frontiers | Frontiers in Tropical Diseases

TYPE Brief Research Report
PUBLISHED 09 May 2023
DOI 10.3389/fitd.2023.1061038

OPEN ACCESS

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RECEIVED 04 October 2022
ACCEPTED 17 April 2023
PUBLISHED 09 May 2023

CITATION
Orlando SA, Paez Martinez K, Sanchez E, de la Cruz C, Calderon J, Arcos F, Torres-Lasso P, Calvopiña M and Garcia-Bereguiain MA (2023) Racehorses from a breeding farm in Tropical Ecuador have a high seroprevalence of anti-*Leptospira* spp. antibodies: a paradigm for leptospirosis management from a One Health perspective.
Front. Trop. Dis 4:1061038.
doi: 10.3389/fitd.2023.1061038

Racehorses from a breeding farm in Tropical Ecuador have a high seroprevalence of anti-*Leptospira* spp. antibodies: a paradigm for leptospirosis management from a One Health perspective

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Background: Leptospirosis is a zoonotic disease of worldwide distribution that affects humans and domestic and wild animals, and it is highly endemic in Ecuador. However, no reports of infections affecting horses have been published in the country.

Methods: This study evaluates the prevalence of anti-*Leptospira* spp. antibodies in racing horses from a breeding farm in the coastal Santa Elena province, southwest Ecuador. Sera were collected from 108 non-vaccinated horses and evaluated for 24 serovars of *Leptospira* spp. using the microscopic agglutination test (MAT).

Results: It was found that 100% of horses were reactive for *Leptospira* spp., most of them for multiple serovars. The most prevalent serovars were *Leptospira kirschneri* serovar Grippotyphosa (100%; 95% CI 99.9% to 100.1%); *L. interrogans* serovars Sejroe (96.3%; 95% CI 96.2% to 96.4%), Saxkoebing (95.4%; 95% CI 95.3% to 95.5%), Canicola (90.7%; 95% CI 90.5% to 90.9%), Icterohaemorrhagiae (80.5%; 95% CI 80.4% to 80.6%), Bataviae (73.1%; 95% CI 73.0% to 73.2%), Australis (75.0%; 95% CI 74.9% to 75.1%), and Bratislava (71.2%; 95% CI 71.1% to 71.4%); and *L. borgpetersenii* serovar Tarassovi (76.8%; 95% CI 76.6% to 77.0%).

Conclusions: We found a high prevalence of anti-*Leptospira* spp. seropositivity in racehorses from a breeding farm in Ecuador. This is the first serologic report for leptospirosis in horses in Ecuador. "One Health"-based sanitary practices for horse-breeding farms are recommended to improve animal and human health.

KEYWORDS

Leptospira, MAT, leptospirosis, horses, Ecuador, One Health

Introduction

Leptospirosis is a zoonotic disease with worldwide distribution although its prevalence is higher in the tropics and poorer regions, and it is endemic in South America (1, 2). Bacteria from the genus *Leptospira* are the causative agent and can infect almost all mammal species (3). Leptospirosis affects domestic and wild mammals, and cross-species transmission, including to humans, occurs through direct contact with urine from infected animals or indirect contact with contaminated soil and water where *Leptospira* spp. can survive for long periods (3, 4). Free-roaming dogs and rats are considered the main reservoir of the disease in urban areas (5), while livestock plays an important role in occupational leptospirosis transmission (6). Leptospirosis is estimated to cause 1.03 million human cases and 58,900 deaths each year worldwide. Although infected individuals can be asymptomatic, severe disease may produce renal or hepatic failure and pulmonary bleeding that can lead to death (7, 8).

