BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Veterinary Microbiology

Occurrence of serological reactions for serogroup Sejroe (CTG and Prajtino) in female buffalo in the state of Pernambuco, Brazil

Pollyanne Raysa Fernandes de Oliveira[a,*], *Larice Bruna Ferreira Soares*[b], *Jonas de Melo Borges*[b], *Noelle de Castro Barrosa*[c], *Hélio Langoni*[c], *Daniel Friguglietti Brandespim*[d], *José Wilton Pinheiro Junior*[d], *Rinaldo Aparecido Mota*[d]

[a] *Universidade Federal Rural de Pernambuco, Pós-graduação em Biociência Animal, Pós-graduação em Veterinária, Recife, PE, Brazil*
[b] *Universidade Federal Rural de Pernambuco – Unidade Acadêmica de Garanhuns, Garanhuns, PE, Brazil*
[c] *Universidade Estadual Paulista, Faculdade de Medicina Veterinária e Zootecnia, Botucatu, SP, Brazil*
[d] *Universidade Federal Rural de Pernambuco, Departamento de Medicina Veterinária, Recife, PE, Brazil*

ARTICLE INFO

Article history:
Received 29 May 2017
Accepted 11 February 2018
Available online 20 March 2018

Associate Editor: Miliane Souza

Keywords:
Leptospirosis
Epidemiology
Bubalusbubalis

ABSTRACT

The objective of this study was to evaluate the occurrence of anti-*Leptospira* spp. antibodies in female buffalo in the state of Pernambuco. A total of 123 female buffalo blood samples were collected from five properties distributed in the state of Pernambuco. The microscopic agglutination test was used to study anti-*Leptospira* spp. antibodies. The occurrence of anti-*Leptospira* spp. antibodies was 28.5% (35/123; CI 20.7–37.3%) and on different properties, the occurrence ranged from 28.6% to 80.0%, with 100% of the properties showing animals with positive results. The serovars of the serogroup Sejroe with a higher incidence were Hardjoprajtino (CTG strain, 49.1%) and Hardjo (Prajtino genotype, 43.2%), followed by serogroup Grippotyphosa with the Grippotyphosa serovar (3.9%), serogroup Pomona with the Pomona serovar (1.9%), and the Icterohaemorrhagiae serovar Copenhageni (1.9%). This was the first record of the occurrence of anti-*Lepstospira* spp. antibodies in female buffalo in the state of Pernambuco. Control measures are necessary to prevent health and economic losses, given that the agent involved affects animal reproduction, triggering drops in conception rates or even clinical cases of abortion.

* *Corresponding author.*
E-mail: pollyanne_raysa_fernandes@hotmail.com (P.R. Oliveira).
https://doi.org/10.1016/j.bjm.2018.02.007

Introduction

Leptospirosis is an anthropozoonosis of wide geographic distribution that occurs in rural and urban areas and is caused by pathogenic bacteria belonging to the Spirochaetales order, Leptospiraceae family, and *Leptospira* genus.[1] In tropical and subtropical countries, it shows a high prevalence as a consequence of the temperature and humidity conditions that favor the persistence of the bacteria in the environment.[2]

In cattle raising, leptospirosis is an important cause of production drops associated with to reproductive issues.[3] In infected pregnant females, the bacteria can cross the placenta at any stage of the pregnancy, causing embryonic losses, abortions, stillbirths, or debilitated births.[4] However, estrus repetition is a characteristic that can be observed for many months.[5]

In Brazil, many studies on the prevalence of different *Leptospira* spp. serovars in buffalo have been conducted, permitting an understanding of the disease's epidemiology, which reflects the ecological relationship between humans and chronically infected mammalian reservoirs.[6] In the state of São Paulo, Vasconcellos et al.[7] isolated *Leptospira santarosai* from buffalo of Vale do Ribeira, whereas Favero et al.[8] found 43.7% positives in 879 serum samples with a higher frequency of the serovars Hardjo (43.3%) and Wolffi (32.5%). In the state of Pará, Silva et al.,[9] researching agglutinins of anti-*Leptospira*, verified a 67.7% positivity rate in 127 analyzed serum samples, of which 15.7% were reactive to serovar Hardjo. Viana et al.,[10] in the state of Amazonas, obtained 80.0% positives (164/205) with a higher frequency of serogroup Autumnalis variant autumnalis and serogroup Sejroe and varieties Hardjo and Wolffi.

