

Estimated prevalence of brucellosis in buffaloes (*Bubalus bubalis*) from the Ribeira Valley region, State of São Paulo, Brazil

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Key words

Buffalo - *Bubalus bubalis* - Brucellosis - Complement fixation test - State of São Paulo - Brazil.

Summary

With the purpose of estimating the brucellosis prevalence among female buffaloes (*Bubalus bubalis*) from the Ribeira Valley region, State of São Paulo, Brazil, 462 animals from 16 herds located in seven municipalities were examined by the complement fixation test. It was observed that 10.39% of the animals were reactors, and the population rate was situated between 7.61 and 13.17%, with 95% confidence limits. The occurrence of reactors was observed in 11 of the 16 herds and in five of the seven municipalities. There was no significant difference in prevalence rates when the type of production or the farming system were used as criteria of the analysis. The prevalence rate among buffaloes that did not cohabit with cattle (13.74%) was significantly higher ($P < 0.01$) than the rate among buffaloes that cohabited with cattle (0.83%). It was also observed that the prevalence rate among animals aged six years or more (18.63%) was higher ($P < 0.01$) than the rate observed among animals up to three years old (8.06%) and among animals aged three to six years. The results suggest that brucellosis may be a serious sanitary problem for the herd studied.

INTRODUCTION

The susceptibility of buffaloes to brucellosis has long been known (12). Infection of this species has been observed in several regions, but reports are more frequent from Asian countries such as India and Pakistan, which contain the world's largest buffalo populations. There are also reports of occurrence of the disease in other countries such as Italy (18) and the United States of America (13).

In Brazil, the occurrence of brucellosis in buffaloes was also confirmed by isolation of the etiological agent or serological investigations carried out in some states. In a study involving two herds in the State of Goiás, Costa *et al.* examined 199 buffalo sera, by the plate agglutination test, and verified that 41 had positive titers (6). In Pará, the state with the largest buffalo herd in Brazil, Láu and Singh observed an 8.6% prevalence of infection, with the proportion of reactors reaching 12.2% on Marajó Island (9). In a study on three herds in the State of Pernambuco, Modesto *et al.* found a 24.0% rate of positivity in one herd, a 6.12% rate in the second, and no positivity in the third, by the plate agglutination test (11).

The occurrence of buffalo brucellosis has also been reported in the State of São Paulo. Santa Rosa *et al.* observed that 27 of 66 buffaloes (40.91%) reacted to the plate agglutination test (17). Ogassawara *et al.*

found 3.77% of reactors among 212 female buffaloes, and also isolated *Brucella abortus* from an articular hygroma observed in one animal (14). Examining 992 buffalo sera from nine herds, Sandoval *et al.* verified that 4.33% of the animals reacted to the plate agglutination test, while 5.69% reacted to the card test (16). These investigators also isolated *B. abortus* from the milk of one reacting animal. In a study comprising the period from 1977 to 1987, Feitosa *et al.* observed that 13.93% of 3167 animals reacted to the plate agglutination test, 28.88% of 2784 animals reacted to the card test and 23.92% of 1321 animals reacted to the mercaptoethanol test (8).

The epidemiology of brucellosis in buffaloes, as in other animal species, may be affected by several factors. Buth and Manchanda, studying the disease in small Indian villages, found low prevalence rates and attributed this fact to the practice of maintaining animals in small groups in isolated holdings (4). Maqsood *et al.*, in Pakistan, observed that the prevalence of infection was higher in older animals (10).

When studying brucellosis in buffaloes, it is also important to consider the effect of vaccination with strain 19 on the diagnosis, since vaccination may induce an increase of serological titers. Domingues *et al.* verified that 3 to 8 year old female buffalo calves vaccinated with the standard dosage may show agglutinating titers for up to 240 days, i.e., for the duration of their study (7). According to Roxo *et al.*, agglutinating titers may persist for five to six months after vaccination (15).

Due to the scarcity of data about the situation of buffalo brucellosis in Brazil, the objective of the present study was to estimate the prevalence of the disease among females of the buffalo herd from the Ribeira Valley, which is the main region of buffalo raising in the State of São Paulo.

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■ MATERIALS AND METHODS

Population

The Ribeira Valley region comprises an area of about 17,000 km² located between parallels 23°45' and 25°15'S and meridians 46°45' and 49°30'W, with 14 municipalities and a part of six other municipalities (figure 1).