Leptospira spp. that infects human and animal populations include pathogenic, intermediate pathogenic, and saprophytic clusters, defined further by the presence of serological characteristics, or serovars. The pathogenic, or interrogans, cluster comprises 16 strains across nine species: *L. interrogans*, *L. kirschneri*, *L. noguchii*, *L. borgpetersenii*, *L. weilii*, *L. santarosai*, *L. alexanderi*, *L. kmetyi*, and *L. alstonii*. The intermediate cluster comprises *L. fainei*, *L. licerasiae*, and *L. wolffii*, which have been associated with mild disease and chronic infections. The saprophytic, or biflexa, cluster comprises 14 non-pathogenic strains, of the species *L. biflexa* and *L. wolbachii*.

Although leptospirosis is mainly subclinical in horses, it can lead to abortion, stillbirth, and neonatal mortality (9, 10). Clinical signs of leptospirosis in horses include moderate fever, anorexia, jaundice, and pulmonary bleeding; death by interstitial nephritis has also been described as indicative of leptospirosis (10–13). Leptospirosis causes economic losses in the racehorse business due to the interruption of training, poor performance, and disqualification in competition, as well as the cost of treatment for sick horses (11). In addition, leptospirosis in racehorses is a threat for zoonotic transmission due to the closeness of horse–human contact. In addition, as racehorses are among the most expensive domestic animals, leptospirosis in racehorses could be considered as a paradigm for evaluating leptospirosis management and concern in a particular region.

Leptospirosis is a neglected tropical disease in Ecuador, with 1,279 human cases reported in 2012 (14–16). A total of 2,584 hospitalizations were recorded from 2000 to 2022 across 22 provinces in Ecuador (17). The few studies addressing leptospirosis in livestock in Ecuador report a high prevalence in cattle, pigs, and dogs (18, 19). Moreover, leptospirosis has never been studied in horses in Ecuador although it is considered endemic in South America, with prevalence values ranging from 4.5% to 90.7% (6). A recent publication from 2019 found a prevalence of 85% using a 24-serovar microscopic agglutination test (MAT) panel on horses from police departments in Colombia (20). There are no public health policies with a "One Health" perspective to address leptospirosis in Ecuador, and even livestock vaccination is scarce. Considering this situation, the aim of this study was to evaluate the seroprevalence of anti-*Leptospira* spp. antibodies in an exploited animal of high economic value, namely, horses from racehorse farms in the coastal region of Ecuador, where leptospirosis is endemic.

Methods

Study design and setting

This study was performed in a racehorse-breeding farm located in the province of Santa Elena, in the southwest coastal region of Ecuador. This is, to our knowledge, the only farm of this kind in the coastal region of Ecuador. The ecological features of this area allow exposure to *Leptospira* spp. through direct contact between horses and free-roaming dogs and cats and wild rodents, or through contaminated water sources.

For the present study, samples were collected from December 2016 to February 2017. None of the animals were vaccinated against leptospirosis. In addition, no signs of leptospirosis were found in any of the horses at the time of sample collection (we were allowed only one quick and superficial animal inspection). The farm veterinarians did not report any horses with signs of leptospirosis during the sample collection period. As this study uses samples collected from domestic animals for diagnosis, in accordance with animal research regulations in Ecuador, IRB approval was waived.

Abbreviations: MAT, microscopic agglutination test.

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Blood sample collection

Horses were managed by certified veterinarians. Blood was collected from the jugular vein. The serum was separated by centrifugation (5,000 rpm for 5 min). A total of 108 samples of sera were collected from all horses older than 1 year present on the farm at the time of the study (Supplementary Material 1).

Microscopic agglutination test for anti-Leptospira spp.

The microscopic agglutination test (MAT) was performed using 24 live antigens. The *Leptospira* species, serogroups, serovars, and strains used for MAT are detailed in Supplementary Table 1. MAT was performed in the Laboratorio Nacional de Referencia para Zoonosis of the Instituto Nacional de Salud Pública e Investigación in Guayaquil. This laboratory focuses on human sample analysis and uses a MAT panel implemented following Pan American Health Organization guidelines.

