Prevalence Survey of Selected Bovine Pathogens in Water Buffaloes in the North Region of Brazil

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Although the largest buffalo herd in the occident is in the north region of Brazil, few studies have been conducted to assess the prevalence of selected parasitic diseases in buffalo herd. The present study was therefore conducted to investigate the epidemiological of Toxoplasma gondii, Neospora caninum, Anaplasma marginale, Babesia bigemina, and Babesia bovis in water buffaloes in the north region of Brazil. A total of 4796 buffalo blood samples were randomly collected from five provinces and simultaneously analyzed by the IFAT and ELISA. The serological prevalence of T. gondii and N. caninum was 41.3% and 55.5% in ELISA and 35.7% and 48.8% in IFAT, respectively. The overall prevalence of A. marginale, B. bovis, and B. bigemina was 63%, 25%, and 21% by ELISA and 50.0%, 22.5%, and 18.8% by IFAT, respectively. This study shows valuable information regarding the serological survey of selected bovine pathogens in water buffaloes in the north region of Brazil which will likely be very beneficial for the management and control programs of this disease.

1. Introduction

Brazil has the biggest western buffalo herd, where approximately 65% of which is located in the north region of this country [1]. Nowadays, the buffalo has been highlighted in the national scenery, showing more than just an alternative to the occupation of lands unsuitable for cattle but becoming an economically important option. As consequence, concern about sanitary management has increased considerably, because the clinical, pathological, and epidemiological studies are still poorly studied in Latin America.

Buffaloes, when compared to other domestic livestock, are generally a resistance animal [2]. Although the reason is not clear, the effect on buffaloes is often less deleterious than that on cattle.

Toxoplasmosis is a widespread zoonosis caused by the coccidian protozoan Toxoplasma gondii which can parasitize human beings and many warm-blooded animals, inducing abortions and neonatal mortality in small ruminants [3]. Neospora caninum is structurally and biologically similar to Toxoplasma gondii [4], causing abortion and neonatal mortality in cattle, sheep, goats, and horses in many countries [5]. Anaplasma marginale, Babesia bovis, and Babesia bigemina are important tick-borne agents of cattle worldwide [6]. Although many studies have been conducted worldwide on the prevalence of these important pathogens in animals, few studies have been conducted on water buffaloes in other regions.
Latin America [7–9]. The present work aimed to assess the prevalence of *N. caninum*, *T. gondii*, *A. marginale*, *B. bovis*, and *B. bigemina* among water buffaloes in the north region of Brazil.

2. Materials and Methods

2.1. Serum Samples. Field samples of blood from water buffalo (*n* = 4,796) were collected from different farms in eight provinces in Marajó island (Soure, Salvaterra, Muaná, Chaves, Ponta de Pedras, Cachoeira do Araú and Santa Cruz do Arari) and five provinces in Continent (Belém, Castanhal, Paragominas, Abaetetuba, and Moju) in the north region of Brazil in 2011. The minimum sample size was calculated by the following formula of the Pan American Zoonoses Center [10]:

\[ N = \frac{p \cdot (100 - p) \cdot Z^2}{(d \cdot p/100)^2}, \]

where *N* is the number of samples; *p* is the expected prevalence; *Z* is the degree of confidence; *d* is the margin of error.

The expected prevalence for selected bovine pathogens of buffaloes was estimated to be 7%. The confidence interval was 95%, and the margin of error was 5%. Thus, the minimum sample size was 3,650 animals, and a total of 4,796 animals were used in this study. Whole blood samples were collected from caudal or jugular vein of individual water buffaloes. For serum samples, blood samples without EDTA were incubated at room temperature and then centrifuged at 3,000 rpm for 15 min; the sera were collected and then stored at −20°C until use.

2.2. ELISA for *B. bovis*, *B. bigemina*, *T. gondii*, *N. caninum*, and *A. marginale*. Briefly, 100 µL of each antigen diluted in 0.05 M carbonate/bicarbonate buffer, pH 9.6, was added to each well of a micro-ELISA plate (Immulon; Dynatech Laboratories Inc.) and protein concentration was adjusted to 5 µg/mL (*B. bovis*, *B. bigemina* and *T. gondii*) or 10 µg/mL (*N. caninum*, and *A. marginale*). The plates were sealed and incubated overnight at 4°C. Plates were blocked for 1 h at 37°C in a humid chamber with 3% skim milk in carbonate/bicarbonate buffer. After five washes with buffer (phosphate buffered saline, pH 7.2, and 0.05% Tween 20, PBS-Tween), 100 µL of diluted bovine sera (1:400) in PBS-Tween plus 5% normal rabbit serum was added in duplicate to the ELISA plate. Plates were incubated at 37°C in a humid chamber for 90 min and then washed five times with PBS-Tween. A 100 µL aliquot of a 1:10,000 dilution of alkaline phosphatase conjugated antibody IgG (Sigma Chemical Co.) was added to each well and the plates were incubated at 37°C under the same conditions for 90 min. Plates were washed five times with PBS-Tween. The appropriate substrate (p-nitrophenyl phosphate) was added and the plates were sealed and incubated for 40 min at room temperature. The plates were then read at 405 nm wavelength on a micro-ELISA reader (BT-100; Embrabio, São Paulo, Brazil). The cutoff values were calculated based on 10 noninfected water buffaloes sera [11].

