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Seroprevalence anti-*Leptospira* spp. antibodies and the risk factors in cattle of the tropical savannah of eastern Colombia

Soroprevalência anti-Leptospira spp. anticorpos e fatores de risco em bovinos da savana tropical do oriente da Colômbia

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ABSTRACT

Leptospirosis is a zoonotic disease in the tropical zone with a broad and endemic distribution. The condition is complex, where different host factors, serovar type, reservoirs, environment, and agricultural practices intervene. In cattle, the disease causes significant damage to the livestock sector, and the surrounding conditions can influence its dynamics. This study aimed to determine the seroprevalence of anti-*Leptospira* spp. antibodies and the risk factors in cattle in the tropical savannah from eastern Colombia. Blood was taken from the coccygeal veins of 1,000 animals. The MAT (Microagglutination) technique was used for the identification of the serogroup *Autumnalis, Bataviae, Bratislava, Canicola, Copenhagen, Cynopteri, Grippotyphosa, Sejroe, Mini, Pomona, Shermani, Tarassovi*, and *Celledoni*. Studies of risk factors were carried out according to the serovar through the calculation of X^2 and OR. Seroprevalence of *Leptospira spp.* was 34.2% with 92.3% of herds. Pomona had the highest frequency of 7.9%, followed by *Hardjo prajit* with 7.0% and *Grippotyphosa* with 6.2%. Antibody agglutination with more than one serovar was seen in 102 (29.8%) samples. The serogroup with the highest correlations were *Pomona* and *Hardjo prajit.*

Keywords: Seroprevalence. MAT. *Leptospira* spp. Bovine. Serodiagnosis.

RESUMO

A leptospirose é uma zoonose de distribuição ampla e endêmica na zona tropical. A condição epidemiologia da doença é complexa, intervém diferentes fatores do hospedeiro, tipo de sorovar, reservatórios, ambiente e as práticas agrícolas. Em bovinos a doenças causa danos significativos ao setor pecuário, e as condições ambientais podem influenciar a sua dinâmica. O objetivo deste estudo foi determinar a seroprevalência anti-*Leptospira* spp. anticorpos e os fatores de risco em bovinos na savana tropical do leste da Colômbia. Foi coletado sangue das veias coccígeas de 1.000 animais e foi utilizada a técnica MAT (Microagglutination) para a identificação do sorogrupo *Autumnalis, Bataviae, Bratislava, Canicola, Copenhagen, Cynopteri, Grippotyphosa, Sejroe, Mini, Pomona, Shermani, Tarassovi*, e *Celledoni*. Estudos de fatores de risco foram realizados de acordo com o sorovar por meio do cálculo de X^2 e OR. A seroprevalência de *Leptospira* spp foi de 34,2% com 92,3% dos rebanhos. *Pomona* teve a maior frequência de 7,9%, seguida por *Sejroe* com 7,0% e *Grippotyphosa* com 6,2%. A aglutinação de anticorpos com mais de um sorovar foi observada em 102 (29,8%) das amostras, o sorogrupo com as maiores correlações foi *Pomona* e *Harjo.*

Palavras-chave: Soroprevalência. MAT. *Leptospira* spp. Bovinos. Sorodiagnóstico.

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Introduction

Leptospirosis is a zoonotic and widely distributed reproductive disease in cattle commonly associated with abortions or subclinical presentation, which causes significant economic losses to the livestock industry (Martins & Lilenbaum, 2017). A spirochete of the genus *Leptospira* spp. causes the condition. Currently, 20 species are known, and around 300 serovars have an affinity for a specific host (Adler & de la Peña Moctezuma, 2010). Leptospirosis affects various animals and is spread through contact with infected animal urine, which acts as a reservoir for the disease, or through exposure to environments contaminated by infected animal urine (Adler & de la Peña Moctezuma, 2010). The disease has many risk factors, including rodents, water, occupational activities, poor sanitary conditions, weather, and existing transmission routes challenging control and prevention actions (Ullmann & Langoni, 2011).

