

Seroepidemiology of *Leptospira* Infection in Backyard Pigs in Durango State, Mexico

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Received: 24 April 2018; accepted: 01 May 2018

Purpose: This study aimed to determine the seroprevalence and correlates of *Leptospira* IgG antibodies in backyard pigs in the northern Mexican state of Durango. We performed a cross-sectional study of 305 backyard pigs. Anti-*Leptospira* IgG antibodies were detected using microscopic agglutination assay (MAT) with a panel of 12 *Leptospira* antigens.

Results: Overall, antibodies against *Leptospira* (any of the 12 *Leptospira* serovars examined) were found in 186 (61.0%) of the 305 pigs studied. Seropositive pigs were found on 80 (70.2%) of the 114 properties surveyed. The predominant serovar was *Leptospira interrogans* Pomona ($n = 55$); followed by *Leptospira noguchii* Louisiana and *Leptospira santarosai* Tarassovi ($n = 53$ each); *L. interrogans* Bataviae ($n = 47$); *Leptospira biflexa* Semarang and *L. interrogans* Hebdomadis ($n = 36$ each); *L. interrogans* Pyrogenes ($n = 30$); *L. interrogans* Djasiman ($n = 20$); *Leptospira borgpetersenii* Ballum ($n = 11$); *L. noguchii* Panama and *L. interrogans* Canicola ($n = 5$ each); and *L. borgpetersenii* Mini ($n = 2$). Logistic regression showed that seropositivity was associated with low (<1000 m above sea level) altitude (odds ratio [OR] = 3.24; 95% confidence interval [CI]: 2.01–5.20; $P < 0.001$).

Conclusions: This is the first report of *Leptospira* exposure in backyard pigs in Mexico and of an association between *Leptospira* exposure in pigs and altitude. Backyard pigs represent a high-risk group for *Leptospira* exposure.

Keywords: *Leptospira*, seroepidemiology, pigs, climatic factors, Mexico

Introduction

Leptospiras are thin helical bacteria classified into at least 12 pathogenic and 4 saprophytic species [1]. Pathogenic *Leptospiras* are the etiological agents of leptospirosis, an emerging zoonotic disease that affects humans and animals worldwide [2]. Leptospirosis may affect many species including domestic and wild animals [3, 4]. In humans, most characteristic early clinical signs include fever of sudden onset, severe general malaise, muscular pain, and conjunctival congestion [5]. Whereas jaundice and hemorrhage are clinical features present in patients with the severe form of leptospirosis [5]. In animals, the acute phase of the *Leptospira* infection is mostly sub-clinical, but chronic infections cause reproductive wastage and great economic losses [6]. More than 300 serovars have been identified among *Leptospiras* [7]. Pigs are generally considered the reservoir host species for Pomona, Bratislava, and Tarrasovi serovars [8]. Leptospirosis is transmitted by direct or indirect contact with an infected animal, and infection with *Leptospira* in pigs may lead to considerable losses due to abortion, stillbirths, weakly piglets, or infertility [8]. The seroprevalence of *Leptospira* infection in pigs varies substantially among countries. For instance, a relatively low (5%–13%) seroprevalence has been reported in

pigs in Trinidad [9], Thailand [10], and feral swine in the USA [11]. Whereas intermedium (20.2%–25%) seroprevalence was found in pigs in Portugal [12], a high (55.9%) seroprevalence was reported in pigs in Córdoba, Colombia [13].

The epidemiology of *Leptospira* infection in pigs in Mexico has been poorly studied. Examination of *Leptospira* infection in pigs is important because contact with *Leptospira*-infected pigs might be a risk for *Leptospira* infection in humans. Epidemiological studies about *Leptospira* infection in pigs are important to get useful information for an optimal planning of preventive measures against this infection and thus to improve the population health. In a previous study, Vado-Solís et al. found a 25% seroprevalence of *Leptospira* infection in pigs in the southern Mexican state of Yucatan [14]. However, the seroprevalence of *Leptospira* infection in pigs in the northern Mexican state of Durango is unknown. Therefore, we sought to determine the seroprevalence of anti-*Leptospira* IgG antibodies and *Leptospira* serovars in pigs in Durango, Mexico. In addition, seroprevalence association with the general characteristics of pigs and their environment was also investigated.

