

GLOBAL PATTERNS OF *LEPTOSPIRA* PREVALENCE IN VERTEBRATE RESERVOIR HOSTS

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ABSTRACT: Leptospirosis is a widespread emerging bacterial zoonosis. As the transmission is believed to be predominantly waterborne, human incidence is expected to increase in conjunction with global climate change and associated extreme weather events. Providing more accurate predictions of human leptospirosis requires more detailed information on animal reservoirs that are the source of human infection. We evaluated the prevalence of *Leptospira* in vertebrates worldwide and its association with taxonomy, geographic region, host biology, ambient temperature, and precipitation patterns. A multivariate regression analysis with a meta-analysis-like approach was used to analyze compiled data extracted from 300 *Leptospira*-related peer reviewed papers. A fairly uniform *Leptospira* infection prevalence of about 15% was found in the majority of mammalian families. Higher prevalence was frequently associated with species occupying urban habitats, and this may explain why climatic factors were not significantly correlated with prevalence as consistently as expected. Across different approaches of the multiple regression analyses, the variables most frequently correlated with *Leptospira* infection prevalence were the host's ability to swim, minimum ambient temperature, and methodologic quality of the study. Prevalence in carnivores was not associated with any climatic variable, and the importance of environmental risk factors were indicated to be of lesser consequence in nonhuman mammals. The dataset is made available for further analysis.

Key words: Climate change, *Leptospira*, leptospirosis, meta-analysis, multivariate regression analysis, phylogeny, reservoir hosts, waterborne.

INTRODUCTION

Leptospirosis is a disease caused by infection with bacteria of the spirochaete genus: *Leptospira*. These bacteria are highly motile, obligate aerobic, helical bacteria with an optimum growth temperature of 28–30 C and optimum pH 6.8–7.4 (Bharti et al. 2003). *Leptospira* has the potential to infect most, if not all, mammalian species, some reptile species (Feigin and Anderson 1975; Calle et al. 2001), and several species of birds (Bernkopf 1948; Jobbins and Alexander 2015).

Following infection, the host can become an asymptomatic long-term carrier of *Leptospira*, by the bacteria chronically infecting the renal proximal tubules. The host can subsequently contaminate the environment with *Leptospira* upon urination (Levett 2001;

World Health Organization 2003; Victoriano et al. 2009; World Health Organization 2011). Chronic asymptomatic carriers are termed “reservoir hosts,” defined by Haydon et al. (2002) as “populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the defined target population.” In our study, the target population is humans. The hosts may also be referred to as “maintenance populations” (Haydon et al. 2002), and each species of host usually hosts a limited number of *Leptospira* serovars, e.g., *Leptospira interrogans* serovar Hardjo in cattle, *Leptospira interrogans* serovars Copenhagenii, Ballum, and Icterohaemorrhagiae in rats (*Rattus* spp.), and *Leptospira interrogans* serovars Canicola and Icterohaemor-

TABLE 1. Overview of article and data search process for compilation of a dataset used to study global prevalence of *Leptospira* infection in vertebrate reservoir hosts. No other search terms (“*,” “?,” etc.) were added to the search.

Search engine	Search words	Search result	No. examined further	Resultant no. of unique articles
Web of Knowledge	Leptospirosis (topic), Prevalence (topic)	367	269	192
Web of Science	Leptospira (topic), Prevalence (topic)	360	23	21
CAB Abstracts	Leptospirosis AND prevalence	322	93	53
<i>Journal of Wildlife Diseases</i>	Leptospirosis (+“full txt” option)	219	24	22
BioOne	Leptospirosis	77	13	12
Sum		1,832	422	300

rhagiae in dogs (*Canis lupus familiaris*; Levett 2001; Bharti et al. 2003; Klaasen et al. 2003).

