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Animal & Human Leptospirosis In A High Transmission Setting In Fiji

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ANIMAL & HUMAN LEPTOSPIROSIS IN A HIGH TRANSMISSION SETTING IN FIJI

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Abstract

Leptospirosis is a neglected zoonotic disease with a worldwide distribution, yet disproportionally affects poor rural subsistence farmers in the tropics. The animal reservoirs for spill-over infection to humans in such settings in the South Pacific have not been well delineated, thus hampering effective control efforts. We conducted a case control investigation among households that participated in a seroprevalence survey for leptospirosis in Western Fiji. We surveyed domestic animals and trapped rodents at 45 cases and 73 control households who had one or more, and no inhabitants with evidence for anti-leptospire agglutinating antibodies. We performed serology among all animals and used polymerase chain reaction to detect *Leptospira* DNA in kidneys of trapped rodents. One or more seropositive animals were identified among 78% of the 96 households with domestic animals or trapped rodents. There was not a significant difference between the presence of seropositive animals between case and control household (67% vs 85%, respectively). Agglutinating antibodies were detected from a high proportion of households with horses (85%) and cattle (73%), indicating that the seroprevalence of leptospirosis in livestock was high in this region. Agglutinating antibodies against serogroup Australis, which was recognized by 64% of the seropositive human inhabitants, were detected from six of the seven animal species. Additionally, a proportional similarity index analysis indicated that cattle, horses, dogs, rodents and humans form a transmission network. There was a non-significant trend for *Leptospira* DNA positive rats to be trapped in case vs control households (OR 5.71, p=0.09). Our studying findings indicate that there exists a complex network of transmission between livestock, domestic animals and rodents in Western Fiji, and the source for human leptospirosis cannot be attributed to a single reservoir species. Therefore, control of leptospirosis in rural Fiji and similar high transmission settings will need to rely on multiple intersectorial strategies that target prevention of leptospirosis in livestock, rodent control, the use of personal protection and barrier approaches, and reduction of high risk behaviors.

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Introduction

Leptospirosis is a neglected zoonotic disease that causes 58,900 deaths and 2.9 million Disability Adjusted Life Years (DALYs) per year.[1, 2] It disproportionally affects underprivileged populations in urban slums and rural subsistence farmers in Africa, South America, Asia and the Pacific [1, 3, 4]. It is a growing burden in urban communities with high population density, as well as in rural agricultural settings.[3, 5, 6] Leptospirosis is caused by spirochete *Leptospira* bacteria, which are transmitted to humans from animal urine or urine contaminated water and soil. [7] It causes symptoms including fever and jaundice, and may lead to a small portion of patients to exhibit severe symptoms such as meningitis, respiratory distress, pulmonary hemorrhage and Weil's disease (serious icteric form). [7] The case fatality ratio of leptospirosis is 7%, although this number is likely a significant underestimation. [2]

Tracing the source of human infection is challenging, due to the complex transmission of leptospirosis which is affected by various animal reservoirs, host behavior and the environment. [8] Reservoirs that contribute to the maintenance and spill-over transmission to humans vary significantly across regions [7]. Reservoirs are inferred by identifying prevalent *Leptospira* serogroups in animals and humans by detecting agglutinating antibodies, or by identifying risk factors related to animal exposures through regression analyses. While transmission in urban slums have been largely attributed to rodents, leptospirosis in rural regions have been linked to exposure to farm animals, dogs, cats and rodents [9 5, 10]

Leptospirosis poses a significant public health burden in the South Pacific, where outbreaks and sporadic cases have been reported from seven countries, and several countries have been classified as hyperendemic. [11 12] In 2012, Fiji experienced an outbreak of leptospirosis following two floods, which caused 576 reported cases and 7% case fatality. [13] Sources of infection to humans living in the South Pacific are mixed. One study found that the most likely reservoirs in several countries in the South Pacific were rodents. [11] On the other hand, a seroprevalence study conducted on human subjects in Fiji found that having pigs in the community and high cattle density were associated with a higher risk of detecting

agglutinating antibodies in humans. [13] Due to the paucity of studies and inconsistent findings, the relative importance of livestock, companion animals and rodents as a transmission source to humans remain unanswered. In order to implement effective interventions, it is imperative to identify important sources of human infections, and tailor prevention and control measures to local transmission characteristics. Challenges are to allocate limited resources to tackle the complex transmission cycle through interventions such as rodent control, public education, improved hygiene around the household, promoting use of protective gear and animal vaccination. [14, 15]

Using a case control design linked to a previous human seroprevalence study, we examined the prevalence of antibodies and pathogenic *Leptospira* DNA in peridomestic animals in a high transmission setting in Ba, Western Fiji. This rural and agricultural region experienced a post-flood outbreak in 2012, and is characterized with a wide variety of prevalent serovars. Our study aims to provide evidence for improving public health interventions in order to mitigate further transmission of leptospirosis and prevent future outbreaks in high transmission settings.

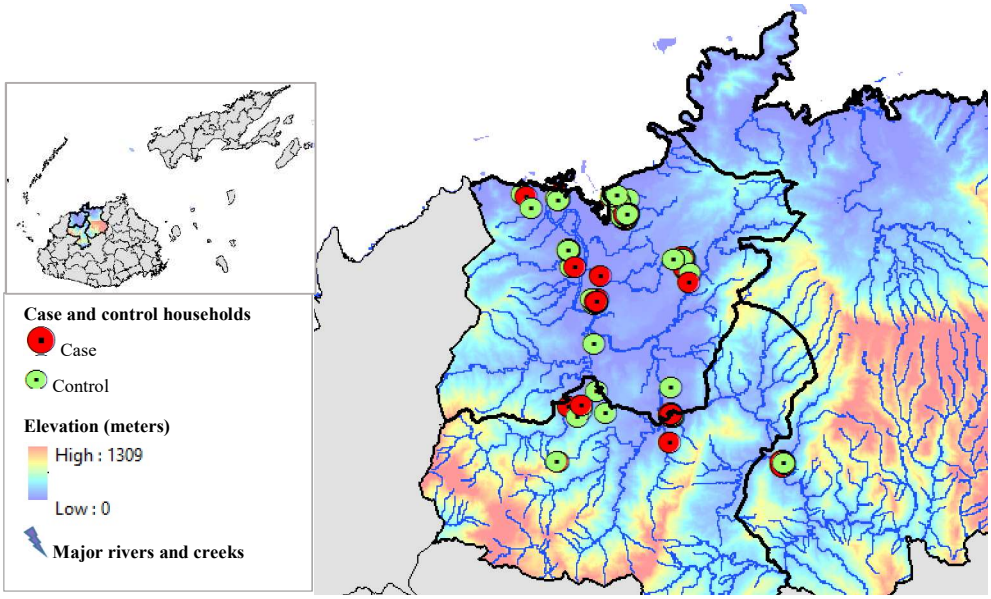
Materials and Methods

Study location and population

This study was conducted in the subdivision of Ba, Western Division of Fiji. Ba is located in the North-West of Viti Levu, one of the main islands of Fiji. The human seroprevalence study by Lau et al.[13], which this study is based on, was conducted in 81 communities across Fiji on three main islands. However, sampling for this study was restricted to Ba for two reasons: a diverse distribution of *Leptospira* serogroups were observed in humans compared to other regions in the study, and a high incidence of leptospirosis was observed during an outbreak in 2012. [13] Sampling was conducted in ten villages and settlements, from Toge, Navala, Veisaru, Lavuci, Naidrodro, Sarava, Sorokoba, Votua, Tabataba and Yalalevu. Agriculture is common in the Ba, where 53% of land is used for crops, coconut farms and pastures. Approximately 50% of cattle farms in Fiji are located in the Western Division, where almost

