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Seroprevalence and risk factors associated with within-flock transmission of *Leptospira interrogans* in transhumant farming systems in Mexico

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SUMMARY

A number of recent reports emphasize the risk of zoonotic diseases and the high degree of prevalence of asymptomatic animals infected with *Leptospira interrogans*. This report sought to assess the prevalence of antibodies to certain serovars of *L. interrogans*, and to describe the association between seropositivity and risk factors associated with within-flock transmission in a mountainous region of Mexico. Overall seroprevalence to *L. interrogans* was 54·5% (95% confidence interval 48·3–60·7); the most frequent serovar was Icterohaemorrhagiae. The accumulation of placentas and fetuses at a site close to lambing paddocks can play a significant role as a risk factor for within-flock transmission of *L. interrogans* in transhumant farming systems in the municipality of Xalatlaco. The high prevalence of *L. interrogans* antibodies supports the hypothesis that natural foci of this zoonosis are present in sheep flocks in this area. These findings emphasize the need for planning and implementation of control programmes for ovine leptospirosis in Mexico and elsewhere.

Key words: Animal pathogens, infectious disease epidemiology, leptospirosis, zoonoses.

INTRODUCTION

Leptospirosis is an infectious disease with zoonotic implications that is distributed widely throughout the world. Although it is currently recognized as a re-emerging disease caused by *Leptospira interrogans* serovars, 13 pathogenic *Leptospira* species have been identified: *L. alexanderi, L. alstoni* (genomospecies 1), *L. borgpetersenii, L. inadai, L. interrogans, L. fainei, L. kirschneri, L. licerasiae, L. noguchi, L. santarosai, L. terpstrae* (genomospecies 3), *L. weilii, L. wolffii*, with more than 260 serovars [1–3]. This disease also causes economic losses in livestock production systems,

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causing reproductive disorders such as abortion and a reduction in the productivity of affected animals. The prevalence of *L. interrogans* antibodies in domestic animals from different areas in Mexico has yet to be fully investigated, but it has been reported that significant regional variations occur in bovine populations and other species. The proportion of seropositive cattle for leptospirosis in the north, west and south of the country has been estimated at between 31% and 49·7% [4, 5], while more than 34% of examined pigs in backyard farms have been reported in the central region [6]. Studies of human infection with leptospirosis have been conducted. Recent findings show that 37·7% of leptospirosis cases have been identified in rural and urban communities in Chiapas [7], and 20·5% in the state of Yucatan [8]. Coincidentally, both types of environments facilitate human exposure to the urine of *Leptospira*-infected mammals, whether from livestock, peridomestic rodents, wild animals or companion animals (especially dogs) [9].

Although *Leptospira* sp. is a human multi-organ pathogen, it is reported as an important reproductive infectious agent in sheep, which are more likely to develop overt disease from infection caused by serovars Hardjo-bovis and Pomona [10]. There are reports of human infection with serovar Pomona in dairy farms where serological evidence of serovars Hardjo and Pomona have been found in cows and milking crews [11]. This observation suggests that sheep may be a maintenance host for one serovar, showing no evidence of infection, yet being susceptible to infection from other pathogenic serovars [12].

Recent epidemiological studies have reported another zoonotic infectious disease in this area. Chlamydial infections have been identified by serology in humans having previous contact with sheep or by polymerase chain reaction in abortion products of sheep [13–15]. Thus, our objectives were: (i) to estimate the prevalence of *L. interrogans* antibodies in ewes from a mountainous region of Mexico; and (ii) to investigate possible associations of the serological response to *Leptospira* with geographical regions, flock size, animals' access to contaminated water supplies, replacement policies, farm management characteristics, and history of abortion.

