoglobulins increased with the age of the animals after have sucking colostrums and even they have mentioned the levels of immunoglobulin by the age of 24 h after birth individually.

In the present study it was used tests of neutralization virus to evaluate the presence of Bovine infectious Rhinotracheitis antibodies. In the situations when the animals presented antibodies for the Bovine infections Rhinotracheitis the titles showed a decrease what demonstrated that

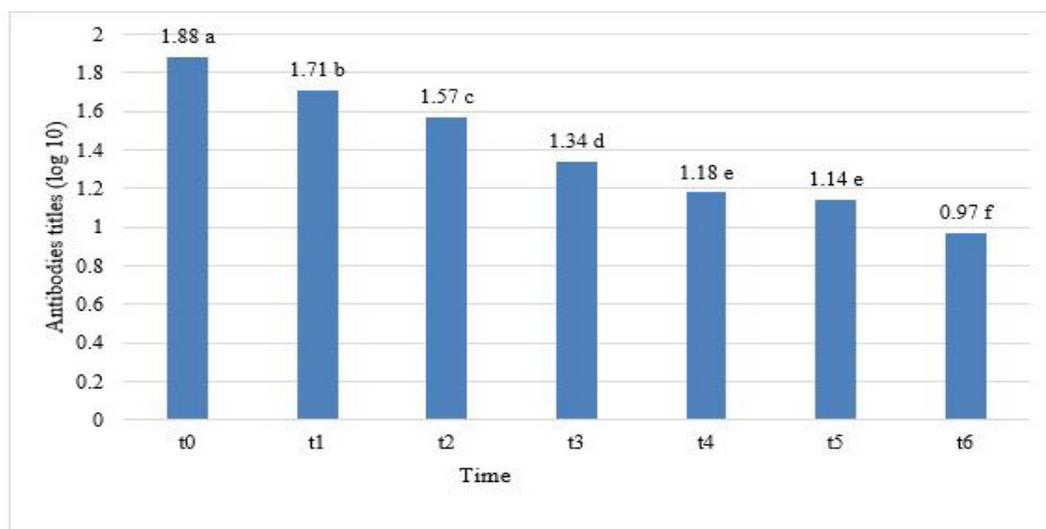


Figure 1. Titles of antibodies (log 10) present in the serum of buffalo calves that were reagent for infectious Rhinotracheitis in bovines (IBR, BoHV-1).

Table 1. Titles that present antibodies in reagent serum blood buffalo calves of virus of bovine infective Rhinotracheitis (IBR, BoHN-1) expressed in  $\log_{10}$ .

Time of collecting	Antibodies title ( $\log_{10}$ )
T0	1.88a
T1	1.71b
T2	1.57c
T3	1.34d
T4	1.18e
T5	1.14e
T6	0.97f
EPM	0.168

\*P-Value<0.0001

Notes: Time 0 (T0) = 24-48 hours from birth; Time 1 (T1) = 15 days; Time 2 (T2) = 30 days; Time 3 (T3) = 45 days; Time 4 (T4) = 60 days; Time 5 (T5) = 75 days; Time 6 (T6) = 91 days.

For qualitative results, any neutralisation at a titre of 1 ( $\pm 0.3 \log_{10}$  units) or above (initial dilution convention) is considered to be positive (OIE, 2016).

the buffalo calves showed passive immunity or else they have received it through their mother's colostrums.

There were not any compatible signal manifestation with IBR/IPV in the experimental group decreasing of weight and the growing up were observed there was a fall of the titles of the antibodies against BoHV-1 what shows that there was not any active infection in these animals by the period they were studied.

With the fall of passive immunity if the virus is circulating, the animals can be infected and present clinical manifestations. In that situation vaccine can be indicated.

## CONCLUSIONS

Evidenced antibodies titles anti-BoHV-1 after sucking the colostrums. The transference of passive immunity occurred in an appropriated way in all the experimental group of calves, a proved fact by the kinetics of antibodies anti BoHV-1 in the neonatal phase, giving the animals protection, at least until three months from birth, period when the mother's antibodies decrease and they become vulnerable to infections by this virus.

Most of male and female buffaloes reached good titles of antibodies from the mother's anti-BoHV-1 through natural sucking.

Most of the calves received mother's antibodies anti-BoHV-1 of naturally infected female buffaloes (that had never received vaccine) showing a high prevalence of IBR/IPV in the studied animals.

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