20,000 cattle are owned.[16] Many subsistence farmers own several animal species in close proximity to each other and to people's homes. Approximately 5% of the population aged 15 and older are unemployed, and the under 5 year mortality rate is over 35%.[17]

Figure 1. Geographic location of case and control households in Ba
a. National map of Fiji and study location, b. Study location with case and control households



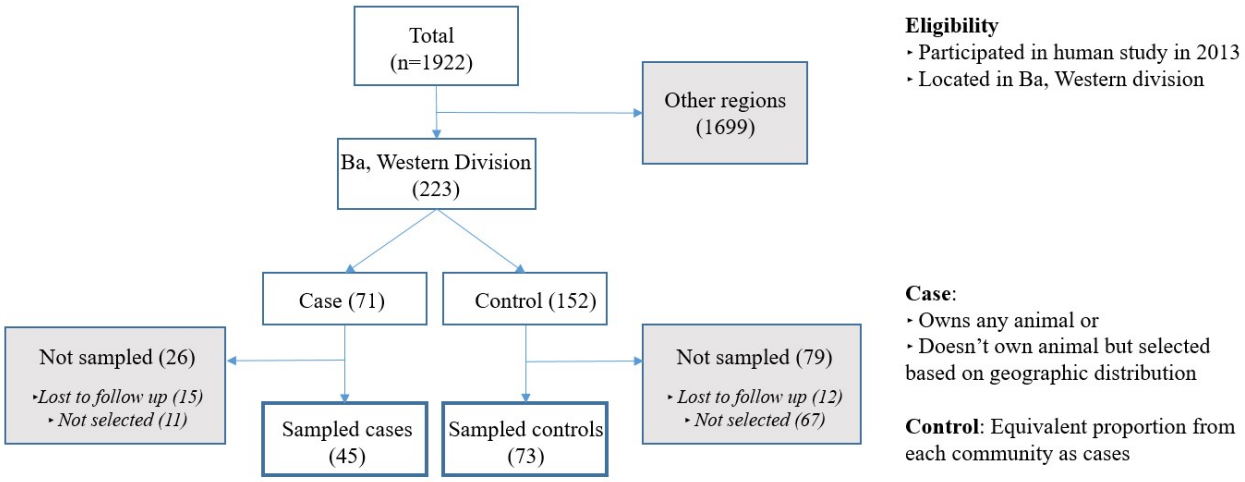
Study design and sampling design

A case control study was conducted based on a previous cross-sectional study of leptospirosis on 2152 human subjects in 2013 [13]. A case household was defined as a household in which at least one member of household was seropositive as determined by Microscopic Agglutination Test (MAT), defined as reactive at a 1:50 titre or higher dilution for one or more serovars. A control household was defined as a household in which no member of the family was seropositive as determined by MAT. Samples were taken from peridomestic animals that were owned by or caught in the proximity of households.

An overview of the sampling design is shown in Figure 2. In the human seroprevalence study, 1922 households were sampled by population-proportionate sampling and purposeful sampling approaches, and up to three randomly selected household members were invited to participate.[13] In Ba, 223 households were sampled, of which 71 were identified as cases and 152 as controls. An initial target to obtain 50

cases and 50 controls was set, and selected by a three-stage sampling scheme (Figures in Appendix). First, 223 households were categorized into cases and controls, and stratified by 10 communities. Second, all case households that owned at least one species were selected, according to the questionnaire from 2013. Then, up to six case households were selected from each community, based on the number of cases available. Finally, 50 control households were selected from 152 potential control households so that the proportion of controls selected from each community was equivalent to the proportion of cases selected from each community.

Figure 2. Sampling scheme of case and control households



During the field operation, households lost to follow up were replaced with other households in the same community, in order to obtain an adequate sample size. Households were identified as lost to follow up if a neighbor confirmed relocation, if the household was uninhabited, or vacant when the team visited two to three times within a two to three-day period. If a case was lost to follow up, it was replaced with another case from the same community, or if unavailable, replaced with a control from the same community. This resulted in a smaller number of cases compared to controls. If a control was lost to follow up, it was replaced with another control from the same community. Ultimately, 45 case and 73 control households included in the study (Figure 2).

Informed consent and ethics approval

Ethical approvals were granted by the Ministry of Health and Medical Services, and the Ministry of Agriculture in Fiji prior to the beginning of field work. The ethics approval granted for the human seroprevalence study was extended and expanded to this study by the Fiji National Research Ethics Review Committee (2013 03). The procedures performed on live animals were approved by the Massey University Animal Ethics Committee (Ref# 15/70).

Questionnaire

A standard questionnaire was conducted in English, Fijian or Hindi depending on the participants' preference. Questions related to ownership of animals, animal handling practices, drinking water source for animals and other potential risk factors for leptospirosis for each household. If available, people who had their blood sampled in 2013 were interviewed. If unavailable, family members of the same household were interviewed. In addition, Geographic Positioning System (GPS) coordinates of the place of residence, using handheld GPS devices.

Sampling from livestock and dogs

At the time of household visit, information on the number of animals owned was collected. All livestock and dogs in the household were sampled unless the animal was inaccessible, or if there were more than 7 animals of the same species, in which case a maximum of 6 animals were sampled. Up to 10 mL of blood was collected by venipuncture using a 10 mL syringe or vacutainer without anticoagulant via a 18 or 22 gauge 1.5-inch needle. Cattle and horses were restrained by tying to trees using ropes around the neck and/or hind legs to restrict movement. Venipuncture was conducted in the coccygeal vein. Pigs and goats were manually restrained by trained staff and blood was collected by venipuncture from the coccygeal vein for goats, and auricular vein for pigs. Dogs were manually restrained using a hand-made muzzle. In some cases, Xylazine hydrochloride was used to sedate the dog, and venipuncture was performed on the cephalic or saphenous vein.

Sampling from rodents

One metal rodent trap was set in each household at the time household visit. Locally acquired bait (roti bread with peanut butter) was placed inside of the trap, and traps were set in the kitchen, bedroom, ceiling or shed. All traps were collected after three nights, regardless of whether or not a rodent was captured. If a rodent was captured before three nights, the field team was contacted, and the rodent was collected the same or following day for euthanasia and dissection. Species of the rodent was determined by a veterinarian based on physical features. In the laboratory, rodents were placed in a plastic bag with halothane impregnated cotton to achieve anesthesia. The rodent was checked for physical signs such as the palpebral reflex to ensure adequate level of anesthesia. The heart was exposed by thoracic median section, and the rodent was euthanized by exsanguination from the right cardiac ventricle using a 5mL syringe and 22 gauge 1.5-inch needle. Almost all rodents were alive and anaesthetized before the dissection. If the rodent had recently died in the laboratory, only the kidney sample was collected. After the rodent was euthanized, complete nephrectomy was performed. After the kidney was extracted, it was immersed in 70% v/v ethanol in at least five times the volume of the kidney. Samples were sent from Fiji to Institute Pasteur in New Caledonia by air courier in compliance with International Air Transport Association (IATA) guidelines.

