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Occurrence of *Brucella abortus*, *Leptospira interrogans* and bovine herpesvirus type 1 in buffalo (*Bubalus bubalis*) herd under extensive breeding system

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The current study aimed to determine the occurrence of *Brucella abortus*, *Leptospira interrogans* and BoHV-1 in buffalo (*Bubalus bubalis*) herd under extensive breeding system. In order to perform the study, blood samples were collected from 306 female buffaloes belonging to a rural property located in São Mateus County – MA. The samples were tested for the presence of specific antibodies. The number of samples collected in such property was estimated by taking under consideration a minimum prevalence of 6% for each infection, with confidence interval of 95%. Of the 306 analyzed female buffaloes' serum samples, 70.58% were positive for one or more *Leptospira interrogans* serovars, with variable titers between 100 and 800. The most prevalent serovars in the current study were Pomona, Butembo, Icterohaemorrhagiae, Sentoti, Copenhageni, Adamanda, Castelonis, Wolffi, Panama and Grippotyphosa. A BoHV-1 occurrence of 87.25% was identified in the evaluated animals. All animals in the study tested negative for brucellosis. The study results indicate that *L. interrogans* and BoHV-1 were widespread microorganisms within the assessed property and they may contribute to a decrease in the production and reproductive rates of the herd.

Key words: Antibodies, BoHV-1, brucellosis, female buffaloes, leptospirosis.

INTRODUCTION

The reproductive infections caused by *Brucella abortus*, *Leptospira* spp. and by Bovine Herpesvirus type 1 (BoHV-1) are among the main causes of cattle productivity losses in Brazil (Frandoloso et al., 2008).

According to the Food and Agriculture Organization (FAO), World Health Organization (WHO) and to the World Organization for Animal Health (OIE) (Al-Majali et al., 2009; Mekonnen et al., 2010; Abernethy et al., 2011), brucellosis is one of the most important and widespread zoonoses in the world. *Brucella abortus* is highly pathogenic and causes severe illness, especially in bovines (Corbel et al., 2006). This species also has clinical and

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epidemiological importance and it is considered as pathogenic for humans (Maurin, 2005).

The damages caused in bovines by brucellosis are countless: the herd undergoes decrease in milk and meat productivity, sale price devaluation regarding the animals and animal products from endemic regions, increased calving intervals, the occurrence of miscarriage in sick female animals, sterility, indication for sacrificing reactive animals and the consequent expense with purchasing other animals for replacement in the herd (Berhe et al., 2007). Buffalo breeding probably undergoes similar losses (Paulin and Ferreira Neto, 2008).

Brucellosis is considered both a foodborne and an occupational disease, and transmission can occur by contact with infected animal parts, consumption of infected unpasteurised milk products and via the airborne route (Pappas et al., 2008).

Leptospirosis is a zoonotic disease that affects domestic and wild animals, and humans, thus representing an important economic and public health problem. Its transmission is facilitated by the presence of water contaminated by bacteria from genus *Leptospira* (WHO, 2011). The consequences of leptospirosis for animal health reaches the economic sphere, in view of the involvement of producing animals such as cattle, sheep, goats, pigs and horses (Brazil, 1995) with it. In these species, economic losses result mainly from reproductive disorders such as infertility, miscarriage, birth of weak calves and temporary decrease in milk production (Cervantes et al., 2002).

Pathogenic leptospires, the bacteria that causes leptospirosis, enter via the mucous membranes or the damaged skin of the host animals and colonize the proximal renal tubules of the kidneys of the carrier (Athanazio et al., 2008; Gamage et al., 2014).