The epidemiological importance of leptospirosis in buffalo must be considered: infected animals may act as sources of infection for other animals raised on the same property as well as for people who manage them because as carriers, buffalo eliminate the agent into the environment, mainly through urine[2] (p601). Among the main risk factors involving leptospirosis in buffalo are their access to diverse ecosystems and their habit of bathing in rivers, creeks, and flooded areas.[11]

Observing the lack of data on this disease in the northeast region of the country and the economic losses related to leptospirosis in buffalo, the objective of this study was to determine the occurrence of anti-*Leptospira* spp. antibodies in buffalo originating from herds in the state of Pernambuco, Brazil.

Methodology

Study area

A transversal study design was carried out through convenience sampling, not probabilities, in five counties distributed in the state of Pernambuco, Brazil. The properties were chosen for convenience, including the counties of Quipapá (5 animals), Ribeirão (42 animals), Canhotinho (21 animals), Agrestina (5 animals), and ÁguaPreta (50 animals).

Sampling

In total, 123 blood samples were collected from female buffalo of reproductive age, with or without a history of reproductive problems, raised on a semi-intensive or extensive regimen, and with no history of vaccination for leptospirosis.

Sample collection

Blood samples were obtained by venipuncture of the coccygeal vein using Vacutainer® disposable 25 × 0.8 mm needles and sterilized 5-mL Vacutainer® tubes. Afterward, the samples were packed into isothermal boxes containing recyclable ice and forwarded to the laboratory where they were centrifuged at 900 × *g* for 10 min. After this procedure, the samples were filled in polypropylene tubes, properly identified, and stored in a freezer at −20 °C until processing.

Serum analysis

For the study of anti-*Leptospira* spp. antibodies, the microscopic agglutination test (MAT) was used. Samples considered positive were those with titrations equal to or higher than 100.[12] When coagglutinations occurred between different serovars with the same titration, both serovars were considered reactive, and in cases of different titrations, the serovar with the higher titration prevailed. Titrations of the seropositive samples were carried out by employing a dilution of 1:100 to 1:3200,[13] employing the serogroups for herbivores, available at the Serviço de Diagnóstico de Zoonoses (SDZ) UNESP Botucatu – São Paulo (Table 1).

Table 1 – Relation of serogroups, serovars, and strain of *Leptospira* spp. utilized in the Serviço de Diagnóstico de Zoonoses (SDZ), UNESP Botucatu – São Paulo, 2016.

Leptospira serovar	Serogroup	Strain
L. interrogans sv Bratislavia	Australis	Jez-bratislava
L. borgpetersenii sv Castellonis	Ballum	Castllon
L. interrogans sv Canicola	Canicola	Hond Utrecht IV
L. interrogans sv Djasiman	Djasiman	Djasiman
L. kirshneri sv Grippotyphosa	Grippotyphosa	Moska v
L. interrognas sv Copenhageni	Icterohaemorraghiae	M 20
L. interrogans sv Icterohaemorraghiae	Icterohaemorraghiae	RGA
L. interrogans sv Pomona	Pomona	Pomona
L. interrogans sv Pyrogenes	Pyrogenes	Salinem
L. interrogans sv Wolffi	Sejroe	3705
L. borgpeterseni sv Tarassovi	Shermani	Perepelicinou Mitis Johnson
L. borgpetersenii sv Hardjo	Hardjobovis	Sponselee
L. borgpetersenii sv Mini	Mini	Sari
L. interrogans sv Hardjo (genoypePrajtino)	Sejroe	Hardjoprajtino
L. interrogans sv Hardjo (strain CTG)	Sejroe	Hardjo CTG
L. interrogans sv Hardjo (genotype Bovis)	Sejroe	Hardjobovis
L. santarosai sv Guaricura	Sejroe	Bov G