Serological analysis

Blood samples were left to clot and centrifuged for 10,000 rpm for 10 minutes. Serum was stored in a freezer (-80ºC) until they were sent to Hopkirk Institute in New Zealand by air courier in compliance with IATA guidelines. Microscopic agglutination tests (MAT) were used to detect anti-*Leptospira* agglutinating antibodies, and determine the putative serogroups associated with past infection. The MAT is the reference serological test recommended by the World Health Organization as the golden standard for serodiagnosis.[18] Seven pathogenic serogroups were selected for the MAT panel for this study and tested per standard protocol: *Leptospira interrogans* serogroups Australis, Canicola, Icterohaemorrhagiae and Pomona, *L. borgpetersenii* serogroup Hardjobovis, Tarassovi and Ballum. Samples were tested at

eight levels of dilution from 1:24 to 1:3072. MAT titers of ≥1:48 were defined as seropositive and indicative of a past infection. This titre was chosen in order to be consistent with the definition used in the human seroprevalence study of ≥1:50.[13] If a sample reacted to multiple serogroups at a MAT titer of ≥1:48, the serogroup with the highest titre was considered to be the reacting serogroup. If a sample reacted to multiple serogroups at the same titre level at 1:48 or above, they were considered to be mixed infections. All seven serovars that were tested in this study were classified into separate serogroups.

DNA detection

Pathogenic *Leptospira* spp. was detected through quantitative polymerase chain reaction (qPCR) targeting the LipL32 gene, which codes an outer membrane lipoprotein and is only present in pathogenic *Leptospira*.[19] DNA was extracted from kidney samples using the QIAmp DNA minikit, and a set of forward (lipL32-45F) and reverse (lipL32-286R) primers and the TaqMan probe lipL32-189P were used to amplify a fragment of 242 bp of *lipL32*. The cycling conditions were as described in the original publication in a LightCycler 480 (Roche Applied Science, New Zealand). A positive sample was defined as detection of 1≤ *lipL32* DNA fragment per μ L of DNA extract. The quantification was achieved using a standard curve from serial dilutions of known number of leptospires and normalized to the initial weight of the piece of kidney used for extraction. For all negative samples, detection of a mammal beta-actin [20, 21] was performed using a similar qPCR procedure as a control for PCR inhibitors.

Data analysis

Data was stored in Microsoft (MS) Excel Spread Sheet program and all analyses were conducted using R version 3.2.1. Household prevalence was calculated by dividing the number of households with at least one positive serum sample by the number of all households tested. In order to examine the serovar distribution among host species, the Proportional Similarity Index (PSI), or Czekanowski index, was calculated to measure the degree of association between host species and serogroups. This measured the

similarity between the frequency of the seven serovars from the seven host species. This index represents a measure of the area of intersection between two frequency distributions [22]. The PSI was estimated such that:

$$PSI = 1 - 0.5\sum_{i} |p_i - q_i|$$

where p_i and q_i represent the proportion of samples belonging to serovar i out of all serovars from hosts p and q [22, 23]. The values for PSI range from zero to one, where zero indicates that two hosts do not share any serovar, and 1 is identical proportions (p, q) of all serovars in two hosts. This analysis measures the tendency of two hosts to harbour similar serovars, which is influenced by host, serovar transmission, and environmental factors such as direct or indirect contact between species. Bootstrap confidence intervals for PSI values were computed [13,14].

Logistic regression was fitted to a binary case-control outcome. Questionnaire and laboratory data were used as exposure variables. Co-linearity and correlation between variables were analyzed by calculating Cohen's kappa value or Phi correlation coefficient value. Univariable logistic regression was fitted to independently assess various risk factors for detecting *Leptospira* antibodies in humans. In the initial multivariable model, variables were included if they were laboratory test results, or if variables had a significance level of $p<0.10$ in the univariable logistic regression. Highly correlated variables were aggregated in a single variable and included in the model. The final multiple logistic regression model was obtained using forward variable selection with the likelihood ratio test, with an inclusion rule of $p<0.10$. In this final step, all variables were considered.

Multiple correspondence analysis (MCA) was carried out including all exposure variables and the case-control status of each household. This analysis was conducted to provide visual representation on the various variables examined in this study. The MCA was used to project a two-dimensional projection of relationships between variables categories and case and control outcomes.

Results

Household characteristics

A total of 45 case and 73 control households were included in this study. A majority of households were Christian and of iTaukei ethnicity (70% and 93%), with a median of four household members. More case households were inhabited by iTaukei families in villages compared to control households ($p<0.01$). A median of three household members were sampled from case households, and two members from control households ($p<0.01$). Among households that owned animals, a median of two animals were present in the household. Case and control households that were sampled in this study were representative of all case and control households included in the previous study with regards to demographics such as religion, community type, urban or rural status, number of household members and number of household members sampled. Based on the questionnaire, animal owners owned one to three animals of each animal species. For rodents, 49 were caught in case households, and 69 were caught in control households.

Table 1: Characteristics of case and control households

	Case household (n=45) Median (1st, 3rd QR) or No. (%)	**Control household (n=73)** Median (1st, 3rd QR) or No. (%)	**Total households (n=118)** Median (1st, 3rd QR) or No. (%)	**P-value**
Religion				<0.01*[a]
Christian	41 (91.11)	42 (57.53)	83 (70.34)	-
Hindu	3 (6.67)	24 (32.88)	27 (22.88)	-
Muslim or other	1 (2.22)	7 (9.59)	8 (6.78)	-
Ethnicity				<0.01*
iTaukei	42 (93.33)	44 (60.27)	86 (72.88)	-
Indofijian or other	3 (6.66)	29 (39.73)	32 (27.12)	-
Community type				<0.01*
Village	28 (62.22)	24 (32.88)	52 (44.07)	-
Settlement	15 (33.33)	40 (54.79)	55 (46.61)	-
Private residential	2 (4.44)	9 (12.33)	11 (9.32)	-
Urban/rural				0.72
Urban	9 (20.00)	18 (24.66)	27 (22.88)	-
Rural	36 (80.00)	55 (75.34)	91 (77.12)	-
Household members				
Number of household members	4 (4, 6)	5 (3, 6)	4 (3.25, 6)	0.68
Number of people sampled	3 (2, 3)	2 (1, 3)	2 (1, 3)	<0.01*[b]
Number of people positive for any serovar	1 (1, 1)	0 (0, 0)	0 (0, 1)	<0.01*
Number of animals owned among animal owners based on survey	2 (1, 4)	2 (2, 7.25)	2 (1.75, 5)	0.04*
Cattle	1 (1, 2)	2 (1, 3.25)	2 (1, 3)	0.23
Goat	1 (1, 3)	4 (2, 6)	3 (1, 6)	0.14
Pig	1.5 (1.25, 1.75)	2.5 (1.75, 3)	2.5 (1.75, 3)	0.21
Horse	1 (1, 1)	1 (1, 1.25)	1 (1, 1)	0.50
Dog	1 (1, 2)	1 (1, 2)	1 (1, 2)	0.35
Number of rodents trapped	49	69	118	-

[a] Fisher's exact test was conducted instead of Chi-square test when one or more of the expected values from the 2x2 tables were less than 5.
[b] Wilcoxon rank sum test was used when sample data was not normally distributed.
* Statistically significant at $\alpha=0.05$ level.