Leptospirosis is found in most of the world (Ullmann and Langoni 2011). Human infection is highest in developing countries with warm humid climates (Levett et al. 2004; Lau et al. 2010), but it is increasingly seen in temperate, developed countries in part due to travelers visiting endemic areas and increased participation in recreational and sports activities that include contact with water (Ullmann and Langoni 2011). Under favorable pH, humidity, rain, and temperature conditions, *Leptospira* may survive in soil for >60 d and several months in fresh water (Rathinam and Namperumalsamy 1999; Levett 2001). Despite the potentially long survival of the free-living bacteria, many human cases of leptospirosis appear to coincide with heavy rainfall and flooding (Su et al. 2011; Coelho and Massad 2012). Thus, climate change affecting weather patterns and extreme weather events may affect the occurrence of leptospirosis (Lau et al. 2010). However, it is still reasonable to assume that transmission among hosts is less dependent on such events because leptospirosis has been reported in arid and high-altitude regions where water is scarce and places where water bodies are not immediately suitable for *Leptospira* survival (such as sea and sewage water; Chang et al. 1948; Trueba et al. 2004), and in subarctic and continental regions (Alaska, Canada, and Mongolia; Jordan et al. 1987; Blair et al. 2004; Odontsetseg et al. 2005; Goldstein et al. 2011).

An important part of disease prevention lies in a better understanding, awareness, and recognition of correlated risk factors and epidemiology of transmission. Thus greater insight, into how the prevalence of infection in hosts is affected by extrinsic factors, such as climate, could prove useful in managing leptospirosis. Herein, we assess the prevalence of *Leptospira* infection among vertebrate hosts with widely differing biology and how prevalence is associated with taxonomic, biologic, and environmental factors.

MATERIALS AND METHODS

We searched four online databases for previously published peer reviewed *Leptospira* records (publications reporting leptospiral prevalence in mammals, birds, and reptiles). Subsequently, we assessed the methodologic quality of the publications (more details are provided in the following) and compiled additional data for independent variables, and from the resultant dataset, we performed a multiple regression analysis of the statistical correlation between prevalence rates, quality of the study, and biologic and environmental variables.

Analytic approach and data sources

The study was based on a large sample of published literature with a substantial biologic and methodologic diversity. To accommodate this challenge, sampling choices and analytic choices were made to ensure data quality and coherency. Scientific peer-reviewed articles were found by using the online search engines: Web of Knowledge, Web of Science, BioOne, CAB Abstracts, and *Journal of Wildlife Diseases*. See Table 1 for search words and outcome (no additional wildcard

TABLE 2. Criteria needed to be fulfilled by an article to earn an individual “Klimisch score” rating that article’s quality. The best score is 1, and the poorest is 3.

Klimisch score	Criteria ^a
Score 3	Article only fulfills the four requirements to gain access to the dataset: location, host species, sample size, and test modality and is free of obvious selection bias
Score 2	Score 3 criteria+info about MAT cutoff titer, vaccination status, sampling year, taxonomy (at least to family), and specific sampling modality
Score 1	Score 2+3+ at least 10 strains tested if MAT, taxonomy to species, appropriate testing method (e.g., MAT, ELISA, PCR, culture, or immunofluorescence), specification of PCR primers, modality in use, season of the year, and method of capture for wild animal

^a MAT = microscopic agglutination test; MAT cutoff titer = reciprocal of serum dilution used to evaluate agglutination under antigen stimulation (=100); ELISA = enzyme-linked immunosorbent assay.