The antigens were prepared from the reference strains detailed in Supplementary Table 1. For the screening of sera, a 1:200 dilution was used initially. Reactive samples were then examined with increasing dilutions from 1:200 to 1:3,200, taking the highest positive dilution to be the titer of the serum. The serum was considered reactive when at least 50% agglutination occurred at a magnification of 40× under the microscope.

Results

All the 108 horses tested were seropositive for 5–15 different *Leptospira* spp. serovars when a MAT titer cut-off value of 200 was set, with titers ranging from 200 to 1,600 (Tables 1, 2). The most prevalent serovars were *Leptospira kirschneri* serovar Grippotyphosa (100%; 95% CI 99.9% to 100.1%); *Leptospira interrogans* serovars Sejroe (96.3%; 95% CI 96.2% to 96.4%), Saxkoebing (95.4%; 95% CI 95.3% to 95.5%), Canicola (90.7%; 95% CI 90.5% to 90.9%), Icterohaemorrhagiae (80.5%; 95% CI 80.4% to 80.6%), Bataviae (73.1%; 95% CI 73.0% to 73.2%), Australis (75.0%; 95% CI 74.9% to 75.1%), and Bratislava (71.2%; 95% CI 71.1% to 71.4%); and *Leptospira borgpetersenii* serovar Tarassovi (76.8%; 95% CI 76.6% to 77.0%). Serogroup and serovar distribution and titers for all horses included in the study are detailed in Supplementary Table 2. No differences in serovar prevalence were found between horses of different ages. As no horses presented signs of leptospirosis at the time that we visited the farm, no association between serovars and signs could be addressed.

We also addressed MAT seropositivity for the 108 horses using a MAT titer cut-off value of 800, as detailed in Table 3 and Supplementary Table 3. In this case, 55 out of 108 horses were positive for at least 1 of 11 different *Leptospira* spp. serovars, and the prevalence obtained was 50.9%. For this cut-off value of 800, the most prevalent serovars were *Leptospira kirschneri* serovar Grippotyphosa (12/108; 11.1%; 95% CI 11.0% to 11.2%), and *Leptospira interrogans* serovars Bataviae (22/108; 22.4%; 95% CI 21.9% to 22.1%) and Canicola (20/108; 18.5%; 95% CI 18.3% to 18.7%).

TABLE 1 Distribution of *Leptospira spp.* serogroups, serovars and strains in the 108 horses included in this study for microaglutination test (MAT) with a cut off titer value of 200.

N°	Serogroup	Serovar	Number of Horses (%; IC 95%)	TiTER RANGE
1	Bataviae	Bataviae	79 (73,1%; 72,94-73,26)	1/200 - 1/800
2	Cynopteri	Cynopteri	48 (44,4%; 44,24-44,56)	1/200
3	Tarassovi	Tarassovi	83(76,9%; 76,74-77,06)	1/200 - 1/800
4	Sejroe	Sejroe	104(96,3%; 96,14-96,46)	1/200 - 1/800
5	Sejroe	Saxkoebing	103(95,4%; 95,24-95,56)	1/200 - 1/800
6	Sejroe	Hardjo	26(24,1%; 23,94-24,26)	1/200
7	Sejroe	Wolffi	23(21,3%; 21,14-21,46)	1/200
8	Pomona	Pomona	64(69,3%; 69,14-69,46)	1/200 - 1/400
9	Canicola	Canicola	98(90,7%; 90,54-90,86)	1/200 - 1/1600
10	Celledoni	Celledoni	7 (6,5%; 6,34-6,66)	1/200
11	Grippotyphosa	Grippotyphosa	108 (100,0%; 99,84-100,16)	1/200 - 1/800
12	Pyrogenes	Pyrogenes	10 (9,3%; 9,14-9,46)	1/200
13	Australis	Bratislava	77(71,3%; 71,14-71,46)	1/200 - 1/1600
14	Australis	Australis	81(75,0%; 74,84-75,16)	1/200 - 1/400
15	Icterohaemorrhagiae	Copenhageni	64(59,3%; 59,14-59,46)	1/200 - 1/800
16	Icterohaemorrhagiae	Icterohaemorrhagiae	87(80,6%; 80,44-80,76)	1/200 - 1/800
17	Djasiman	Djasiman	8(7,4%; 7,24-7,56)	1/200 - 1/800

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TABLE 2 Number and percentage of seropositive horses for multiple *Leptospira* spp. serovars.

