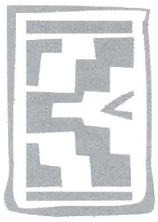
Onderstepoort Journal of Veterinary Research, 68:275–279 (2001)



Seroprevalence of bovine leptospirosis in Garanhuns municipal district, Pernambuco State, Brazil

A.A.F. OLIVEIRA[1], R.A. MOTA[1], G.C. PEREIRA[1], H. LANGONI[2], M.I. SOUZA[1], W.A. NAVEGANTES[2] and M.E.P. SÁ[2]

ABSTRACT

OLIVEIRA, A.A.F., MOTA, R.A., PEREIRA, G.C., LANGONI, H., SOUZA, M.I., NAVEGANTES, W.A. & SÁ, M.E.P. 2001. Seroprevalence of bovine leptospirosis in Garanhuns municipal district, Pernambuco State, Brazil. *Onderstepoort Journal of Veterinary Research*, 68:275–279

The prevalence of *Leptospira interrogans* serovars in dairy cattle was determined by analyzing 464 serum samples from cows on 15 properties in Garanhuns municipal district, Pernambuco State, Brazil. A microscopic seroagglutination test including 12 serovars of *Leptospira interrogans* as antigens was used. Samples with titres ³ 100 were considered positive. Two hundred and twenty-one (47.63%) of the samples were positive to one or more serovars. The prevalence of the serovars was *hardjo* (21.98%), *bratislava* (15.73%), *castellonis* (11.64%), *tarassovi* (10.56%), *pyrogenes* (1.72%), *icterohaemorrhagiae* (1.08%), *pomona* (0.86%), *wolffi* (0.86%), *grippotyphosa* (0.86%), *djasiman* (0.43%), *canicola* (0.21%), and *copenhageni* (0.21%).

Keywords: Brazil, cattle, leptospirosis

INTRODUCTION

Leptospirosis is a serious zoonosis caused by *Leptospira interrogans* with more than 200 different serotypes, which affects mainly cattle, pigs, horses, and dogs (Amatredjo & Campbell 1975). Cattle susceptibility varies according to factors such as age, physiological state and population density (Brod & Fehberg 1992). In the Americas, the predominant serotypes are *hardjo, pomona* and *gryppotyphosa* (Ellis 1984). Prevalence varies considerably between herds, regions and countries; it is highest in tropical countries with heavy rainfall and neutral or alkaline soils (Guimarães 1982/83).

Bovine leptospirosis is endemic in Brazil and quite frequent in cattle. It causes abortions, placental retention and perinatal mortality, congenital abnormalities, milk production loss, and mastitis (Lilenbaum, Santos & Dos Santos 1995). The last two are generally associated with the *hardjo* serotype, which is related to the "Milk Drop Syndrome" (Higgins, Harbourne & Little 1980; Pearson, Mackie & Ellis 1980).

The aim of this study was to determine the seroprevalence of *L. interrogans* serovars known to affect cattle in the studied region.

MATERIALS AND METHODS

Herd description

Four hundred and sixty-four adult cows were used in this study. The animals were crossbred Holstein-Friesian, Jersey and Brown Swiss either dry or in various lactation stages. Some animals presented reproductive disturbances. They were maintained under semi-intensive management systems on 15 farms in

[1] Federal Rural University of Pernambuco, Department of Veterinary Medicine, Av. Dom Manuel de Medeiros, s/n, Dois Irmãos, Recife-PE, Cep: 52.171-900, Brazil. E-mail: deaalice@zipmail.com.br

[2] São Paulo State University, Department of Veterinary, Hygiene, and Public Health, Rubiao Junior District s/n, Rubiao Junior, Botucatu, São Paulo, Cep: 18.618-000 Brazil. E-mail: mepsa@zipmail.com.br

Accepted for publication 17 October 2001—Editor

the municipal district of Garanhuns, Pernambuco State, northeastern Brazil, for more than a year without being vaccinated against leptospirosis. The sampling fraction was determined according to the Pan-American Center of Foot-and-Mouth Disease, considering a 10% possibility of error and 49.37% of expected prevalence (calculated based on total population) (Astudillo 1979).

