Prevalence and spatial analysis of antileptospiral agglutinins in dairy cattle – Microregion of Sete Lagoas, Minas Gerais, 2009/2010

[*Prevalência e análise espacial de aglutininas antileptospira em gado leiteiro – Microrregião de Sete Lagoas, Minas Gerais, 2009/2010*]

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ABSTRACT

The aim of this study was to evaluate the seroprevalence of leptospirosis in the dairy herds from Minas Gerais, Brazil, during the years 2009 and 2010. A total of 2,915 serum samples were collected from the lactating cows of 151 properties in eleven municipalities located in the Sete Lagoas region. The Microscopic Agglutination Test was used to detect antileptospiral agglutinins. An individual animal prevalence of 20.7% (95% CI = 17.1% − 24.3%) and a herd prevalence of 80.8% (95% CI = 73.8% = 87.7%) were determined. The most prevalent serovars were *hardjoprajitno* at 19.4%; *hardjoprajitno* strain Norma at 17.4%; and *hardjo-bovis* at 17.4%. These results show the significance of the *hardjo* serovar in bovine leptospirosis cases in Minas Gerais.

Keywords: Leptospirosis, prevalence, Sete Lagoas, dairy cattle, Brazil

RESUMO

Avaliou-se a prevalência de aglutininas antileptospira em rebanhos leiteiros durante os anos de 2009 e 2010. Foram coletadas 2.915 amostras de soro de vacas lactantes de 151 propriedades localizadas na região de Sete Lagoas − MG. Foi utilizado o teste de aglutinação microscópica (MAT) para detecção de aglutininas antileptospira. Prevalência animal de 20,7% (95% CI = 17,1%-24,3%) e prevalência de rebanho de 80,8% (95% CI = 73,8% = 87,7%) foram reportadas. Os sorotipos mais prevalentes no estudo foram hardjoprajitno *(19,4%);* hardjoprajitno *estirpe Norma (17,4%) e* hardjo-bovis *(17,4%). Estes resultados demostram a importância da sorovariedade* hardjo *nos casos de leptospirose bovina do estado de Minas Gerais.*

Palavras-chave: Leptospirose, prevalência, Sete Lagoas, rebanho leiteiro, Brasil

INTRODUCTION

Leptospirosis is a bacterial zoonotic disease of major economic importance to dairy farming that causes abortions, stillbirths, infertility and decreased milk production in dairy livestock (Bennett, 2003). This disease occurs worldwide and is caused by infection with spirochetes from the genus *Leptospira*. Pathogenic leptospire were previously classified as members of the *Leptospira interrogans* species. The genus was recently reorganized, and leptospira were classified into 12 pathogenic species with over 250 serovars (Adler and De La Peña Moctezuma, 2010). Leptospira microorganisms are identified worldwide by surface-antigen and genomic testing (Levett, 2001; Bolin, 2003).

Worldwide, *hardjo* is the most commonly reported serovar that causes infection in cattle. Two types of the *hardjo* serovar that are indistinguishable serologically but are genetically distinct have been identified: *Leptospira interrogans* serovar *hardjo,* strain hardjoprajitno; and *L. borgpetersenii* serovar

hardjo, strain hardjo-bovis (Faine *et al.,* 2000; Bolin, 2003). Several studies have demonstrated that leptospirosis in cattle causes large economic losses in the milk supply chain. The direct and indirect costs of the disease include reproductive losses, decreased milk production and increased veterinary expenses, including vaccinations and laboratory tests (Bennett, 2003).

One of the greatest problems encountered in controlling leptospirosis infection is environmental contamination. Leptospires actively penetrate mucosa (ocular, digestive, respiratory and genital), broken skin and even intact skin when animals stand in contaminated ponds of water for an extended amount of time. Spirochete elimination in the urine occurs intermittently, and this process may persist for extended periods of time and can cause outbreaks of disease in healthy animals due to direct contact with urine from infected animals. Aborted fetuses and placentas are also important transmission routes (Faine *et al*., 2000).

One method for controlling leptospirosis combines reducing the prevalence of infection by serovars maintained in the population and decreasing the degree of ecological exposure to leptospira maintained by free-living animals (Faine *et al*., 2000). In veterinary practice, this control is achieved by systematically vaccinating the herd, and preventing direct contact between diseased and healthy animals (Faine *et al*., 2000).

In Brazil, the presence of antileptospiral agglutinins has been reported in every state and always highly prevalent in the individual animal and herd levels. Favero *et al*. (2001) conducted a study from 1984 to 1997 in 21 Brazilian states, analyzing more than 31,000 bovine serum samples. They found a 37.9% prevalence of agglutinins in the animals overall and 41.3% of the animals in the state of Minas Gerais. Araújo *et al*. (2005) performed a study with more than 39,000 bovine serum samples and found an individual animal prevalence of 23.7% in the state of Minas Gerais. These studies also demonstrated the importance of the *hardjo* and *hardjbovis* strains in leptospirosis cases.

