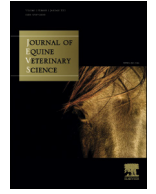




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Original Research

Seroprevalence of *Leptospira* spp. in Working Horses Located in the Central Region of Chile

Tamara A. Tadich*, Constanza Tapia, Daniela González

Departamento de Fomento de la Producción Animal, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile

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ABSTRACT

Urban working horses live in close contact with their owners. They are usually kept in periurban areas of big cities and cohabit with other animals under precarious sanitary conditions, whereas army horses are kept under controlled management and work. These characteristics leave urban working horses in higher risk of exposure to *Leptospira* spp. and could become a zoonotic risk for their owners. The aim of this study was to determine the frequency of seropositive working horses to diverse serovars of *Leptospira* spp. and compare them to a group of army horses. The microscopic agglutination test was used to assess the serum of 426 horses (160 working horses and 266 army horses) against two serovars of *Leptospira borgpetersenii* (Hardjo and Ballum) and four of *Leptospira interrogans* (Pomona, Canicola, Icterohaemorrhagiae, and Autumnalis). In the urban working horses group, 30.63% of horses were positive to at least one serovar at titers above 1:100, whereas 23.31% of the army horses were positive. The most frequent serovar in the working horse group was Ballum followed by Canicola, whereas in the army group was Autumnalis followed by Ballum. The serovars Hardjo, Pomona, and Icterohaemorrhagiae were not present in the army horses, whereas all serovars studied were detected in urban working horses. Although no horses studied presented clinical signs of leptospirosis, the study confirms exposure to *Leptospira* spp. and the importance of studying in more detail the livelihood conditions in which working horses are kept and possible risk of transmission to their owners.

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1. Introduction

Horses present a limited number of diseases that can be transmitted to humans; however, the bond established between horses and their owners allows a closer contact with them than with other large animals. This closer bond can increase the exposure rate to *Leptospira* spp., for example, leptospirosis is a worldwide zoonosis caused by the infection of *Leptospira interrogans* serovars [1]. The pathogenic serovars can be transmitted through infected urine, contaminated soil or water, and other bodily

fluids [2], affecting humans, domestic animals, and wildlife. Although leptospirosis is a systemic disease more common in humans and domestic animals, mainly dogs, cattle, and swine [3], it can also occur in horses.

Most horses' present unremarkable signs and can go by unnoticed, although the prevalence of the infection can be greater than for other species. Horses with clinical disease can present uveitis [4], abortions, and other reproductive problems. The subclinical forms are perhaps more common with chronically infected animals that can be carriers for years to life, becoming a public concern [5]. Humans, on the other hand, are not host adapted to any particular serovar and are accidental hosts that acquire the bacteria by contact with infected environmental sources (water or soil), contact with infected wildlife or pets, or occupational exposure [2].

* Corresponding author at: Tamara A. Tadich, Departamento de Fomento de la Producción Animal, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santa Rosa 11735, La Pintana, Santiago, Chile.

E-mail address: tamaratadich@u.uchile.cl (T.A. Tadich).

In developing countries, many families still depend on animal traction, particularly on equids, for the generation of income, either at small-scale farms or in entrepreneurial businesses in urban areas [6]. Most urban working horses in Chile live in the urban and periurban areas of the main cities (slums) where access to basic services is limited for them and their owners and are many times surrounded by rubbish dumps [6]. This results in the creation of an environment where horses, owners, dogs, rodents, and many times pigs and cattle live in close contact, sharing resources such as water and space, and with minimum hygiene standards, perfect for the maintenance of *Leptospira* spp.

During the year 2011, three cases of leptospirosis in horses were notified in Chile through the Agricultural and Animal Service [7]; for the same period, four human cases were reported at national level [8]. Although these figures are low, they are probably underestimated due to lack of recognition of the disease in its subclinical form, especially for horses where the economic impact could be difficult to establish.

In Chile, several studies have been conducted in relation to leptospirosis in cattle [9], rodents [10], pets [11,12], and humans [13], but little research has been done with horses [14]. Because of the close contact that urban working horses have with their owners and the environment in which they are managed, they have an increased rate of exposure compared with other groups of equines. This is why the aim of this study was to estimate the seroprevalence of several *Leptospira* spp. in a group of urban working horses and compare it to the seroprevalence in working horses kept in a controlled system (army horses).

