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High seroprevalence of anti-*Leptospira* spp. antibodies in domestic and wild mammals from a mixed use rescue center in Ecuador: Lessons for “One Health” based conservation strategies

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ABSTRACT

Leptospirosis is a zoonotic disease of worldwide distribution that affects humans, domestic and wild animals. This study evaluates the frequency of anti-*Leptospira* spp. antibodies in companion, livestock and wild mammals from a mixed use rescue center on Guayaquil, the main city of Ecuador. Sera were collected from 23 domestic and 6 wild mammals. All animals tested were seropositive for *Leptospira* spp. using Microscopic Agglutination Test (MAT), most of them for multiple serovars. *Leptospira interrogans* serovars Canicola, Hardjo and Icterohaemorrhagiae were the most frequent ones. We conclude that the presence of domestic animals at this mixed use rescue center may cause the exposure of wild animals, considering the high frequency of *Leptospira* spp. seropositivity already reported for livestock and companion animals in Ecuador. This is the first serological survey for leptospirosis including wild animals rescued from illegal traffic in Ecuador and point out the urgent need of exclusive rescue and conservation units for wild species and the potential role as *Leptospira* spp. reservoir for wild mammals.

1. Introduction

Leptospirosis is a zoonotic infectious disease with a morbidity above one million people per year. Although infected individuals can be asymptomatic, severe disease may produce renal or hepatic failure and pulmonary bleeding that can lead to death [1–3]. Leptospirosis has a worldwide distribution, although its prevalence is higher in the tropics and poor regions, and it is considered endemic in South America [4,5]. Bacteria from the genus *Leptospira* are the causative agents and can infect almost all mammals [2]. Leptospirosis has been reported on companion, livestock and wild mammals, and transmission among animals or to humans happens by direct contact with urine from infected animals or indirect contact with contaminated soil and water where *Leptospira* spp. can survive for long periods [7,8]. Free roaming dogs and rats are considered the main reservoir of the disease in urban areas [2,9] while livestock animals play an important role for occupational leptospirosis transmission [2,6]. However, on the context of developing countries, livestock animal raised for self consumption are an important source for community acquired leptospirosis on rural areas [6,10]. Also,

wild mammals can play an important role in the epidemiology of leptospirosis, because they can carry and spread the bacteria over long distances [8,11].

Leptospirosis is a neglected tropical disease in Ecuador despite up to 1279 cases in humans were reported in 2012 [12]. Although a few studies regarding this zoonotic disease have been reported, showing a high prevalence in febrile patients, cattle, pigs and dogs [10,13–16], no control and prevention strategies based on a “One Health” approach have ever been carried out by public health authorities. Additionally, Ecuador is among the countries with higher biodiversity in the world, including several endemic endangered mammal species. Wild animals illegal traffic is a common practice, particularly on rural areas where these animals are exposed to humans, companion and livestock animals. Limited budget from environmental conservation authorities is allocated to rescue and reintroduction of wild animals from illegal traffic, and rescue and conservation units normally rely on non profit organizations. No standard protocols, poor infrastructure and mixed used for wild and domestic animals are common at this rescue and conservation centers. However, neither the potential role of wild

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mammals as potential reservoir for *Leptospira* spp. or the conservation threat of transmission of *Leptospira* spp. serovars from domestic to wild mammals has ever been studied in Ecuador. Considering this scenario, the aim of this study was to evaluate the frequency of anti-*Leptospira* spp. antibodies in animals from a mixed use conservation center on the city of Guayaquil, the most populated one in Ecuador.

2. Materials and methods

2.1. Study area

This study was performed at the rescue and conservation center “Centro de Rescate Narayana-Chongón”, located 24 km west from the city of Guayaquil, on the southwest of the country. Characteristics of this and surrounding areas confer direct contact between humans, domestic and wild animals from the pacific rainforest biomes. Wild animals are kept on cages at the rescue center; domestic animals have free movement within the facility. For the present study, samples were collected during august 2017.

2.2. Blood sample collection

Companion and livestock animals were managed by veterinarians from the conservation center and their blood was collected from the cephalic vein. The wild mammals were captured using live-traps and anaesthetized with ketamine and xylazine for blood samples to be collected. Wild animals were taxonomically identified according to morphologic characteristics.