The buffalo herd is formed by 11,461 animals raised on 102 farms located in nine municipalities. This herd is predominantly crossbred and destined mainly to meat or milk and meat production. Management is typically extensive, with the animals being reared on pasture, with a low use of technology. Serological tests for brucellosis control are performed by 63.33% of the farmers, and vaccination with strain 19 is done in 44.00% of the herds (3).

Sampling

The sample size was calculated according to the criteria recommended by the Centro panamericano de zoonosis (5), using the following formula:

$$n = \frac{p \cdot (100 - p) \cdot z^2}{\left(\frac{p \cdot d}{100}\right)^2}$$

n = number of animals to be examined

p = expected prevalence (%)

z = degree of confidence

d = expected level of error (%)

$$\begin{aligned} n &= \frac{11 \cdot (100 - 11) \cdot 1.96^2}{\left(\frac{11 \cdot 26}{100}\right)^2} \\ &= \frac{3760.9264}{8.1796} = 460 \end{aligned}$$

A total of 462 samples from adult female buffaloes were obtained at random from January 1992 to June 1993 in 16 farms located in seven municipalities (Pariquera-açu, Cananéia, Registro, Eldorado, Iguape, Sete Barras, Jacupiranga). When the herd had up to 30 females, all of them were sampled, and when the number of animals was more than thirty, 10 to 30% of the animals were sampled.

Serological tests

The microtechnique of the complement fixation test with incubation at 37°C during both phases and five hemolytic units of complement (5 CH 50%) was performed as recommended by Alton *et al.* (1). Sera were tested in double dilutions from 1/2. The antigen was a heat killed suspension of *B. abortus* strain 1119/3, and guinea pig serum was used as complement. To estimate the prevalence, sera with a titer equal to or higher than 1/4 in the complement fixation test were considered to be positive.

Statistical analysis

The limits of the confidence interval to estimate the population prevalence were determined according to Astudillo and Wanderley (2). The frequency of reactors observed in the various categories analyzed was compared by the chi-square test.

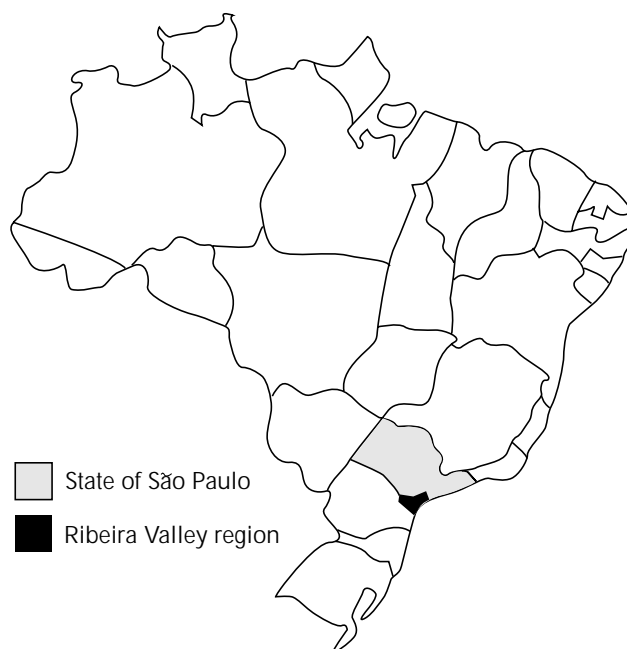


Figure 1: Location of the Ribeira Valley region, State of São Paulo, Brazil.

■ RESULTS

The results showed that 10.39% of the animals examined had positive reaction to brucellosis. The prevalence rate for the population, with 95% confidence limits, was 7.61 to 13.17%.

Eleven of the 16 herds (73.33%) studied had reacting animals. In the herds with infected animals, the reactor frequency ranged from 3.75 to 35.71%.

Five of the seven municipalities under study had reacting animals, while in two of them, Eldorado and Jacupiranga, there was no animal with a serological titer in the complement fixation test. The highest frequency was 35.71%, observed in Cananéia (table I).

The percentage of reactors in dairy herds was similar to that observed in beef herds, 6.67 and 5.17%, respectively, while in the herds for milk and meat production the percentage of reactors was 12.42%, but the chi-square test did not show a significant difference between these categories (table II).

