The Potential Role of Captive Collared Peccary (*Tayassu tajacu*) as a Leptospirosis Reservoir in the Peruvian Amazon

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Abstract
A serological survey was conducted in a collared peccary farm in the Peruvian Amazon to investigate variations in leptospiral seroprevalence over a period of 18 months. The first survey in 2003 revealed a prevalence of 100% of leptospirosis in the captive population (n=27) with a total of 9 different serovars (sv) identified. Leptospira *spp.* Varillal 010 was detected in 100% of the population reaching titers of 1:1600 and 8 other strains were detected sometimes at high titers. A second survey in 2005, confirmed a prevalence of leptospirosis in 86.4% of the sampled population (n=22) and revealed serological conversion to two new serovars in 40% of the herd. The absence of clinical symptoms and serological conversion to previously detected or to new serovars in more than 90% of the herd confirms the circulation of spirochaetes within the captive herd and the possible role the collared peccary as a potential reservoir of leptospirosis.

Introduction
Leptospirosis is a zoonotic disease of worldwide distribution caused by pathogenic spirochetes. The disease is endemic in tropical South America and it appears as a significant public health problem for people living in some regions of the Amazon Basin, particularly in Peru, where outbreaks of severe acute leptospirosis, have been reported in the past few years (Bharti *et al*. 2004; Segura *et al*. 2005). In addition, the collared peccary (CP) -*Tayassu tajacu*-, represents a major source of meat and income for local inhabitants in tropical forest areas and is one of the most hunted species in the Amazon region. As a result, it has been considered an interesting species for captive breeding in order to produce local protein in forested areas and several attempts to develop its production are in progress in Latin America (Nogueira Filho & Nogueira 2004). Previous reports showed different antibody titres against leptospira in captive and wild populations of CP (Mayor *et al*. 2006; Mendoza *et al.* 2006). In the Peruvian Amazon, spirochetes have been found in renal tissue of several wild animals (Bharti *et al*. 2004), but game animals such as the CP had not been tested. In 2003, a survey in 4 different farms, sometimes very distant, showed that 64.6% of the captive population of CP had antibodies to one or more *Leptospira* sp. serovars (strains). Among positive animals, 28.1% from the samples reacted to more than one leptospiral strain (Mendoza *et al*. 2006).
The objective of this paper was to recover additional information on the evolution of leptospirosis among a specific population of captive CP in order to confirm the maintenance and circulation of the agent by seroconversion in the monitored herd and its potential role as possible as reservoirs of leptospirosis in the Amazon Basin.

Methods
The study was conducted on a commercial CP farm from Iquitos, the capital city from the Department of Loreto. The climate is typically equatorial with an average annual temperature of 22-36ºC and a daily variation of 4ºC. The relative humidity varies between 80 and 100%. Annual rainfall oscillated between 1500 mm and 3000 mm. Experimental animals had been maintained in captivity for several years in the monitored farm. Animals were kept outdoors in large enclosures with a natural environment. Available space per animal was 100 to 125 m2.
Two surveys were performed at a 16 month interval. The first survey was performed in May 2003 and 90% of the population was sampled (n=27) including different age categories. In that occasion, personnel from the farm (n=3) and domestic animals (two dogs and one cat) were also tested.

The second survey took place in between August and September 2005 and 30% of the herd was sampled (n=22), including some individuals sampled in the first survey (n=11) and animals born in the farm since the first survey (n=11). Blood samples were collected from the cephalic or saphenous vein into sterile tubes for preparation of serum. The serum was removed and frozen at -20ºC ºC until analyses. In all animals, physical examinations were performed in order to assure the health status of the animals. Haemolytic and contaminated samples were discharged and not included in the study. The microscopic agglutination test (MAT) was performed for 22 Leptospiral serovars in the National Leptospirosis Reference Laboratory. Positive sera were considered to the end point of 50% agglutination, and micro agglutination titers of • 1:100 were considered evidence of previous exposure and varied between 1:100 and 1:6400.

Results

During the 2003 survey, 9 different serovars were identified. The whole population was positive Leptospira *spp* Varillal 010. This strain, isolated from a human patient in the Peruvian Amazon is currently pending of taxonomic classification (Segura *et al*. 2005). Other serovars identified were Bratislava (19%), Borincana (19%), Tarassovi (11%), Autumnalis (11%), Icteroheamorragiae (7.4%) , Ballum s102 (7.4%), Bataviae (7.4%) and Copenhagenii (3.7%). Maximum titers were recorded for Tarassovi (1:6400) and Varillal 010 (1:1600). Juveniles and subadults were all positive to sv Varillal 010 but at low titers (1:100). Personnel (n=3) from the farm tested negative to leptospiral serovars. However, one of the dogs was found positive to sv. Icteroheamorrhagiae

During the 2005 survey, 6 different serovars were detected two of which (sv Cynoptery and sv Panama) were different from those reported in the 2003 survey. Equally, five serovars from the previous survey were not detected this time. The most prevalent Leptospiral serovar was *Leptospira interrogans* icteroheamorrhagiae which was detected in 50% of the population sample. Other serovars identified were Cynopteri (32%) Varillal 010 (27%), Bratislava (23%) and Panama (13%).