2. Material and Methods

2.1. Animals

A total of 426 horses were assessed, corresponding to two groups depending on their working function. The first group corresponds to 160 urban working horses from the Metropolitan and Maule regions, all working pulling carts, between aged 1.5 and 20 years, mares, geldings, and stallions. All working horses are only housed during night, usually within the same owners house, share space with dogs, pigs, and cattle, have no rodents control system, and are fed with hay and vegetables residues from markets; water source is not drinkable and is usually mixed with sewerage water.

The second group corresponds to 266 working horses from the army that are kept in the same stud farm in Valparaíso region. Horses are all under the same housing, feeding and husbandry conditions, and with a fixed sanitary program that includes influenza vaccination and internal parasite control.

2.2. Sampling

Blood samples were obtained by jugular venipuncture by a veterinarian, while horses were held by their owner or keeper. Later on serum was obtained by centrifugation at 3,000 rpm for 10 minutes and stored at -20°C for posterior serology.

2.3. Serology

The research of *Leptospira* spp. antibodies was performed by microscopic agglutination test (MAT) at the Biochemistry and Microbiology Institute of the Science Faculty at the Universidad Austral de Chile. Samples were tested against four live serovars of *L. interrogans* (Pomona, Canicola, Icterohaemorrhagiae, and Autumnalis) and two live serovars of *Leptospira borgpetersenii* (Hardjo and Ballum), according to previous reports and other species cohabiting with horses. Serum samples were initially diluted at 1:100; serums that presented over 50% agglutination against serovars tested were considered as positives. In a second phase, positive serums were continuously diluted until a dilution of 1:1,600.

2.4. Statistical Analysis

Descriptive statistics were applied to establish proportions, distributions, and prevalence. Chi square test was applied to establish an association with the working activity of the horse (cart pulling or army horse).

2.5. Ethics Approval

The study was certified by the animal care and use committee from the Faculty of Veterinary Sciences and Animal husbandry from the Universidad de Chile. All owners signed an informed consent to take the blood samples.

3. Results

A total of 426 horses were evaluated to determine the seroprevalence of *Leptospira* spp. From these, 160 were urban draught horses and 266 were horses that belong to the army.

From the working horses group, 30.63% were positive to at least one of the serovars studied with 6.88% being positive to two of the six serovars studied, whereas for the army group, 23.31% of horses were positive, with only 0.38% being positive to two serovars (Table 1). According to the chi square test, there was a tendency for an association between the urban work activity and being seropositive to *Leptospira* spp. ($\chi^2 = 2.78$; $P = .096$).

The most frequent serovar in the urban working horse group was Ballum followed by Canicola, whereas in the army group was Autumnalis followed by Ballum. The serovars Hardjo, Pomona, and Icterohaemorrhagiae were

Table 1

Number and percentage of horses seropositive to one or two serovars of *Leptospira* spp. according to activity performed.

Horses positive to:	Working Horses (n = 160)		Army Horse (n = 266)	
	n	%	n	%
1 serovar	38	23.75	61	22.93
2 serovars	11	6.88	1	0.38
Positive horses	49	30.63	62	23.31

not present in the army horses group, whereas all serovars studied were present in the working horses group (Table 2).

Lower titrations were the most frequent ones in both groups (1:100 and 1:200), whereas higher titrations ($\geq 1:400$) were more common in urban working horses than in the army group (Table 3).

4. Discussion

In humans, leptospirosis is usually acquired from an animal source [3], and in the case of urban environments, the major potential reservoir has frequently been limited to rats and dogs [15], but livelihood involved with the use of working horses should also be explored as a potential risk for leptospirosis.

Working horses do not only present themselves as an occupational risk for their owners [3,16], but also are associated with conditions of slum living, which has also been pointed out as a risk [16], and coexistence with other hosts of *Leptospira* spp. including rodents, dogs, swine, and cattle.

In the present study, 26.1% ($n = 426$) of the horses were positive to serovars of *L. interrogans* and *L. borgpetersenii*, at titers of 1:100 or more, using the microscopic agglutination test (MAT) (Tables 1 and 3). The MAT is the most widely used test, being specific for serovars, although it cannot discriminate antibodies resulting from infection from those resulting from vaccination [3]. Because horses in Chile are not vaccinated against *Leptospira* spp., the presence of the antibodies can only be explained by occurrence of sub-clinical infections, confirming exposure to pathogenic serovars by both groups.