As shown in table III, the prevalence in herds raised under conditions of extensive management (10.57%) was close to that observed in the semi-intensive raising system (9.68%) ($P > 0.05$).

The prevalence of brucellosis was 0.83% in buffalo herds cohabiting with cattle and 13.74% in herds that did not cohabit with cattle (table IV), with a significant difference between values ($P < 0.01$).

The frequency of reactors among animals up to three years old (8.06%) was practically the same as that observed among animals aged three to six years (8.05%), but the prevalence among animals older than six years was 18.63% (table V), and the chi-square test showed that this difference was statistically significant ($P < 0.01$).

■ DISCUSSION

The prevalence rate of brucellosis among Ribeira Valley buffaloes observed in the present study was 10.39%. Other studies performed in the State of São Paulo have shown rates ranging from 4.33% (18)

Table I

Brucellosis positive buffaloes in the Ribeira Valley region, State of São Paulo, 1993, according to the municipality

Municipality	Num. of herds	Animals		
		Examined	Reactors	%
Pariquera-açu	3	53	5	9.43
Cananéia	1	28	10	35.71
Registro	4	186	19	10.22
Eldorado	1	19	0	0.00
Iguape	4	118	3	2.54
Sete Barras	2	44	11	25.00
Jacupiranga	1	14	0	0.00
Total	16	462	48	10.39

Table II

Brucellosis positive buffaloes in the Ribeira Valley region, State of São Paulo, 1993, according to the type of herd

Herd production	Num. of herds	Animals		
		Examined	Reactors	%
Meat	4	90	6	6.67
Milk	3	58	3	5.17
Meat and Milk	9	314	39	12.42
Total	16	462	48	10.39

Table III

Brucellosis positive buffaloes in the Ribeira Valley region, State of São Paulo, 1993, according to management

Management	Num. of herds	Animals		
		Examined	Reactors	%
Extensive	12	369	39	10.57
Semi-intensive	4	93	9	9.68
Total	16	462	48	10.39

to 13.93% (8), obtained by the plate agglutination test. Other investigators, testing a small number of animals, obtained rates from 3.77% (14) to 40.91% (17), using the plate agglutination test. The rate observed in the present study was not very different from the one obtained by Láu and Singh (9) for Marajó Island, State of Pará (12.2%), another important buffalo raising center in Brazil. The proportion of reactors varied greatly, being influenced by the representativity of the sampling and by the serological test used for diagnosis.

Of the 16 herds examined, five had no reacting animal, while in the other 11 herds the prevalence ranged from 3.57 to 35.71%. Sandoval *et al.* (16), examining 936 buffaloes from nine herds in the State of São Paulo, did not find any reactor in three of the herds, while in the other six herds the prevalence ranged from 0.47 to 60.0% of the animals examined by the plate agglutination test, reaching 90.0% when the animals were examined by the card test.

Table IV

Brucellosis positive buffaloes in the Ribeira Valley region, State of São Paulo, 1993, according to cohabitation or not with cattle

Cohabitation with cattle	Num. of herds	Animals		
		Examined	Reactors	%
Yes	5	120	1	0.83
no	11	342	47	13.74
Total	16	462	48	10.39

Table V

Brucellosis positive buffaloes in the Ribeira Valley region, State of São Paulo, 1993, according to the animal age

Age (years)	Examined	Reactors	%
< 3	186	15	8.06
3 - 6	174	14	8.05
> 6	102	19	18.63
Total	462	48	10.39

Analysis of the data according to municipality showed great variation, with no reactor found in two of the seven municipalities and with a frequency of reactors from 2.54 to 35.71% in the other five. In Registro, where the highest number of animals was studied, the rate was 10.22%, close to that for the sample as a whole.

Although the prevalence rate observed in herds producing meat and milk was nearly double that observed in beef herds and in dairy herds, statistical analysis did not show a significant difference among these categories, with meat and milk herds representing most of the sample and having a prevalence rate close to that for the sample as a whole.

The raising system also did not influence the prevalence of brucellosis since the rate for herds raised in the extensive system was close to that for herds raised in the semi-intensive system, although some workers state that the raising system may influence the occurrence of the disease (4).