Effective control measures need to be economically efficient. However, economic losses related to leptospirosis are unknown in Brazil. Estimating the prevalence is the first step towards quantifying economic losses.

The purpose of this study was to measure the prevalences of antileptospiral agglutinins at the animal and herd levels in eleven municipalities at Sete Lagoas microregion in the state of Minas Gerais, Brazil. This work was a completely randomized, probabilistic study specifically designed to determine the prevalence.

MATERIAL AND METHODS

The state of Minas Gerais is the largest milk producer in Brazil, over 205,773 families are financially supported from this activity and the region has more than 223,073 dairy farms. In 2010, milk production in Minas Gerais surpassed eight billion liters, accounting for 28% of the Brazilian production, according to the Instituto Brasileiro de Geografia e Estatística (IBGE, 2010). The real gross value added by milk production in 2005 was R$3.8 billion, according to IBGE. The exports by dairy have also grown. In 2006, there was US$66.4 million in exports: an increase of 74% over the previous year (IBGE, 2010).

The Sete Lagoas region is located 90 km from Belo Horizonte, MG, 1,446 families are part of the familiar farms, producing over 24 million liters of milk per year, yielding approximately 12 million Reais in 2006. Samples were collected from 11 counties which produced more than 73 million liters of milk in 2006. The studied municipalities have 1,500 milk-producing properties with approximately 36 thousand animals (IBGE, 2006).

To determine the appropriate sample size, 1,500 milk-producing properties from 11 municipalities (IBGE, 2006) were used. An expected herd prevalence of 70% with a maximum error of 10% was used, resulting in the lowest expected prevalence of 63%. The confidence level used in this study was 95%. Using the StatCalc module from the EpiInfo 6.04d® (CDC, USA) program, it was calculated that a minimum of 148 herds was necessary to estimate the prevalence.

An average of 25.4 cows per property in the region was previously determined (IBGE 2006). Therefore, the number of sampled cows per property was set at 20. Using the Herdacc 3.0® program (Statistics, University of Guelph, USA) with sensitivity and specificity levels set at 99%, population size of 25 animals, animal prevalence

levels of 15% and a cutoff of 1, a herd sensitivity of 98.7% and a specificity of 100% (with 20 animals per property) were calculated.

A total of 2,915 samples were collected from 151 dairy herds from November 2009 to July 2010; herds were chosen randomly from the farm registry at the Instituto Mineiro de Agricultura (IMA). All of the properties were geographically mapped using a Global Positioning System.

The Microscopic Agglutination Test (MAT), as recommended by the World Health Organization was used for diagnosis in accordance with the protocol described by Cole *et al*. (1973) and modified by Herrmann *et al*. (2004). The eight serovars most commonly found in dairy herds in the studied region (Favero *et al*., 2001; Araújo *et al*., 2005) were included in the MAT. Table 1 shows the serovars utilized and their reference strains.

All properties were georeferenced during sample collection (latitude and longitude). To perform spatial cluster detection analysis, a Bernoulli model was used in which all of the herds were classified either as a case (positive) or control (negative) to determine clusters in the municipalities. A case herd was defined using the same criteria used for the serum prevalence: at least one positive animal. All of the calculations were performed using SaTScan® version 8.2 (Statistics, Harvard Medical School and Harvard Pilgrim Health Care, USA) (Kulldorff *et al*., 1998). The Terra View 3.5.0® (Statistics, INPE, Brazil) program was used for map creation.

Table 1. Serovars used in the MAT assay for dairy herds in the Sete Lagoas region, Minas Gerais, 2009-2010.

Serogroup	Serovar	Reference Strain
Serjoe	*Hardjo*	Hardjoprajitino
	hardjobovis	Sponcelee
	hardjo	Hardjoprajitino
	wolffi	Norma
Pomona	*pomona*	3705
Grippotyphosa	*grippotyphosa*	Pomona
-	-	Moska V
-	-	Bolivia*
		Lagoa*

*Strains isolated and under genetically classification by Universidade Federal de Minas Gerais.

The estimated serum prevalences were determined for the proportions of animals and herds positive for antileptospiral agglutinins using data analysis and by adjusting the sample weight (Dargatz and Hill, 1996) of each farm and each animal with the Stata 11® (Statistics, Stata Corporation, USA) software package.