Seroprevalence

Among 45 case households, a majority of inhabitants of the household were reactive against *Leptospira interrogans* serogroup Australis (64%), followed by serogroup Canicola (13%), and mixed infection, where the maximum titre was shared by two or more serogroups (11%). Few case households had inhabitants who were positive for serogroups Icterohaemorrhagiae (7%), and Ballum (4%), and no households had people who tested positive for serogroup Hardjo. No control households had MAT seropositive participants, as per case definition.

Table 2. Microscopic Agglutination Test results for animals and humans by serogroup

	Aggregated animal result				Human result
	Case household No. (%)	**Control household** No. (%)	**Total household** No. (%)	**OR (95%CI)**	**Case household** No. (%)
	n=36	n=60	n=96		n=45
Positive MAT for any serovar (serogroup)	**24 (66.67)**	**51 (85.00)**	**75 (78.12)**	**0.35 (0.13,0.96)**	**45 (100.00)**
Pohnpei (Australis)	-	-	-	-	29 (64.44)
Australis (Australis)	2 (5.56)	3 (5.00)	5 (5.21)	1.12 (0.17, 7.20)	0 (0.00)
Ballum (Ballum)	4 (11.11)	8 (13.33)	12 (12.50)	0.81 (0.22 2.97)	2 (4.44)
Canicola (Canicola)	0 (0.00)	0 (0.00)	0 (0.00)	-	6 (13.33)
Copenhageni (Icterohaemorragiae)	7 (19.44)	14 (23.33)	21 (21.88)	0.79 (0.28, 2.23)	3 (6.67)
Hardjo (Sejroe)	0 (0.00)	1 (1.67)	1 (1.04)	0.54 (0.00, 0.75)	0 (0.00)
Pomona (Pomona)	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Tarassovi (Tarassovi)	0 (0.00)	1 (1.67)	1 (1.04)	0.54 (0.00, 0.75)	-
Mixed	11 (30.56)	24 (40.00)	35 (36.46)	0.66 (0.27, 1.61)	5 (11.11)
Negative	12 (33.33)	9 (15.00)	21 (21.88)	2.83 (1.04,7.73)	-

Positive MAT (1:48 or higher) for mixed serovars indicates that the maximum titre was shared with more than one serovar.

Serum samples were collected from 367 animals from seven species: cattle, horses, goats, pigs, dogs, rats and mice from 36 case and 60 control households (Species-stratified table in appendix). Serogroup Australis, which was the predominant serogroup recognized in humans (64%), was detected from cattle, horses, goats, dogs, rats and mice. Rats were sampled from the highest number of households, followed by dogs and cattle (48, 38 and 33 households, respectively). Pigs were only sampled from four households. Among a total of 96 households, 78% had at least one animal that tested positive for any serovar. Predominant serogroups for different animal species were serogroups Sejroe and Ballum for cattle (18% and 12%), serogroup Ballum for horses and goats (30% and 19%), and serovar Icterohaemorrhagiae for dogs, rats and mice (31%, 21% and 30%). Only one cow tested positive for serogroup Tarassovi, and no animals tested positive for serogroup Pomona. More controls tested positive

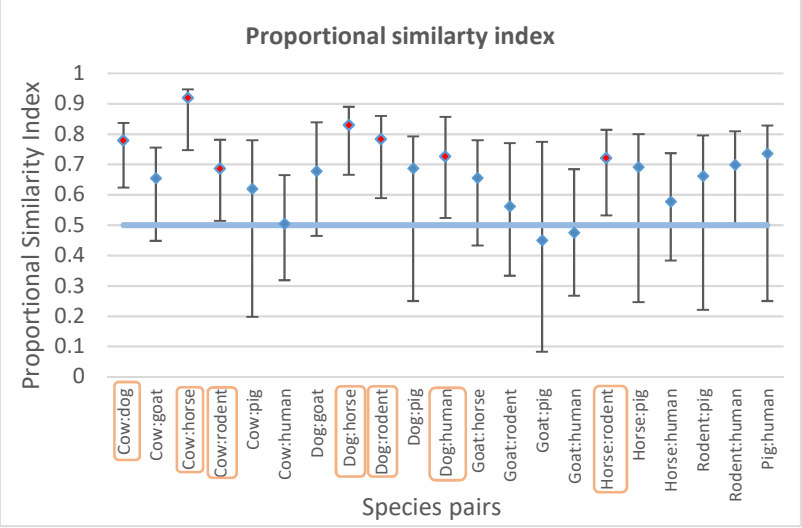
for at least one animal for any serovar compared to cases (85% vs 67%, p=0.04). When stratified by animal species, 85% of households with horses were seropositive and 73% of households with cattle were seropositive.

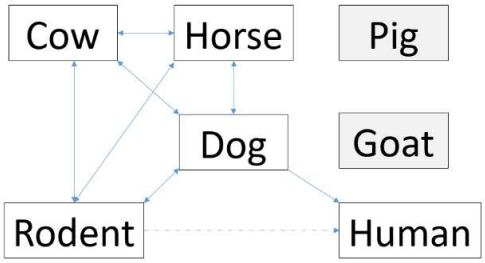
Proportional Similarity Index

A pairwise comparison of animal species using the proportional similarity index showed significant correlation between the following seven pairs of animals out of 21 possible pairs; cows and goats, cows and horses, cows and rodents, dogs and horses, dogs and rodents, dogs and humans, horses and rodents. The association between humans and livestock were not significant. The association between humans and rodents was marginally non-significant. However, humans were significantly associated with dogs. In this sample, pigs and goats were not significantly associated with any other animal species.

Figure 3. Pairwise comparisons of animals and humans by serovar distribution

a. Proportional similarity indices, b. Relationship of significant pairs based on proportional similarity indices





Detection of pathogenic Leptospira *DNA*

A total of 119 rodents, including 51 rats, 56 mice and 11 unidentified rodents were caught from 80 households. Unidentified rodents indicate rodent which could not be specified between rats and mice

without further examination. Pathogenic *Leptospira* DNA was detected from 34% of households. One mouse captured in a control household showed PCR inhibition and was excluded from the analysis. *Leptospira* DNA was detected from more rodents caught in case households than in controls, although not statistically significant (42% vs 29%, p=0.32). The prevalence of households with PCR positive rodents was higher in mice (19%) compared to rats (10%).

Table 3. Number of households with pathogenic *Leptospira* DNA

Number of households with PCR positive rodent	**Case household** No. positive (%)	**Control household** No. positive (%)	**Total household** No. positive (%)	**OR of cases and controls (95% CI)**
	n=31	**n=49**	**n=80**	
Total rodent	13 (41.94)	14 (28.57)	27 (33.75)	1.81 (0.70, 4.69)
Rat	5 (16.13)	3 (6.12)	8 (10.00)	2.91 (0.52, 20.24)
Mice	7 (22.58)	8 (16.33)	15 (18.75)	1.49 (0.47, 4.68)
Unidentified species	1 (3.23)	3 (6.12)	4 (5.00)	0.52 (0.01, 6.76)

[a] Fisher's exact test was conducted instead of a Chi-square test if the expected cell count was less than 5.
[b] Unidentified rodent indicates a rodent which could not be differentiated from rats and mice without further examination, and was beyond the scope of this study.