Materials and Methods

Study Design and Pigs Surveyed. A cross sectional study using residual sera from a *Toxoplasma gondii* serosurvey in pigs in Durango State, Mexico [15] was performed. Inclusion

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criterion of pigs was backyard pigs raised in Durango State. Exclusion criterion was pigs with insufficient serum sample or missing information about their general characteristics. In total, 305 randomly selected backyard pigs were included in the study. Pigs were raised in eight municipalities in three geographical regions of Durango State, Mexico (Table 1). Pigs were apparently healthy and raised in 114 homes. A median of three pigs (range, 1 to 6) per property were surveyed. Of the 305 pigs, 117 (38.4%) were juveniles (4- to 8-month-old) and 188 (61.6%) were adults (9-month-old to 4-year-old); 204 (66.9%) were females and 101 (33.1%) were males. The great majority ($n = 269$) of pigs were mixed-breed, 35 were Yorkshire, and one was Landrace. Environment characteristics of the municipalities surveyed including climate, mean annual temperature and rainfall, and altitude (meters above sea level) were obtained.

Detection of Anti-*Leptospira* IgG Antibodies. Serum samples of pigs were analyzed for anti-*Leptospira* IgG antibodies using 2-fold serial dilutions from 1:25 with the microscopic agglutination assay (MAT). A titer of 1:100 was used as cut-off for seropositivity in MAT. A panel of 12 leptospiral antigens was used: *Leptospira biflexa* Semarang, *Leptospira noguchii* Panama, *Leptospira interrogans* Djasiman, *L. noguchii* Louisiana, *Leptospira borgpetersenii* Ballum, *Leptospira santarosai* Tarassovi, *L. interrogans* Pyrogenes, *L. interrogans* Bataviae, *L. interrogans* Pomona, *L. interrogans* Hebdomadis, *L. interrogans* Canicola, and *L. borgpetersenii* Mini.

Statistical Analysis. Results were analyzed with the aid of the software Microsoft Excel 2010, Epi Info 7 (Centers for Disease Control and Prevention: <http://wwwn.cdc.gov/epiinfo/>), and SPSS 15.0 (SPSS Inc., Chicago, Illinois). We assessed the association of *Leptospira* seropositivity and the general and environment characteristics of pigs with the Pearson's χ^2 test or the two-tailed Fisher's exact test (when values were small). *Leptospira* seroprevalence was correlated with two groups of mean annual temperatures of the municipalities surveyed: $<20^\circ\text{C}$, and $\geq 20^\circ\text{C}$. In addition, *Leptospira* seroprevalence was correlated with three groups of altitudes: <1000 , 1000 – 2000 , and >2000 m above sea level. We also assessed the association between *Leptospira* seroprevalence and two groups of mean annual rainfalls: ≤ 600 mm and >600 mm). Variables with a P value of ≤ 0.05 obtained by bivariate analysis were further analyzed by multivariate analysis. Odds ratio (OR) and 95% confidence interval (CI) were calculated by stepwise regression analysis using the backwards elimination method. A P value of <0.05 was considered statistically significant.

Ethics. The Bioethics and Animal Welfare Commission (BAWC) of the Veterinary Medicine and Animal Husbandry School (Facultad de Medicina Veterinaria y Zootecnia) of the State University of Veracruz (Universidad Veracruzana) approved this project. Owners of pigs provided verbal consent to study their animals. Blood sampling in pigs was performed according to guidelines of the BAWC.

Results

Overall, antibodies against *Leptospira* (any of the 12 *Leptospira* serovars examined) were found in 186 (61.0%) of the 305 backyard pigs studied. The seroprevalence of *Leptospira* infection did not vary with age, sex, or breed of the pigs. Table 2 shows a correlation between *Leptospira* seroprevalence and general characteristics of pigs. Seropositive pigs were found on 80 (70.2%) of the 114 properties surveyed. Positive anti-*Leptospira* titers to all 12 *Leptospira* serovars examined were found. Sera of *Leptospira*-positive pigs showed reactivity to one or up to 6 *Leptospira* serovars. Seroreactivities to 1, 2, 3, and 4 *Leptospira* serovars were found in 79, 59, 39, and 7 pigs, respectively, whereas seroreactivities to 5 and 6 serovars were found in one pig each. The predominant serovar was *L. interrogans* Pomona ($n = 55$), followed by *L. noguchii* Louisiana and *L. santarosai* Tarassovi ($n = 53$ each); *L. interrogans* Bataviae ($n = 47$); *L. biflexa* Semarang and *L. interrogans* Hebdomadis ($n = 36$ each); *L. interrogans* Pyrogenes ($n = 30$); *L. interrogans* Djasiman ($n = 20$); *L. borgpetersenii* Ballum ($n = 11$); *L. noguchii* Panama and *L. interrogans* Canicola ($n = 5$ each); and *L. borgpetersenii* Mini ($n = 2$).