RESULTS

The serum prevalence level in animals from the eleven municipalities in the Sete Lagoas region, MG, Brazil, was 20.7% (95% CI = 17.1% – 24.3%). The prevalences of the serovars used in this study confirmed the importance of *hardjo* and *hardjobovis* serovars in bovine infection cases. Serovar *hardjo* strain Hardjoprajitno was the most prevalent at 19.4%, followed by the Lagoa isolate (19.3%), the Bolivia isolate (18.8%), the *Hardjo* strain Norma (17.4%) and *hardjobovis* (17.4%). The Lagoa and Bolivia strains were recently isolated by the Departamento de Medicina Veterinária Preventiva da Universidade Federal de Minas Gerais and these strains are under genetic classification process. The measured prevalence is listed in Table 2.

Table 2. Prevalence of each tested serovar in dairy herds in the Sete Lagoas region, Minas Gerais, 2009-2010

Serovar	Analyzed Samples	Positive	Prevalence %
Hardjobovis	2,915	562	17.4
hardjo strain Hardjoprajitino	2,915	590	19.4
hardjo strain Norma	2,915	546	17.4
bolivia *	2,915	601	18.8
*lagoa**	2,915	610	19.3
Pomona	2,915	003	0.006
Wolffi	2,915	371	11.2
grippotyphosa	2,915	016	10.01

*Strains isolated and under genet classification by Universidade Federal de Minas Gerais.

The most prevalent serovars also had the highest titers in positive samples, Tab. 3, confirming the presence of these serovars in the studied herds.

The herd-level prevalence was 80.7% (95% CI = 73.8% - 87.7%). The analysis based on the Bernoulli model did not identify any clusters in the region for either positive or negative cases, owing mainly to the high prevalence found. Figure 1 shows the geographical location.

Table 3. Antileptospiral agglutinin titers of positive samples from dairy herds in the Sete Lagoas region, Minas Gerais 2009-2010

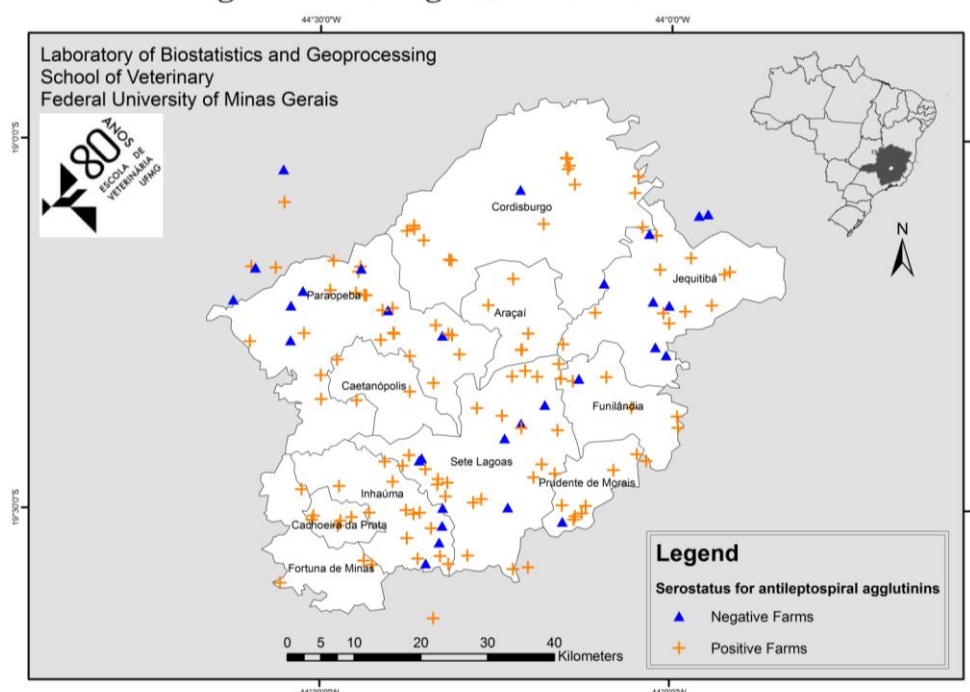| Serovar	Titers						
	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400
hardjobovis	67.0%	13.8%	9.1%	6.5%	2.7%	0.5%	0.4%
hardjo strain Norma	70.8%	17.6%	6.7%	3.1%	1.3%	0.5%	-
Hardjoprajitino	73.1%	14.2%	8.1%	3.8%	0.5%	-	0.2%
bolivia	60.1%	16.4%	12.2%	5.4%	2.9%	2.8%	0.2%
lagoa	58.2%	23.8%	10.5%	4.2%	3.3%	-	-
wolffi	87.0%	6.8%	5.5%	0.4%	0.3%	-	-
grippotyphosa	84.0%	-	16.0%	-	-	-	-
pomona	100%	-	-	-	-	-	-

Figure 1. Geographical locations of positive and negative farms for anti-Leptospiral agglutinins at the region of Sete Lagoas, Minas Gerais, Brazil, 2010.