The serum was separated by centrifugation (5000 rpm, 5 min). A total of 29 sera were analyzed: 10 of companion animals (4 dogs, 3 horses and 3 lions; lions were rescued from owners that bought them illegally as pets), 13 of livestock animals (3 cows, 3 sheeps, 3 pigs, 3 rabbits and 1 guinea pig) and 6 of wild animals (2 *Nasua nasua*, 1 *Nasuella olivacea*, 1 *Leopardus tigrinus* and 2 primates: *Lagothrix lagothricha* and *Cebus aequatorialis*).

2.3. Serology

The Microscopic Agglutination Test (MAT) was performed using 21 live antigens. *Leptospira borgpetersenii* serovars: Castellonis, Javanica, Tarassovi; *Leptospira interrogans* serovars: Australis, Autumnalis, Bataviae, Bratislava, Canicola, Copenhageni, Hardjo, Hebdomadis, Pomona, Pyrogenes, Icterohaemorrhagiae, Wolfii, Sejroe; *Leptospira kirschneri* serovars: Cynopteri, Grippytyphosa; *Leptospira noguchii* serovar: Panama and *Leptospira santarosai* serovars: Shermani; *Leptospira biflexa* serovar: Patoc.

The antigens were prepared from reference strains maintained at the Laboratorio de Referencia de Zoonosis from Instituto Nacional de Salud Pública e Investigación “Leopoldo Izquieta Pérez” at the city of Guayaquil. For the screening of sera a 1:100 dilution was the starting one used. Reactive samples were then examined with increasing dilutions from 1:100 to 1:3200, taking the highest positive dilution to be the titer of the serum. The serum was taken as reactive when at least 50% of agglutination occurred at 40× under the microscope.

3. Results

All the 29 animals tested were seropositive for at least one *Leptospira* serovar with titers ranging from 200 to 3200. Additionally, 28 animals were seropositive for multiple serovars, from 2 to 11 different serovars. Most animals have a higher titer serovar and animals of the same species usually have the same serovar. For example, all pigs

had a higher titer (1/400) for serovar Bataviae and all horses presented the same title (1/800) for serovar Icterohaemorrhagiae.

The most frequent *Leptospira* spp. serovars were Canicola (24/29; 82.8%), Hardjo (21/29; 72.4%) and Icterohaemorrhagiae (19/29; 65.5%). All serovars tested are present on companion, livestock or wild animals, with the exception of serovar Wolfii only present on wild animals (4/6; 66.7%). Serovars distribution and titers for all the animals included on the study are detailed on Table 1.

4. Discussion

The present study reports a striking case of a rescue center in Ecuador where all the animals present at the time of the sample collection were seropositive for *Leptospira* spp. This rescue and conservation unit hosts on the same facility companion, livestock animals and wild mammals rescued from illegal traffic. This conservation center has the permits to operate from the environmental authorities (Ministerio del Ambiente de Ecuador) that actually are responsible for wild animal delivery to the center, although there are neither quarantine nor isolation areas for wild mammals.

With the only exception of a dog, the rest of the animals on the conservation unit were positive for multiple *Leptospira* spp. serovars, spread all over the animals tested no matter if they were domestic or wild species. There are three reports regarding *Leptospira* spp. serovars prevalence on companion and livestock animals in Ecuador, showing high prevalence in dogs (70%), cattle (from 35.4 to 74%; up to 97% at herd level) and pigs (up to 67%) [10,14,15]. Additionally, all the *Leptospira* serovars reported on those studies were found on our study; even the serovar Wolfii, only found in wild animals in our study, has been already reported at a prevalence over 9% in cattle from the neighbor Ecuadorian province of Manabi [10,14,15]. Wildlife could act as reservoirs of pathogenic *Leptospira* spp. and participate on the transmission of leptospirosis from wild to domestic environments [16]. However, no information regarding *Leptospira* spp. prevalence on wild animals in Ecuador is available but a recent report in Brazil show a low prevalence of 1.51% among wild mammals at conservation units indicating a minor role for wild mammals as a reservoir for *Leptospira* spp. [8]. So, although we cannot ruled out neither exposure on the wildness or by human or domestic animal contact previous to rescue, it is tempting to speculate that *Leptospira* spp. exposure could have occurred at the mixed use conservation unit due to the presence of infected domestic animals. For instance, the extremely high prevalence of some *Leptospira interrogans* serovars could be explained by the presence of a maintenance host of this serovar among animals at rescue center. It is important to notice that although wild animals are kept on cages, domestic animals (particularly dogs), have free movement within the rescue facility included on this study. Also, the rescue and conservation unit is located in Guayaquil where the occurrence of floods is usual favoring the transmission on *Leptospira* spp., where it would be possible that urine from infected animals spread around all the unit during raining season. Actually, raining season has been associated to higher frequency of leptospirosis among livestock animals in Ecuador [10].