Blood sampling and serological testing

A serum sample from each cow was obtained by jugular venipuncture, using disposable needles (40 x 16 mm) after disinfecting the skin with 3% iodine alcohol. Approximately 10 ml of blood was obtained from each animal. The blood samples were collected in sterile tubes that were placed at an incline at ambient temperature to facilitate blood serum acquisition after blood clotting. The blood was then centrifuged at 804.9 *g* for 10 min, the serum decanted and stored in cryotubes at –18 °C until testing was performed.

The test was the standardized microscopic seroagglutination, read under a dark-field microscope, according to WHO (Myers 1985) and the Ministry of Health—National Health Foundation (Brasil 1995). The batch of living antigens used was based on recommendations of FIOCRUZ/RJ (Anon. 1995) and included 12 serovars considered important for cattle (Table 1). Dilutions of the antigens used were 1:100, 1:200, 1:400, 1:800, 1:1 600, and 1:3 200, as proposed by Myers (1985) and Anon. (1995).

TABLE 1 Serovars used in the microscopic seroagglutination test

Serogroups	Serovars	Sample of reference
Australis	*bratislava*	Jez-bratislava
Ballum	*castellonis*	Castellón
Canicola	*canicola*	Hond Utrecht IV
Djasiman	*djasiman*	Djasiman
Grippotyphosa	*grippotyphosa*	Moskva V
Icterohaemorrhagiae	*Icterohaemorrhagiae*	RGA
	copenhageni	M 20
Pomona	*pomona*	Pomona
Pyrogenes	*pyrogenes*	Salinum
Sejroe	*hardjo*	Hardjoprajtino
	wolffi	3705
Shermani	*tarassovi*	Perepelicin

TABLE 2 Numbers of seropositive cows according to the farm of origin

Farms	Examined animals	Reacting animals	
		Positive*	%
A	17	7	41.18
B	5	1	20.00
C	28	16	57.14
D	30	20	66.67
E	31	16	51.61
F	30	22	73.33
G	42	25	59.52
H	21	13	61.90
I	30	12	40.00
J	38	13	34.21
K	18	10	55.56
L	41	16	39.02
M	26	9	34.61
N	38	15	39.47
O	69	26	37.68
Total	464	221	47.63

* Antibody titres ⊕ 100

Investigative questionnaire

When each batch of serum samples was collected, the farmers concerned were requested to complete a questionnaire in order to acquire information about the farms and animals. Farm management practices such as feed, semi-intensive system, water quality and the type of terrain as well as sanitary data like vaccination, presence of reproductive disturbances and the use of anthelmintics were evaluated.

Statistical analysis

The samples were analyzed using descriptive analysis. Absolute and relative frequencies of positive animals on the studied farms and the agglutination titres related to *L. interrogans* serovars were evaluated.

The questionnaires were analyzed by the EPI-INFO and Chi-square tests to determine associations between levels of prevalence and independent variables.

RESULTS

A seropositive prevalence of 47.63% was obtained for one or more of the serovars with titres of ⊕ 100, while 52.37% of the animals tested seronegative.

Seropositive animals were found on all 15 farms (Table 2), and many serotypes of *L. interrogans* were present. These results revealed the widespread prevalence of infection, which demonstrates a high capacity for the organism to survive in the environment, ease of transmission and possibly the contribution of many other susceptible species of animals in facilitating the maintenance of the agent in nature. In this study, samples of the serum antibodies to the following 12 serovars were detected: *hardjo* (22.00%), *bratislava* (15.73%), *castellonis* (11.00%), *tarassovi* (10.66%), *pyrogenes* (1.72%), *icterohaemorrhagiae* (1.08%), *grippotyphosa* (0.86%), *pomona* (0.86%),

TABLE 3 Distribution of the agglutination titres in cow sera, with dilutions 1:100 to 1:3200 for 12 *Leptospira interrogans* serovars