DISCUSSION

The Prevalence studies allow the generalization of results to a larger population than the previously sampled, but this extrapolation requires a complex and rigorous sampling design so that the result found is not biased (Dargatz and Hill, 1996). The studies must correctly set the target population and the population to be sampled, so the sampled population must represent the target population. A very common problem is convenience samples, such as a bank of serum or properties, which biases the samples. It is simple to understand that the use of a serum bank from a University would increase the estimated prevalence, because the animals that perform an examination of leptospirosis are suspected positives. The present study has selected both the properties and the animals, which are completely randomized, each property and animal sampled had the same probability of being sampled in relation to non-sampled. The sampling weight calculation defines how an animal sampled from the population represents

the population target (Dargatz and Hill, 1996). Each one of the 11 municipalities sampled has different populations, so each animal sampled had its sampling weight related to its corresponding population, adjusting appropriate prevalence. Thus, a study of prevalence with the methodology presented in a state like Minas Gerais would be costly financially and operationally, so smaller dairy region studies are important for the knowledge of the actual dispersion of leptospirosis in different counties and to propose control measures geared specifically for each region. It does not occur when the region studied is aggregate and the result shows the average of all small regions. In science the saying "local actions, can generate overall results" is true.

Serological studies to determine the prevalence of antileptospiral agglutinins have been conducted in Brazil, particularly in dairy herds. The relationships among species of leptospire and preferred hosts vary according to the region, however, in general, like this work, the records are prevalent in dairy cattle for serovars *hardjo, hardjobovis* and *wolffi* (Vasconcellos, 1997; Favero *et al.*, 2001). This result has a special significance, since serovar *hardjo* is considered the most widespread worldwide and cause great economic impact on livestock, as a consequence of abortion and milk drop syndrome (Ellis, 1984). The results depend on the collections of antigens used and the criteria used for interpreting results, however, the proportions of positives have been high and ranged from 15-66% in dairy cattle (Moreira *et al*., 1979; Vasconcellos *et al.,*1997; Favero *et al*., 2001; Lilenbaun and Souza, 2003; Araújo *et al*., 2005).

Small properties are part of local reality, equipped with low technological development, absence of biosafety, especially regarding the handling of animals, few investments in genetics, poor sanitary control especially for the control of leptospirosis, and low productivity of the herd. Opposing this scenario for livestock with low technification, a demand is growing for products that meet the minimum requirements in technical regulations for quality and biosecurity. With a herd expansion characterized by heterogeneity of the properties, disease controls have a great importance to maintain and enable the animal production in optimum levels and sustainability for production systems. In addition to the productive aspect, the reproduction of livestock can be severely affected by infectious agents, particularly the complex leptospirosis, compromising the economic and epidemiologic efficiency of the entire system.

The economic viability of milk production depends on a high reproductive efficiency in order to produce the healthiest animals, which will be used for replacement breeding stock, as well as the direct sale of these animals, increase of productivity and milk production quality. Thus, the results found show that a large economic loss is affecting the region's properties, and efforts to control and especially prevent bovine leptospirosis should be taken.

Data from the 2006 agricultural census shows that the familiar farmers with dairy cows had the gross income of approximately R$948 in one year, a countless difference when compared to unfamiliar farmers, where dairy cows had a gross income of 3,515 in a year. This great difference can also be observed in the milk production of each animal. The familiar dairy farmer has produced an average of 3 liters of milk per cow in 305 days while the non-familiar cow produced over 11 liters of milk per day in 305 days (IBGE, 2006).

This low productivity in familiar agriculture can be explained by the low technicization, no genetic selection of animals and poor sanitary control, especially for the leptospira complex, within the properties studied. Only 30% of the properties perform vaccination for leptospirosis, 74% do not test animals purchased for leptospirosis and only 3% test their animals for leptospirosis, the vast majority only requires testing for brucellosis and tuberculosis. This occurs due to the existence of the Brazilian National plan for the Control and Eradication of Brucellosis and Tuberculosis (PNCEBT). This plan was established in 2001 by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) in order to lessen the impact of these diseases. The PNCEBT requires a negative diagnosis test of all animals and vaccination certificate only for females for any transit purpose (Brazil, 2006).

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The high prevalence observed, particularly for serovar hardjo, demonstrates that large economic losses may be reaching the herds of the Sete Lagoas region, mostly in family farms.

Studies aimed at knowing the risk factors for the disease and models simulating the economic impacts and their interactions with the controlling methods of the disease must be carried out aiming to improve the knowledge about how the disease behaves in herds and the actual economic impact for the region's producers.

CONCLUSIONS

The Sete Lagoas region, MG, is endemic for leptospirosis, having high animal and herd prevalence of the disease. High economic impacts must be affecting producers in the region, especially the so-called familiar, and for this reason works that simulate economic impacts should be done. The disease is randomly distributed in the studied region, probably due to its high prevalence.

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