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SEROLOGY FOR *LEPTOSPIRA* SP. IN CATTLE OF THE STATE OF PARAÍBA, BRAZIL

A.P. Lage[1], R. de M.H. Leite[1,2], J.A. Thompson[3], D.A. Bandeira[2], G.P. Herrmann[4], É.C. Moreira[1], V.S.P. Gonçalves[5]

[1]Universidade Federal de Minas Gerais, Escola de Veterinária, Departamento de Medicina Veterinária Preventiva, Núcleo de Pesquisa em Saúde Animal, Laboratório de Bacteriologia Aplicada, CP 567, CEP 30123-970, Belo Horizonte, MG, Brasil. E-mail: alage@vet.ufmg.br

ABSTRACT

A survey was conducted to estimate the frequency of *Leptospira* sp. infection in cattle in the State of Paraíba, Brazil. A random sample of 6 municipalities was chosen from each of 3 ecoregions of the state: the Agreste / Litoral, the Cariri / Curimatau and the Sertão. These 18 municipalities were broken down into quadrants and one property was selected at random from each quadrant. Random samples were collected from at least eight animals at each property, in four-age categories, summing at least 32 animals per property. All municipalities that were included in the study (100%) and 87.75% of the properties had at least one positive sample for one of the 15 different *Leptospira* sp. serovars (16 strains) studied. From 2,343 samples, 759 showed positive reaction to, at least, one of the 16 strains tested. Four hundred and seventy animals were positive to multiple strains. The serovar Hardjo, of the Sejroe serogroup, was the most frequent and accounted for 16.05% (95% CI - 12.69% to 19.41%) of the positive results (376 positive reactions). There were differences in the frequency of positive results according to the two strains of serovar Hardjo used: strain Norma (15.62% - 95% CI - 12.38% to 18.86%), isolated in Brazil, and reference strain Hardjoprajitino (2.43% - 95% CI - 1.12% to 3.75%). The remaining serovars tested had frequencies lower than 2.8%. Results obtained in the present study confirmed that *Leptospira interrogans* serovar Hardjo is widely spread in the State of Paraíba, Brazil.

KEY WORDS: Leptospirosis, *L. interrogans* serovar Hardjo, epidemiology, cattle, Paraíba, Brazil.

RESUMO

SOROLOGIA PARA *LEPTOSPIRA* SP. EM BOVINOS DO ESTADO DA PARAÍBA, BRASIL. Foi realizada a estimativa de freqüência de infecção por *Leptospira* sp. em bovinos do Estado da Paraíba, Brasil. Seis municípios foram escolhidos ao acaso em cada uma das 3 ecorregiões do Estado: Agreste / Litoral, Cariri / Curimatau e Sertão. Estes 18 municípios foram divididos em quadrantes e uma propriedade foi selecionada por quadrante. As amostras de sangue foram coletadas aleatoriamente de, pelo menos, 32 animais por propriedade. Todos os municípios incluídos no estudo (100%) e 87,75% das propriedades apresentaram no mínimo uma amostra positiva para pelo menos uma das 15 diferentes sorovariedades de *Leptospira* sp. (16 amostras) testadas. Dos 2.343 animais examinados, 759 reagiram positivamente para pelo menos uma das 16 amostras de *Leptospira* sp. testadas. Quatrocentos e setenta animais reagiram positivamente para múltiplas amostras. A sorovariedade Hardjo, do sorogrupo Sejroe, foi a mais freqüente com 16,05% (95% IC – 12,69% a 19,41%) dos resultados positivos (376 reações positivas). Houve diferenças na freqüência dos resultados positivos para as amostras da sorovariedade Hardjo testadas: amostra Norma (15,62% - 95% IC – 12,38% a 18,86%), isolada no Brasil, e a amostra de referência Hardjoprajitino (2,43% - 95% IC – 1,12% a 3,75%). As demais sorovariedades apresentaram freqüências inferiores a 2,8%. Os resultados obtidos no presente estudo confirmam que *Leptospira interrogans* serovar Hardjo é amplamente distribuída no Estado da Paraíba, Brasil.