Risk factors

A total of 50 independent variables regarding household characteristics, animal interaction and laboratory results were assessed in the univariable logistic regression. Eleven variables that were statistically significant in the univariate analysis were considered for the multiple logistic regression. Three significantly correlated variables, ethnicity, religion and community type were combined as a new variable 'household type' (iTaukei in villages, iTaukei in non-villages, and non-iTaukei in non-villages). There were no non-iTaukei in villages. The final model included three variables; household type, detection of *Leptospira* antibodies in rat serum and detection of pathogenic *Leptospira* DNA in rat kidneys. Households that were of iTaukei ethnicity located in villages were 12.0 times as likely to be case households compared to non-iTaukei households that were not located in villages ($p<0.01$). Similarly, households that were iTaukei located in a settlement or other communities were 6.3 times as likely to be cases ($p<0.01$). Adjusting for household type, households with DNA positive rats were 5.7 times as likely to be cases compared to households with DNA negative rats (p=0.09). Households that captured rats that

tested positive for antibodies were 66% less likely to be a case household compared to households that captured antibody negative rats, although not statistically significant.

Table 4. Risk factors for human leptospirosis

Variable	Category	Univariable Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)
Household type [a]	iTaukei, village	-	11.95 (3.44, 57.09)*
	iTaukei, non-village	-	6.27 (1.74, 30.19)*
	Non-iTaukei, non-village	-	1.00
Ethnicity	iTaukei	9.23 (2.99, 40.57)	-
	Non-iTaukei	1.00	-
Community type	Village	3.36 (1.57, 7.43)	-
	Non-village	1.00	-
Rat MAT	Positive	0.85 (0.25, 2.80)	0.34 (0.06, 1.56)
	Not tested	1.13 (0.46, 2.90)	0.92 (0.29, 2.90)
	Negative	1.00	1.00
Rat DNA	Positive	2.95 (0.67, 15.33)	5.71 (0.86, 50.67)*
	Not tested	1.03 (0.45, 2.32)	1.34 (0.46, 4.08)
	Negative	1.00	1.00

[a] The variable "household type" included in the multiple logistic regression is a combination of "Ethnicity" and "Community type" variables in the univariable logistic regression.
* Statistically significant at alpha=0.10 level

Multiple Correspondence Analysis

The multiple correspondence analysis was conducted for four different categories of variables; (1) household characteristics and animal ownership, (2) laboratory test results, (3) drinking water source for animals, and (4) animal birth, slaughter and rodent sightings (Figures in Appendix). The analysis found that rodent DNA detection, village dwellers, being Christian and iTaukei (indigenous Fijian), having a stream near the household, household flooded between 2010 and 2013 were closely associated with a household being a case compared to a household being a control, relative to other variables. Results of this analysis were similar to that derived from the multiple logistic regression.

Discussion

Implementing effective interventions for rural leptospirosis in a high transmission setting has been a challenge, due to the lack of information on animal risk factors. In this community-based case control study in Fiji, we found that 78% of households with animals had serologic evidence for a prior *Leptospira* infection. The high overall household seroprevalence of leptospirosis indicates that *Leptospira* is highly

prevalent in various animal species in Fiji. Other animal seroprevalence studies found that 12% of livestock were MAT reactive in Thailand, and 2% of rodents, 8% of cattle and 38% of dogs were MAT reactive in Tanzania. [24, 25] The predominant serogroup in humans, serogroup Australis, was detected in six animal species. In addition, our Proportional Similarity Index analyses found that pairs of serogroup distribution between cows, horses, dogs, rodents and humans were significantly associated. Therefore, in a rural and high transmission setting, leptospirosis transmission cannot be attributed to a single species, but instead forms a complex network between humans and animals, including livestock, dogs and rodents. To our knowledge, this study is the first to investigate the risk factors for human leptospirosis among livestock, companion animals and rodents, thereby comprehensively examining various exposures of people in Pacific Island Countries.

The previous study in humans indicated that pigs in the community and high cattle density in the district are significant risk factors in Fiji [13]. In our study, 73% of households that owned cattle were MAT reactive. Moreover, cattle were significantly associated with the infection of horses, dogs, and rodents in the proportional similarity index analysis. Although the density of cattle measured in the previous human study were predominantly commercial cattle, whereas this study were cattle from subsistence farming, both results indicate that cattle may play an important role as a risk factor for leptospirosis. Regions with heavy dairy farming have been associated with higher incidence of leptospirosis in New Zealand. [26] A large proportion of households with horses were MAT reactive (85%). On the other hand, there were only four households that owned pigs that were available to be sampled in Ba, and only two households owned pigs that had signs of previous infection of leptospirosis. Furthermore, pigs were not significantly associated with other animal species, although risk factors related to pigs remain inconclusive due to the small sample size. It can be interpreted that certain livestock, such as cattle and horses, play an important role in the spread of human leptospirosis in this region.

The proportion of MAT reactive animals that were sampled from case households were significantly lower compared to MAT reactive animals that were sampled from control households (67% vs 85%, p=0.04). This may have been caused by the tested serovar or case classification. First, *L. interrogans* serovar Pohnpei was not included in the MAT panel used for animal samples in this study due to logistic challenges. Serovar Pohnpei was the predominant serovar that accounted for 64% of reactive MATs in the human study, and it is possible that testing of serovar Pohnpei would have yielded a higher prevalence in case and control households. When a follow-up MAT is conducted for serovar Pohnpei in 2017, study findings may change. Secondly, there is a possibility that some households may have been misclassified as control households, despite having MAT positive members in the household. Given the fact that up to three people were sampled from each household, and that cases had a significantly higher number of people tested (median of 3 tested per household vs. 2, $p<0.01$), it is possible that some control households were true case households, had more household members been tested.

In this study, rodents were tested for antibodies in sera and *Leptospira* DNA in the kidney, which yielded different results. In the multiple logistic regression, while DNA detection from rats was positively associated with a case household, antibody detection from rats was negatively associated with a case household, although not significant. The results may have differed in directionality if some rodents that were infected with *Leptospira* had a weak immune response to the bacteria and only produced lower levels of antibodies. Alternately, qPCR is a more sensitive laboratory test compared to Microscopic Agglutination Tests, and there may have been more MAT false negatives compared to qPCR false negatives.

Behavioral factors have been associated as risk factor for leptospirosis.[13] In the multiple logistic regression analysis, there were several factors in the model that were significantly associated with a household being a case, but were not included in the final model because they were intervening factors. For example, variables for households that were ‘flooded between 2010 and 2013’ and ‘located near a

stream' were significantly associated with being iTaukei (indigenous Fijian), whereas 'having a goat born in the household', 'slaughtering animals in the past 12 months', and 'seeing mongoose in the household, farm or the community' were significantly associated with being a non-iTaukei household. This can be attributed to the distinct differences in life style, animal handling practices, housing structures and religion between iTaukei and non-iTaukei households.