METHODS

Study sites

The study was conducted in an 845·2 km^2 area in the southern region of the state of Mexico, Mexico, comprising three geographical regions: valley, intermountain and mountain (19° 05′–19° 15′ N, 99° 20′–99° 35′ W). The geographical characteristics of the area indicate an extensive flat land at 2670 metres above sea level (masl), with high hills, mountains and slopes extending from 2890 to 3273 masl. Agrotechnical activities are widely distributed in the valley region, which can support mechanized agriculture for almost two agricultural cycles per year. No agricultural machinery can be used in transition and mountain regions, but they are suited to seasonal manual agriculture. These lands have a large gradient of up to 30%, with soil derived from volcanic ash capable of fixing phosphorus. Soil depth is >20 cm, with obstruction by rocks in less than 20% and moderate erosion. Water supplies are small lakes, watering places and irrigation canals. In the past 30 years, climatic factors were regularly recorded by the meteorological station 'Almoloya del Rio' (at 2670 masl). Minimum and maximum temperatures ranging between 3·7 °C and 20·2 °C were recorded (mean temperature 11·9 °C), as well as an annual average rainfall of 991·9 mm. Mean rainfall in spring (April–June), summer (July–September), autumn (October–December) and winter (January–March) is 83·2 mm, 175·5 mm, 24·1 mm and 11·8 mm, respectively [16]. The regular movement of flocks is a widespread practice in agricultural areas with a strong environmental impact of the human population (transhumance is the seasonal movement of livestock to regions of different climate). The classic cycle is from low ground in winter to mountain pastures in summer and autumn; the latter is associated with alpine herbage, which is seldom covered by snow. In winter, when animals can barely gather sufficient feed from natural grazing, the sheep consume the edible components and stalks of maize and oats. An ovine census, carried out by the local sheep-farmers' association in 2000, recorded 3762 ewes and 3818 rams and lambs. The ewes were mainly of the Hampshire breed or Suffolk-Pelibuey cross.

Sample size for stratified random sampling

A cross-sectional study was conducted from November 2008 to March 2010 to select a random sample of unvaccinated ewes; sheep flocks were the primary sampling unit. The separated sera were transferred to CENID-Microbiology INIFAP-SAGARPA (Mexico City, Mexico) and stored at −20 °C for serological tests. Seventy-five flocks included in the ovine

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census at the time of sampling were considered for the study. The sampling method was stratified random sampling with proportional allocation [17]. Stratification used flock size as the variable; flock-size comprised three groups: (i) <50 animals; (ii) 51–140 animals; and (iii) >141 animals. The flocks sampled had a 27·8% within-flock prevalence for group (i), 39·4% for group (ii) and 44·4% for group (iii), respectively. Assuming a 95% level of confidence and an error limit of 5%, about 10% of the animals (or all ewes in flocks <10 animals) were randomly sampled from each flock using a random-number generator. To provide accurate estimates, the design effect (D_{eff}) was used to determine the difference in variance between the sample design actually used to obtain the data and a simple random sample of animals. The 35 flocks included in the sample were distributed uniformly throughout the study area and blood samples were collected from 367 animals in selected flocks.

Interview of participating owners

The aim of the study and its confidential character were described to the local sheep-farmers' association. Letters of invitation, along with the proposed sampling schedules, were given to each of the selected farmers. The farmers were required to answer questions in an interview form that recorded management data about each animal and flock (see Supplementary Appendix). This interview form, based on a literature review, included questions about leptospirosis [18, 19]. This study was performed in strict accordance with the recommendations of the Technical Specifications for the Production, Care and Use of Laboratory Animals of the Norma Oficial Mexicana (NOM-041-ZOO-1995) [20]. The protocol was approved by the Institutional Committee of Research and Advanced Studies of the Animal Health Center at the UAEM, Toluca, Mexico (protocol number 2230/2006U).

Serological testing

Antibodies were detected in sera using the microscopic agglutination test (MAT) [19]. The antigens used were live cultures of reference strains of *L. interrogans* serovars Bratislava, Pyrogenes, Grippotyphosa, Pomona, Wolffi and Tarassovi. Owing to the low immunogenicity of some serovars included in the antigen panel, the field strains Icterohaemorrhagiae, Hardjo and Canicola, isolated from animals in different regions of Mexico, were used for serological diagnosis. These field strains were typed by restriction endonuclease analysis of DNA [21]. The minimum serum dilution was 1:50; titres $\geqslant$1:100 were considered positive [22, 23]. The endpoint reading of the microscopic agglutination reaction was reported as the serum dilution at which 50% of the leptospires were agglutinated according to direct visual observation by dark-field microscopy. The sensitivity and specificity of the MAT for detection of *Leptospira*-specific antibodies in animals were 98·2% and 96·4%, respectively [24].