The seroprevalence of *Leptospira* infection varied significantly ($P = 0.001$) among geographical regions, being the highest

Table 2. General characteristics of the 305 pigs surveyed and seroprevalence of *Leptospira* infection

Characteristics	Pigs tested no.	Seroprevalence of <i>Leptospira</i> infection		P value
		No.	%	
Age				
4 to 8 months	117	72	61.5	0.87
9 months to 4 years	188	114	60.6	
Gender				
Male	101	66	65.3	0.27
Female	204	120	58.8	
Breed				
Pure	36	23	63.9	0.70
Mixed	269	163	60.6	

Table 1. Seroprevalence of *Leptospira* infection and environment correlates in backyard pigs in Durango, Mexico

Region ^a	Municipality ^b	Climate ^c	Meters above sea level ^d	Mean annual temperature (°C)	Mean annual rainfall (mm)	Homes surveyed	Homes with positive pigs		Pigs tested no.	Seropositivity to <i>Leptospira</i>	
							No.	%		No.	%
Valleys	Durango	Semi-arid	1885	16.9	529	15	14	93.3	67	44	65.7
	Vicente Guerrero	Subhumid	1950	17.4	500–600	15	10	66.7	23	16	69.6
	Coneto de Comonfort	Dry	2000	15.3	450	17	17	100	51	45	88.2
	Peñon Blanco	Subhumid	1800	18	450	9	4	44.4	20	8	40
	All					56	45	80.3	161	113	70.2
Semi-desert	Cuencame	Semi-arid	1580	21.1	243.7	16	11	68.8	45	24	53.3
	Santa Clara	Dry	2050	14.9	300–600	10	4	40	20	4	20
	All					26	15	57.7	65	28	43.1
Mountains	Pueblo Nuevo	Temperate	2560	18	1300	20	9	45	45	17	37.8
	San Dimas	Warm subhumid	520	23.7	1050	12	11	91.7	34	28	82.4
	All					32	20	62.5	79	45	57
All						114	80	70.2	305	186	61

^aStatistically significant ($P = 0.001$) difference among regions.

^bStatistically significant ($P < 0.001$) difference among municipalities.

^cStatistically significant ($P = 0.001$) difference among climates.

^dStatistically significant ($P < 0.001$) difference among altitudes.

(70.2%) in pigs raised in the Valleys region (Table 1). In addition, the seroprevalence of *Leptospira* infection varied significantly ($P < 0.001$) among municipalities, being the highest (88.2%) in pigs raised in Coneto de Comonfort municipality. The seroprevalence also varied significantly ($P = 0.001$) among climates, being the highest (82.4%) in a warm sub-humid climate. Seroprevalence did not vary ($P = 0.39$) among mean annual rainfall groups: 62.4% in ≤ 600 mm and 57.0% in > 600 mm. Seroprevalence (59.3%) in places with mean annual temperatures of < 20 °C was similar ($P = 0.30$) to that (65.8%) found in places with mean annual temperatures ≥ 20 °C. Seroprevalence decreased significantly ($P < 0.001$) with altitude (meters above sea level): 82.4% in < 1000 , 66.5% between 1000 and 2000, and 32.3% in > 2000 .

Logistic regression analysis of variables likely ($P < 0.05$) associated with *Leptospira* infection obtained by bivariate analysis showed that seropositivity was associated with low altitude (< 1000 m above sea level; OR = 3.24; 95% CI: 2.01–5.20; $P < 0.001$), whereas other variables including region, municipality, and climate were not associated with seropositivity by logistic regression.