PALAVRAS-CHAVE: Leptospirose, *L. interrogans* serovar Hardjo, epidemiologia, bovinos, Paraíba, Brasil.

[2]Empresa Estadual de Pesquisa Agropecuária da Paraíba, João Pessoa, PB, Brasil
[3]Texas A&M University, College of Veterinary Medicine, Department of Large Animal Medicine and Surgery, College Station, USA.
[4]Universidade Federal de Santa Maria, Departamento de Microbiologia e Parasitologia, Santa Maria, RS, Brasil.
[5]Universidade de Brasília, Faculdade de Agronomia e Medicina Veterinária, Brasília, DF, Brasil.

INTRODUCTION

Leptospirosis is caused by one of the seven pathogenic species of *Leptospira*, which are distributed in 25 serogroups, divided into more than 190 serovars defined by antigenic affinity (LEVETT, 2001). Several serovars of *Leptospira* sp. have been isolated from cattle in Brazil, however, the serovar Hardjo has recently been found to be the most frequent serologically and the one that causes greater impact in the reproductive efficiency of cattle herds (VASCONCELLOS *et al*., 1997). Infected animals can develop either an acute or chronic form of the disease. Infected herds can present low reproductive performance, temporary infertility, abortions, fetal mummifications and birth of weak calves, mastitis and reduced milk production (ELLIS, 1984, PRESCOTT; ZUERNER, 1993). All those manifestations can lead to major economical losses in Brazil due to its wide distribution (VASCONCELLOS et al., 1997), as already reported in the State of Paraíba (LEITE et al., 2000).

The Brazilian State of Paraíba is the most eastern point of the Americas. Paraíba holds a population of 3,305,616 inhabitants, covers an area of 56,584.6 km^2, contains 223 municipalities and is located in a tropical climate. Cattle production is one of the major agricultural activities, with a total herd size of 1,327,826 animals (IBGE, 1998). Cattle production is mostly extensive, on semi arid soils with poor vegetation and low rain indices. According to the Comitê de Planejamento Agropecuário do Estado da Paraíba, there are three types of cattle regions in the State in line with the three ecological areas: the Agreste / Litoral region, the Cariri / Curimatau region and the Sertão region.

The objective of the present study was to estimate the occurrence of *Leptospira* sp. infecton in commercial cattle herds in the State of Paraíba, Brazil.

MATERIAL AND METHODS

Sampling

A sample frame was developed containing a list of potential participants from both dairy and beef production properties (farm). To be eligible for inclusion in the study the property would have to hold at least 50 animals. Eligible properties were required to have information concerning health status, reproduction and management. Therefore, the sample frame was restricted to commercial cattle herds. Small-scale subsistence herders were not targeted by the present survey. Property managers were pre-screened for their willingness to participate. A random sample of six municipalities was chosen from each of three ecoregions (cattle breeding regions): the Agreste / Litoral region (1), the Cariri / Curimatau region (2) and the Sertão region (3) (Fig. 1). Six municipalities were randomly selected from each ecoregion: Alagoinha, Bananeiras, Fagundes, João Pessoa, Tacima and Umbuzeiro from ecoregion 1; Boqueirão, Campina Grande, Gurjão, Monteiro, Serra Branca and Soledade from ecoregion 2; Conceição, Patos, Piancó, Pombal, Riacho dos Cavalos and Souza from ecoregion 3. These 18 municipalities were divided in quadrants and one property was selected at random from each quadrant.

Table 1 - Serovars of *Leptospira* sp. used in the Microscopic Agglutination Test (MAT) for testing cattle from the State of Paraíba, Brazil.