Statistical analysis

The overall prevalence of *L. interrogans* antibodies and the 95% confidence interval (CI) were calculated based on the following equation:

$$\hat{P} = \sum_{h=1}^{L} \frac{N_h}{N} \hat{P}_h,$$

where L is the number of strata in the population, N the number of observations in the population, N_h the number of observations in stratum h of the population, and P_h the true proportion in stratum h of the population

The variance of the estimated population total is given by:

$$\text{Var}[\hat{P}] = \sum_{h=1}^{L} W_h^2 \left(1 - \frac{n_h}{N_h}\right) \frac{\hat{P}_h(1-\hat{P}_h)}{n_h - 1},$$

where n_h is the number of observations in stratum h of the sample and W_h the sampling fraction, N_h/N. Univariate odds ratios (OR) with 95% CIs were estimated for selected factors that could be relevant for *L. interrogans* seropositivity. Factors related to the response variable were identified during the reduction process; factors with a P value <0·25, estimated by the Wald test, were included in the entry model. A multilevel mixed-effects logistic regression (MMELR) was used to model the seropositivity of *Leptospira* and possible risk factors associated with within-flock transmission. The MMELR is a mixed-effects model that takes into account the hierarchical nature of the data and within- and between-subject heterogeneity. The ovine population was considered to have a two-level hierarchical structure, with lower level units at level 2 (animals), nested within the groups at level 1 (flocks). The percentage variance explained by the higher-level hierarchy was

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estimated by the variance partition coefficient. To control the flock as a random effect on the response variable (seropositivity) in the absence of other explanatory variables, the MMELR was adjusted by considering a variance component of zero according to the likelihood ratio test. The random-effects assumption is that the individual specific effects are uncorrelated with the independent variables of the flocks. Variables that exhibited multicollinearity were excluded from the model; this decision was based on an assessment of the potential functional relationships between the variables and a consideration of the effects of *Leptospira* in ewes.

A variable selection algorithm was used to facilitate the incorporation of variables into the final MMELR. The procedure for selecting variables was similar to a stepwise elimination of covariates [25]. The model was built and applied in four steps. (1) The variable 'municipality' was introduced as the first variable of integration. (2) A new variable in the model, 'deviance', was assessed by comparing the current model with the previous one. The additional variable was maintained if the P value of the Wald test was <0·10 based on Schwarz Bayesian (BIC) and Akaike's (AIC) Information Criteria. (3) If any variable in the previous model did not show statistical significance in the presence of a new factor, it was removed from the new model. (4) The procedure was repeated until none of the variables entered or left the final model. The confounding effects of the variables that were not included in the final model were evaluated by successively replacing each variable in the model and assessing the percentage change in the OR of the factors retained. A variable was considered as a confounder if there was a >20% change in the estimated OR [26]. The data were analysed using Intercooled Stata v. 13·0 (StataCorp., USA).

RESULTS

Serum samples from 367 ewes were tested for antibodies against selected *Leptospira* serovars. Of the tested animals, 48% were mainly of Hampshire breed, while 42% were cross-breeds (Hampshire, Suffolk and Pelibuey). The overall seroprevalence of *L. interrogans* was 54·5% (95% CI 48·3–60·7, D_{eff} 1·36) and varied significantly in flock size with respect to the geographical region. Seropositivity increased with flock size, from 37% (95% CI 23·7–50·3) in the <50 animals group to 59·62% (95% CI 52·7–66·5) in the 51–140 animals group and 58·7% (95% CI 46·2–71·2) in the >141 animals group.

Antibodies were detected in the sera of 200 ewes. The MAT results only showed questionable titres in seven (1·9%) other sera (1:50 in sera that tested negative). The prevalence of agglutinins was detected, mostly against serovars Icterohaemorrhagiae (54·5%, 95% CI 48·3–60·7), Bratislava (40%, 95% CI 33·5–46·6), Canicola (19·1%, 95% CI 14·4–23·8), and Tarassovi (15·8%, 95% CI 11·7–19·9). In all, 37% of the seropositive ewes had high antibody titres ($\geqslant$400) to these serovars, and only a correlation between history of abortion and *Leptospira* serovars Icterohaemorrhagiae ($r = 0{\cdot}19$, $P < 0{\cdot}002$) or Tarassovi ($r = 0{\cdot}25$, $P < 0{\cdot}01$) was determined. All the sera that tested positive had antibody titres from 1:100 to 1:6400, with the strongest reactions to Canicola (Fig. 1).