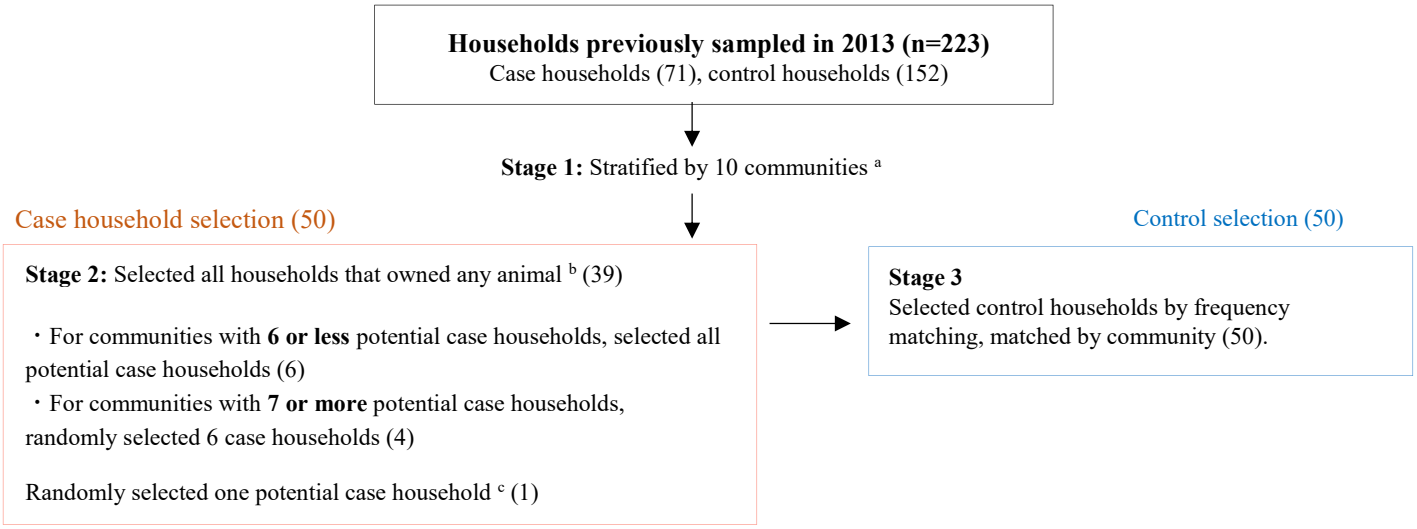
A limitation of our study was the sampling time frame and generalizability. Firstly, since human sera and animal sera were collected two years apart in 2013 and 2015, it is possible that animal ownership and prevalence of *Leptospira* antibodies changed significantly during this period. However, variables that were assessed as potential risk factors, such as ethnicity, religion, household distance from a stream, animal slaughtering practices are unlikely to change in a short period of time. Additionally, since *Leptospira* may persist in the environment for several months and animals are kept in close proximity, animals that were newly introduced to the household would have seroconverted within the two-year period, if the bacteria was present in the household environment [27]. Secondly, we found that Leptospira transmission was associated with interaction of factors associated with animal exposure. Since the study was focused in Ba, Western Fiji, our findings should be extrapolated to other regions with caution. However, subsistence farming practices, flood-prone environments and high leptospirosis transmission settings similar to that of Ba can be found in many parts of the world.

Exposure to various animal species which were found to be transmission factors for *Leptospira* in this study can be addressed by improving rodent control, personal protection measures and reducing behavioral risk. In Fiji, several animals are kept in close proximity to each other and to humans, indicating high animal to human interaction. This environment calls for short term and long term interventions in order to minimize risk of infection. In the short term, using protective gear such as boots and gloves for subsistence farming, altering high risk behaviors such as swimming in stream close to where animals are kept, and controlling rodents in the household should be recommended. In addition to

this, the agriculture sector should educate farmers, who are occupationally at high risk, about the risks and burden of leptospirosis. In the long term, efforts are required to engage environmental, agricultural and public health sectors to collaboratively prevent and control the disease.

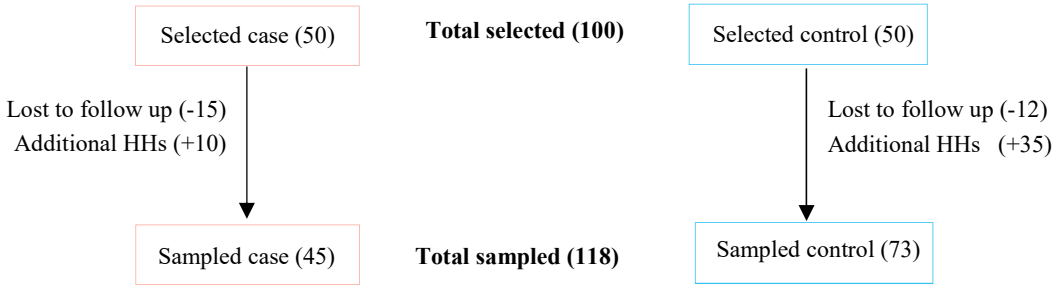
Appendix

Supplemental figure. Sample selection of households.



*Parentheses indicate the total number of households selected at each stage.
a: In the human seroprevalence study in 2013, households were from ten communities: Toge, Navala, Veisaru, Lavuci, Naidrodro, Sarava, Sorokoba, Votua, Tabataba and Yalalevu.
b: Based on questionnaire data obtained from human study in 2013.
c: This was done in order to achieve sampling goal of 50 case households

Supplemental figure: Overview of sampling strategy used in study



*Additional households include replacements for households that were lost to follow up (LTF), and households that were added later in the study from Yalalevu, and communities that were revisited.
*There are more additional control households because some case LTF households were replaced with control households, and more control households were sampled in Yalalevu.

Supplemental table. Household characteristics regarding animal interaction and observations