Serovars	Dilutions/titres								
	1:100	1:200	1:400	1:600	1:800	1:1600	1:3200	Total	%
Hardjo	34	41	13	1	9	3	1	102	33.22
Bratislava	43	18	11	–	1	–	–	73	23.78
Castellonis	34	17	2	–	1	–	–	54	17.59
Tarassovi	26	15	5	1	2	–	–	49	15.96
Pyrogenes	5	1	–	–	2	–	–	8	2.61
Icterohaemorrhagiae	5	–	–	–	–	–	–	5	1.63
Pomona	3	–	1	–	–	–	–	4	1.30
Wolffi	3	1	–	–	–	–	–	4	1.30
Grippotyphosa	2	1	–	–	1	–	–	4	1.30
Djasiman	2	–	–	–	–	–	–	2	0.65
Canicola	–	1	–	–	–	–	–	1	0.33
Copenhageni	–	1	–	–	–	–	–	1	0.33
Total	157	96	32	2	16	3	1	307	100.00

wolffi (0.86 %), *djasiman* (0.43 %), *copenhageni* (0.21 %) and *canicola* (0.21 %).

Table 3 shows the distribution of the agglutination titres in relation to the *L. interrogans* serovar antigens used. It can be seen that a higher frequency of agglutination titres was obtained at dilutions 1:100 (157) and 1:200 (96).

The analysis of the questionnaires did not reveal any association between reproductive disturbances and the level of leptospirosis prevalence in the animals.

DISCUSSION

Results of the prevalence of seropositive animals obtained in this study are in agreement with similar studies performed in other regions of Brazil (Abuchaim & Dutra 1985; Ribeiro, Gouveia & Silva 1988; Brod, Martins, Nussbaum & Fehberg 1995; Negrão, Mólnar & Mólnar 1999) and in other countries (Miller, Wilson & Beran 1991; Niang, Will & Kane 1994; Cantu-Covarrubias & Banda-Ruiz 1995), but the titres obtained are higher than those of Girio, Mathias, Silva & Franceschini (1990) and Rodrigues, Muller & Freitas (1999) and lower than those obtained by Lilenbaum *et al.* (1995) and Lilenbaum & Santos (1996). The prevalence of antibodies to *L. interrogans* varies considerably between countries, regions, areas and even farms, which might explain the different prevalence rates found in various regions or in one region.

Animal susceptibility varies according to age, physiological state, population density, presence of rodents and wildlife on the farm, access to water and feed, as well as climatic and environmental factors (Brod & Fehberg 1992). In this study, however, a correlation between prevalence of infection and the variables population density, type of soil and conditions of water and feed troughs was not obtained. The semi-intensive management system was used on all studied farms.

The prevalence of bovine leptospirosis in Brazil has been studied by many researchers, the first reference dating back to 1958 (Guida & Barros 1958) when 3.7 % of animals were reported positive. Subsequent studies demonstrated a higher incidence of infection in different regions of Brazil (Abuchaim & Dutra 1985; Ribeiro *et al.* 1988; Brod *et al.* 1995; Lilenbaum *et al.* 1995; Lilenbaum & Santos, 1996; Negrão *et al.* 1999; Rodrigues *et al.* 1999).

A positive correlation between rainfall and leptospirosis in cattle has been reported, indicating that during the current study period (October to December 1998), when there was heavy rainfall, an increase in the number of seropositive animals would be seen. This, however, did not occur.

On the 15 farms a high prevalence of seropositive animals to the *hardjo, bratislava* and *castellonis* serovars was seen and a low or no prevalence to the *copenhageni, canicola, djasiman* and *grippotyphosa* serovars. This even distribution may contribute to a characterization of the disease in the studied municipal district. The *wolffi, pomona, icterohaemorrhagiae, pyrogenes* and *tarassovi* serovars showed an average level of reactors amongst the animals.