Serogroups	Serovar	Strain
Australis	Australis	Ballico
	Bratislava	Jez Bratislava
Autumnalis	Autumnalis	Akiyamy A
Ballum	Castelonis	Castellon 3
Bataviae	Bataviae	Swart
Canicola	Canicola	Hond Utrecht IV
Grippotyphosa	Grippotyphosa	Moskva V
Hebdomadis	Hebdomadis	Hebdomadis
Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
Mini	Szwajizak	Szwajizak
Pomona	Pomona	Pomona
Pyrogenes	Pyrogenes	Salinem
Sejroe	Hardjo	Hardjoprajitno
		Norma
	Wolffi	3705
Tarassovi	Tarassovi	Perepelicin

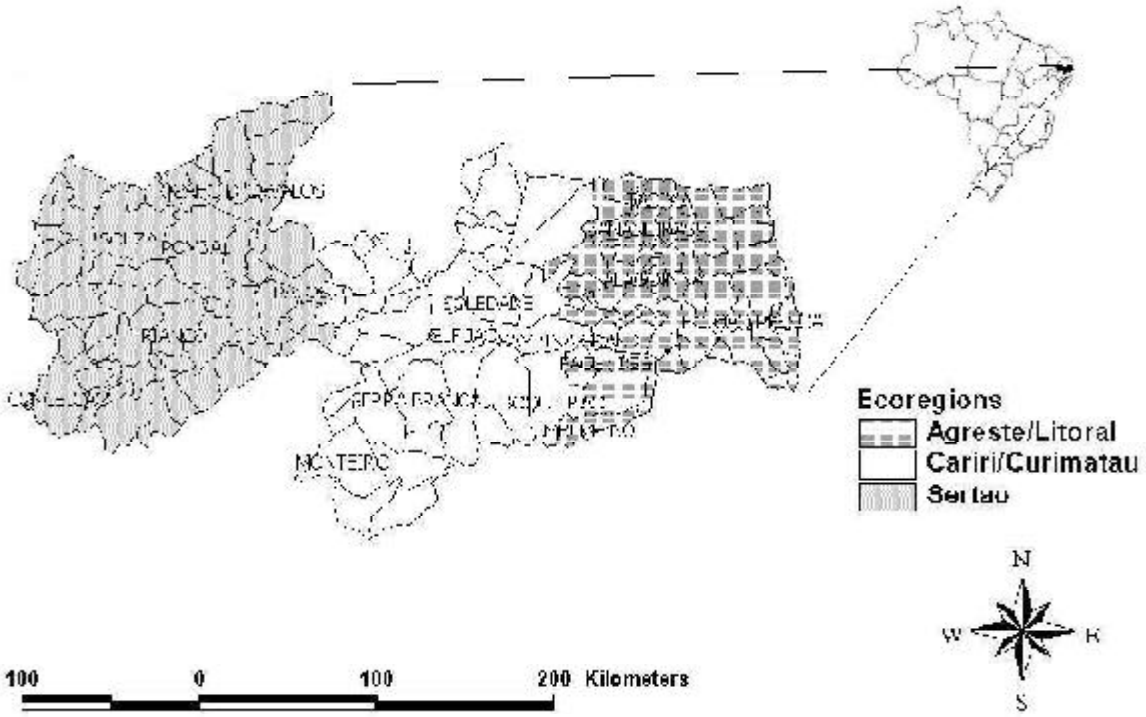

Fig. 1 - Ecoregions and Municipalities sampled for the study in the State of Paraíba, Brazil.

Animals

Blood samples, totaling at least 32 animals per property, were randomly collected from at least eight animals of each property of the following age categories: (1) 0 to 6 months, (2) 7 to 18 months, (3) 19 to 30 months and (4) more than 30 months From march to September of 2000 serum samples were collected through jugular venopunction and stored at minus 20º C until tested.

Serological testing

A battery of 15 different serovars (16 strains) of *Leptospira* sp. (Table 1) was used as antigen. Each strain was harvested from EMJH medium, after a seven-day incubation period at 27º C, with approximately 1-2 X 10^8 bacteria / mL, free of contaminants and of auto-agglutination. The choice of the serovars to be used as antigens was made based on reports of the most frequent leptospiral serovars serologically found in Brazil. Serum samples were tested by the Microscopic Agglutination Technique (MAT) (COLE et al., 1973; OIE, 2000). Reactions were read directly on microplate (Nunc, Denmark) with a microscope equipped with a dry condenser and a dark field, long distance objective (Axiolab, Carl Zeiss, Germany). Negative and positive sera and culture control standards were run in each assay. Sera were screened at 1:100 dilution and diluted on a two-fold basis up to obtain the final titer. Sera presenting 50% or more agglutinated *Leptospira* sp. per microscopic field at a given dilution were considered positive.