Bovine herpesviruses (BoHV) are the etiological agents of many impacting diseases in livestock, and they are responsible for major economic losses in cattle breeding (Silva et al., 2000). Bovine herpesvirus type 1 (BoHV-1) is related to respiratory manifestations, such as infectious bovine rhinotracheitis (IBR), genital manifestations (infectious pustular balanoposthitis and vulvovaginitis), reproductive failures, return to estrus and miscariages (Del Médico Zajac et al., 2010). The bovine species is the natural host of bovine herpesvirus type 1 (BoHV-1) and type 5 (BoHV-5). However, serological studies have suggested that buffaloes may be susceptible to BoHV-1 (Cavirani et al., 1997; Galiero et al., 2001) and to other genetically related alpha herpes viruses (Thiry et al., 2007).

BoHV-1 is shed in nasal discharge for 10–14 days during acute respiratory infection and transmission occurs by contact with mucosal droplets from infected cattle. The virus is also shed following reactivation from latency. BHV-1 can also be transmitted to susceptible animals through contaminated materials including semen (Biswas et al., 2013).

Thus, the current study aimed to determine the occurrence of *Brucella abortus*, *Leptospira interrogans* and BoHV-1 in buffalo (*Bubalus bubalis*) herds under extensive breeding system.

MATERIALS AND METHODS

Study area

The study was conducted in São Mateus County, which is located in the Central Mesoregion of Maranhão State, Brazil, and has a land area of 783.335 km² (IBGE, 2011). The studied area was selected according to the existence of buffalo beef cattle ranching. The county has a herd of 26,801 bovids distributed among 304 rural properties (Maranhão, 2012).

Animals

The studied population consisted of female beef cattle buffaloes, over the age of 24 months and vaccinated against brucellosis only. The study excluded postpartum females, females 15 days before or 15 days after delivery, thus following the technical regulations of the National Program for the Control and Eradication of Brucellosis and Tuberculosis – PNCEBT (Brazil, 2001; Brazil, 2004; Brazil, 2006).

Sampling design

The number of blood samples collected from the property was estimated by using Win Episcope 2.0 (Blas et al., 2004) software. The prevalence of at least 6% for each disease, and 95% probability of detecting at least one positive animal was predicted. The number of female buffaloes in this property is of 896 and the total number of analyzed samples was of 306.

Blood samples collection

Blood was collected from the animals' jugular vein by using a system of tubes containing vacuum. After clot retraction, the samples were centrifuged at 3.000 rpm (1,512 g) for 15 min to extract serum and the sera were stored at -20°C until the completion of serological tests (Subharat et al., 2012). An epidemiological questionnaire was applied in order to obtain information pertaining to the management and the health status of the evaluated animals. The current study was approved by the Ethics Committee on Animal Experimentation - CEEA at the Veterinary Medicine School of the State University of Maranhão, Protocol 037/2011.

Diagnostic tests

Brucellosis

The Buffered Acidified Antigen (BAA) was performed as a screening test for detecting anti-*Brucella abortus* antibodies by using an antigen produced by the Laboratory of Technology of Paraná - TECPAR. The reactive samples in BAA were simultaneously subjected to 2-Mercaptoethanol (2-ME) and Slow Serum-agglutination in Tubes (SSA) by using antigen produced by TECPAR, in titers of 1:25, 1:50, 1:100 and 1:200. Results interpretation was performed according to the current legislation (Brazil, 2004).

Leptospira interrogans

The blood sera were submitted to the study of anti-leptospira

Table 1. Ocurrence of antibodies against *Brucella abortus* in 306 buffaloes under extensive breeding system according to the diagnostic test.

Diagnostic tests	Positive animals N (%)	Negative animals N (%)
BAA	27 (8.82)	279 (91.18)
SSA	27 (8.82)	279 (91.18)
2-ME	0 (0)	306 (100)

N = Number of animals; % = Percentage of animals; BAA = Buffered Acidified Antigen; SSA = slow serum-agglutination in tubes; 2-ME = -Mercaptoethanol.

agglutinins against 24 serovars from the *Leptospira* complex kept by the Laboratory of Bacterial Zoonoses, Veterinary Medicine Graduation Course, University of São Paulo (FMVZ-USP). The procedure was performed by using Microscopic Agglutination Test (MAT), according to the standards by the Ministry of Health (Brazil, 1995).