Leptospirosis is a chronic and persistent infection of the reproductive tract that causes infertility, makes conception more difficult, extends the time between births, causes spontaneous abortions, and causes morbidity and mortality (Grooms, 2006). Once it is a subclinical and silent disease, it causes reproductive problems with a high economic impact. Erectile dysfunction probably occurs due to inflammation in response to colonization by *Leptospira spp.*, which alters implantation or embryonic damage (Aymée et al., 2021). The serogroup determines the disease presentation. Some studies have linked embryonic death and estrus repetition to *Leptospira* spp. of the *Sejroe* serogroup, while abortions have been linked to *L. icterohaemorrhagiae* (Oliveira et al., 2010). The disease in cattle is frequently misdiagnosed and underreported, and it is also a more neglected reproductive disease in livestock.

In the tropics, Leptospirosis is an endemic disease. The climate, temperatures, rainfall, and diversity of wild species carrying strains can favor the presence and survival of *Leptospira* spp. (Martins & Lilenbaum, 2017; Ullmann & Langoni, 2011). In Colombia, cases in people and animals have increased (Bello et al., 2013; Carreño Buitrago et al., 2017). Leptospirosis in humans is a notifiable disease in Colombia. However, it is still highly underreported, and few studies allow us to understand the behavior of the disease and its interface with the animal environment.

In Latin America, high seroprevalence of Leptospirosis in cattle rates with 75.0% at the herd level and 44.2% at the individual animal level have been reported, with a predominance of *Sejroe* serogroup strains (80.3%) (Pinto PS et al., 2016). In Colombia, reproductive diseases significantly impact the livestock sector, resulting in significant losses (Betancur & Rodas, 2008). The country's rising food demand and the rising cost of post-COVID livestock production need the development of new control and prevention strategies to mitigate economic losses. The eastern Orinoquia accounts for 21% of Colombia's cattle population, making it an essential center of livestock production for the country's economy. The objective of this study was to determine the seroprevalence of anti-*Leptospira* spp. antibodies and risk factors in the tropical savannah of eastern Colombia.

Materials and Methods

Study area and population

The research was carried out in the Villavicencio municipality of Meta, with a total area of 124,890 hectares, of which 122,421 are in the rural area. It has an intertropical savannah climate, a gallery forest, and a diverse wildlife population. The average altitude is 380 msl, the temperature is 27°C, the humidity is 80%, and the annual precipitation is between 3,500 and 4,000 mm (Instituto de Hidrología, Meteorología y Estudios Ambientales, 2014). The climatic conditions present rain patterns characterized by periods of precipitation (March-December) followed by periods of lesser rainfall (December-March). The sample size for the study was calculated using the postulates (Thrusfield, 2018), considering a prevalence of 74.5% reported in Córdoba, Colombia (Ensuncho-Hoyos et al., 2017), a confidence limit of 95%, an accepted error of 2.7%, and a bovine population of 108,109 cattle reported by the ICA (Instituto Colombiano Agropecuario, 2016). The calculations were performed using SPSS software version 20 for a sample of 1,000 cattle (SPSS Inc., Chicago, IL, USA).

Animals that had been vaccinated against Leptospirosis were not included in the study. The study was carried out in 2017 in five territorial subdivisions of the rural and 29 farms in the municipality. The UDCA 02-2017 ethics committee submitted and approved the study.

MAT test

Blood samples (5.0 mL) were collected from the coccygeal veins of each animal. The samples were placed in sterile tubes without anticoagulants (vacutainer®) and transported to the University de los Llanos Veterinary Faculty laboratory in isothermal coolers. After centrifuging the samples at 5,000 g for 10 min, the serum was extracted and stored at -70°C until analysis. The technique implemented used MAT (Microagglutination) with the 13 *Leptospira* spp. serogroup: *Autumnalis, Bataviae, Bratislava, Canicola, Copenhagen, Cynopteri, Grippotyphosa, Sejroe, Mini, Pomona, Shermani, Tarassovi*, and *Celledoni*. A titer of 1:100 or higher was considered seropositive, indicating a previous infection.

Risk analysis and statistics

The general prevalence, as well as the prevalence by sex and territorial subdivisions of the rural and farm, was calculated using the Epiinfo TM version 7 (CDC) software, with a 95% confidence interval. For epidemiologic map design, the prevalence data was entered into the software Arc Gis 10. 1 (ESRI). The non-parametric Spearman test was used to analyze correlations between serogroups identified by the MAT test, and positive results were interpreted with a $p \leq 0.05$.