search terms were added to the searches). Initial crude selection only excluded human studies, and inclusion required that the publications clearly specified location, host species sampled, sample size, testing modality, and the study had to be void of nonrandom sampling that could influence prevalence (e.g., the inclusion of sick animals and individuals suspected of suffering from leptospirosis). The literature search ceased when 300 papers were collected. In recognizing that the accuracy of prevalence estimates may vary among studies according to methodology (Klimisch et al. 1997), we scored publications according to the quantitative or qualitative details provided and their method of *Leptospira* detection. We refer to this as the “Klimisch score” of a publication. Publications in which authors provided convincing evidence of highly accurate and sensitive methodology were given a score of 1 (see Table 2). These would include the use of PCR or microscopic agglutination test (MAT) with a wide array of *Leptospira* serovars (>10) and with clear and detailed information on the time and location of sampling, capture method, and analytical methodology. We gave higher scores to publications with limited relevant information and of lower quality. Publications that only provided the number of positive animals, the total number of animals investigated, the type of methodology, and location and time of sampling, were given the Klimisch score 3. This scoring represented a simple measure of potential bias that could be included in our statistical analyses.

Our database includes a combination of prevalence records of *Leptospira* infection determined by PCR, culture (direct diagnostics of *Leptospira* infection), and serology-based tests (indirect diagnostics). Nevertheless, all these diagnostic methods were accepted as equally valid assessments of *Leptospira* infection. Because this approach raises concerns about the comparability of prevalences given by different diagnostic

methods, the mean prevalence generated by each method was compared statistically.

We performed another literature search in 2015 specifically for the brown rat (*Rattus norvegicus*), the second most represented species in our dataset after the domestic dog. That search yielded five additional references, of which two were published after our literature search, suggesting that our dataset could be increased by about 5%.

Taxonomic, biologic, and environmental variables

Information about each variable for each observation was retrieved from the publication or suitable literature and online databases. Table 3 provides an overview of variables included in the dataset and information source (again, only if it was not given in the article from which the observation originated).

Sample characteristics

The 300 publications led to a compilation of 740 observations (one observation=one leptospirosis prevalence estimate from one sampling of one species). The observations totaled 328,452 animals, sampled from six continents and 107 countries. For continents, the highest number of observations came from Latin America (184), followed by Europe (145). The three most frequently reported countries were Brazil (61 observations), Australia (55), and New Zealand (36). Reports of mammals (717) clearly outnumbered reports from other taxonomic classes (717). The three most frequent orders were Artiodactyla, Rodentia, and Carnivora (Table 4); and within these, the most frequently reported families were Bovidae, Muridae, and Canidae. Sample sizes in families that included livestock were larger than in families that only represented wildlife species. If sample sizes were pooled through the dataset, the

TABLE 3. Sources of taxonomic, biologic, environmental, and geographic data used in our dataset and meta-analysis.

Data	Variables	Sources
Taxonomy	Class, order, family, genus, species	Integrated Taxonomic Information System 2016
Biology	Herbi-, carni-, pisci-, insecti-, frugi-, or omnivorous, “ability to swim” (natural ability to move in water, thus also including animals that are not 100% water living) and if associated with water, whether this was mainly salt or fresh water, and finally pH of urine with an upper and lower reference interval	Urine pH reference interval: Parfentjev and Perlzweig 1932; Minnich 1972; Long 1982; Styles and Phalen 1998; Carpenter 2005. Data defining other biological variables for each species were, if not already defined within the given publication, retrieved from Nowak 1991.
Environmental data	Information of domestication status: wild, domesticated, or in captivity; natural habitat: forest, open land, urban area, or rural; average rainfall: yearly average minimum and yearly average maximum for the location; temperature regime: yearly average minimum, yearly average maximum, and the temperature amplitude for the location; season of sampling; and beginning and end of sampling period	As specified in the given article or from World Meteorological Organization (http://www.worldweather.org/index.htm). This Web site provides weather information for a 30-yr period (1971–2000) for multiple locations of each country. The location of the study, or the closest location, was used for the climatic variables in the dataset.
Geography	Continent and United Nations region and country	United Nations Statistics Division 2013

highest number of sampled animals, by family, were Bovidae, Canidae, and Suidae.