	Number of serovars										
	5	6	7	8	9	10	11	12	13	14	15
Number of horses (%)	1 (0.9)	4 (3.7)	16 (14.8)	13 (12)	17 (15.7)	11 (10.2)	6 (5.5)	17 (15.7)	14 (13.0)	8 (7.4)	1 (0.9)

TABLE 3 Distribution of *Leptospira spp*. serogroups, serovars and strains in the 108 horses included in this study for microaglutination test (MAT) with a cut off titer value of 800.

N°	SEROGROUP	SEROVAR	NUMBER OF HORSES (%; IC 95%)	TITER RANGE
1	Bataviae	Bataviae	22(27.5%; 27,34-27,66)	1/800
2	Tarassovi	Tarassovi	2 (2,5%; 2,34-2,66)	1/800
3	Sejroe	Sejroe	9(11,25%; 11,09-11,41)	1/800
4	Sejroe	Saxkoebing	2 (2,5%; 2,34-2,66)	1/800
5	Canicola	Canicola	20(25%; 24,84-25,16)	1/800 - 1/1600
6	Grippotyphosa	Grippotyphosa	12(15%; 14,84-15,16)	1/800
7	Australis	Bratislava	8(10%; 9,84-10,16)	1/800 - 1/1600
8	Icterohaemorrhagiae	Copenhageni	2(2,5%; 2.34-2,66)	1/800
9	Icterohaemorrhagiae	Icterohaemorrhagiae	2(2,5%; 2.34-2.66)	1/800
10	Djasiman	Djasiman	1(1,25%; 1.09-1.41)	1/800

Discussion

This study reports on the striking case of a racehorse-breeding farm in Ecuador, where 100% of the horses were seropositive for *Leptospira* spp. at a MAT cut-off titer value of 200. Furthermore, most horses were positive for multiple *Leptospira* spp. serovars. Cross-reactivity between different *Leptospira* spp. serovars has been described for the MAT technique; therefore, we also addressed anti-*Leptospira* spp. antibody seroprevalence using a MAT titer cut-off value of 800 (21). Although there was a remarkable reduction in seropositivity and serovar distribution (some serovars were not present at the 800 titer cut-off value), we cannot totally rule out the possibility that positive MAT results for titers ranging from 200 to 800 are due to cross-reactivity. Moreover, anti-*Leptospira* spp. antibody seroprevalence was over 50% even for a high specific cut-off titer value of 800. In addition, for a titer cut-off value of 800, the main infective serovars were Bataviae (20.4%) and Canicola (18.5%), which belong to the pathogenic cluster. We found six horses that were positive for both serovars, belonging to different serogroups, so either coinfection with multiple serovars or successive infections could have occurred.

We could not find any differences associated with age for *Leptospira* spp. serovar distribution. The differences in serovars between horses could be partially explained by the different origins of the horses (some of them were brought from neighboring countries such as Peru, Chile, and Argentina) or frequent travel to competitions. However, this information was not provided in detail by the farm and could not be evaluated. Another limitation of our study was the scarce information regarding signs of leptospirosis in the horses, as we were allowed only one quick evaluation of the horses prior to sample collection; furthermore, no clinical records were provided by the farm, and the veterinarians did not recall any horses with signs of leptospirosis. Therefore, no associations between signs of leptospirosis and specific serovars could be addressed.