Microscopic agglutination test is the method recommended by OIE for the diagnosis of leptospirosis in animals. This test detects predominantly IgM and helps to identify the infecting serovar, which is essential for the establishment of a vaccination program (FAINE et al., 1999, OIE, 2000). Vaccination, like infection, can stimulate specific anti-*Leptospira* sp. IgM shortly after use (GODDAR et al., 1991). In the present study, only a small percentage of herds vaccinated the animals against leptospirosis (LEITE et al., 2004) and none of these herds were sampled less than three month after vaccination.

In the present study, only in a small percentage of herds, the animals were vaccinated against leptospirosis (LEITE et al., 2004) and none of these herds were sampled before three month after vaccination. Therefore, the results obtained were not influenced by vaccine use.

Statistics

Frequencies, confidence intervals (CI), design effects and intracluster correlation coefficients were calculated according to BENNETT et al. (1991).

RESULTS

All municipalities that were included in this study (100%) and 87.75% of the properties had at least one positive sample for one of the 15 different *Leptospira* sp. serovars studied (Table 2). Out of the 2,343 samples, 759 animals showed positive reaction to one of the 16 strains tested. Four hundred and seventy animals tested positively for multiple strains.

Table 2 - Seroprevalence of *Leptospira* sp. serovars in 2,343 cattle in the State of Paraíba, Brazil.

Serovar	Positive	%	95% CI[1]	
			min	max
Australis	5	0.21	0.00	0.43
Autumnalis	34	1.45	0.46	2.44
Bataviae	21	0.90	0.53	1.26
Bratislava	54	2.30	1.40	3.21
Canicola	7	0.30	0.00	0.66
Castelonis	16	0.68	0.23	1.13
Grippothyphosa	3	0.13	0.00	0.27
Hardjo	376	16.05	12.69	19.41
strain Norma	366	15.62	12.38	18.86
strain Hardjoprajitino	57	2.43	1.12	3.75
Hebdomadis	31	1.32	0.60	2.05
Icterohaemoragiae	5	0.21	0.00	0.43
Pomona	10	0.43	0.04	0.81
Pyrogenes	13	0.55	0.17	0.94
Szwajizak	65	2.77	1.19	4.36
Tarassovi	38	1.62	0.72	2.52
Wolffi	34	1.45	0.71	2.19

1 – 95% CI – 95% confidence interval of the mean

The serovar Hardjo, of the Serjoe serogroup, was the most frequent and accounted for 16.05% (95% CI -12.69% to 19.41%) of the positive results (376 positive reactions) obtained by summing positives for strain Norma (366) with positives for strain Hardjoprajitino (57) and positives for both (47). The serovar Hardjo Norma strain contributed to 15.62% of the reactions (95% CI - 12.38% to 18.86%) and the reference strain Hardjoprajitino to 2.43% (95% CI - 1.12% to 3.75%). Forty-seven animals tested positive for both serovar Hardjo strains, Norma and Hardjoprajitino, but all presented higher agglutinating titers for Norma strain.

Lower frequencies of infection were found for serovars Szwajizak (2.77%), Brastilava (2.30%), Tarassovi (1.62%), Wolffi (1.45%), Autumnalis (1.45%) and Hebdomadis (1.32%). The remaining serovars tested had frequencies of less than 1.0% (Table 2).

DISCUSSION

Some studies carried out in Brazil estimated the prevalence of *Leptospira* sp. infection in targeted higher-risk populations (Moreira et al., 1979; Doria et al., 1980; Madruga et al., 1980; Ribeiro et al., 1988; Lilenbaum et al., 1995; Vasconcellos et al., 1997). In the most recent ones, Lilenbaum et al. (1995) and Vasconcellos et al. (1997), the serovar Hardjo was the most prevalent followed by serovar Wolffi.