Discussion

The seroepidemiology of *Leptospira* infection in pigs in Mexico has been scantily studied. Few studies about the seroprevalence of *Leptospira* infection in pigs in Mexico have been reported. Cisneros-Puebla et al. studied 1970 swine from a number of Mexican farms and found a 39.8% seroprevalence of *Leptospira* infection, and the most frequent serovars were Bratislava (22.5%), Icterohaemorrhagiae strain Palo Alto (14.5%), and Portland-vere strain Sinaloa ACR (13.8%) [16]. In another study, Vado-Solis et al. examined 353 pigs from farms and ranches of the southern Mexican State of Veracruz and found a 25% seroprevalence of *Leptospira* infection, and the most frequent serovar was Bratislava (51%) followed by icterohaemorrhagiae (12%) and Panama (10%) [14]. However, there is no report about the seroepidemiology of *Leptospira* infection in pigs in the northern Mexican state of Durango. Therefore, this study was aimed to determine the seroprevalence and correlates of *Leptospira* infection in pigs in Durango, Mexico. We found that more than half (61%) of the pigs examined had anti-*Leptospira* antibodies. In fact, the seroprevalence found in our study is the highest reported so far. The seroprevalence found is higher than seroprevalences (25%–39.8%) of *Leptospira* infection reported in pigs in two previous Mexican studies [14, 16]. In an international context, the seroprevalence found in our study is higher than seroprevalences reported in pigs in Trinidad (5%) [9], (8.17%) Vietnam [17], Thailand (11.3%) [10], Portugal (20.2%) [12], and Colombia (55.9%) [13]. Studies of *Leptospira* infection in feral swine in the USA have found seroprevalences ranging from 13.0% to 48.9% [11, 18, 19]. In a study in Poland, 10.4% of 377 wild boars examined were found seropositive for *Leptospira* [20]. In contrast, in a study in New South Wales, researchers found antibodies to *L. interrogans* serovar Pomona in 53% of 239 feral pigs surveyed [21]. Furthermore, a high seroreactivity rate (48.6%) to *Leptospira* species was found in feral pigs in Queensland, Australia [22]. It is not clear why pigs in our study had a higher seroprevalence than pigs in other studies. The same MAT was used in all studies. However, differences in the serovars tested and in environment or raising characteristics might have influenced the seroprevalences among the studies. We studied backyard pigs, whereas farm or feral pigs were examined in the other studies. The three predominant serovars in pigs in our study were *L. interrogans* Pomona, *L. noguchii* Louisiana, and *L. santarosai* Tarassovi, whereas

the serovars Bratislava, Icterohaemorrhagiae, and Portland or Panama were predominant in pigs in other Mexican studies [14, 16]. The difference in predominant serovars can be explained by differences in the serovars tested among the studies. To the best of our knowledge, there is only one previous report about the seroepidemiology of *Leptospira* infection in backyard pigs. Anampa et al. found an 82.1% seroprevalence of *Leptospira* infection in 133 backyard pigs slaughtered in two abattoirs in Lima, Peru [23].

We searched for the association between *Leptospira* seropositivity and environment characteristics of pigs. Multivariate analysis showed that *Leptospira* seropositivity was associated with decreasing altitude. Pigs raised in places with low altitude (< 1000 m above sea level) had the highest seroprevalence rate. To the best of our knowledge, there is no previous report about this association. It is not clear why pigs raised in places with low altitude had a higher seroprevalence of *Leptospira* infection than those raised in places with higher altitude. It is likely that places with low altitude have a more suitable environment for *Leptospira* growing than places with higher altitudes. *Leptospira* survives in moist environments [24]. It is possible that contact with contaminated water source in low altitude places may favor *Leptospira* transmission.

The present survey has some limitations. All pigs examined were raised in backyards; therefore, further studies to determine the seroprevalence of *Leptospira* infection in farm pigs raised in Durango State are needed. All pigs tested were apparently healthy, and seroprevalence might vary in ill pigs. It is unknown whether maternal antibodies might have influenced the seroprevalence of *Leptospira* infection in very young pigs. We did not include the testing of some *Leptospira* serovars; however, inclusion of more serovars would increase the seroprevalence.

Our results suggest that *Leptospira* infection is very common in backyard pigs in the northern Mexican state of Durango. Remarkably, all municipalities and the majority (70.2%) of the houses sampled had seropositive pigs. Further research to determine risk factors associated with *Leptospira* exposure in pigs in northern Mexico should be conducted. It is recommended to avoid contact with *Leptospira*-infected backyard pigs, their urine, or contaminated environment to prevent *Leptospira* infection in humans. Wearing protective clothing and covering skin lesions when handling backyard pigs, as well as disinfecting their contaminated areas, are measures to prevent *Leptospira* transmission.

Conclusions

This is the first report of *Leptospira* exposure in backyard pigs in Mexico and of an association between *Leptospira* exposure in pigs and altitude. Results of the present study suggest that backyard pigs represent a high-risk group for *Leptospira* exposure. Results may be useful for the planning of optimal measures against *Leptospira* infection in pigs.

Funding Sources

This study was financially supported by Juárez University of Durango State, Durango, Mexico, and State University of Veracruz (Universidad Veracruzana), Veracruz, Mexico.

Authors' Contributions

C.A.E. designed the study, obtained the samples and data of the pigs, performed the statistical analysis, analyzed the data, and wrote the article. A.C.R. and D.R.S. performed the data analysis and the laboratory assays. A.O.A.F. performed the statistical analysis and analyzed the data. S.S.M., J.H.T., and L.F.S. performed the data analysis and wrote the article.

Conflicts of Interest

The authors declare no conflicts of interest.

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