Seropositivity rates and serovars present in horses vary among studies. Worldwide, the reported prevalences range from 1.5% to 79% [17,18], and the reported serovars vary between *Icterohaemorrhagiae*, Bratislava, Copenhageni, Sejroe, Australis, and Pomona [14,17–22]. Differences in seroprevalence may be related to the number of horses tested, the detection of nonpathogenic serovars, and the inclusion of titers $\geq 1:20$ as positive samples. In the present study, 26.1% of horses were positive to *Leptospira* spp. when considering titers $> 1:100$ and only testing for six pathogenic serovars. There has been a characteristic association of particular serovars with certain species of animals as carriers, but the association is not absolute and the

molecular basis for this maintenance host “specificity” is unknown [3].

In the particular case of urban working horses, 30.63% of horses were positive with 6.88% positive to two serovars (Table 1). All serovars studied were found in at least one urban working horse, being the most prevalent serovar Ballum, followed by Canicola and *Icterohaemorrhagiae*; Hardjo was the less common serovar with only one positive horse (Table 2). This differs from a study in cart horses in Curitiba, Brazil, where *Icterohaemorrhagiae* was the most frequent serovar found [23], and from the cart horses studies done in Cuba, where Australis was the most frequent serovar [24,25]. It is important to point out that in Suárez et al [24], 3.2% of the cart horse’s owners presented Immunoglobulin M (IgM) antibodies at the hemolytic test for *Leptospira* spp.

The biodiversity of leptospires in the environment is affected by geography, climate, biotic interactions, and anthropogenic activities [16], which could explain the diversity in serovars across studies in equines. Working horses are mainly kept in slum living conditions where rodents are common and could explain the high prevalence of *L. borgpetersenii* serovar Ballum and *L. interrogans* serovar *Icterohaemorrhagiae* [16]. The high number of free roaming dogs in these areas [26], plus the horse owner’s dogs, could explain the prevalence of serovar Canicola; on the other hand, serovars Pomona and Hardjo could be related to the maintenance of cattle and pigs [27] as subsistence farming animals.

In the case of the army horses, a lower prevalence of seropositive horses was expected because these horses are confined and sanitary practices are routinely performed, including pest control (rats and mice), bedding changes, cleaning of the stables, and use of drinking water for horses. Barwick et al [28] have pointed out how the more frequently stalls are cleaned up, fewer opportunities for the horse to be exposed to microorganisms, even so 23.31% of horses were seropositive (Table 1). Interestingly, although located in the same geographical area of the country than the urban working horses sampled, the prevalence of serovars differs, with Autumnalis being the most frequent one in the army horses, and with no horses positive to serovars *Icterohaemorrhagiae*, Pomona, and Hardjo. The relationship between warfare and leptospirosis has been described as “an occupational disease for soldiers” [29], and attention should be given to preventive practices in these sites or revision of the current ones.

The serovar Autumnalis has been associated with raccoons as a natural host, raccoons are not found in Chile, but the serovar has also been isolated from rodents including *Rattus rattus* and from dogs [30]. Because preventive measures are taken within the army, the high prevalence of serovar Autumnalis could be explained because although dogs are vaccinated, the vaccine does not include this serovar, protecting only against Canicola, *Grypthofosa*, *Icterohaemorrhagiae*, and Pomona, the last two serovars were not identified within the army horse population. This is contrary to our findings in the urban working horses, where the serovars included in the vaccine were present (Table 2). Special attention in this last case should be given to the high prevalence of serovars *Icterohaemorrhagiae* and

Table 2
Proportion of horses positive and prevalence according to activity (urban working horse or army horse) and serovar.

Serovar	Urban Working Horses ($n = 160$)		Army Horses ($n = 266$)	
	Positive Horses	Prevalence	Positive Horses	Prevalence
Hardjo	1	0.63	0	0
Pomona	3	1.88	0	0
Canicola	16	10.00	10	3.76
Ballum	24	15.00	25	9.40
<i>Icterohaemorrhagiae</i>	9	5.63	0	0
Autumnalis	6	3.75	28	10.53

Table 3

Incidence of titration found according to serovar and activity of the horses (urban working horse = WH, army horse = AH).