More than nine diagnostic test methods were represented, but MAT dominated with 554 observations, followed by PCR 77 observations and culture (56). The range of cutoff values used with MAT was 1:10–1:1600, with the cutoff value of 1:100 being most frequently represented (325/554 observations). The variables for temperature and precipitation from monthly aggregated, 30-yr average climate indices (mean daily minimum and maximum temperature or precipitation over a month) for the given locations ranged from –30.8 C to 46.9 C and 0 mm to 646.6 mm, respectively. The monthly average temperature amplitude ranged from 7.2 C in Thailand to 56.8 C in Mongolia.

Statistical analysis

The effect of potential risk factors on the prevalence of *Leptospira* infection was evaluated by logistic regression. In particular, our response was whether or not an animal was positive for *Leptospira*, and we consequently regressed the probability of being found positive on a logit scale.

To accommodate the sample heterogeneity and produce robust inference on overall (population

averaged) effects, we applied a working independence generalized estimation equation (GEE) approach (Liang and Zeger 1986). The GEE is particularly well suited to assess overall (population averaged) effects in heterogeneous samples consisting of data from different studies. As the method adapts correctly to this heterogeneity the GEE approach has been advocated when performing meta-analysis (e.g., Ringquist 2013).

Estimated effects were reported as odds ratios and accompanied by robust Wald 95% confidence intervals. Confidence intervals were calculated on the log-odds scale and backtransformed. *P* values corresponded to robust score test statistics. The GEE analyses were performed by using PROC GENMOD in SAS 9.3 (SAS Institute, Cary, North Carolina, USA).

Model selection and reduction was carried out with and without prescreening for marginal correlation (i.e., variables with $P > 0.1$ were, with prescreening, excluded from the subsequent final multivariate model), with and without subsequent backwards stepwise elimination of nonsignificant variables from the multivariate model (variables with $P > 0.05$ eliminated). In combination, these lead to four different analytical multiple regression approaches. In all approaches, the models

TABLE 4. Prevalences of *Leptospira* infection in for the three most commonly represented families in eight taxonomic orders in terms of frequency of representation in our database. Prevalence is given as mean, SE, number of observations (obs), and total number animals, where one obs=one leptospirosis prevalence estimate from one sampling of one species.

Mammalian order	Family	Mean prevalence±SE	No. obs	No. animals
Perissodactyla ^a		16.4±1.03	19	9,945
	Tapiridae	17.2±12.4	2	29
	Equidae	16.4±4.28	17	9,916
Artiodactyla		15.9±1.44	217	196,809
	Bovidae	16.6±1.83	139	161,318
	Cervidae	6.1±1.67	34	9,611
	Suidae	11.8±2.48	31	22,214
Carnivora		11.6±0.93	149	90,508
	Felidae	12.7±4.01	18	809
	Canidae	10.9±1.13	73	84,616
Chiroptera		34.8±8.52	17	844
		12.2±1.86	46	1,408
	Phyllostomidae	8.9±2.57	28	832
	Pteropodidae	14.1±3.22	7	222
	Vespertilionidae	16.8±3.67	7	186
Diprotodontia		37.5±4.54	18	2,035
	Macropodidae	19.0±9.87	6	231
	Phalangeridae	41.3±6.46	7	1,723
	Vombatidae	10.6±4.19	2	66
Lagomorpha ^b		11.8±3.38	14	1,817
	Leporidae	11.8±3.38	14	1,817
Primates		27.9±4.40	12	888
	Cercopithecidae	28.8±6.49	5	734
	Cebidae	20.4±9.6	3	25
	Callitrichidae	36.8±6.02	2	76
Rodentia		17.8±1.34	209	23,640
	Cricetidae	15.3±3.07	31	1,151
	Muridae	17.1±1.58	155	19,824
	Soricidae	10.6±3.63	13	273

^a Only represented by two families in the dataset.