In 2005, overall prevalence of leptospiral antibodies in the monitored population was still high, passing from 100% to 86.4% of the herd. All of the negative animals were young or subadult and positive subadult animals (n=3) did not present titers higher than 1:100. All the adult animals in the 2003 survey remained positive in the second survey although for different serovars. Seven individuals out of the total sample (32%) presented titers • 1:400 to one or more servovars. Maximum titers (1:800) were detected for most (4/6) of the serovars.. Despite Icteroheamorragiae was the most prevalent strain in the 2005 survey, the peruvian strain Varillal 010 was well represented in almost 30% of the sampled population however to the exception of one individual, titers were not higher than 1:100.

Discussion

The CP, one of the most popular game species in the Amazon Basin, is receiving increasing attention these last years as a candidate for the production of meat and hides in the neotropics (Nogueira Filho & Nogueira 2004). If this new promising activity develops, the risk of emerging zoonosis may increase due to the presence of high densities of wild animals in contact men or domestic animals. Despite the pathology of captive peccaries in its natural environment is not well known, leptospirosis seroprevalence has been reported repeatedly in that species (Mayor et al. 2006; Mendoza et al. 2006). This is the first report of seroconversion of leptospiral strains in a captive colony of CP, confirming that the agent was maintained and circulated within the herd population through the period of the study. The low titers registered in younger animals during both surveys suggest that individuals get probably infected during their adult life in our study.

In Vietnam, Boqvist *et al*. (2005) noticed significant variations in seroprevalence in sows over the year, seroprevalence for some serovars such as Icterohaemorrhagiae being higher after a period of high precipitation. The 2005 survey was taken during the dry season in Iquitos, while the first survey was performed during a period of high precipitations. However, we do not know how if such variations can occur in the Amazon Basin.

The MAT is considered a confirmative test for positive diagnosis in pigs if the initial titer in an individual animal is • 1:400 (Boqvist et al. 2005). Despite this test has not been specifically validated for the CP, at least 27% of the sampled herd presented titers • 1:400 in the 2005 survey. This was particularly true for sv Icterohaemorrhagiae for which titers were • 1:400 in 45% of the positive animals. In tropical regions, wild mammals are commonly infected by a wide diversity of leptospiral strains (Bharti, *et al.* 2003) and this seems to be confirmed in our farm: A total of 11 different serovars were detected, sometimes in high titers and all of them were of clinical importance for human patients (Bharti *et al*. 2003, Segura *et al*. 2005). During our survey, attempts to detect spirochaetes by PCR from the urine bladder of a positive animal were unsuccessful, so excretion of spirochaetes into the environnement could not be confirmed. However, the examined individual did not present high titers (1:100 to sv. Varillal).
The farm in our study was also hosting a population of capybaras (*Hydrocaheris hydrochaerys*) living in ponds, which in 2000 had tested positive for different serovars in 71,4% of the sampled population (n=42) in a different laboratory (Muñoz et al. 2000). New samples of these animals are currently under analysis in our laboratory in order to identify possible epidemiological links between the two species. The absence of clinical signs and the observation of seroconversion in a majority of the CP colony confirm that this species is a potential maintenance host for leptospirosis. This fact should not be a reason to stop efforts towards the development of CP production in the tropics which is considered a promising activity as a source of meat and income. Indeed, this infectious agent is common among many other domestic animals in the tropics, particularly in tropical areas where animals are exposed to outdoor environment and where water is abundant (Boqvist, 2005). However, precautions should be taken in order to limit the potential risks of transmission to domestic animals and human beings.

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Table 1: Prevalence of anti-leptospiral agglutinins per positive serovar in the sampled population during the 2003 and 2005 surveys.

Serovar	**December 2003 (n=27)**		**August 2005 (n=22)**	
	Seropositive (%)	**Max Titer**	**Seropositive (%)**	**Max Titer**
Varillal 010	27 (100)	1:1600	6 (27.2)	1: 800
Bratislava	5 (18.5)	1:100	5 (22.7)	1:600
Borincana	5 (18.5)	1:100	0 (0)	-
Autumnalis	3 (11.1)	1:100	0 (0)	-
Icterohaemorrhagiae	2 (7.4)	1:100	11(50.0)	1:800
Bataviae	2 (7.4)	1:200	0 (0)	-
Copenhageni	1 (3.7)	1:100	0 (0)	-
Ballum S102	2 (7.4)	1:100	0 (0)	-
Tarassovi	3 (11.1)	1:6400	1 (4.5)	1:400
Cynopteri	0 (0)	-	7 (31.8)	1:800
Panama	0 (0)	-	3 (13.6)	1:800

Table 2. Serovars detected by the microscopic agglutination test (MAT) in our study

Species	Serogroup	Serovar	*Strain*
L. interrogans	Australis*	Bratislava	Jez Bratislava
L. interrogans	Autumnalis*	Autumnalis	Akiyami A
L. borgepetersenii	Ballum*	Ballum	S 102
L. kischneri	Cynopteri*	Cynopteri	3522 C
L. santarosai	Hebdomadis	Borincana	Hs 622
L. interrogans	Icterohaemorrhagiae*	Copenhageni	M 20
L. interrogans	Icterohaemorrhagiae*	Icterohaemorrhagiae	RGA
L.interrogans	Panama*	Panama	
L. borgepetersenii	Tarassovi*	Tarassovi	Perepelicin
	*	Varillal 010**	

**Serovar in process of typification by the National Health Institute (Lima, Peru)

* Serogroups of clinical importance in human health