To analyze risk factors, an epidemiological survey was carried out in collaboration with the official authorities of the Colombian Agricultural Institute (ICA). General information was collected from the animals, including sex, age, breed, and presence of animal species, as well as health information on productive activities, technical assistance, sanitation, and infrastructure. The epidemiological surveys were entered into databases and analyzed using descriptive statistics. Four serogroups were selected with more frequencies and identified in this study in cattle (*Sejroe*), pigs (*Pomona*), canines (*Canicola*), and rodents (*Grippotyphosa)*. They were associated with the variables studied through an analysis of risk factors. For the statistical analysis, a 2x2 table, the Chi-square (X^2) test, and Odd Ratio (OR) were used, using SPSS version 20 software (SPSS Inc) with statistical significance $p \leq 0.05$.

Results

The seroprevalence anti-*Leptospira* spp. antibodies were 34.2% (342/1000) (95% CI: 31.2 –37.2%), 92.3% of the herd, and it had at least one positive animal. The seroprevalence anti-*Leptospira* spp. antibodies according to sex were similar. In females, it was 34.0%, and for males, 35% (Table 1). The analysis according to age range shows 27.4% seropositivity was under one year, 32.4% between 1 to 3 years, 31.7% between 3 to 4 years, and 37.5% older 4-year-olds. The territorial subdivisions of the rural with the highest seroprevalence anti-*Leptospira* spp. antibodies were Amor 40.6% and Barcelona 35.3% (Figure 1). The farm with the highest seropositivity was Bariri, with 71.4%, and the property with the lowest was Marsella, with 7.7%.

Table 1 – The seroprevalence of anti-*Leptospira* spp. antibodies in cattle according to population characteristics from Villavicencio, Colombia, 2017

General variables	Total animals	Positive Animals	Prevalence (%)	IC 95%*
Sex				
Female Sex	803	273	34.0%	30.7 – 37.3%
Male sex	197	69	35%	28.0 – 42.1%
Age ranges				
Under 1 year old	226	62	27.4%	21.7 – 33.7%
Between 1 to 3 years	108	35	32.4%	23.7 – 42.0%
Between 3 to 4 year	85	27	31.7%	22.0 – 42.7%
Over 4 years old	581	218	37.5%	33.5–41.6%
Village				
Amor	197	80	40.6%	33.6 – 47.82%
Barcelona	221	78	35.3%	29.0 – 41.9%
Apiay	169	41	24.2%	18.0 – 31.4%
Bella suiza	317	112	35.3%	30.0 – 40.8%
Cocuy	96	31	32.2%	23.1 – 42.6%

*Confidence Intervale 95%.

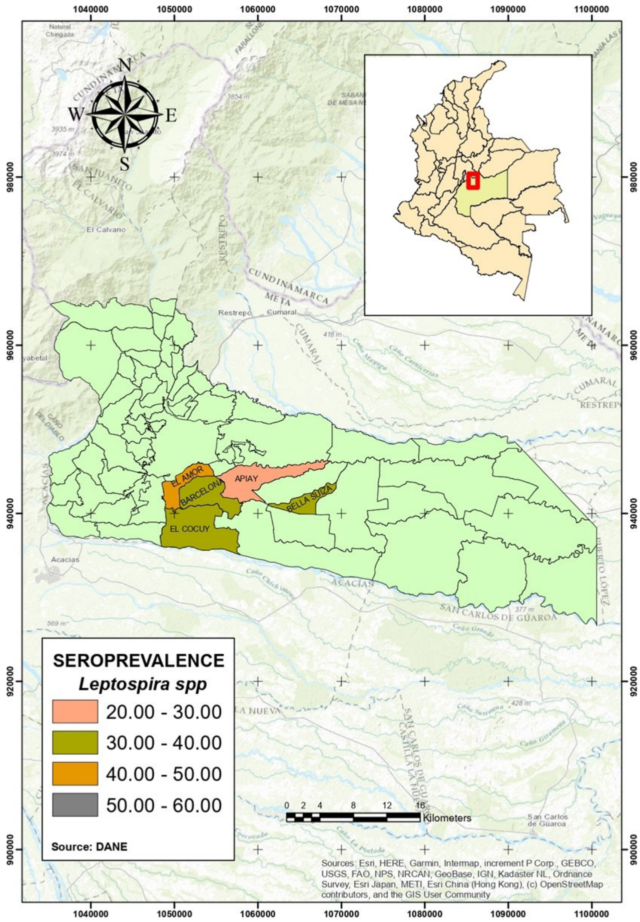

Figure 1 – Seroprevalence of antibodies to *Leptospira* spp. in cattle of the tropical savannah of eastern Colombia, 2017. The seroprevalence of *Leptospira* spp. calculated for the different territorial subdivisions of the rural area in the municipality of Villvicencio is presented on the map.