Each serum sample was first diluted at 1:100 in phosphate buffered saline solution, pH 7.2, and tested against 24 serovars: Andamana, Patoc, Shermani, Cynopteri, Butembo, Panama, Hardjobovis, Castelonis, Whitcombi, Tarassovi, Javanica, Australis, Autumnalis, Bataviae, Bratislava, Canicola, Copenhageni, Grippothyphosa, Hebdomadis, Icterohaemorrhagiae, Pomona, Sentot, Wollfi, Pyrogenes.

Samples with agglutination ≤50% were considered as positive for *Leptospira interrogans* when they were compared with the control. The positive samples in the initial titer (screening) were tested again in order to set the antibody titer for each serovar by using increasing dilutions from 1:100 up to 1:800. Samples with titer equal to or less than 100, with 50% agglutination or cells disappearing from the field were considered as positive, according to the dark field microscopy (Brazil, 1995; Santa Rosa et al., 1970). The reactive sera were titrated in geometric series of four dilutions of ratio two, and the titer was the reciprocal of the highest dilution in which there was agglutination.

Bovine Herpesvirus type 1

The qualitative detection of anti-BoHV-1 antibodies was performed by using Enzyme-Linked Immunoabsorbent Assay (ELISA) technique by applying the commercial kit for Indirect ELISA Test (CHEKIT IBR – SERO - Dr. BOMMELI AG/Liebefeld – Bern – Swiss).

Data analysis

The information from the questionnaires, as well as the serology results were stored in a database by using Microsoft Access®.

RESULTS AND DISCUSSION

The property assessed in the current study is a prototype of rural property for beef cattle buffalo breeding currently found in the State of Maranhão, Brazil. The property has Murrah buffaloes and Murrah-crossbred ones reared under extensive production system. The buffaloes feed on pastures and their diet is supplemented with minerals only. The breeders do not apply artificial insemination and, for sanitary control, they vaccinate females for brucellosis only and sporadically test animals for brucellosis and tuberculosis only.

The introduction of new animals in the property is usually made without considering health aspects, and without performing quarantine. Given this scenario, there is a great possibility of introducing animals infected by *B. abortus*, *L. interrogans* and BoHV-1.

The current study investigated the occurrence of *B. abortus*, *L. interrogans* and BoHV-1 in a buffalo herd in São Mateus County, where there was no previously reported epidemiological data on such diseases.

Of the 306 examined serum samples from female buffaloes, 8.82% (n=27/306) tested positive for brucellosis in the screening test (BAA). Of these, 100% were positive in the SSA test and negative in the 2-ME test regarding the four tested dilutions (1:25, 1:50, 1: 100 and 1:200) (Table 1).

No positive result was found for brucellosis in buffaloes from the assessed property. Only vaccination antibodies were detected in the examined animals. The absence of positive animals in the current study may be associated with a set of official health actions performed over the last decades in almost all Brazilian regions covering the control and eradication of such disease.

Of the total serum samples from the analyzed female buffaloes, 70.58% (n = 216/306) were positive for one or more *Leptospira* serovars, with titers ranging from 100 to 800. Of the 24 serovars investigated in the current study, 100% of them were detected, and the most common serovars were: Pomona (29.41%), Butembo (25.49%), Icterohaemorrhagiae (24.50%), Sentoti (22.54%), Copenhageni (20.58%); Adamanda (20.58%), Castelonis (19.60%), Wolffi (18.62%), Panama (18.62%) and Grippotyphosa (17.64%) (Table 2).

In the current study, the occurrence rate of *L. interrogans* found in the buffalo species, regardless the serovar, was higher than the rates reported for buffaloes by some researchers in the country, whose studies showed values ranging between 37.70% (Langoni et al., 2000) and 67.72% (Silva et al., 2009) for measures taken in the States of São Paulo and Pará, respectively.