As shown in Table 1, factors affecting the serological status to *L. interrogans* revealed that flock-management characteristics, such as municipality, grazing time, place of lambing, paddock design, cleaning of lambing paddocks and disposition of fomites, were significant risk factors in univariate analyses. A comparison of the 14 possible models is shown in Table 2. The best model, according to BIC and AIC, was the seropositivity of *L. interrogans* as a function of municipality and accumulation of fomites. The mixed-effect coefficients of this model were significantly different from zero (municipality, $P = 0{\cdot}01$; accumulation of fomites, $P = 0{\cdot}02$). The reduction of the variance of the random effect between the intercept-only (variance = 0·55) and the best AIC–BIC model (variance = $1{\cdot}42 \times 10^{-29}$) indicated that the within-flock correlation of the results was well accounted for by the mixed-effects model. In the final multilevel mixed-effects logistic model, seropositivity was found to be significantly associated with the accumulation of fomites (placentas and fetuses) at a site close to lambing paddocks (OR 1·3, 95% CI 1·1–1·7) in transhumant farming systems of Xalatlaco municipality (OR 1·8, 95% CI 1·2–2·7) (see Table 3).

DISCUSSION

The zoonotic potential of pathogenic *Leptospira* serovars is well known and might be a threat to people handling abortive materials from sheep. The overall seroprevalence of *L. interrogans* appears to be very high, with 54·5% of animals in all flocks testing

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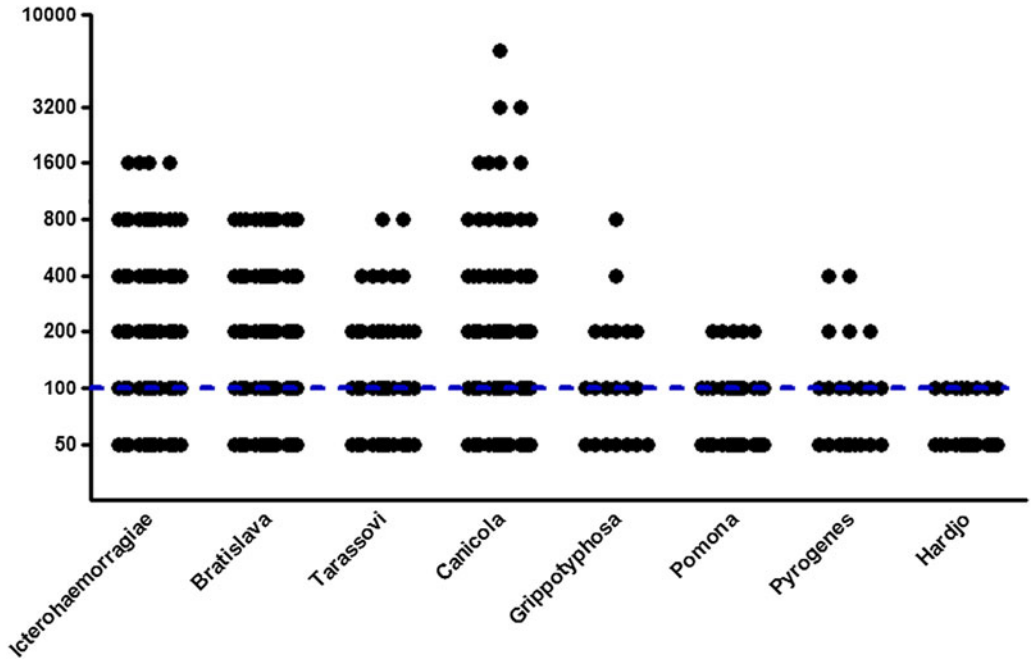

Fig. 1. Distribution of *Leptospira interrogans* antibody titres; cut-off 1:100, according to selected serovars.