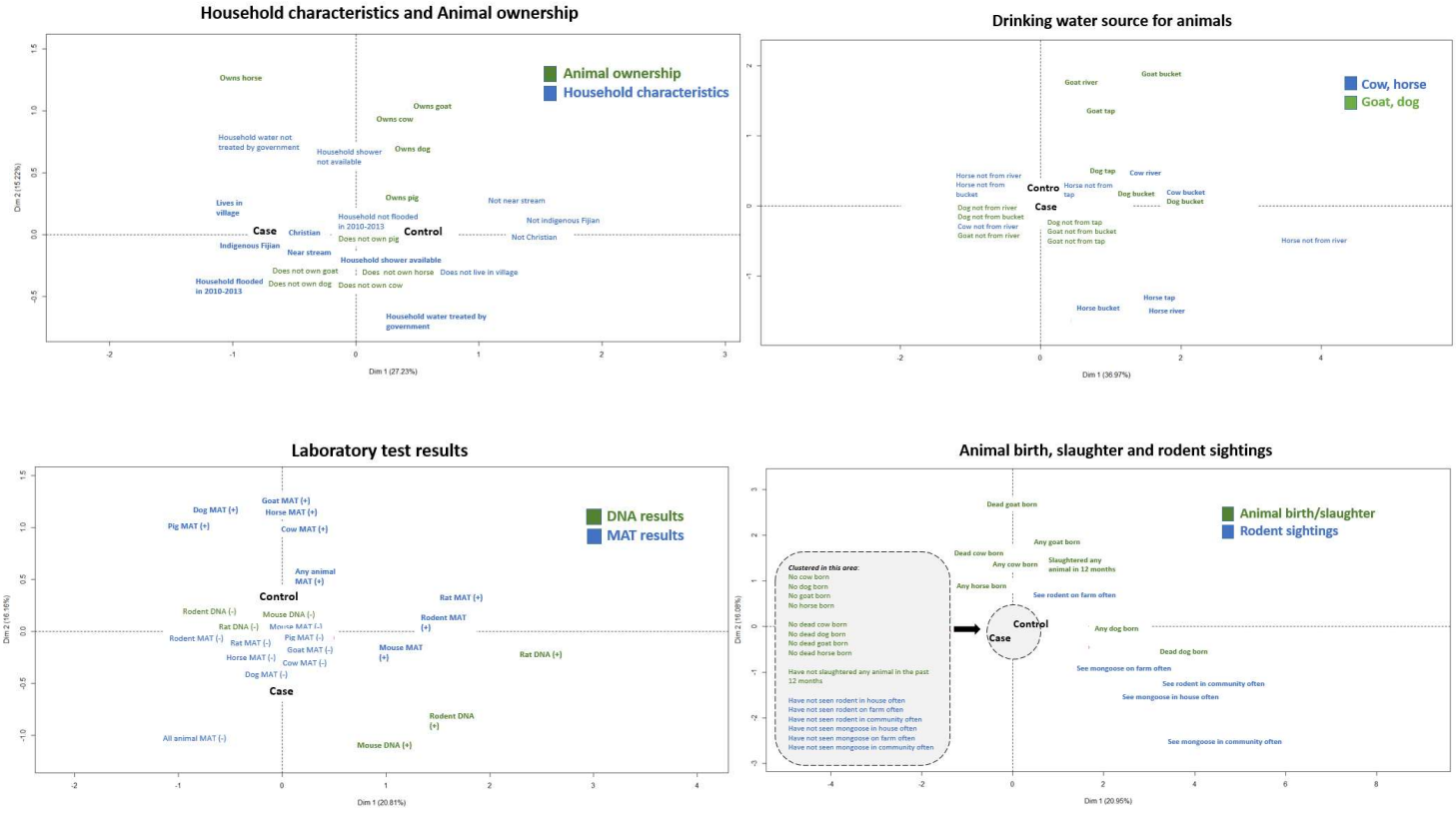
	Case household Median (1st, 3rd QR) or No. (%)	**Control household** Median (1st, 3rd QR) or No. (%)	**Total household** Median (1st, 3rd QR) or No. (%)	**OR (95% CI) or difference (CI)**	**P-value of t-test or X^2**
Animal interaction					
1)Livestock slaughter: Number of HHs that slaughtered animals in the past 12 months					
Any animal	3 (6.67)	17 (23.94)	20 (17.24)	0.22 (0.05, 0.73)	0.032*
Cattle	0 (0.00)	3 (2.54)	3 (2.54)	0.00 (0.00, 3.92)	0.286 [a]
Goat	3 (2.54)	16 (13.56)	19 (16.10)	0.25 (0.06, 0.82)	0.053
Pig	0 (0.00)	1 (0.85)	1 (0.85)	0.00 (0.00, 63.21)	1.000 [a]
Chicken	0 (0.00)	2 (1.69)	2 (1.69)	0.00 (0.00, 8.65)	0.524 [a]
2)Animal movement at HH level in the past 12 months					
Has left or has been added	9 (23.29)	17 (23.29)	26 (22.03)	0.82 (0.32, 2.01)	0.849
Has left HH	12 (26.67)	17 (23.29)	29 (24.58)	1.20 (0.50, 2.81)	0.846
3)Seen rodents or mongoose (separate data for rodents and mongoose available)					
Around the house	0 (0.00)	1 (1.39)	1 (0.85)	0.00 (0.00, 62.34)	1.000 [a]
Never					
Sometimes	19 (42.22)	33 (45.83)	52 (44.44)	0.86 (0.40, 1.83)	0.848
Often	26 (57.78)	38 (52.78)	64 (54.70)	1.22 (0.58, 2.61)	0.736
On the farm	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Never					
Sometimes	41 (91.11)	57 (79.17)	98 (83.76)	2.70 (0.90, 10.00)	0.148
Often	4 (8.89)	15 (20.83)	19 (16.24)	0.37 (0.10, 1.11)	0.148
In the community	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Never					
Sometimes	44 (97.78)	67 (93.06)	111 (94.87)	3.26 (0.35, 158.66)	0.404 [a]
Often	1 (2.22)	5 (6.94)	6 (5.13)	0.31 (0.01, 2.88)	0.404 [a]
Symptoms observed in cattle or goats at HH level					
Red/brown urine	1 (0.85)	3 (2.54)	4 (3.39)	0.53 (0.01, 6.88)	1.000 [a]
Premature death/abortion	0 (0.00)	1 (0.85)	1 (0.85)	0.00 (0.00, 63.21)	1.000 [a]
Water source for animals					
Cattle	20 (44.44)	30 (41.10)	50 (42.37)	1.15 (0.54, 2.43)	0.098
Tap					
River	18 (40.00)	27 (36.99)	45 (38.14)	1.14 (0.53, 2.43)	0.895
Bucket	18 (40.00)	23 (31.51)	41 (34.75)	1.45 (0.67, 3.15)	0.458
Goat	13 (28.89)	22 (30.14)	35 (29.66)	0.94 (0.41, 2.11)	1.000
Tap					
River	11 (24.44)	14 (19.18)	25 (21.19)	1.36 (0.55, 3.34)	0.654
Bucket	11 (24.44)	13 (17.81)	24 (20.34)	1.49 (0.60, 3.71)	0.526
Horse	16 (35.56)	16 (21.92)	32 (27.11)	1.97 (0.86, 4.52)	0.160
Tap					
River	16 (35.56)	14 (19.18)	30 (25.42)	2.33 (1.00, 5.47)	0.077
Bucket	16 (35.56)	14 (19.18)	30 (25.42)	2.33 (1.00, 5.47)	0.077
Dog	16 (35.56)	36 (49.32)	52 (44.07)	0.57 (0.26, 1.21)	0.204
Tap					
River	9 (20.00)	20 (27.40)	29 (24.58)	0.66 (0.26, 1.58)	0.492
Bucket	9 (20.00)	16 (21.92)	25 (21.19)	0.89 (0.34, 2.20)	0.988

Supplemental table. Seroprevalence of animals, stratified by species.