The differences found among the results obtained in the current study and some results published in the literature can be understood, in part, through the number and types of serovars used in the serologic evaluation, herds' hygienic-sanitary handling, as well as in the degree and type of exposure to other domestic and wild animals and to synanthropic rodents that are known by

Table 2. Serovars ocurrences of MAT-positive and percentages in 216 buffaloes under extensive breeding system.

Serovars	Ocurrences N (%)
1 – Pomona	90 (29.41)
2 – Butembo	78 (25.49)
3 – Icterohaemorrhagiae	75 (24.50)
4 – Sentoti	69 (22.54)
5 – Copenhageni	63 (20.58)
6 – Adamanda	63 (20.58)
7 – Castelonis	60 (19.60)
8 – Wolffi	57 (18.62)
9 – Panama	57 (18.62)
10 – Grippotyphosa	54 (17.64)
11 – Patoc	51 (16.67)
12 – Autuminallis	45 (14.70)
13 – Hebdomadis	39 (12.74)
14 – Batavae	36 (11.76)
15 – Brastilava	36 (11.76)
16 – Australlis	30 (9.80)
17 – Canícola	30 (9.80)
18 – Javanica	30 (9.80)
19 – Hadjo	27 (8.82)
20 – Taransovi	27 (8.82)
21 – Cynope	24 (7.84)
22 – Pyrogenes	18 (5.88)
23 – Shermani	15 (4.90)
24 – Whitcombi	9 (2.94)

MAT = Microscopic agglutination test.

their interference in the epidemiology of this disease, as highlighted by Linhares et al. (2005).

Silva et al. (2009) investigated the occurrence of anti *Leptospira* agglutinins in blood sera from buffaloes in Pará State and they identified Hardjo (hardjioprajitno) Grippotyphosa and Pomona serovars as the most prevalent ones. Langoni et al. (1999) tested 403 sera from buffaloes and found that their samples were positive for Wolffi (44.80%), Icterohaemorrhagiae (33.60%), Hardjo (33.60%) and Castellonis serovars (16.5%). These data partly resemble those obtained in the current study, since Hardjo serovar, despite having reacted in the tested samples, did it less frequently (8.82%) in the current study.

As it is considered that buffaloes' physiology is similar to that of bovines, the current study draws a parallel with the seroepidemiological investigation conducted by Silva (2011) who analyzed 2582 serum samples from cows in reproductive age in the state of Maranhão. The authors reported a seroprevalence of 24.32%, in which Hardjo serovars were identified as the most prevalent ones (24.32%), followed by Wolffi (22.00%), Patoc (12.42%), Shermani (8.85%) Grippotyphosa (8.21%) and hebdomadis serovars (7.35%). These differences may be related to the degree and type of exposure to sources of infection that affect leptospirosis epidemiology.

The large number of *Leptospira* serovars identified in the assessed buffalo herd, such as Pomona, Icterohaemorrhagiae, Sentot, Copenhageni, Andamana, castellonis, Panama and Grippotyphosa, reinforces the suspicion of frequent and intense presence of wild and free living animals in the studied rural property.

Several studies demonstrate the large number of serovars affecting wild and free living animals (Silva, 2011). Silva et al. (2010) reported that opossums (*Didelphis albiventris*) and cervids can be Patoc, Autumnalis, Icterohaemorrhagiae, Andamana and Canicola serovar reservoirs to domestic animals such as cattle, goats, sheep, pigs, horses and dogs.

Incidental serovars such as Castellonis, Sentot and Andamana, which were detected in the current study and which descriptions are related to wild animals (Santa Rosa et al., 1975; Santa Rosa et al., 1980), raise suspicions on the involvement of wild animals as reservoirs of these serovars among the evaluated buffaloes.