2.3. IFAT for *T. gondii*, *N. caninum*, *A. marginale*, *B. bovis*, and *B. bigemina*. Briefly, a 10 µL field serum sample diluted in PBS (1:40) was applied as the first antibody on the fixed slides. After washing with PBS three times, the fluorescein isothiocyanate (FITC)-conjugated sheep antibovine IgG antibody (Sigma, St. Louis, MO, USA) was applied as a secondary antibody (1:300) and then incubated for 1 h at 37°C. The slides were washed three times with PBS and then examined using a fluorescent microscope (E-400 Eclipse, Nikon, Kawasaki, Japan). Positive and negative control sera were added to each slide.

2.4. Statistical Analysis. The kappa coefficient was calculated to evaluate the agreement among the ELISA and IFAT. The chi-square test was used to evaluate significant differences (*P* < 0.05) of infection rate (*T. gondii*, *N. caninum*, *A. marginale*, *B. bovis*, and *B. bigemina*) in animals of different breed, reproductive status, and locations. The operational procedures were done using the R statistical software (R Foundation for Statistical Computing, version 2.12.2, 2011).

3. Results and Discussion

IgG antibodies of *T. gondii* were detected in 41.33% (1982/4796) and 35.77% (1715/4796) of sampled buffaloes, by ELISA and IFAT, respectively (Table 1).

### Table 1: Summary of the serological detection of *T. gondii*, *N. caninum*, *A. marginale*, *B. bovis*, and *B. bigemina* using ELISA and IFAT. The results of ELISA were cross-tabulated with those of IFAT.

| Agent                | ELISAa | IFATb | ELISA/IFATc |
|----------------------|--------|-------|-------------|
| *Toxoplasma gondii*  | (+)    | 1934  | 1672 262 1936 |
|                      | (−)    | 2862  | 2 2860 2860 |
|                      |        | 4796  | 1674 3122 4796 |
| *Neospora caninum*   | (+)    | 2599  | 2285 314 2601 |
|                      | (−)    | 2197  | 2 2195 2195 |
|                      |        | 4796  | 2287 2509 4796 |
| *Anaplasma marginale*| (+)    | 2997  | 2393 604 3002 |
|                      | (−)    | 1799  | 5 1794 1794 |
|                      |        | 4796  | 2398 2398 4796 |
| *Babesia bigemina*   | (+)    | 1092  | 891 201 1100 |
|                      | (−)    | 3704  | 8 3696 3696 |
|                      |        | 4796  | 899 3897 4796 |
| *Babesia bovis*      | (+)    | 1193  | 1075 118 1197 |
|                      | (−)    | 3603  | 4 3599 3599 |
|                      |        | 4796  | 1079 3717 4796 |

*a* Number of positive and negative buffaloes in ELISA assays.

*b* Number of positive and negative buffaloes in both ELISA and IFAT assays.

*c* The frequencies of positive and negative samples of combined ELISA and IFAT results.
Few reports have been done about the occurrence of *T. gondii* among buffaloes in Brazil [7]. Previous studies have reported a lower seroprevalence of this agent when compared to that observed among cattle. For instance, seroprevalence rates of 3.85%, 3.2%, and 1.1% were found among buffaloes in states of Bahia [3], São Paulo [12], and Pará [12], respectively. A lower seroprevalence of *T. gondii* among buffaloes has also been reported in Turkey [13], Egypt [14], Vietnam [15], and Iran [16].

Antibodies of *N. caninum* were detected in 55.55% (2665/4796) and 48.88% (2345/4796) of sampled buffaloes, by ELISA and IFAT, respectively (Table 1). Antibodies titers detected in seropositive animals ranged from 100 (cutoff) to 800: 100 (50%), 200 (25%), 400 (13.6%), and 800 (11.4%). Previous studies in the state of Pará have reported a seroprevalence of *N. caninum* ranging from 40.9% [17] to 70.15% [18] among buffaloes. On the other hand, a seroprevalence of 36.5% of *N. caninum* has been found among buffaloes from the state of Bahia [3]. The seroprevalence found here was higher than that found among buffaloes from Italy (34.6%) [19] but lower than that found in Egypt (68%) [14] and Argentina (64%) [20]. However, it is noteworthy to mention that differences in reported seroprevalence could be explained also by the test utilized.