The serogroup study showed a higher frequency of *Pomona* at 7.9% (79), followed by *Hardjo prajit* with 7% (70%), *Grippotyphosa* at 6.2% (62), *Tarassovi* 5.8% (58), *Bratislava* 5.3% (53), *Canicola* 5.2% (52). In the middle of 342 positive samples, 102 (29.8%) presented antibody agglutination with more than one serogroup, 70 (20.5%) with two, 28 (8.2%) with three, 4 (1.2%) with four. The results of the correlation analysis showed several statistically significant positive correlations $p \leq 0.05$ between serogroups (Table 2). The serogroup with the highest correlations were *Pomona* with *Hardjo prajit* ($Rs = 0.135$) and *Canicola* with *Pomona* ($Rs = 0.135$), *Copenhageni* with *Bratislava* ($Rs = 0.160$) and *Hardjo prajit* with *Grippotyphosa* ($Rs = 0.163$).

The risk factors identified were similar, considering the seroprevalence of anti-*Leptospira* spp. antibodies *Canicola, Grippotyphosa,* and *Pomona***,** the presence of canines, burial of dead animals, and artificial insemination as risk factors (Table 3). Lack of rodent control was a risk factor for *Canicola* and *Grippotyphosa*. Water from wells obtention (OR= 5.26 CI: 1.259-21.867) and poor food storage (OR= 1.808 CI: 1.028-3.180) were risk factors associated with *Canicola.* Animals more than four years old (OR= 1.757 CI: 0.984-3.138) and burial of dead animals were risk factors for the presence of *Hardjo prajit,* while animals younger than one year (OR= 0.239 CI: 0.086-0.667) were a protective factor. Some risk factors of commercial and sanitation practices, such as the sale of animals (OR= 2.044 CI: 1.144-3.652), were identified for the serovar *Hardjo prajit.*

Table 2 – Correlations between serovars among MAT-positive samples in cattle by nonparametric Spearman correlation test, 2017.

	Autumnalis	***Bataviae***	***Bratislava***	***Canicola***	***Copenhageni***	***Cynopteri***	***Grippotyphosa***	***Hardjo prajit***	***Mini***	***Pomona***	***Shermani***	***Tarassovi***	***Celledoni***
Autumnalis	1												
Bataviae	-0.11	1											
Bratislava			1										
Canicola				1									
Copenhageni			0.16		1								
Cynopteri		-0.11				1							
Grippotyphosa					0.35		1						
Hardjo prajit							0.16	1					
Mini			-0.12						1				
Pomona				0.13				0.10		1			
Shermani											1		
Tarassovi									-0.10			1	
Celledoni													1

The result of the coefficients with significance ($p \leq 0.05$).

Table 3 – Analysis of risk factors associated with the seroprevalence of the four *Leptospira* spp. serovars in cattle, 2017

***Leptospira* spp Serovar**	**Risk Factor**	**X²**	**P**	**Odd Ratio**	**Confidence Interval**
Canine host	Presence of canines	7.922	0.005	3.955	1.411-11.085
Canicola	Burial of dead animals	7.886	0.005	6.021	1.452-24.971
	Lack of rodent control	6.643	0.010	2.777	1.238-6.233
	Obtaining water from wells	6.515	0.011	5.268	1.259-21.867
	Poor food storage	4.339	0.037	1.808	1.028-3.180
	Artificial insemination	7.432	0.006	2.183	1.230-3.872
Rodent host	Presence of canines	4.395	0.036	2.205	1.034-4.703
Grippotyphosa	Burial of dead animals	3.476	0.062	2.216	0.940-5.224
	Lack of rodent control	4.263	0.039	1.993	1.024-3.881
	Artificial insemination	3.585	0.058	1.640	0.978-2.750
Pig host	Older 4-year-old	5.337	0.021	1.796	1.087-2.969
Pomona	Lack of pens on the property	3.854	0.050	1.090	1.069-1.111
	Presence of canines	4.693	0.030	2.034	1.057-3.913
	Burial of dead animals	4.068	0.044	2.126	1.005-4.497
	Artificial insemination	5.369	0.021	1.719	1.082-2.731
Cattle host	Under 1 years	8.763	0.003	0.239	0.086-0.667
Hardjor	Older 4-year-old	3.709	0.054	1.757	0.984-3.138
	Sale of animals	6.037	0.014	2.044	1.144-3.652
	Burial of dead animals	4.050	0.044	2.521	0.994-6.397

Only risk factors with statistical significance are presented $p \leq 0.05$, $p \leq 0.10$.

