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Communication

[*Comunicação*]

Prevalence and identification of *Salmonella* spp. in water buffaloes from São Paulo State, Brazil

[*Prevalência e identificação de Salmonella spp. em búfalos do Estado de São Paulo, Brasil*]

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Salmonella spp. are gram-negative bacteria and an important cause of economic losses in livestock besides being a zoonotic agent responsible for foodborne illness (Borrielo *et al*., 2012). There are two species of *Salmonella*: *S. bongori* and *S. enterica* (subdivided into six subspecies: *arizonae*, *diarizonae*, *enterica*, *houtenae*, *indica* and *salamae*), with more than 2,500 known serotypes. The infection of warm-blood animal species is caused by several serotypes of *S. enterica* subspecies *enterica* (Strockbine *et al*., 2015).

Bovine salmonellosis is caused predominantly by *S. enterica* subspecies *enterica* serotype Dublin (*S*. Dublin) and *S. enterica* subspecies *enterica* serotype Typhimurium (*S*. Typhimurium), often associated with systemic infection and enteritis, respectively (Silva *et al*. 2008; Ávila *et al*., 2011).

Salmonella spp. have been reported worldwide in buffaloes, with prevalence rate between 1% to 25% (Borrielo *et al*., 2012; Anwarullah *et al*., 2014; Yousif and Al-Hashimi, 2014, Hadimli *et al*., 2017), but the serotypes isolated from buffaloes are poorly characterized in some studies. The aim of this study was to investigate the occurrence of *Salmonella* spp. in fecal samples of buffaloes from São Paulo State, Brazil, and to identify the serotypes isolated.

A total of 116 rectal swab samples of Jafarabadi and Murrah buffaloes (water buffaloes), collected in triplicate, from six rural properties located in Central, Midwest and Northeast of São Paulo State, Brazil, were examined. To evaluate the presence of *Salmonella* spp., rectal swabs were enriched in 10mL of selenite cystine broth (CM0699, Oxoid), Muller-Kauffmann tetrationate broth (CM0343, Oxoid) and Rappaport-Vassiliadis broth (CM0866, Oxoid) and incubated at 37°C for 24h. The broths were then seeded on plates containing modified brilliant green agar (CM0329, Oxoid) and xylose lysine tergitol 4 (XLT4) agar (223420 and 235310, BD Difco) and incubated at 37°C for 24h. Colonies with morphologic characteristics of the genus *Salmonella* (Quinn *et al*., 2005) were inoculated in tubes containing triple sugar iron (TSI) (CM0277, Oxoid) and lysine iron agar (LIA) (CM0381, Oxoid) and incubated at 37ºC for 24h. After biochemical confirmation, slide agglutination tests were performed using somatic and flagellar polyvalent *Salmonella* antiseras (Probac do Brasil). Positive samples in slide agglutination test were inoculated in tubes containing nutrient agar (CM003, Oxoid) and sent to the Laboratory of Enterobacteria of the Instituto Oswaldo Cruz – IOC/FIOCRUZ (Manguinhos, Rio de Janeiro, Brazil) for serotyping. The study was approved by the Animal Research Ethics Committee of Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal – SP (protocol number 010885/08).

Of the 116 rectal swabs examined, eight samples (6.90%; 8/116) were positive for *Salmonella* spp. (Figure 1), characterized by four serotypes: *S*. Panama (50%; 4/8), *S*. Agona (25%; 2/8), *S*.

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Newport (12.5%; 1/8) and *S*. Saintpaul (12.5%; 1/8) (Figure 2). Of the eight positive samples of *Salmonella* spp., two samples (25%; 2/8) were isolated from selenite cystine broth, four samples (50%; 4/8) were isolated from Muller-Kauffmann tetrationate broth, one sample (12.5%; 1/8) was isolated from Rappaport-Vassiliadis broth, and one sample (12.5%; 1/8) was simultaneous isolated from Muller-Kauffmann tetrationate broth and Rappaport-Vassiliadis broth. All eight samples were isolated simultaneous from modified brilliant green agar and XLT4 agar.

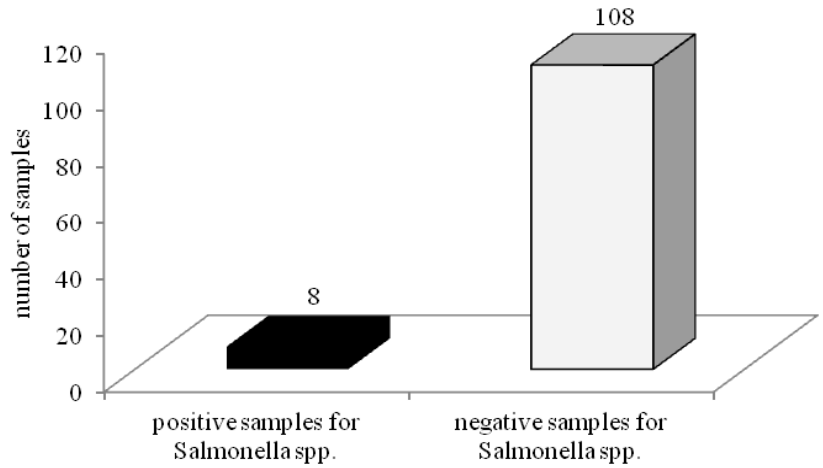

Figure 1. Number of rectal swab samples of water buffaloes from rural properties located in São Paulo State, Brazil, positive or negative for *Salmonella* spp.