Leptospira hardjo was the most prevalent serovar in the studied region, which is in agreement with studies in Brazil by Abuchaim & Dutra (1985), Ribeiro *et al.* (1988), Lilenbaum & Santos (1996), Brod *et al.* (1995) and Negrão *et al.* (1999). These authors emphasized the importance of bovine as reservoirs in various regions of the country. Since the 1980's when the *hardjo* serovar was first included in the batch of antigens tested, it has proven to be the predominant serovar in cattle (Miller *et al.* 1991; Brod *et al.* 1995;

Tedesco 1997; Ribeiro *et al.* 1998). It is considered to be maintained in cattle (Ellis 1984) and as being the major responsible serovar for reproductive disorders. In this study, however, the infectious problems related to fertility could not be properly clarified, since none of the farms recorded adequate sanitary data. This made it impossible to obtain important information such as births, service periods, calving intervals and reproductive efficiency.

Serological monitoring can provide indirect evidence of the association between infection with the *hardjo* serovar and reproductive disturbances, mainly abortions, when high titres are found after the detection of clinical signs. This may suggest that in this study, the occurrence of reproductive disturbances in some analyzed animals may be associated with *Leptospira interrogans*, despite the high prevalence of the *hardjo* serovar. However, other diseases that cause reproductive problems should be considered and properly studied.

The prevalence of the *bratislava* serovar was 23.78%, the second highest, which is in agreement with the findings of Lilenbaum & Santos (1996) and Negrão *et al.* (1999). These authors stated that this serovar has increased in the last few years in Brazil. Rodrigues *et al.* (1999) found 10.24% of animals seropositive to the *bratislava* serovar, although it was only the seventh most prevalent in this study. Thirteen of the 15 farms had animals positive to this serovar, which may be related to incidental infections involving contact of bovine with other species, mostly swine, since the farmers adopt a semi-intensive management system and some raise cattle together with other species.

The *castellonis* and *tarassovi* serovars represented 17.59% and 15.96% respectively, of the seropositive animals, confirming the findings of Caldas, Sampaio, Tishcenko & Viegas (1977) and Negrão *et al.* (1999). The *castellonis* serovar was considered by Langoni, Marinho, Baldini & Da Silva (1995) the second most prevalent in sheep. This suggests that infection of cattle by these serovars is related to the presence of different species on seven of the farms. However, a better knowledge of the importance and ecology of these serovars and others, such as *pyrogenes, djasiman* and *copenhageni*, is needed. The last named three are generally reported as of low incidence and their significance in cattle is not well-known. Prospective studies should focus on the correlation of seroconversion or high antibody titres and clinical disease associated with *L. interrogans* serovars.

ACKNOWLEDGEMENTS

We thank the staff of Garanhuns Bovine Clinical for their support and Dr Helio Langoni and his team for their assistance with the laboratory analyses.

REFERENCES

ABUCHAIM, D.M. & DUTRA, N.F.L. 1985. Prevalência da leptospirose em bovinos da bacia leiteira de Porto Alegre, RS. *Arquivos Faculdade de Veterinária UFRGS*, 13:55.

AMATREDJO, A. & CAMPBELL, R.S.F. 1975. Bovine leptospirosis. *Veterinary Bulletin*, 43:875–891.

ASTUDILLO, V.M. 1979. *Procedimentos para estudos de prevalência por muestreo.* Rio de Janeiro: Centro Panamericano de Fiebre Aftosa.

ANONYMOUS. 1995. *Manual de Leptospirose*, 2 ed. Brasília: Fundação Nacional de Saúde: 1–98.

BROD, C.S. & FEHBERG, M.F. 1992. Epidemiologia da leptospirose em bovinos. *Ciência Rural*, 22:239–245.

BROD, C.S., MARTINS, L.F.S., NUSSBAUM, J.R. & FEHBERG, M.F.B. 1995. Leptospirose bovina na região sul do Estado do Rio Grande do Sul. *Hora Veterinária*, 14:15–20.

CALDAS, E.M., SAMPAIO, M.B., TISHCENKO, L.M. & VIEGAS, E. 1977. Aglutininas antileptospira em hemosoros de animais. *Arquivos Escola Medicina Veterinária da UFBA*, 2:83–98.