positive. This result, demonstrating previous exposure to *Leptospira* is surprising compared to previously reported seroprevalences in countries, such as Australia (32·3%), Nigeria (23·5%), Iran (18·4%), Greece (13·6%), Brazil (3%), and England and Wales (26·3%) [27–32]. The high seroprevalence in animals is not consistent with the small number of cases of human leptospirosis in Mexico. In the past 10 years, 49 human confirmed cases with positive serological reaction were recorded by the Mexican Ministry of Health [33]. Owing to the lack of diagnostic laboratories and a limited reporting system, leptospirosis is one of several neglected diseases in Mexico and this may be one of the reasons why few cases were identified over this period, despite the high carriage of *Leptospira* in domestic animals.

Of all the sheep serum samples submitted for this study, 200 showed positive reactions to *L. interrogans* according to MAT, reacting against the following serovars: Icterohaemorrhagiae, Bratislava, Canicola and Tarassovi. Cross-agglutination and even paradoxical reactions were observed using MAT, but the presence of high-titre seropositivity ($\geqslant$1:400) in 36·6% of seropositive sheep suggests the possibility of infection with these serovars. This feature of the MAT probably resulted in overestimates of overall seroprevalence. The comparisons of seroprevalence estimates should be made with caution, due to different animal populations, serological assays and criteria for seropositivity that are used. The latter is especially important in population surveys, although estimates of prevalence obtained by other research groups for the serological diagnosis of leptospirosis demonstrate that a high concordance of test results does not reflect the complexity of the population from which the sample was obtained. The variances of the estimates produced by our stratified random two-stage sampling method were caused by these features, with potential consequences on the accuracy of estimates [34].

Cluster sampling of specific groups, or primary sampling units, is usually performed rather than simple random sampling, mainly as a means of saving money when the population is widespread and universal sampling is not possible. However, elements in the same cluster are likely to be somewhat similar to one another, resulting, in theory, in an increased intraclass correlation. If an additional element is selected from the same cluster, this observation adds less new information than would an independent selection based on simple random sampling. The post-estimation adjustment that should be used to determine survey sample size and intraclass correlation is the design effect (D_{eff}). In this survey, the D_{eff} for seroprevalence of leptospirosis in sheep was about 1·36; this means that the actual variance under the sampling method actually used is 1·36 times as large as it would have been had we obtained these results from a simple random sample. Estimates of D_{eff} for the prevalence of

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Table 1. Leptospira interrogans *antibodies (%) in the population of ewes and univariate analysis* of the factors associated with seropositivity to* L. interrogans

Variable	No. (%) of ewes		OR (95% CI)	*P* value
	Seropositive ($n = 79$)	Seronegative ($n = 288$)		
Municipality†				0·04
Chapultepec	9 (50)	9 (50)	1·1 (0·3–4·7)	
Santiago-Tianguistenco	18 (45)	22 (55)	0·7 (0·2–1·7)	
Capulhuac	35 (46·1)	41 (53·9)	0·4 (0·2–0·8)	
Xalatlaco	138 (59·2)	95 (40·8)	2·2 (1·3–3·9)	
Production type				0·13
Meat only	33 (46·5)	38 (53·5)	Reference	
Reproduction	167 (56·4)	129 (43·6)	0·7 (0·4–1·1)	
Grazing time				0·04
Permanent	190 (56)	149 (44)	Reference	
Occasional	10 (35·7)	18 (64·3)	2·3 (1·03–5·1)	
Supply of water				0·11
Tap water	121 (58·2)	87 (41·8)	Reference	
Fresh drinking water from lake	79 (49·7)	80 (50·3)	1·4 (0·9–2·1)	
Place of lambing				0·05
Pasture lambing	27 (69·2)	12 (30·8)	Reference	
Shed lambing	173 (52·7)	155 (47·3)	2 (1–4·1)	
Number of ewes in paddocks†				0·03
<5 animals	116 (57·1)	87 (42·9)	1·7 (0·4–7·3)	
6–15 animals	36 (66·7)	18 (33·3)	1·8 (0·9–3·4)	
>16 animals	32 (71·1)	13 (28·9)	2·2 (1·1–4·5)	
Building design of paddocks				0·05
Brick, timber and steel	144 (58·1)	104 (41·9)	Reference	
With easily removable materials	56 (47·1)	63 (52·9)	1·6 (1–2·4)	
Number of ewes housed at night†				0·09
<10 animals	51 (52·6)	46 (47·4)	0·9 (0·6–1·4)	
11–15 animals	100 (50·8)	97 (49·2)	1·1 (0·7–1·8)	
>16 animals	49 (67·1)	24 (32·9)	1·9 (1·1–3·3)	
Cleaning of bed				0·02
No	181 (52·8)	162 (47·2)	Reference	
Yes	19 (79·2)	5 (20·8)	0·3 (0·1–0·8)	
Frequency of cleaning of lambing paddock				0·04
Never	64 (47·4)	71 (52·6)	Reference	
Twice a week	136 (58·6)	96 (41·4)	0·6 (0·4–0·9)	
Disposition of excrement				0·09
Spread as fertilizer	162 (52·6)	146 (47·4)	Reference	
Accumulation of excrement at a nearby place	38 (64·4)	21 (35·6)	0·6 (0·3–1·1)	
Disposition of fomites				0·03
In meadows	136 (79·1)	36 (20·9)	Reference	
Rubbish collection	64 (32·8)	131 (67·2)	1·3 (1·03–1·7)	