Although it is difficult to compare those studies with the current one because of differences in sample design and *Leptospira* sp. serovars tested, this study also found serovar Hardjo to be the most frequent and widely disseminated in cattle in the State of Paraíba, Brazil. Conversely, there was a lower frequency of many other serovars such as Szwajizak, Brastilava, Tarassovi, Wolffi, Autumnalis and Hebdomadis (Table 2) already detected at lower rates in other studies in brazilian cattle (Guida et al., 1959; Santa Rosa et al., 1961; Moreira et al., 1979; Giorgi et al., 1981, Moreira, 1994). These results confirm the status of cattle as the usual reservoir for serovar Hardjo and reinforce that its high frequency may interfere with cattle production (Prescott et al., 1993; Faine et al., 1999; Leite et al., 2000).

Using the same sampling frame of this study, Leite *et al.* (2004) reported that 10.1% to 19% of the herds experienced reproductive problems as abortions, stillbirths, and weak calves. Although these signs could be related to leptospirosis, this disease was not known by herd owners and workers in Paraíba and only 8.7% of the studied herds were vaccinated against leptospirosis (Leite at al., 2004). Recognition of the disease is the first and one of the most important steps in a control program. This absence of disease recognition, which precludes the adoption of control strategies in affected herds, is in sharp contrast to the leptospirosis 16% frequency found in the present study and the of disease outbreaks detection ,with reproductive and bloody mastitis signs in the Paraíba State (Leite *et al*., 2000). Hence, control programs for leptospirosis in Paraíba must be preceded by educational programs. Hence, in Paraíba, the control of leptospirosis, must be preceded by an educational program.

Results for the two serovar Hardjo strains used were markedly different, with strain Norma yielding most of positive animals. Diagnostic differences between Hardjo strains Norma and Hardjoprajitino, the reference strain, were already observed, with lesser or greater intensity, in other cattle and sheep populations from other states in Brazil (Moreira, 1994, Herrmann *et al*., 2004). Strain Norma was isolated in the State of Minas Gerais, Brazil and was identified by monoclonal antibodies and PCR as *L. interrogans*

serovar Hardjo genotype Hardjoprajitino (Korver – personal communication[1]) (MOREIRA, 1994). The differences found were probably due to the low passage and origin of strain Norma. Being isolated in 1991, strain Norma has a smaller number of "in vitro" passages than strain Hardjoprajitino that was isolated in 1938 from a man in Sumatra (WOLFF, 1969). Furthermore, the antigenic relationship between strain Norma and *L. interrogans* serovar Hardjo infecting cattle in Brazil is probably closer than that of strain Hardjoprajitino, because it was isolated in the same country and from cattle (MOREIRA, 1994). FONTANALS *et al*. (2001) studying different isolated of *L. interrogans* serovar Pomona from cattle found similar results. FONTANALS *et al.* (2001) found similar results studying different cattle isolates of *L. interrogans* serovar Pomona. These results suggest an increase in sensitivity of diagnosis of infected cattle by the use of a local isolated strain of serovar Hardjo.

Risk analysis on the spatial and age distribution has already been performed in the same set of data for serovar Hadjo infection (THOMPSON *et al*., 2006). The effect of ecoregion was observed; higher prevalence was reported in the Agreste / Litoral region, which is closer to the coast, showing higher humidity than the other regions studied (SEMARH / LMRS-PB, 2002). An age effect was also observed. Animals younger than seven months have a lower prevalence rate than older ones. The prevalence rate increases with age in animals older than 6 month, which is compatible with a greater time of exposure in older animals.

In conclusion, the present survey confirms that serovar Hardjo is the most frequent in cattle in the State of Paraíba, Brazil, followed by other serovars with lower frequencies, namely Szwajizak, Brastilava, Tarassovi, Wolffi, Autumnalis and Hebdomadis. Likewise, results strengthen the use of serovar Hardjo strain Norma as the most sensitive for the diagnostic of *Leptospira* sp. infection in cattle in Brazil.

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