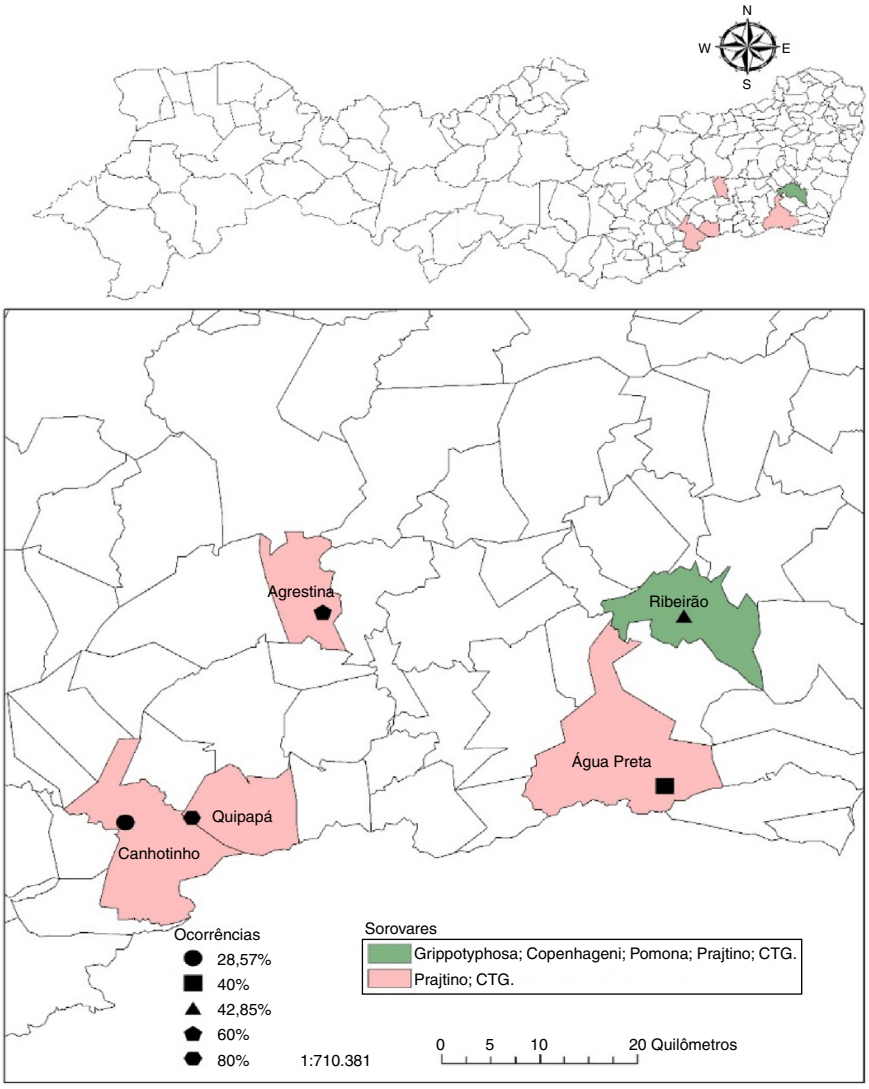

Fig. 1 – Distribution and occurrence of anti-*Leptospira* spp. in buffaloes of the state of Pernambuco, Brazil.

Georeferencing

The location of the properties was obtained with the help of a Global Positioning System. For mapping, the georeferenced data were entered in TerraView 3.1.3 software.

Statistical analysis

Descriptive statistical analysis was carried out to calculate the relative and absolute frequencies of the results obtained in the serological test. Fisher's exact test was used to identify associations between abortion history and the serological examination. This involved univariate analysis of the variables of interest. Epi Info, version 3.5.1 software was used (CDC).

Results

The occurrence of anti-*Leptospira* spp. antibodies was verified in 28.5% (35/123; CI 20.7–37.3%) of the samples analyzed in Pernambuco. When the occurrence of antibodies in buffalo on different properties was analyzed, a range of 29.0–80.0% was observed with at least one positive animal perserovar. The geographical distribution of positive buffalo is shown in Fig. 1 and Table 2.

Among the positive samples, 14 (40.0%) reacted to more than serovar. The serovars of serogroup Sejroe with higher occurrences were Hardjo (strain CTG – 49.1%) and Hardjo (genotype Prajtino – 43.2%), followed by serogroup Grippotyphosa with the serovar Grippotyphosa (3.9%), serogroup Pomona (1.9%), and Icterohaemorrhagiae serovar

Table 2 – Occurrence of anti-*Leptospira* spp. according to serogroups in buffaloes distributed by municipalities of the state of Pernambuco.