OR, Odds ratio; CI, confidence interval.

* Univariate multilevel mixed-effects logistic regression with leptospiral seroreactivity as the outcome, and flock as a random effect.

† Dummy explanatory variables.

agglutinins against *Leptospira* serovars were low, reflecting the regular availability of serum samples in the multilevel design. To determine what sample size to use, it is necessary to take into account that statistical power depends on the total sample sizes for each level. It is normally desirable to have as many units as possible at the top level of the multilevel hierarchy. Generally, in a well-designed survey, D_{eff} ranges from 1 to 3 [35]. However, it is not uncommon for D_{eff} to be much higher, up to 7 or 8, or even 10 [36].

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Table 2. *Schwarz Bayesian and Akaike's Information Criteria for 14 mixed-effect logistic regression models of the serological prevalence of* Leptospira interrogans *in 367 ewes from 35 flocks*

Mixed-effect logistic regression models†	BIC	AIC	*P* value
munic*	513·3	505·5	0·04
MUNIC* + prod	516·3	504·6	0·03
MUNIC* + prod + grazing	304	290·6	0·06
MUNIC* + prod + paddock	304	290·6	0·06
MUNIC** + prod + lambing	302	288·6	0·03
MUNIC* + lambing + water	303·2	289·7	0·05
MUNIC* + lambing + anicor	303	289·2	0·04
MUNIC* + lambing + encierro	302·6	289·1	0·04
MUNIC* + lambing + cleaning	302·9	289·6	0·05
MUNIC** + lambing + flambing	302·4	289	0·03
MUNIC** + lambing + excrement	301	287·5	0·02
MUNIC** + excrement + fomites*	301·1	287·7	0·02
MUNIC* + excrement	298·9	288·8	0·03
MUNIC** + fomites*	296·3	286·2	0·01

BIC, Schwarz Bayesian Information Criterion; AIC, Akaike's Information Criterion.
MUNIC, Municipalities; PROD, sheep production; GRAZING, grazing time; PADDOCK, design of lambing paddock; LAMBING, place of lambing; WATER, supply of water; ANICOR, ewes in paddock; ENCIERRO, number of ewes housed at night; CLEANING, cleaning of bed; FLAMBING, frequency of cleaning of lambing paddock; EXCREMENT, disposition of excrement; FOMITES, disposition of placentas and fetal remains.
† With flock as the random effect.
* $P < 0·05$
** $P < 0·01$.

Table 3. *Final multilevel mixed-effect logistic regression model for risk factors associated with seropositivity to* Leptospira interrogans *in ewes from transhumant farming systems in Mexico*

Variable	S.E.	OR (95% CI)	*P* value
Xalatlaco municipality	0·39	1·8 (1·2–2·7)	0·01
Accumulation of placentas and fetal remains at a site close to lambing paddocks	0·16	2·4 (1·2–4·7)	0·02
Intercept	0·14	0·5 (0·3–0·9)	0·02

S.E., Standard error; OR, odds ratio; CI confidence interval.