Serovar	Titration Incidence											
	WH		AH		WH		AH		WH		AH	
	100	100	200	200	400	400	800	800	1,600	1,600		
Hardjo	0	0	1	0	0	0	0	0	0	0	0	
Pomona	0	0	2	0	0	0	0	0	1	0		
Canicola	5	2	4	3	3	2	2	2	2	1		
Ballum	5	14	7	5	9	3	3	1	0	2		
Icterohaemorrhagiae	5	0	2	0	1	0	1	0	0	0		
Autumnalis	3	21	1	5	1	1	0	1	1	0		
Total	18	37	17	13	14	6	6	4	4	3		

Canicola that are known to cause clinical disease in humans.

Little attention is commonly given to the potential role horses play in zoonotic diseases [28]. This study provides evidence that infection of horses with *Leptospira* spp. is common and that serovars causing clinical disease in humans are present. The lack of official reports is probably due to the subclinical form of the disease in this species, form that could affect the working capacity of the horses and with this affect the family's sole source of income generation. Epidemiologic studies have suggested equines as shedders of *Leptospira* spp., becoming a potential threat for humans [28]. On the other hand, Hamond et al [31] were able to identify by PCR leptospires in urban horses, being the first report to our knowledge, confirming that these animals can spread the bacteria in the environment, with important implications for public health. Efforts should be taken to provide urban working horse owners with preventive measures to avoid possible human infection. Special attention should be given to housing practices, cohabitation with other animals (included production animals, pets, and pests), and water sources; all practices that urban working horse's owners commonly keep.

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References

- [1] Park YG, Gordon JC, Bech-Nielsen S, Slemmons RD. Factors for seropositivity to leptospirosis in horses. *Prev Vet Med* 1992;13:121–7.
- [2] Verma A, Stevenson B, Adler B. Leptospirosis in horses. *Vet Microbiol* 2013;167:61–6.
- [3] Adler B, de la Peña Moctezuma A. *Leptospira* and leptospirosis. *Vet Microbiol* 2010;140:287–96.
- [4] Rohrbach B, Ward D, Hendrix D, Cawrse-Foss M, Moyers T. Effect of vaccination against leptospirosis on the frequency, days to recurrence and progression of disease in horses with equine recurrent uveitis. *Vet Ophthalmol* 2005;8:171–9.
- [5] OIE. World Organisation for animal health. Leptospirosis. In: *Manual of Diagnostic tests and vaccines for Terrestrial animals*. 5th edition, Volume 1. Paris, France: OIE Biological Standards Commission and adopted by the International Committee of the OIE. Office International des epizooties; 2004. p. 316–27.
- [6] Tadich TA, Stuardo Escobar L. Strategies for improving the welfare of working equids in the Americas: a Chilean example. *OIE Sci Tech Rev* 2014;33:203–11.
- [7] SAG. Situación sanitaria equina en Chile. Resultados Vigilancia epidemiológica, primer semestre 2001. Chile: Ministry of Agriculture; 2011. p. 1–5.
- [8] MINSAL. Leptospirosis (CIE 10:A27), situación epidemiológica, semanas 1 a 52, año 2012. Departamento de Epidemiología, Ministerio de Salud, Gobierno de Chile; 2012. p. 3.
- [9] Salgado M, Otto B, Sandoval E, Reinhardt G, Boqvist S. A cross sectional observational study to estimate herd level risk factors for *Leptospira* spp. Serovars in small holder dairy cattle farms in southern Chile. *BMC Vet Res* 2014;10:126.
- [10] Zamora J, Riedemann S, Cabezas X. Leptospirosis de roedores silvestres en el área rural de Valdivia. Pesquisa de *Leptospira* interrogans mediante inmunofluorescencia e inmunoperoxidasa. *Arch Med Vet* 1995;27:115–8.
- [11] López J, Abarca K, Cerda J, Valenzuela B, Lorca L, Olea A, Aguilera X. Surveillance system for infectious diseases of pets, Santiago, Chile. *Emerg Infect Dis* 2009;15:1674–6.
- [12] Lelu M, Muñoz-Zanza C, Higgins B, Galloway R. Seroepidemiology of leptospirosis in dogs from rural and slum communities of Los Rios Region, Chile. *BMC Vet Res* 2015;11:31.
- [13] Perret C, Abarca K, Dabanch J, Solari V, García P, Carrasco S, Olivares R, Avalos P. Prevalencia y presencia de factores de riesgo de leptospirosis en una población de riesgo de la Región Metropolitana. *Rev Med Chil* 2005;133:426–31.
- [14] Troncoso I, Toro J, Guzmán A, Fuentealba J, Fischer C. Evaluación serológica de *Leptospira* interrogans en equinos pertenecientes a un centro ecuestre de la provincial de Linares, Chile. *Rev CES Med Zootec* 2014;8:101–7.
- [15] Ko AI, Galvao Reis M, Dourado Ribeiro CM, Johnson WD, Riley LW. Urban epidemic of severe leptospirosis in Brazil. Salvador leptospirosis study group. *Lancet* 1999;354:820–8255.
- [16] Bharti AR, Nally JE, Matthias MA, Diaz MM, Lovett M, Levett PN, Gilman RH, Wilig MR, Gotuzzo E, Vinetz JM. Leptospirosis: a zoonotic disease of global importance. *Lancet Infect Dis* 2003;3:757–71.
- [17] Ebani V, Bertelloni F, Pinzauti P, Cerri D. Seroprevalence of *Leptospira* spp. and *Borrelia burgdorferi* sensu lato in Italian horses. *Ann Agric Environ Med* 2012;19:237–40.
- [18] Houwers DJ, Goris MGA, Abdoel T, Kas JA, Knobbe SS, van Dongen AM, Westerduin FE, Klein WR, Hartskeerl RA. Agglutinating antibodies against pathogenic *Leptospira* in healthy dogs and horses indicate common exposure and regular occurrence of subclinical infections [Letter to the Editor]. *Vet Microbiol* 2011;148:449–51.
- [19] Hamond C, Pinna A, Martins G, Lilienbaum W. The role of leptospirosis in reproductive disorders in horses. *Trop Anim Health Prod* 2014;46:1–10.
- [20] Jung BY, Lee KW, Ha TY. Seroprevalence of *Leptospira* spp. in clinically healthy racing horses in Korea. *J Vet Med Sci* 2010;72:197–201.
- [21] Báverud V, Gunnarson A, Olsson Engvall E, Franzén P, Egenvall A. *Leptospira* seroprevalence and associations between seropositivity, clinical disease and host factors in horses. *Acta Vet Scand* 2009; 51:15.
- [22] Ellis WA, O'Brien JJ, Cassells JA, Montgomery J. Leptospiral infection in horses in Northern Ireland: serological and microbiological findings. *Equine Vet J* 1983;15:317–20.
- [23] Finger MA, de Barros Filho I, Leutenegger C, Estrada M, Ullman LS, Langoni H, Kikuti M, Dornbush PT, Deconto I, Biondo AW.