City	*n*	Serogroup				
		Grippotyphosa	Icterohaemorraghiae[a]	Pomona	Sejroe[b]	Sejroe[c]
Quipapá	5				2 (40.0%)	2 (40.0%)
Ribeirão	42	2 (4.8%)	1 (2.4%)	1 (2.4%)	9 (21.4%)	5 (12.0%)
Canhotinho	21				2 (10.0%)	4 (19.0%)
Agrestina	5				2 (40.0%)	1 (20.0%)
Água Preta	50				7 (14.0%)	13 (26.0%)

n, total of samples analyzed; F.A., absolute frequency; F.R., relative frequency.
[a] *L. interrognas* sv Copenhageni.
[b] *L. interrogans* sv Hardjo (genoype Prajtino).
[c] *L. interrogans* sv Hardjo (strain CTG).

Table 3 – Occurrence of serogroup by use of the microscopic agglutination test in female buffalo of the state of Pernambuco.

Leptospira serovar	Serogroup	Positives	Occurrence
L. kirshneri sv Grippotyphosa	Grippotyphosa	2	3.9%
L. interrognas sv Copenhageni	Icterohaemorraghiae	1	1.9%
L. interrogans sv Pomona	Pomona	1	1.9%
L. interrogans sv Hardjo (genotype Prajtino)	Sejroe	22	43.2%
L. interrogans sv Hardjo (strain CTG)	Sejroe	25	49.1%
Total	–	51	100%

Table 4 – Distribution of serovars of *Leptospira* spp. by titles in buffaloes of the state of Pernambuco, Brazil.

Leptospira serovar	Title				Total
	100	200	400	800	
L. kirshneri sv Grippotyphosa	1	–	–	1	2
L. interrognas sv Copenhageni	1	–	–	–	1
L. interrogans sv Pomona	1	–	–	–	1
L. interrogans sv Hardjo (genotype Prajtino)	11	6	5	–	22
L. interrogans sv Hardjo (strain CTG)	14	9	2	–	25
Total	28	15	7	1	51

Copenhageni (1.9%) (Table 3). These serovars showed titrations varying from 100 to 800 (Table 4).

No significant difference was observed for the variable history of abortion ($p = 0.405$) associated with MAT; however, a higher occurrence of 31% of seropositive animals was observed in the properties with a history of abortion.

Discussion

This is the first study of serological reaction by *Leptospira* serogroup Sejroe (CTG and Prajtino) in female buffalo in the state of Pernambuco, Brazil. Results are similar to those found for the occurrence of anti-*Leptospira* spp. antibodies in buffalo formerly reported in the country by Brasil et al.[14] in Paraíba with 27.9% of seropositive animals; however, some serogroups found by those authors were distinct from the ones observed in this study, for example Bratislava (11.0%) and Canicola (5.9%). Silva et al.[9] (p543) investigated the occurrence of anti-*Leptospira* spp. antibodies in buffalo in the state of Pará and identified 67.7% of animals as seropositive, highlighting serovar Hardjo genotype Prajtino, showing the importance of this serovar to the species.

Among the herds of the studied municipalities, Ribeirão was the one that presented a greater variety of serogroups, Grippotyphosa, Sejroe (CTG and Prajtino), Pomona and Icterohaemorrhagiae (Copenhageni), suggesting the contact of the buffaloes with wild animals and of production that function as reservoirs for these serovars.[15]

Despite the low occurrence of the serogroup Grippotyphosa serovar Grippotyphosa, the latter presented a reagent sample with an 800 titer. According to OIE,[12] the occurrence of any serovar with titers between 100 and 200 may indicate infection or vaccine antibody residue, however higher titers may disease and/or the emergence of a carrier that can eliminate the agent in the urine in the stage of leptospiruria that contaminates the environment, acting as a source of infection for other animals and humans. It is noteworthy that the animals sampled in this study had no history of vaccination and therefore the high titration indicates recent infection, and the positive animals are considered sources of infection.