	Case HH No. (%)	Control HH No. (%)	Total HH No. (%)	OR (95%CI)	P value		Case HH No. (%)	Control HH No. (%)	Total HH No. (%)	OR (95% CI)	P value
Any animal	n=36	n=60	n=96			**Cattle**	n=9	n=24	n=33		
Positive MAT for any serovar	24 (66.67)	51 (85.00)	75 (78.12)	0.35 (0.13,0.96)	0.04 *	***Positive MAT for any serovar***	7 (77.78)	17 (70.83)	24 (72.73)	1.44 (0.22,9.39)	0.69
Australis	2 (5.56)	3 (5.00)	5 (5.21)	1.12 (0.17, 7.20)	0.91	Australis	1 (11.11)	0 (0.00)	1 (3.03)	8.65 (NA, NA)	0.60
Ballum	4 (11.11)	8 (13.33)	12 (12.50)	0.81 (0.22 2.97)	0.75	Ballum	1 (11.11)	3 (12.50)	4 (12.12)	0.88 (0.07,10.69)	0.91
Canicola	0 (0.00)	0 (0.00)	0 (0.00)	-	-	Canicola	0 (0.00)	1 (4.17)	1 (3.03)	0.82 (NA, NA)	0.60
Copenhageni	7 (19.44)	14 (23.33)	21 (21.88)	0.79 (0.28, 2.23)	0.66	Copenhageni	0 (0.00)	2 (8.33)	2 (6.06)	0.47 (NA, NA)	0.94
Harjo	0 (0.00)	1 (1.67)	1 (1.04)	0.54 (0.00, 0.75)	0.80	Harjo	3 (33.33)	3 (12.5)	6 (18.18)	3.50 (0.52,23.74)	0.19
Pomona	0 (0.00)	0 (0.00)	0 (0.00)	-	-	Pomona	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Tarassovi	0 (0.00)	1 (1.67)	1 (1.04)	0.54 (0.00, 0.75)	0.80	Tarassovi	0 (0.00)	1 (4.16)	1 (3.03)	0.82 (NA, NA)	0.60
Mixed	11 (30.56)	24 (40.00)	35 (36.46)	0.66 (0.27, 1.61)	0.36	Mixed	2 (22.22)	7 (29.17)	9 (27.27)	0.69 (0.11,4.52)	0.69
Negative	12 (33.33)	9 (15.00)	21 (21.88)	2.83 (1.04,7.73)	0.04 *	***Negative***	2 (22.22)	7 (29.17)	9 (27.27)	0.69 (0.11,4.52)	0.69
Horse	n=8	n=12	n=20			**Pig**	n=2	n=2	n=4		
Positive MAT for any serovar	7 (87.5)	10 (83.33)	17 (85.00)	1.40 (0.09,22.42)	0.80	***Positive MAT for any serovar***	1 (50.00)	1 (50.00)	2 (50.00)	1.00 (0.00,5460.55)	1.00
Australis	2 (25.00)	1 (8.33)	3 (15.00)	3.67 (0.23,59.42)	0.34	Australis	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Ballum	2 (25.00)	4 (33.33)	6 (30.00)	0.67 (0.08,5.69)	0.70	Ballum	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Canicola	0 (0.00)	0 (0.00)	0 (0.00)	-	-	Canicola	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Copenhageni	1 (12.50)	1 (8.33)	2 (10.00)	1.57 (0.07,36.31)	0.77	Copenhageni	1 (50.00)	1 (50.00)	2 (50.00)	1.00 (0.00,5460.55)	1.00
Harjo	0 (0.00)	2 (16.67)	2 (10.00)	0.25 (0.00, NA)	0.65	Harjo	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Pomona	0 (0.00)	0 (0.00)	0 (0.00)	-	-	Pomona	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Tarassovi	0 (0.00)	0 (0.00)	0 (0.00)	-	-	Tarassovi	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Mixed	2 (25.00)	2 (16.67)	4 (20.00)	1.67 (0.16,17.73)	0.66	Mixed	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Negative	1 (12.50)	2 (16.67)	3 (15.00)	0.71 (0.05,11.44)	0.80	***Negative***	1 (50.00)	1 (50.00)	2 (50.00)	1.00 (0.00,5460.55)	1.00
Goat	n=9	n=18	n=27			**Dog**	n=12	n=27	n=39		
Positive MAT for any serovar	3 (33.3)	7 (38.89)	10 (37.04)	0.79 (0.14,4.59)	0.78	***Positive MAT for any serovar***	7 (58.33)	19 (70.37)	26 (66.67)	0.59 (0.14,2.54)	0.47
Australis	0 (0.00)	1 (5.56)	1 (3.70)	0.61 (NA, NA)	0.72	Australis	1 (8.33)	2 (7.41)	3 (7.69)	1.14 (0.09,15.11)	0.92
Ballum	2 (22.22)	3 16.67)	5 (18.52)	1.43 (0.17,11.70)	0.73	Ballum	1 (8.33)	3 (11.11)	4 (10.26)	0.73 (0.06,8.46)	0.79
Canicola	0 (0.00)	1 (5.56)	1 (3.70)	0.61 (NA, NA)	0.72	Canicola	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Copenhageni	1 (11.11)	0 (0.00)	1 (3.70)	6.53 (NA, NA)	0.72	Copenhageni	3 (25.00)	9 (33.33)	12 (30.77)	0.67 (0.14,3.25)	0.61
Harjo	0 (0.00)	0 (0.00)	0 (0.00)	-	-	Harjo	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Pomona	0 (0.00)	0 (0.00)	0 (0.00)	-	-	Pomona	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Tarassovi	0 (0.00)	0 (0.00)	0 (0.00)	-	-	Tarassovi	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Mixed	0 (0.00)	2 (11.11)	2 (7.41)	0.35 (NA, NA)	0.80	Mixed	2 (16.67)	5 (18.52)	7 (17.95)	0.88 (0.14,5.67)	0.89
Negative	6 (66.67)	11 (61.11)	17 (62.96)	1.27 (0.22,7.43)	0.78	***Negative***	5 (41.67)	8 (29.63)	13 (33.33)	1.70 (0.39,7.32)	0.47
Rat	n=17	n=31	n=48			**Mouse**	n=13	n=17	n=30		
Positive MAT for any serovar	7 (41.18)	14 (45.16)	21 (43.75)	0.85 (0.25,2.91)	0.79	***Positive MAT for any serovar***	6 (46.15)	10 (58.82)	16 (53.33)	0.60 (0.13,2.75)	0.50
Australis	0 (0.00)	1 (3.23)	1 (2.08)	0.58 (0.00,0.75)	0.76	Australis	1 (7.69)	1 (5.88)	2 (6.67)	1.33 (0.07,26.80)	0.85
Ballum	4 (23.53)	5 (16.13)	9 (18.75)	1.60 (0.35,7.27)	0.54	Ballum	1 (7.69)	2 (11.76)	3 (10.00)	0.63 (0.05,8.68)	0.72
Canicola	0 (0.00)	0 (0.00)	0 (0.00)	-	-	Canicola	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Copenhageni	3 (17.65)	7 (22.58)	10 (20.83)	0.74 (0.16,3.45)	0.69	Copenhageni	4 (30.77)	5 (29.41)	9 (30.00)	1.06 (0.21,5.52)	0.94
Harjo	0 (0.00)	0 (0.00)	0 (0.00)	-	-	Harjo	0 (0.00)	1 (5.88)	1 (3.33)	0.41 (0.00,0.75)	0.89
Pomona	0 (0.00)	0 (0.00)	0 (0.00)	-	-	Pomona	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Tarassovi	0 (0.00)	0 (0.00)	0 (0.00)	-	-	Tarassovi	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Mixed	0 (0.00)	1 (3.23)	1 (2.08)	0.58 (0.00,0.75)	0.76	Mixed	0 (0.00)	1 (5.88)	1 (3.33)	0.41 (0.00,0.75)	0.89
Negative	10 (58.82)	17 (54.84)	27 (56.25)	1.18 (0.34,4.02)	0.79	***Negative***	7 (53.85)	7 (41.18)	14 (46.67)	1.67 (0.36,7.64)	0.50

Supplemental figure. Multiple correspondence analysis

a.Household characteristics and animal ownership, b. drinking water source for animals, c. laboratory test results, d. animal birth, slaughter and rodent sightings`



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