Herein, 64.7% (3073/4796) and 50.0% (2399/4796) of sampled buffaloes showed IgG antibodies to *A. marginale*, by ELISA and IFAT, respectively (Table 1). Although previous studies reported the occurrence of *Babesia* sp. among buffaloes [9, 11], those regarding the prevalence of *A. marginale* are scarce. In Brazil, seroprevalence of *A. marginale* among buffaloes (ranging from 31.1 to 80%) is much different from that found among cattle (reaching in most cases 100%) [21, 22]. In the present study, sampled buffaloes did not show clinical signs of anaplasmosis. Although clinical anaplasmosis is not usually seen among *A. marginale* infected buffaloes, manifestation of diseases such as hemolytic anemia, inapten- tence, depression, emaciation, melena, tachycardia, tachypnea, constipation, jaundice, and pale mucous was reported in buffaloes from India showing a low parasitaemia [23].

IgG antibodies to *B. bovis* and *B. bigemina* were detected in 24.9% (1193/4796) and 22.76% (1092/4796) by ELISA and 22.5% (1079/4796) and 18.74% (899/4796) by IFAT of sampled buffaloes, respectively (Table 1). Thirteen percent (623/4796) of animals were seropositive to both *B. bovis* and *B. bigemina*.

The low seroprevalence of *B. bovis* and *B. bigemina* found in the present study suggests a low transmission rate in the studied area due to the habitat where buffaloes are maintained, characterized by woods isolated from cattle infested by *Babesia* sp. infected ticks. A low exposure of buffaloes to babesiosis agents could be explained by the fact that those animals live submerged in muddy waters, diminishing the probability of attachment of ticks and consequently the transmission of studied pathogens [8, 11]. These findings classify the studied area as endemically stable for babesiosis agents in buffaloes, suggesting an elevated risk to babesiosis outbreaks in bovines, mainly in regions where cattle is raised closed to buffaloes or even when new animals are introduced in such herds.

The present study provides important information about the prevalence of *T. gondii*, *N. caninum*, *A. marginale*, *B. bovis*, and *B. bigemina* infections in water buffaloes. The real role of water buffaloes on the epidemiology of these diseases and, consequently, the impact of management and control programs targeting these animals should be determined.

**Conflict of Interests**

None of the authors of this work have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of this paper.

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**References**

[1] Instituto Brasileiro de Geografia e Estatística (IBGE), [www.ibge.gov.br](http://www.ibge.gov.br).

[2] W. R. Cockrill, “The water buffalo: a review,” *British Veterinary Journal*, vol. 137, pp. 8–16, 1981.

[3] L. F. P. Gondim, R. M. Souza, J. Guimarães, and M. A. O. Almeida, “Frequência de anticorpos contra *Neospora caninum* em búfalos criados no Estado da Bahia,” in *Proceedings of the Seminário Brasileiro de Parasitologia Veterinária*, vol. II, p. 227, Colégio Brasileiro de Parasitologia Veterinária, 1999.

[4] J. P. Dubey, A. L. Hattel, D. S. Lindsay, and M. J. Topper, “Neonatal *Neospora caninum* infection in dogs: isolation of the causative agent and experimental transmission,” *Journal of the American Veterinary Medical Association*, vol. 193, no. 10, pp. 1259–1263, 1988.

[5] J. P. Dubey, G. Scharf, and L. M. Ortega-Mora, “Epidemiology and control of neosporosis and *Neospora caninum*,” *Clinical Microbiology Reviews*, vol. 20, no. 2, pp. 323–367, 2007.

[6] K. M. Kocan, J. de la Fuente, E. F. Blouin, J. F. Coetzee, and S. A. Ewing, “The natural history of *Anaplasma marginale*,” *Veterinary Parasitology*, vol. 167, no. 2–4, pp. 95–107, 2010.

[7] J. B. Silva, A. H. Fonseca, S. J. T. Andrade, A. G. M. Silva, C. M. C. Oliveira, and J. D. Barbosa, “Prevalence of babesiosis anti-Toxoplasma gondii em búfalos (Bubalus bubalis) no Estado do Pará,” *Pesquisa Veterinária Brasileira*, vol. 33, pp. 581–585, 2013.