- Serological and molecular survey of *Leptospira* spp among cart horses from an endemic area of human leptospirosis in Curitiba, southern Brazil. *Rev Inst Med Trop Sao Paulo* 2014;56:473–6.
- [24] Suárez M, Johnson D, Sánchez A, Gorgoy V, Alfaro I, Vega L. *Leptospira* y *brucella* en equinos y humanos vinculados a vehículos de tracción animal. *A Vet (murcia)* 1996;11:29–35.
- [25] Castillo JC, Cepero O, Silveira EA, Casanova R, González Y, Gutiérrez I. Leptospirosis prevalence in draught horses in Santa Clara city, Cuba. *REDVET* 2007;7:1–4.
- [26] Ibarra L, Morales MA, Acuña P. Demographic aspects of dog and cat populations in Santiago City, Chile [in Spanish]. *Av Cs Vet* 2003;18:13–20.
- [27] Fentahun T, Alemayehu M. Leptospirosis and its public health significance: a review. *Eur J Appl Sci* 2012;4:238–44.
- [28] Barwick R, Mohammed HO, McDonough PL, White ME. Epidemiologic features of equine *Leptospira interrogans* of human significance. *Prev Vet Med* 1998;36:153–65.
- [29] Johnston JH, Lloyd J, McDonald J, Waitkins S. Leptospirosis—an occupational disease of soldiers. *J R Army Med Corps* 1983;129:111–4.
- [30] Davis MA, Evermann JF, Petersen CR, Vander Schalie J, Besser TE, Huckabee J, Daniels JB, Hancock DD, Leslie M, Baer R. Serological survey for antibodies to leptospira in dogs and raccoons in Washington State. *Zoonoses Public Health* 2008;55:436–42.
- [31] Hamond C, Martins G, Lawson-Ferreira R, Medeiros MA, Lilenbaum W. The role of horses in the transmission of leptospirosis in an urban tropical area. *Epidemiol Infect* 2013;141:33–5.