CANTU-COVARRUBIAS, A. & BANDA-RUIZ, V.M. 1995. Seroprevalencia de leptospirosis bovina en tres municipios de sur de Tamaulipas, México. *Tecnica Pecuaria Mexico*, 33:121–124.

ELLIS, W.A. 1984. Bovine leptospirosis in the tropics: prevalence, pathogenesis and control. Preventive *Veterinary Medicine*, 2: 411–421.

GIRIO, R.J.S., MATHIAS, L.A., SILVA, R.A.P. & FRANCESCHINI, P.H. 1990. Estudo da possível influência da leptospirose sobre determinadas características reprodutivas em fêmeas bovinas leiteiras. *Ciência Veterinária Jaboticabal*, 4:27–28.

GUIDA, V.D. & BARROS, W.M. 1958. Pesquisa de aglutininas e lisinas anti-leptospira em soros de bovinos aparentemente normais e sacrificados no matadouro. *O Biológico*, 26–27.

GUIMARÃES, M.C. 1982/83. Epidemiologia e controle da leptospirose em bovinos: papel de portador e seu controle terapêutico. *Revista Faculdade Medicina Veterinária e Zootecnia da USP*, 6/7:21–34.

HIGGINS, R.J., HARBOURNE, J.F. & LITTLE, T.W.A. 1980. Mastitis and abortion in dairy cattle associated with *Leptospira* serotype *hardjo*. *The Veterinary Record*, 107:307–310.

LANGONI, H., MARINHO, M., BALDINI, S., & DA SILVA, A.V. 1995. Pesquisa de aglutininas antileptospíricas em soros de ovinos no Estado de São Paulo, Brasil, utilizando provas de Macroaglutinação em placa e Soroaglutinação Microscópica. *Revista Brasileira de Medicina Veterinária*, 17:264–268.

LILENBAUM, W., SANTOS, M.C.R. & DOS SANTOS, M.R.C. 1995. Leptospirose em reprodução animal: III. Papel do sorovar *hardjo* nas leptospiroses bovinas no Rio de Janeiro, Brasil. *Revista Latino Americana de Microbiologia*, 37:87–92.

LILENBAUM, W. & SANTOS, M.C.R. 1996. Effect of management systems on the prevalence of bovine leptospirosis. *The Veterinary Record*, 138:570–571.

MILLER, D.A., WILSON, M.A. & BERAN, G.W. 1991. Survey to estimate prevalence of *Leptospira interrogans* infection in mature cattle in the United State. *American Journal Veterinary Research*, 52:1761–1765.

MYERS, D.M. 1985. *Manual de Métodos para el diagnostico de laboratorio de leptospira.* Buenos Aires: Centro Panamericano de Zoonosis (Nota Técnica no. 30).

NEGRÃO, A.M.G., MOLNÁR & E., MOLNÁR, L. 1999. Dados sorológicos da leptospirose bovina em algumas regiões do

Estado do Pará. *Revista Brasileira Reprodução Animal,* 23: 406–407.

NIANG, M., WILL, L.A. & KANE, M. 1994. Seroprevalence of leptospiral antibodies among dairy cattle kept in communal corrals in periurban areas of Barnako, Mali, West Africa. *Preventive Veterinary Medicine,* 18:259–265.

PEARSON, J.K.L., MACKIE, D.P. & ELLIS, W.A. 1980. Milk Drop Syndrome resulting from *Leptospira hardjo. The Veterinary Record,* 107:135–136.

RIBEIRO, S.C A., GOUVEIA, M.A.V. & SILVA, P.L. 1988. Levantamento sorológico em dois surtos de leptospirose bovina em Uberlândia, triângulo mineiro. *Arquivo Brasileiro Medicina Veterinária e Zootecnia,* 40:415–423.

RODRIGUES, C.G., MULLER, E.E. & FREITAS, J.C. 1999. Leptospirose bovina: Sorologia na bacia leiteira da região de Londrina, Paraná, Brasil. *Ciência Rural,* 29:309–314.

TEDESCO, L.A. 1997. Leptospirose: uma doença que se expande e assusta. *Balde Branco:* 44–48.