Discussion

The present study identified a seroprevalence anti-*Leptospira* spp. antibodies in cattle of 34.2% in the tropical savannah of eastern Colombia, where there is a high biodiversity of fauna and wildlife. In the eastern Andean region of Colombia, a higher seroprevalence was identified at 60.9% (Ochoa et al., 2000). However, in the central Andean region, a seroprevalence of 39.9% was identified (Taddei et al., 2021), showing variability within the same area. In rural Ciénaga de Oro, Córdoba, 325 bovines of reproductive age were studied, finding high seroprevalence rates of 74.5% (Ensuncho-Hoyos et al., 2017). Slaughterhouse animals were analyzed on the island of San Cristóbal in the Caribbean, identifying a seroprevalence of *Leptospira* spp. 79.8% (Shiokawa et al., 2019). In dairy farms on the coast and mountains of Peru with drier climates, a low prevalence between 14.8% and 12.3% is identified, showing a difference between territories in the tropics (Llanco et al., 2017). In the Brazilian Amazon, a 46.6% seroprevalence of *Leptospira* spp. was identified (Guedes et al., 2019), while other regions of the country have shown low prevalence, as in the state of Santa Catarina, 6.44% (Fávero et al., 2017).

In other tropical savannah countries of the eastern hemisphere, such as Madagascar, a seroprevalence of *Leptospira* spp. of 59.3% higher than that identified in this study has been reported (Schafbauer et al., 2019). Three hundred seventy-three animals with reproductive problems were analyzed in India, reporting a *Leptospira spp.* prevalence of 70.51% (Balamurugan et al., 2018). Low prevalence rates of Leptospira spp. in countries outside the tropics are reported. In Canada, a prevalence of 0.8% was identified (Van De Weyer et al., 2011). In Bosnia, 1,197 cattle were analyzed, and 1.59% were positive (Rifatbegović & Maksimovic, 2011). In New Zealand, a prevalence between 10.5%-16.7% was identified for serovars such as *Ballum, Copenhageni,* and *Tarassovi*, with low prevalence rates, though that deserves attention (Wilson et al., 2021).

In Latin America, bovine Leptospirosis is associated with the *Sejroe* serogroup, particularly *Hardjo* serovar (Pinto PS et al., 2016). In our study, the most frequent *Leptospira* spp. serogroup identified was *Pomona,* followed by *Sejroe*. However, other serogroups were also presented in a high frequency, such as *Grippotyphosa, Tarassovi*, *Bratislava, and Canicola,* affecting other species, suggesting possible transmission by wildlife and domestic animals. The results found many agglutinations and evident correlations that identified the diversity of serogroup of *Leptospira* spp. that may occur in cattle in a limited area. Other studies identified similar results on the island of San Cristóbal. Serovars other than *Sejroe* were also identified in slaughterhouse cattle, such as *Mankarso, Djasiman, Autumnalis, Ballum, and Bratislava* (Shiokawa et al., 2019).

Results of *Leptospira* spp. isolations in urine and vaginal fluids of animals without symptoms in a slaughterhouse in Brazil also show the presence of other strains, different from *Harjod*, suggesting that they can be maintained and excreted (Pinto et al., 2017). It is essential to consider that Leptospirosis caused by serogroup *Sejroe* can be associated with commonly adapted infections with subclinical presentation and minor pathological damage. However, in the disease caused by strains of wild or other domestic animals, it causes an accidental infection resulting in acute and severe systemic forms, often associated with climatic conditions and poor sanitary measures (Loureiro & Lilenbaum, 2020), could be found in the herds studied the two types of clinical presentation.