These results show the importance of intensifying the studies on leptospirosis in wild and free-living animals that live in the surroundings of rural properties in the State of Maranhão and humans. So, management measures can be implemented in order to reduce the presence of these reservoir species in herds and, thus, prevent and control this disease in a most efficient manner.

Leptospirosis is a globally important zoonotic disease. Humans contract leptospirosis mainly from infected animal sources (human-to-human transmission has yet to be reported). Pathogenic leptospires, the bacteria that causes leptospirosis, enter via the mucous membranes or the damaged skin of the host animals and colonize the proximal renal tubules of the kidneys of the carrier. The known carriers of leptospires are wild and domestic animals, such as rodents, cattle, buffaloes, pigs and dogs (Gamage et al., 2014).

It is worth emphasizing that Icterohaemorrhagiae serovar, which is of great relevance to public health (Brazil, 2005) and is frequently isolated from rodents (Acha and Szyfres, 2001), showed high incidence (24.50%) in the evaluated female buffaloes. Similar results were obtained by Juliano et al. (2000) who reported the occurrence of 20.6% of this serovar among reactive animals in Goiania.

Besides Icterohaemorrhagiae, other serovars foun in the current study, such as Grippotyphosa, may be of importance to public health if the environment is contaminated by urine from infected animals and if the infecting dose is high within an exposed population. In addition, a pre-existing immunodeficiency condition in an individual or a group of individuals exposed to environments by

contaminated other serovars can generate a serious clinical condition in these individuals, even when the infectious dose is low, as outlined by Silva (2008).

Regarding the analyzed period and herd, BoHV-1 antibodies were found in 87.25% (n = 267/306) of the analyzed samples. The occurrence of BoHV-1 was highly diagnosed in the studied property.

This is the only data about BoHV-1 occurrence in female buffaloes in the State of Maranhão, Brazil. The BoHV-1 occurrence observed in the current study was higher than that reported by Medeiros et al. (2011) in southern Rio Grande do Sul, who found a 43.75% prevalence in a total of 80 analyzed samples. Such difference may be related to the sample size used in the research. However, the results from both studies indicate that the infection by this virus occurs in significant percentages of buffalo herds and, despite the claims by many professionals involved in buffalo breeding that this virus is exotic in Brazil, the results from the two studies say otherwise.

Studies by Scicluna et al. (2010) indicate that the buffalo species is susceptible to infection by bovine herpesvirus, thus demonstrating the possible role of buffaloes as hosts or reservoirs of this virus.

There are different reasons for the high occurrence and distribution of *Leptospira sp*. and BoHV-1 in the studied property. However, both diseases have in common the fact that they are caused by microorganisms able to definitively establish themselves in the animals, whether by persistent infection (*Leptospira* spp.) or by latent infection (BoHV-1). Thus, with respect to any of the diseases, the introduction of a single animal contaminated by the microorganism in the property would be enough for the further spread and perpetuation of the infection in the buffaloes. This is a common practice performed in the studied property, as stated by the respondent producer.

In addition to such scenario, the neglect of buffaloes' health aspects in this property and the lack of knowledge on the pathogenicity of the reproductive infections associated with diagnosis difficulties and costs inhibit initiatives to implement control programs prior to the introduction of new animals in the property, or to the investigation of the infectious cause's related to reproductive disorders.

Conclusions

By analyzing the results from the current study, it can be inferred that *B. abortus* was not found in the assessed herd. On the other hand, *L. interrogans* and BoHV-1 were found and showed high occurrence rates. The presence of such microorganisms in the studied population may constitute an important factor to the reduced productivity rates of female buffaloes in São Mateus County, State of Maranhão, Brazil.

Considering that buffalo breeding is important to the State of Maranhão, it is recommended that efforts should be focused on conducting studies that are more comprehensive, with epidemiologically acceptable standards, involving larger numbers of herds and samples. It is worth emphasizing the importance of conducting health education programs.

Conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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