^b Only represented by one family in the dataset.

were adjusted by sample size and quality using the Klimisch score. The four analytical approaches were applied to all observations in the dataset and also to specific taxonomic subgroups within the dataset (i.e., Muridae, Bovidae, Canidae, Rodentia, and Muridae+Bovidae+Canidae, respectively). This allowed us to assess the uniformity of given correlations across taxa. A quality score was included as a predictor variable in all analyses to adjust for the methodologic quality of a specific study. Only the more conservative approach based upon prescreening and no backwards elimination is reported in full here. The results produced by the other three approaches, as well as the full dataset, are presented in the Supplementary Material.

RESULTS

Leptospira was common in all eight orders and 21 families of the class Mammalia (Table 4). Prevalence among orders, ranged from 11.6% for Carnivora to 37.5% for Diprotodontia. Among families, the mean prevalence ranged from 6.1% for Cervidae to 41.3% for Phalangeridae (Table 4).

Families Phalangeridae, Callitrichidae, and Cercopithecidae, found in subtropical or tropical regions had higher prevalences than families generally sampled in temperate regions, including Cervidae, Soricidae, and

Muridae (Table 4). Some families sampled in the temperate regions, however, families such as Mustelidae, showed high prevalence despite a relatively low ambient temperature. The apparent effect of differences in temperature nevertheless appeared to remain within Mustelidae by presenting prevalences between 0% and 10% in areas with monthly average ambient temperatures ranging -11 to 15 C and prevalences of 35–89% in association with monthly average ambient temperatures of 2 to 36 C (Alaska: $n=316$, MAT tested, cutoff: 100, France and Spain: $n=346$, MAT tested, cutoff 80–100) for Mustelidae. As such, Mustelidae represented a family with a relatively high prevalence that was nevertheless still under the influence of ambient temperature.

The differences we saw in prevalences among the nine diagnostic tests represented in the dataset were not statistically significant (analysis of number positive or number tested modeled by “method of detection” in the SAS procedure: PROC GENMOD; P values 0.41–0.94).

In prescreening for marginal correlation without subsequent backwards stepwise elimination of nonsignificant ($P>0.05$) variables, we found a significant effect of the biologic variable “ability to swim” in two of the six analyses for each taxonomic group, including one analysis without taxonomic discrimination (Table 5; see Table 3 for a reference to this variable’s definition in the dataset). In three of the six analyses, minimum temperature had an effect on prevalence, while quality of the study had a significant effect on prevalence in four of the six models.

The three other statistical approaches and their results have been summarized in the Supplementary Material. These approaches overall supported the results given here. We would nevertheless still encourage interested readers to review these supplementary results.

DISCUSSION

Our most important finding was that the majority of higher taxonomic groups had fairly

uniform prevalences of $15\pm 5\%$ but also that environmental variables, in some cases, were associated with differences in *Leptospira* prevalence but not consistently so.

Synurbization and diverging responses within taxonomic groups

A large number of observations for brown rats (*Rattus norvegicus*) allowed a detailed evaluation of within-species response to environmental factors, which could negatively impact our ability to interpret the results from the statistical analyses. This showed that prevalences in general were around 20–25% in brown rats but also that a few studies found far higher prevalences (50–90%; e.g., Krøjgaard et al. 2009; Himsforth et al. 2013; Jensen and Magnusson 2015). The latter records originated from cities in the northern hemisphere and thus raised concern that synurbization (adaptation of wildlife populations to urban environments) and associated changes (e.g., smaller home range and increased aggression; Luniak 2004; Parker and Nilon 2012) was contributing to elevated prevalence. This further indicates that diverging within-group responses to temperatures exist, because the effect of synurbization was only clear under cooler climates. This issue was further complicated by the fact that not all urban rat populations showed increased prevalences (Krøjgaard et al. 2009; Himsforth et al. 2013), allowing speculation that dispersal abilities and community size under some circumstances may have critical impact on reservoir potential (Haydon et al. 2002).