A significantly higher prevalence rate in herds that did not cohabit with cattle was observed in the present study. Although cattle may act as a source of brucellosis infection, transmitting the disease to buffaloes, the results suggest that this species may maintain the disease, independently of cohabiting with cattle. These data differ from those observed by Sandoval *et al.*, who found a higher prevalence of brucellosis among buffalo herds cohabiting with cattle (16).

The present results show an association between the age of the animals and the prevalence rate, except for the fact that the rate for animals up to three years old was the same as that for animals aged three to six years, being important to consider that the prevalence rate observed for younger animals was certainly influenced by strain 19 vaccination, which also in buffaloes induces a serological response that may persist for some time, as observed by several workers (7, 15). The relationship between age and prevalence rate may be explained by the greater probability of older animals to be exposed to the disease when compared with younger animals, as also observed by Maqsood *et al.* in a study on buffalo herds in Pakistan (10).

■ CONCLUSION

The present results show that brucellosis may be an important sanitary problem for the buffalo herd in the Ribeira Valley region, a problem that justifies the implementation of more detailed studies and sanitary measures in order to fight the disease.

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Résumé

Mathias L.A., Del Fava C., Girio R.J.S., Samara S.I. Estimation de la prévalence de la brucellose chez des buffles (*Bubalus bubalis*) de la région de la vallée du Ribeira, Etat de São Paulo, Brésil

Afin d'estimer la prévalence de la brucellose chez des buffles (*Bubalus bubalis*) de la région de la vallée du Ribeira, Etat de São Paulo, Brésil, 462 animaux provenant de 16 troupeaux et localisés dans sept districts municipaux, furent examinés au moyen de la réaction de fixation du complément. Parmi les animaux examinés, 10,39 p. 100 étaient positifs et le taux de positivité de la population a varié de 7,61 à 13,17 p. 100, avec un intervalle de confiance de 95 p. 100. Des animaux positifs ont été trouvés dans 11 des 16 troupeaux et dans cinq des sept districts municipaux. Il n'y a pas eu de différence significative des taux de prévalence de la brucellose en prenant pour critères le type de production et le système d'élevage. Le taux de prévalence de la brucellose était significativement plus élevé ($P < 0,01$) chez les buffles ne vivant pas en commun avec d'autres bovidés (13,74 p. 100) que chez celles qui étaient élevées avec d'autres bovidés (0,83 p. 100). Il a aussi été observé que la prévalence de la maladie chez les animaux âgés de plus de six ans (18,63 p. 100) était plus importante ($P < 0,01$) que celle des animaux de trois à six ans et de moins de trois ans (8,06 p. 100). Les résultats indiquent que la brucellose pourrait être un problème sanitaire important pour le troupeau étudié.

Mots-clés : Buffle - *Bubalus bubalis* - Brucellose - Réaction de fixation du complément - Etat de São Paulo - Brésil.

Resumen

Mathias L.A., Del Fava C., Girio R.J.S., Samara S.I. Prevalencia estimada de la brucelosis en búfalos (*Bubalus bubalis*) de la región del Valle Ribeira, estado de Santo Paulo, Brasil

Con el fin de estimar la prevalencia de la brucelosis en las hembras búfalo (*Bubalus bubalis*) en la región del Valle Ribeira, estado de Santo Paulo, Brasil, se examinaron mediante fijación de complemento 462 animales provenientes de 16 hatos localizados en siete municipalidades. Se observó una reacción en 10,39% de los animales y la tasa de la población se situó entre 7,61 y 13,17% con límites de confianza de 95%. Los animales reactivos se observaron en 11 de los 16 hatos y en cinco de las siete municipalidades. No hubo diferencia significativa en las tasas de prevalencia cuando se analizaron los criterios tipo de producción o sistema de crianza. La tasa de prevalencia entre los búfalos que no cohabitaron con ganado (13,74%) fue significativamente mayor ($p < 0,01$) que la tasa de los búfalos que cohabitaron con ganado (0,83%). También se observó que la prevalencia entre los animales de seis o más años (18,63%) fue mayor ($p < 0,01$) que la tasa observada entre animales hasta tres años de edad (8,06%) y entre animales de tres a seis años. Los resultados sugieren que la brucelosis puede ser un problema sanitario serio para los hatos estudiados.

Palabras clave: Búfalo - *Bubalus bubalis* - Brucelosis - Prueba de fijación del complemento - Estado de Santo Paulo - Brasil.