[8] J. B. Silva, M. R. André, A. H. Fonseca et al., “Molecular and serological prevalence of *Babesia bovis* and *Babesia bigemina* in water buffaloes in the north region of Brazil,” *Veterinary Parasitology*, vol. 197, pp. 678–681, 2013.

[9] J. B. Silva, M. R. André, A. H. Fonseca et al., “Molecular and serological prevalence of *Anaplasma marginale* in water buffaloes in the north region of Brazil,” *Ticks and Tick-Borne Diseases*, 2013.

[10] Centro Panamericano de Zoonoses, “Prueba de doble difusión arco 5 para el diagnóstico de la hidatidosis humana,” Nota Técnica, Centro Panamericano de Zoonoses, Buenos Aires, Argentina, 1979.

[11] M. A. Terkawi, N. X. Huyen, C. Shinuo et al., “Molecular and serological prevalence of *Babesia bovis* and *Babesia bigemina* in...
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water buffaloes in the northeast region of Thailand,” Veterinary Parasitology, vol. 178, no. 3-4, pp. 201–207, 2011.

[12] T. U. Fujii, N. Kasai, S. M. Nishi, J. P. Dubey, and S. M. Gennari, “Seroprevalence of Neospora caninum in female water buffaloes (Bubalus bubalis) from the southeastern region of Brazil,” Veterinary Parasitology, vol. 99, no. 4, pp. 331–334, 2001.

[13] J. P. Dubey and C. P. Beattie, Toxoplasmosis of Animals and Man, CRC Press, Boca Raton, Fla, USA, 1988.

[14] J. P. Dubey, S. Romand, M. Hilali, O. C. H. Kwok, and P. Thulliez, “Seroprevalence of antibodies to Neospora caninum and Toxoplasma gondii in water buffaloes (Bubalus bubalis) from Egypt,” International Journal for Parasitology, vol. 28, no. 3, pp. 527–529, 1998.

[15] L. T. T. Huong, B.-L. Lundström, A. Uggla, and C. Björkman, “Prevalence of antibodies to Neospora caninum and Toxoplasma gondii in cattle and water buffaloes in southern Vietnam,” Veterinary Parasitology, vol. 75, no. 1, pp. 53–57, 1998.

[16] S. Navidpour and N. Hoghooghi-Rad, “Seroprevalence of anti-Toxoplasma gondii antibodies in buffaloes in Khoozestan province, Iran,” Veterinary Parasitology, vol. 77, no. 2-3, pp. 191–194, 1998.

[17] S. P. Silva, R. A. Mota, E. B. Faria et al., “Occurrence of IgG antibodies anti-Neospora caninum and Toxoplasma gondii in female water buffaloes (Bubalus bubalis) raised in the Brazilian state of Pará,” Pesquisa Veterinaria Brasileira, vol. 30, no. 5, pp. 443–446, 2010.

[18] S. M. Gennari, A. A. R. Rodrigues, R. B. Viana, and E. C. Cardoso, “Occurrence of anti-Neospora caninum antibodies in water buffaloes (Bubalus bubalis) from the Northern region of Brazil,” Veterinary Parasitology, vol. 134, no. 1-2, pp. 169–171, 2005.

[19] A. Guarino, G. Fusco, G. Savini, G. Di Francesco, and G. Cringoli, “Neosporosis in water buffalo (Bubalus bubalis) in southern Italy,” Veterinary Parasitology, vol. 91, no. 1-2, pp. 15–21, 2000.

[20] C. M. Campero, A. Pérez, D. P. Moore et al., “Occurrence of antibodies against Neospora caninum in water buffaloes (Bubalus bubalis) on four ranches in Corrientes province, Argentina,” Veterinary Parasitology, vol. 150, no. 1-2, pp. 155–158, 2007.

[21] F. N. Corrêa, Estudo Epidemiológico de Borrelia burgdorferi, Babesia bovis, Babesia bigemina e Anaplasma marginale em Búfalos (Bubalus bubalis) do Estado do Rio de Janeiro [Tese de Doutorado], Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil, 2011.

[22] M. C. Vidotto, O. Vidotto, G. M. Andrade, G. Palmer, T. McElwain, and D. P. Knowles, “Seroprevalence of Anaplasma marginale in cattle in Paraná State, Brazil, by MSP-5 competitive ELISA,” Annals of the New York Academy of Sciences, vol. 849, pp. 424–426, 1998.

[23] R. Srivastava and S. S. Ahluwalia, “A clinical case of anaplasmosis in buffalo," Indian Veterinary Journal, vol. 51, pp. 371–374, 1974.