Based on our results, mixed use conservation units for wild and domestic animals should be improved on an epidemiological scenario like Ecuador, where *Leptospira* spp. seroprevalence among livestock and companion animals is high. Guidances and recommendations for better practices in rescue centers including the use quarantine of animals before sharing spaces, treatment of sick animals and vaccination of healthy animals should be develop by environmental authorities. This would be particularly crucial if the final aim for wild animals rescue is the reintroduction from conservation units because of the potential negative effects on wild populations. Also, transmission of *Leptospira*

Table 1

Titers found for the MAT test for serovars of *Leptospira* spp. in sera from domestic and wild animals at the rescue center “Centro de Rescate Narayana-Chongón” in Guayaquil (Ecuador). Only serovars with positive titers are included.

Sample	canicola	copenhageni	sejroe	cynopteri	hardjo	pomona	autumnalis	icterohaemorrhagiae	wolfii	gripotyphosa	bataviae	tarassovi	australis
Dog 1	200							200		200			
Dog 2	200				200			200					
Dog 3	200				200								
Dog 4	200												
Lion 1	800	400	400										
Lion 2	400	800	800	400	3200	800							
Lion 3	400	200		400	400	800							
Horse 1	400	200					200	800		200	200		
Horse 2				400	200			800		200	200		
Horse 3	200		200	200	200	200	200	800		200	200	400	200
Cow 1	200	800	200		400		200	400			200	200	200
Cow 2	200		200		200		200	400			200	200	
Cow 3	200		200		200		200	200					
Sheep 1					200					200			
Sheep 2								200		200			
Sheep 3	200							200					
Pig 1	200				200			200				200	
Pig 2	200				200								
Pig 3	200				200								
Rabbit 1					200			800					
Rabbit 2	200				200			800		200			
Rabbit 3	200				200			800		200			
guinea pig								800					
<i>Nasua nasua 1</i>	1600				800	200	1600	200	200	800			
<i>Nasua nasua 2</i>	800	200	200				800	200	400		200		200
<i>Nasuella olivacea</i>	400	200			800		200						
<i>Leopardus tigrinus</i>	200	400	200	200	800	200	200	400	200				
<i>Cebus aequatorialis</i>	1600	200		200	1600						800		
<i>Lagothrix lagotrichia</i>	200			200	200			800	200	200	200		
total positive animals	24	9	8	7	21	5	9	19	4	10	13	4	3

spp. from domestic to wild animals could create new wild reservoirs that would facilitate the pathogen spread. “One Health” based strategies should be implemented when dealing with wild animals rescued from illegal traffic not only from a public health perspective but also for successful conservation policies.

In conclusion, exposure to *Leptospira* spp. among wild mammals is reported for the first time in Ecuador. All wild animals tested were seropositive, the most frequent serovars have being widely described in domestic animals and the animals were kept rescued from illegal traffic, supporting the idea of transmission of *Leptospira* spp. from livestock or companion animals.

Finally, the authors of this study do not pretend to dismiss the good will and generous efforts from non profit rescue and conservation units operating in Ecuador under extremely difficult conditions. Moreover, we hope that this work call for the action of environmental and conservation authorities in Ecuador to allocate the proper resources for a successful rescue and reintroduction of wild animals from illegal traffic.

5. Animal rights

According to national regulations in Ecuador, the need for ethics approval is unnecessary for sample collection for the diagnosis of domestic animals (“Ley Orgánica de Sanidad Agropecuaria” 2017, Asamblea Nacional, República del Ecuador. For wild animals, Centro De Rescate Narayana-Chongon have the permits from Ministerio del Ambiente to conduct test to improve a better health diagnosis.

Authors' contributions

- All authors contributed towards sample collection and data analysis.
- SAO and MAGB composed the manuscript.
- All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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