Differences found between the results obtained in the present study and those reported by Carvalho et al.[2] (p600) (70.58%), Langoni et al.[16] (37.70%), Silva et al.,[9] (p543) (67.70%), and Viana et al.[10] (p455) (80.00%) on the occurrence of anti-*Leptospira* spp. antibodies in buffalo may be partially attributed to the quantity of types and serogroups utilized in serological

screening and possible environmental differences related to the studied region, such as the hygienic/sanitary practices adopted in the management of the herd; the level and type of exposure to other domestic, wild, and synanthropic animals; and also rodents that participate in the epidemiological chain of leptospirosis.[17] Otherwise, a comparison between the results obtained in different studies is difficult to analyze, because the MAT technique may vary among laboratories according to their collection of antigens.[18]

This study found a higher occurrence of serogroup Sejroe with serovar Hardjo (Prajtino and CTG). The strain CTG was isolated for the first time in samples from cattle in Brazil by Moreira,[19] being typified in the Royal Tropical Institute, Amsterdã, Holanda. Since 1991, many studies in Brazil have highlighted the importance of serogroup Sejroe in bovine leptospirosis, including the serovar of this serogroup in the antigen panel[18] (p242). According to those authors, a high prevalence of infection has been demonstrated in the herd (75%) and individuals (44.2%) with a predominance of serovars of the serogroup Sejroe (80.3%). Infection in buffalo by this serogroup may be associated with their proximity to bovines, because on 80.0% (4/5) of the properties studied, bovines were present.

The elevated occurrences of seropositive female buffalo for serogroup Sejroe serovar Hardjo (CTG and Prajtino) showed the importance of that species in the epidemiology for leptospirosis involving those serovars, because these animals may act as carriers and contribute to environmental contamination through urine elimination, acting in this way as a source of infection of other animals and humans[2] (p601). The presence of seropositive animals may assure the persistence of this serogroup in the herds,[3] (p311) besides causing an economic impact, generating reproductive disturbances as well as hemolytic cases[6] (p217).

Like bovines, buffalo suffer abortions associated with infection by *Leptospira* spp.[6] (p215). It is known that serovars of the serogroup Sejroe, the Hardjo and its strains and genotypes are described as causes of abortion in that species.[20] Regarding the abortion history, no significant association was observed in this study. However, it was verified that the property with a history of miscarriages (20%, 1/5) presented buffaloes infected the different serogroups (Grippotyphosa; Icterohaemorraghiae; Pomona; Sejroe) which may indicate the participation of *Leptospira* spp. as agent responsible for reproductive disorders in this herd.

In the literature, there are few seroepidemiological studies in buffalo. Therefore, it is necessary to execute other studies by using isolation and molecular techniques to investigate the importance of this serogroup as a cause of abortion in buffalo. Pinto et al.[18] (245) reinforce the necessity of studies in different countries and a greater standardization of the diagnosis of *Leptospira* spp. infection.

Conclusion

This is the first record of the occurrence of anti-*Leptospira*-specific antibodies against the serogroup Sejroe, serovar Hardjo (CTG and Prajtino) in buffalo in the state of Pernambuco, Brazil. Control measures are necessary to prevent sanitary and economical losses, because the agent involved affects animal reproduction and poses hazards to public health.

Conflicts of interest

None declared.