Flock size is a feature of transhumant farming systems, and stratification was a way of breaking up the population of ewes into different mutually exclusive groups. Once the strata were defined, a sample from each stratum was tested as if it were independent of all the other strata. This is why the probability weight for animals from different flock-size strata is expected to be different. A large number of primary sampling units from each stratum was required to reduce the standard error of the estimates in our epidemiological survey [37].

The sampling and probability weight were established to expand the sample to the population level represented by the sample. In our two-stage design, the probability weight was calculated as the product of two probabilities; the inverse of the sampling fraction (number of elements in the population and number of animals in the sample) for the first stage was multiplied by the inverse of the sampling fraction for the second stage. Ewes were selected with a probability of 0·04, and the animals sampled represented 25 animals of the entire population.

The final MMELR appeared to fit the data adequately (Pearson's goodness-of-fit test statistic = 2·41, $P = 0·49$). A significant (deviance) statistic would show that the MMELR is inappropriate for the data. The area under the ROC curve (0·64) was significantly different from 0·5, since the *P* value was 0·000, indicating that the MMELR classified the group significantly better than chance. Our MMELR had an acceptable predictive ability; 213 (58·04%) of the ewes sampled were correctly classified by sensitivity (80%) and specificity (31·8%). The accumulation of fomites (placentas and fetal remains) at a site close to the lambing paddocks in the municipality of Xalatlaco remained independently associated with seroreactivity to *L. interrogans*, suggesting that these two factors together were the strongest independent predictors in the evaluation of epidemiological risk factors. The increase in leptospirosis seroreactivity was closely associated with municipality; this was consistent with findings of other seroprevalence studies [38]. Xalatlaco municipality is located in the valley region, where frequency of contact between sheep and other animals in these flocks is necessary to ensure transmission and, hence, to maintain an endemic focus. Ovine transhumant farming systems are mixed-species enterprises that also contain cattle, dogs, rats or wild animals, some species of which are maintenance hosts for *Leptospira* serovars [39]. Compared to sheep flocks from other municipalities, the highest seroprevalence estimate was 59·2% (95% CI 51·4–68·6) in flocks from Xalatlaco municipality.

Ewes were at higher risk of seroreactivity to *Leptospira* when there was an accumulation of placentas and fetal remains at a site close to lambing

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paddocks. In this case, relative differences in sanitation between flocks contributed to increase the risk of seroreactivity, along with characteristics of the environment in which the sheep reside. Seropositivity increased in those flocks where placentas and abortion remains were scattered in the meadow, from 50% (95% CI 41·9–58·1) to 69% (95% CI 55·6–82·4) when fomites remained very close to the lambing pens. Some farmers keep their sheep outside year-round and lambing paddocks are small pens with an open yard and little drainage in areas susceptible to frequent flooding. The rise in water level of up to 1 metre during flood events and inadequate drainage in these areas due to waste obstruction could be related to a mechanism of indirect transmission, where healthy ewes are exposed to water contaminated by abortion products or the urine of other infected animals [40–42]. Leptospires may be excreted with placentas or fetal remains into the environment, where they can survive for several months, depending on environmental conditions. Human infection may occur via direct contact with the carrier's urine (companion animals) or indirectly through a urine-contaminated environment. A probable transmission route can be established based on our results. Canicola, Pomona and Icterohaemorrhagiae serovars are frequently transmitted to humans via dogs, but transmission sources require further investigation [8].

The high prevalence of *L. interrogans* antibodies supports the hypothesis that natural foci of this zoonosis are present in transhumant sheep farming systems in the southern region of the state of Mexico. However, the impact of the transmission of this zoonotic pathogen from ovine livestock to humans is unknown. The conclusion that the geographical region where the flocks are located and an unhealthy environment contribute to the risk of *L. interrogans* infection suggests that prevention and control of leptospirosis in ewes in transhumant farming systems must combine an improvement in sanitation and the control of shedding animals, both of which cause unequal animal health outcomes.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268814003549.

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DECLARATION OF INTEREST

None.

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