The results of our study coincide with previous reports showing that horse leptospirosis is endemic in South America, with prevalence values ranging from 4.5% to 90.7% (6). In addition, a recent publication, from 2019, found a prevalence of 85% using a 24-serovar MAT panel on horses from police departments in Colombia (20). However, it is important to note that the most prevalent serovars in our study (Grippotyphosa, Bataviae, Sejroe, Canicola, Bratislava, and Tarassovi) differed from the most prevalent ones reported in South America (Icterohaemorrhagiae, Australis, and Pomona) (6) or in Colombia in particular (Djasiman) (20). These differences may be explained by environmental differences, such as the amount of rain or the duration of the rainy season, or by the presence of different transmission vectors depending on the area of study. Notably, the *Leptospira* spp. serovar MAT panels used by different countries in South America may differ, which could be an additional

methodological reason for the difference in serovars reported in this study (22). Moreover, a recent study from New Zealand of 499 racing horses from 25 different breading farms found a *Leptospira* spp. seroprevalence of 25%, despite only five serovars being tested (23). These results indicate that leptospirosis is not just a problem for horse health in low- and middle-income countries.

The ultrahigh seroprevalence of anti-*Leptospira* spp. antibodies reported in our study coincide with values reported in previous publications regarding leptospirosis prevalence in livestock and domestic animals in Ecuador: 70% in dogs, 35.4%–74% in cattle, and 67% in pigs (16, 18, 19). Although those studies reported a high prevalence of leptospirosis, they were performed using PCR or a MAT panel comprising only eight serovars for diagnosis; these methodological differences could explain the higher prevalence found in our study. Moreover, a recent report from our laboratory using a MAT panel of 24 serovars for diagnosis also found 100% seroprevalence for *Leptospira* spp. in domestic and wild animals from a mixed-use rescue center in the coastal region of Ecuador (22). Although this is the first report of leptospirosis in horses from Ecuador, the *Leptospira* spp. serovars reported in those studies were also found in our study, suggesting the widespread distribution of serovars among livestock.

These horses were not vaccinated against *Leptospira* spp., despite the high economic value of this kind of horses in the market (some of the most prevalent serovars are included in the vaccine formulation, such as Grippotyphosa and Canicola). The farm lacked proper sanitary conditions, and free-roaming dogs, cats, and wild rodents were reported by the farm workers. Moreover, the farm is located in the coastal region of Ecuador, where seasonal floods are frequent and could facilitate leptospirosis transmission (18).

Future directions of our research will include a deeper "One Health" approach, identifying the *Leptospira* spp. serovars associated with free-roaming dogs, cats, and rodents found in farms, and also with water sources, to identify a potential transmission route. Finally, it is also necessary to increase awareness among the public health authorities of the risk of environmental exposure to *Leptospira* spp. in farm workers and other high-risk groups in Ecuador, in order to develop guidelines for leptospirosis surveillance and prevention according to the "One Health" concept.

Conclusions

Although the main limitation of our study was that only a single horse-breeding farm was included, a "One Health"-based management approach to horse breeding must be improved in an epidemiological context such as that in Ecuador, where anti-*Leptospira* spp. antibody seroprevalence in livestock and companion animals is extremely high, especially considering the close human–horse contact that racehorse breeding and training implies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Universidad de Las Americas. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

SO contributed to study conceptualization, logistics and funds allocation, data collection, experimental analysis, drafting the first manuscript, and reviewing the final version. KP contributed to data collection, experimental analysis, and reviewing the final version. ES contributed to data collection, experimental analysis, drafting of the first manuscript, and reviewing the final version. CC contributed to data collection, experimental analysis, and reviewing the final version. FA contributed to logistics and funds allocation, data collection, and reviewing the final version. PT-L contributed to logistics and funds allocation, data collection, and reviewing the final version. MG-B contributed to study conceptualization, logistics and funds allocation, publication funds allocation, data collection, experimental analysis, and drafting of the first and final version of the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgments

The authors of this work thank the staff from the horse-breeding farm for their support in carrying out this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fitd.2023.1061038/full#supplementary-material

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