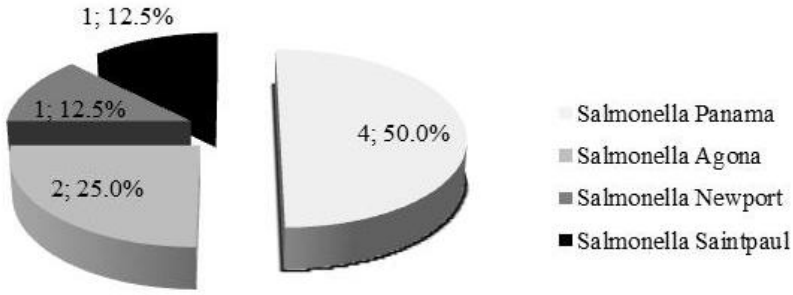

Figure 2. *Salmonella* serotypes isolated in rectal swabs samples of water buffaloes from rural properties located in São Paulo State, Brazil.

The serotypes *S*. Panama (n= 4), *S*. Newport (n= 1) and *S*. Saintpaul (n= 1) were isolated from Murrah buffalo calves between 11 to 26-day-old while *S*. Agona (n= 2) were isolated from Jafarabadi lactanting buffaloes, both without clinical signs of salmonellosis.

Of the six rural properties evaluated, only in two farms (33.3%; 2/6) *Salmonella* spp. was not detected. According to IBGE data (Produção…, 2016), the Brazilian buffalo population in 2015 was 1,365,636 animals, and São Paulo State accounts for the third largest herd of the country, with 90,873 buffaloes.

However, in Brazil there are few studies about the prevalence of *Salmonella* in buffaloes and in none of these studies *Salmonella* spp. were detected (Ribeiro *et al*., 2000; Fortes, 2013), unlike the present study in which a prevalence rate of 6.90% was reported and four different serotypes were identified (*S*. Panama, *S*. Agona, *S*. Newport and *S*. Saintpaul).

While bovine salmonellosis is caused predominantly by *S*. Dublin and *S*. Typhimurium (Silva *et al*. 2008; Ávila *et al*., 2011), many other serotypes have been isolated from buffaloes (Borrielo *et al*., 2012; Yousif and Al-Hashimi,

2014, Hadimli *et al*., 2017), as also observed in the present study.

Salmonella spp. was more isolated in fecal samples from asymptomatic newborn buffalo calves (75%; 6/8). Adult buffaloes positive for *Salmonella* spp. (25%; 2/8) did not show clinical signs either, indicating the importance of asymptomatic animals as a source of infection to other animals and humans. Other important aspect of epidemiology of *Salmonella* infection in buffaloes is that in four farms (66.7%; 4/6) *Salmonella* spp. were detect. These results can be associated with the use of more than one selective enrichment broth (selenite cystine broth, Muller-Kauffmann tetrationate broth and Rappaport-Vassiliadis broth) and culture medium (modified brilliant green agar and XLT4 agar) for *Salmonella* isolation, as recommended by Fernandes *et al*. (2004).

The prevalence of *Salmonella* spp. in water buffaloes from São Paulo State was 6.90% and four different serotypes were identified: *S*. Panama, *S*. Agona, *S*. Newport and *S*. Saintpaul. Positive animals did not present clinical signs of salmonellosis indicating the importance of the asymptomatic animals as a source of infection to other animals and humans.

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Keywords: Bubalus bubalis, *enrichment broth, feces*, Salmonella, *serotype*

RESUMO

O objetivo do estudo foi investigar a prevalência de Salmonella *spp. em amostras de fezes de búfalos do estado de São Paulo, Brasil, e identificar os sorotipos isolados. Foram examinadas 116 amostras de suabes retais de búfalos das raças Jafarabadi e Murrah, coletadas em triplicata, em seis propriedades rurais localizadas nas regiões Central, Centro-Oeste e Nordeste do estado de São Paulo, Brasil. Para avaliar a presença de* Salmonella *spp., foram utilizados três diferentes caldos de enriquecimento (caldo selenito cistina, caldo tetrationado Muller-Kauffmann e caldo Rappaport-Vassiliadis) e dois diferentes meios de cultura (ágar verde brilhante modificado e ágar XLT4). Das 116 amostras de suabes retais examinadas, oito amostras (6,90%; 8/116) foram positivas para* Salmonella *spp., incluindo quatro sorotipos: S. Panama (50%; 4/8), S. Agona (25%; 2/8) , S. Newport (12,5%; 1/8) e S. Saintpaul (12,5%; 1/8), todos isolados de búfalos sem sinais clínicos de salmonelose, indicando a importância dos animais assintomáticos como fonte de infecção para outros animais e seres humanos. Das seis propriedades rurais avaliadas, apenas em duas fazendas (33,3%; 2/6) não foi detectada* Salmonella *spp. O uso de mais de um caldo de enriquecimento seletivo e de mais de um meio de cultura é indicado para o isolamento de* Salmonella.

Palavras-chave: Bubalus bubalis, *caldo de enriquecimento, fezes*, Salmonella, *sorotipo*

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