The uncertainties regarding the effect of synurbization also apply to the family with the highest (41%) prevalence, Phalangeridae. The Phalangeridae are small marsupials that are variably arboreal and mainly folivorous (Nowak 1991). However, in this study, the common brushtail possum (*Trichosurus vulpecula*) from Australia (Victoria and New South Wales) and the North Island of New Zealand accounted for the majority of observations in this family. The common brushtail possum populations encountered in the dataset were predominantly animals that were potentially affected by synurbization, and as in the case of

TABLE 5. Results of one approach to multivariate regression analysis to identify variables associated with high prevalence of infection with *Leptospira* in mammal taxa. The analysis was conducted with prescreening for marginal correlation and without subsequent backwards stepwise elimination of nonsignificant variables ($P > 0.05$). Only variables with a significant correlation with *Leptospira* infection prevalence are shown.^a

Group ^b	No. obs/no. animals ^c	Biology ^d	Environment ^e (variable <i>P</i> value)	Sampling (variable <i>P</i> value)	Quality ^f (variable <i>P</i> value)
All obs	696/251,688			x	x
Variable		Swimmer	Minimum temperature		
Pos/neg corr		+	+		
<i>P</i> value		0.031	0.0092		
OR (CI)		0.49 (0.29–0.85)	1.05 (1.01–1.09)		
Rodentia	190/19,753		x	x	
Variable		Swimmer			K score
Pos/neg corr		+			–
<i>P</i> value		0.011			0.048
OR (CI)		0.42 (0.25–0.70)			0.32 (0.17–0.61)
Muridae	140/16,376	x	x	x	
Variable					K score
Pos/neg corr					–
<i>P</i> value					0.027
OR (CI)					0.30 (0.16–0.59)
Bovidae	139/155,755	x		x	
Variable			Minimum temperature		K score
Pos/neg corr			+		+
<i>P</i> value			0.0031		0.025
OR (CI)			1.14 (1.05–1.24)		3.93 (1.90–8.11)
Canidae	66/17,722	x	x	x	x
Variable					
Pos/neg corr					
<i>P</i> value					
OR (CI)					
Can+Bov+Mur	340/189,853	x		x	
Variable			Minimum temperature		K score
Pos/neg corr			+		+
<i>P</i> value			0.0035		0.033
OR (CI)			1.09 (1.02–1.16)		3.69 (1.65–8.26)

^a Results are given for all observations in the dataset and then by the use of taxonomic discrimination for families Canidae (Can), Bovidae (Bov), and Muridae (Mur) and for Can+Bov+Mur collectively. A “+” indicates that the variable had a significant positive correlation with higher prevalence, while “–” indicates a negative correlation with higher prevalence. “x” indicates that no significant variables were found.

^b Pos = positive; neg = negative; OR = odds ratio; CI = 95% confidence interval.

^c Obs = observation (one leptospirosis prevalence estimate from one sampling of one species).

^d Swimmer = a species with natural ability to swim (see Table 3 for definition).

^e Lower temperatures were negatively correlated with prevalence (continuous variable).

^f K score = Klimisch score. A score of 2 compared to 1 was associated with a lower prevalence; a score of 3 compared to 1 was associated with higher prevalence.

brown rats previously described, they seem to become more omnivorous through eating pet food, food scraps, and small vertebrates (Nowak 1991; Brown et al. 1993; Harper 2005) when living in urban habitats. Habitat and diet alterations associated with synurbiza-

tion or increased encounters with other urban-dwelling species may be contributing to the high *Leptospira* prevalence in brushtail possums. Similar considerations appeared for other families with high prevalences, including the primate families Callitrichidae (36.8%

prevalence) and Cercopithecidae (28.8%; Table 4). Baboons (genus *Papio*) were the most frequent cercopithecids in the dataset, and baboons are also attracted to urban zones where they become omnivorous (Nowak 1991; Hoffman and O’Riain 2011; Kaplan et al. 2011; Swedell and Saunders 2012). Thus, species with higher prevalences were frequently small- to medium-sized omnivorous mammals that often live in close proximity with human populations or in urban environments.