The high diversity of serogroup may be related to the biodiversity of fauna in this region. In Kenya, the transmission potential of *Leptospira* spp. associated with the presence of wildlife was identified, compared to other pathogens such as *Brucella* spp. and Bovine Viral Diarrhea Virus (BVDV) (Rajeev et al., 2017). Three serogroups were identified in bovines associated with pigs, such as *Pomona, Tarassovi, and Bratislava*. The study by Shiokawa et al. (2019) evidences the correlation between bovine and pig serovars. This may be associated with mixed productions, low-technification of livestock, and the backyard culture of these animals in Colombian homes. The serogroup of *Canicola* and *Grippotyphosa* present in canines and rodents highlights these species' role in transmitting the disease in livestock production. In Colombia, the existence of *Leptospira* spp. has been identified in bats with the presence of different serovars (Monroy et al., 2021), in pig production water (Giraldo de León et al., 2002), and the relationship of Leptospirosis in humans with the climate has been evidenced (Builes-Jaramillo & Arias-Monsalve, 2019). In tropical areas, the disease dynamics are complex, and disease control is challenging for livestock, including the interface between livestock, the environment, and wildlife.

The water obtained from a well was a risk for *Canicola*. Therefore, in Brazil, the relationship of *Leptospira* spp. to water from rivers was identified (Fávero et al., 2017), and in Jordan (Ismail et al., 2019), the water source as a risk factor must be analyzed according to water contamination, water uses, water treatment, flow, among others. Poor food storage was also a risk factor for this serogroup. In Brazil, inadequate food storage conditions with rodent access are also identified as a risk (Fávero et al., 2017). Rodents were a risk factor for four serogroups in the study. In Nepal, the relationship between rodents and the disease is reported (Gompo et al., 2020); in Brazil (Fávero et al., 2017). It is a persistent problem in livestock production and significantly impacts leptospirosis. Burial practices such as the final disposal of dead animals were identified as a risk for several serogroups. In northeast Brazil, it is reported that burying or burning abortion products is a risk factor necessary to implement better sanitary measures (Oliveira et al., 2010). The study analyzed the association of the disease with different species of animals. However, only association with canines was identified. Further studies identified an association with other domestic species, sheep, goats, and cats (Schoonman & Swai, 2010). In Nepal, *Leptospira* spp. is associated with the presence of canines (Gompo et al., 2020), an essential reservoir of the disease, especially when there are no entry controls for this species. The study suggests that the serogroup *Canicola* and *Grippotyphosa* correlated more with environmental factors. However, more studies are required.

The analysis evidenced that age was a risk factor for *Hardjo prajit.* Adult animals were more likely to be positive. Similar results are reported in Brazil (Fávero et al., 2017) and in Ireland, the prevalence of seroprevalence of *Leptospira* spp. increased with age (Ryan et al., 2012). This may be associated with increased exposure over time and weak immune states. Artificial insemination was a risk factor in this study. No association was identified in northeast Brazil, where this factor was addressed. However, studies report the transmission of *Leptospira* spp. through semen and reproductive problems of this etiological agent (Givens, 2018). Some commercial and sanitary practices were identified as risk factors in serogroups *Pomona* and *Hardjo prajit*, even though they were statistically weak.

Identifying serovars allows us to guide the vaccine production since vaccination against *Leptospira* spp. creates specific immunity for serovar, and also considers that vaccines formulated with international reference strains may not be adequate for protection against infections determined by local strains (Martins & Lilenbaum, 2017).

In Colombia, Leptospirosis in humans is one of the primary zoonotic diseases and is an endemic disease. A study review reports a prevalence between 6% and 35% for humans (Carreño Buitrago et al., 2017). Human studies have reported mainly serovars *Icterohaemorrhagiae, Grippotyphosa,* and *Canicola* (Carreño Buitrago et al., 2017) and *Bratislava, Hebdomadis, Sejroe,* and *Icterohaemorrhagiae* (Bello et al., 2013), some of these were found in the cattle which tested positive for leptospirosis in this study. Therefore, livestock can be a crucial sector for preventing Leptospirosis in humans.

Conclusion

The present study identifies the serogroup circulating in the tropical savannah of eastern Colombia and the risk factors associated with specific serogroup. The seroprevalence of *Leptospira* spp. was 34.2% with 92.3% of herds. The serogroup with the highest frequency was *Pomona* at 7.9%, *Hardjo prajit* at 7.0%, and *Grippotyphosa* at 6.2%. Animals under one year, older 4-year-olds, presence of canines, burial of dead animals, lack of rodent control, obtaining water from wells, poor food storage, artificial insemination, lack of pens on the property, and sales of animals were risk factors.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Statement

The Faculty of Agricultural Sciences of the UDCA Ethics Committee approved the study. N. 001–2017. On the properties, informed consent was obtained from the owners.

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