REFERENCES

1. Quinn PJ, Markey BK, Carter ME, Donnelly WJC, Leonard FC, Maguire D. *Microbiologia Veterinária e Doenças Infecciosas*. 2ª ed. São Paulo: Artmed; 2005.
2. Carvalho OS, Gonzaga LNR, Albuquerqu AS, Bezerra DC, Chaves NP. Occurrence of *Brucella abortus*, *Leptospira interrogans* and bovine herpesvirus type 1 in buffalo (*Bubalus bubalis*) herd under extensive breeding system. *Afr J Microbiol Res*. 2015;9:598–603.
3. Marianelli C, Tarantino M, Astarita S, Martucciello A, Capuano F, Galiero G. Molecular detection of Leptospira species in aborted fetuses of water buffalo. *Vet Rec*. 2007;161:310–311.
4. Menezes AT, Contador TL, Rodrigues RV, Barcelos F, Bonadia GO. A Leptospirose e seus Efeitos na Reprodução. *Rev Cient Eletrônica Med Vet Publicação científica da faculdade de medicina veterinária e zootecnia de GARÇA/FAMED*. 2006;3:1–4. http://faef.revista.inf.br/imagens_arquivos/arquivos_destaque/4bmL2jajqmHOrxJ_2013-5-20-15-37-49.pdf Accessed 19.01.17.
5. Hafez ESE. *Reprodução Animal*. 4ª ed. São Paulo: Manole; 1982.
6. Faine S, Adler B, Bolin C, Perolat P. *Leptospira and Leptospirosis*. 2ª ed. Melbourne: MediSci; 1999.
7. Vasconcellos SA, Oliveira JCF, Morais ZM, et al. Isolation of *Leptospira santarosai*, serovar Guaricura from Buffaloes (*BubalusBubalis*) in Vale do Ribeira, São Paulo, Brazil. *Braz J Microbiol*. 2001;32:298–300.
8. Favero ACM, Pinheiro SR, Vasconcellos SA, Morais ZM, Ferreira F, Ferreira Neto JS. Sorovares de Leptospiras predominantes em exames sorológicos de bubalinos, ovinos caprinos, equinos, suínos e cães de diversos estados brasileiros. *Cienc Rural*. 2002;32:613–619. http://www.scielo.br/scielo.php?pid=S0103-84782002000400011&script=sci_abstract&tlng=pt Accessed 19.01.17.
9. Silva GR, Moraes CCG, Melo KCN, et al. *Distribuição de anticorpos para Leptospira sp. em búfalos (Bubalusbubalis) da região nordeste do Estado do Pará, Brasil. Ciência Animal Brasileira – Suplemento 1, 2009 – Anais do VIII Congresso Brasileiro de Buiatria*; 2009:540–545.
10. Viana RB, Del Fava C, Moura ACB, et al. Ocorrência de anticorpos anti-Neosporacaninum, *Brucella* sp. e *Leptospira* spp. em Búfalos (*Bubalus bubalis*) criados na Amazônia. *Arq Inst Biol, São Paulo*. 2009;76:453–457.
11. Nardi Júnior G, Genovez ME, Ribeiro MG, Castro V, Jorge AM. Interference of vacinal antibodies on serological diagnostic of leptospirosis in vaccinated buffalo using two types of commercial vaccines. *Braz J Microbiol*. 2007;38:363–368.
12. OIE. World organisation for animal health. Leptospirosis. Chapter 2.1.9.2008. htlm: http://www.oie.int/fileadmin/home/eng/health_standards/tahm/2.01.09_LEPTO.pdf Accessed 19.01.17.
13. Brasil. Ministério da Saúde. *Manual de Leptospirose. Fundação Nacional de Saúde. Centro Nacional de Epidemiologia. Coordenação de Controle de Zoonoses e Animais Peçonhentos*. 2ªed. rev Brasília: Fundação Nacional de Saúde; 1995.

14. Brasil AWL, Parentoni RN, Costa DF, et al. *Occurrence of anti-Brucella abortus and anti-Leptospira spp. antibodies in buffaloes from Paraíba state, Northeastern Brazil*. 36. Semina: Ciências Agrárias, Londrina; 2015:2005–2012. http://www.uel.br/portal/frm/frmOpcao.php?opcao=http://www.uel.br/revistas/uel/index.php/semagrarias Accessed 19.01.17.
15. Gamage CD, Koizumi N, Perera AK, et al. Carrier status of leptospirosis among cattle in Sri Lanka: a zoonotic threat to public health. *Transbound Emerg Dis*. 2014;61:91–96.
16. Langoni H, Del Fava C, Cabral KG, Silva AV, Chagas SAP. *Aglutininas Antileptospíricas em Búfalos do Vale do Ribeira, Estado de São Paulo*. 29. Santa Maria: Cienc. Rural; 1999:305–307. http://www.scielo.br/scielo.php?pid=S0103-84782002000400011&script=sci_abstract&tlng=pt Accessed 19.01.17.
17. Linhares GFC, Girio RJS, Linhares DCL, Mondeiro LC, Oliveira APA. Sorovares de *Leptospira interrogans* e respectivas prevalências em cavalos da microrregião de Goiânia, GO. *Ciênc Anim Bras*. 2005;6:255–259. https://www.revistas.ufg.br/vet/article/view/376/351 Accessed 22.01.17.
18. Pinto PS, Libonati H, Penna B, Lilenbaum W. A systematic review on the microscopic agglutination test seroepidemiology of bovine leptospirosis in Latin America. *Trop Anim Health Prod*. 2016;48:239–248.
19. Moreira EC. Avaliação de métodos para erradicação de leptospiroses em bovinos 1994. 94f. Tese (Doutorado em Medicina Veterinária) – Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte.
20. Levett PN. Leptospirosis. *Clin Microbiol Rev*. 2001;14:296–326.