Our methods were inadequate for further investigation of the possible effect of synurbization because the associated behavioral alterations of a particular species are impossible to extract from published studies. Also, the ecologic adjustment of a species cannot be predicted from human densities or landscape features. Thus, it seems likely that our statistical models could only identify main predictors of *Leptospira* prevalence across taxa and regions. It may be more fruitful to perform focused evaluations on species that have frequently been examined and in a stepwise manner build a database for comparative analyses. This would improve the quality of statistical evaluation and provide a better impetus for the interpretation. Our analysis serves, however, as a reference for how *Leptospira* prevalence is associated with higher taxa.

Multivariate regression analyses

Our statistical analysis of the compiled records identified risk factors that, a priori, we expected to find and thus are in agreement with the general epidemiologic ideas relating to *Leptospira* transmission. The factors “ability to swim,” “minimum temperature,” and “Klimisch score” (defined in Tables 2 and 3), were also found in the three other analytical approaches that we executed (see Supplementary Material), so we believe that they are robust and real factors for predicting variation in prevalence in our dataset. The Canidae, for which no variables were found to be significantly correlated with prevalence (for the statistical approach presented here and in

three out of four approaches altogether) are an exception. We speculate that this is because, rather than climatic factors, canids may be more affected by their prey or, in the case of the domestic dog, by companionship with man, which often requires urban living. In our dataset, Canidae was represented by domestic dogs in 43 of 73 observations. For other groups, such as Rodentia and its family Muridae, the lack of association with environmental factors is more difficult to explain, but again synurbization could replace the missing environmental effect (also observed in the results of the other statistical approaches; see Supplementary Material). Hence, we assume, that factors not accounted for here (e.g., sampling characteristics and population density) are responsible or that rodents, or specifically murids, are unresponsive or show diverging within-group responses to given environmental factors.

The analytical approach we present here may be too conservative, because prescreening by univariate analyses can be biased due to unadjusted confounding. It may also be that we could have chosen independent variables with greater precision (e.g., weather data for the particular habitat and lifetime of the sampled species, instead of regional climate data). Nevertheless, it seems that the absence of relevant variables was the main limitation, not the accuracy of the variables included.

Finally, >9 test methods are represented in the dataset, and these all have different sensitivity and specificity characteristics (Merien et al. 1995; Ooteman et al. 2006). The differences in prevalence between the dominating test methods (MAT, PCR, and culture), were not statistically significant. This does not exclude that differences in prevalence may occur as a result of methodology but only that it appears insignificant in the presence of other contributors to differences in prevalence.

We recognize that the large sample based on 300 published papers contained heterogeneous data that was difficult to analyze. Even with these uncertainties, it would be reasonable to suggest that climatic correlations did not demonstrate consistent effects across taxa.

Rather, correlations differed significantly between taxonomic groups, suggesting different sources and risk factors for transmission, possibly even at a subpopulation level. Future studies of leptospirosis epidemiology, including management and control, should, therefore, focus on species that frequently show high prevalence of infection that cannot be explained by climatic conditions. These should be conducted across an urban gradient because such studies should be able to resolve the issue of synurbization, which represents the primary obstacle in interpreting our data. Nonetheless, other detailed studies (rather than simple surveys) of any species that stands out by having high prevalence in an otherwise low-prevalence environment, is likely to contribute to understanding *Leptospira* epizootiology. This also includes further scientific research regarding the effect of flooding, which was not specifically addressed in our analyses. We generally know little of the driving forces or maintenance of leptospirosis in wild animals and a deeper insight could inform management.

Our dataset is available from the lead author and colleagues in the field are invited to perform further and more exhaustive analyses thereupon.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at <http://dx.doi.org/10.7589/2014-10-245>.

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