Evaluation of environmental risk factors for leptospirosis in dogs: 36 cases (1997–2002)

Michael P. Ward, BVSc, MSc, MPVM, PhD; Lynn F. Guptill, DVM, PhD, DACVIM; Ching Ching Wu, DVM, PhD

Objective—To identify environmental risk factors for leptospirosis.

Design—Retrospective study.

Animals—36 dogs with leptospirosis and 138 dogs seronegative for leptospirosis as determined by microscopic agglutination test for antibodies against *Leptospira* spp.

Procedures—Medical records of dogs evaluated for leptospirosis from 1997 though 2002 were identified. Owner address was used to geocode locations of dogs, and location-specific environmental risk factor data were obtained by use of a geographic information system. Risk of leptospirosis was estimated by odds ratios, controlling for potential confounding by dog age, sex, and breed.

Results—Leptospirosis in 19 of the 30 dogs in which an infecting *Leptospira* serovar could be identified was associated with *Leptospira kirschneri* serovar grippotyphosa infection. Dogs in which a diagnosis of leptospirosis was made, and dogs with leptospirosis caused by *L kirschneri* serovar grippotyphosa, were more likely to have addresses located in areas classified as rural in 1990 but urban in 2000. By use of information on recent urbanization and a logistic regression model, the status of 81.6% and 89.8% of dogs with leptospirosis and leptospirosis caused by serovar grippotyphosa, respectively, were correctly classified. Other environmental variables (proximity to streams, recreational areas, farmland, wetlands, areas subject to flooding, and areas with poor drainage; annual rainfall; and county cattle or pig population) did not significantly improve accuracy of classification.

Conclusions and Clinical Relevance—Dogs in periurban areas are at greater risk of leptospirosis. Vaccination of dogs in these areas to protect against leptospirosis should be considered. (*J Am Vet Med Assoc* 2004;225:72–77)

Leptospires are spirochete bacteria that infect many animal species and humans. More than 200 different *Leptospira* serovars, grouped into several species, have been described. At least 1 primary host species acts as the reservoir for each serovar, maintaining the disease. For example, raccoons may act as the reservoir for *Leptospira kirschneri* serovar grippotyphosa and rats as the reservoir for *Leptospira interrogans* serovar icterohaemorrhagiae.[1,2] *Leptospira* organisms are shed in the urine of infected animals and may survive for long periods in surface water. Susceptible animals and humans are infected through contact with contaminated water. Bacteria enter through damaged skin or mucous membranes.[2] Localized outbreaks of leptospirosis can occur when susceptible animals and humans are infected through contaminated environments.[3] Human leptospirosis is an occupational health hazard (eg, sanitary and agriculture workers), and some outbreaks have been associated with recreational activities such as swimming and endurance competitions.[4] A common-source outbreak caused by leptospires of the Grippotyphosa serogroup has been reported in a Tennessee community[5]; patients were apparently infected while swimming in a local stream.

Leptospirosis in dogs may be associated with acute renal and hepatic failure and coagulation abnormalities.[1,6] Age (4 to 10 years), sex (male), and breed (herding dogs, hounds, working dogs, and mixed-breed dogs) are risk factors for leptospirosis.[7] Leptospirosis caused by *Leptospira interrogans* serovars canicola and icterohaemorrhagiae has been reported in dogs in the United States for more than 100 years.[3] More recently, leptospirosis caused by other serovars (*Leptospira interrogans* serovar bratislava, *L kirschneri* serovar grippotyphosa, and *Leptospira interrogans* serovar pomona) have been recognized. These serovars have a range of reservoir species, including pigs (serovars bratislava and pomona), horses (serovar bratislava), skunks and opossums (serovars grippotyphosa and pomona), and voles and raccoons (serovar grippotyphosa).[1-3,8-10] Since 1983, the prevalence of leptospirosis in dogs has increased,[7] possibly because of cases associated with serovar grippotyphosa.[1,6,11-14] The reason for this increasing prevalence is unknown, but may include urbanization of rural areas (increasing during the 1980s and 1990s) that provides more opportunity for contact between dogs and wildlife, such as skunks, raccoons, and opossums.[1,2,15,16] Through the use of **geographic information system (GIS)** technology, developed in the earth sciences, landscape features that are associated with leptospirosis can be identified. Identifying characteristics of localities at which there is an increased risk of leptospirosis could be used to implement control programs to reduce disease in dogs. The purpose of the study reported here was to identify environmental risk factors for leptospirosis and specifically determine if urbanization was associated with diagnosis of leptospirosis in dogs evaluated at a veterinary teaching hospital.

Criteria for Selection of Cases

Medical records of dogs examined at the Purdue University Veterinary Teaching Hospital from January 1, 1997, through December 31, 2002, with a diagnosis of leptospirosis were identified by searching the Veterinary Medical Database (diagnosis code 010017200).[17]

From the Departments of Veterinary Pathobiology (Ward, Wu) and Veterinary Clinical Sciences (Guptill), School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907-2027.
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Address correspondence to Dr. Ward.

Leptospirosis was diagnosed by a hospital clinician on the basis of history, clinical signs, CBC and serum biochemical analyses, radiography, and response to treatment, and supported by **microscopic agglutination test (MAT)** results or histologic examination. Six dogs were not tested with the MAT, but leptospires were detected by silver staining of liver and kidney tissue specimens collected at necropsy (1 dog) or by fluorescent antibody test performed on urine (1 dog); liver and kidney tissue (1 dog); or urine and liver and kidney tissue (1 dog). In 30 dogs that were serologically tested, a 4-fold increase in MAT titers of paired sera was detected in 11 dogs, 3- and 2-fold increases in MAT titers of paired sera were detected in 2 dogs, and an MAT titer ≥ 1:800, or 1:400, 1:200, 1:100, and < 1:100 was detected in a single serum sample from 8, 4, 1, 2, and 2 dogs, respectively. Dogs admitted to the same hospital during the same period and tested seronegative (MAT titer < 1:100) for *Leptospira interrogans* serovar hardjo, *L interrogans* serovars bratislava, canicola, icterohaemorrhagiae, and pomona, and *L kirschneri* serovar grippotyphosa antibodies were identified through a laboratory reporting system.[a] The date of diagnosis (cases) or of serum collection (controls) was recorded for all dogs. Information on age, sex, breed, and owner addresses was extracted from recorded information for all dogs selected for inclusion in the study.

Procedures

Dogs examined at the teaching hospital with a diagnosis of leptospirosis or that were seronegative for *Leptospira* serovars were considered as cases and controls, respectively. From recorded information, the following variables were created: age (≤ 1, 1.1 to 1.9, 2 to 3.9, 4 to 6.9, 7 to 10, and > 10 years), sex (male, male castrated, female, and female spayed), and breed (companion dogs, gun dogs, herding dogs, hounds, terriers, working dogs, and mixed-breed dogs), as previously described.[7] From recorded address information, locations were geocoded[b] by use of a 5-digit zip code, street and street number, and a line file of roads created from the US 2000 national census data.[18] Locations were mapped[b] by use of a Universal Transverse Mercator grid system (zone 16) shape file of Indiana counties.[18] A range of environmental factors at study locations was identified by overlaying location data on polygon, line, or point layers. Primary data sources included the State Soil Geographic data base for Indiana,[19] metropolitan areas,[18] National Wetlands Inventory,[20] Indiana recreation facilities inventory,[21] and the 1961 to 1990 average annual precipitation.[22] Several data sets, including urbanized areas from 1990 to 2000 (areas that were rural according to the 1990 national census, but that were metropolitan according to the 2000 census), county hog density (county hog population divided by county land area), county cattle density (county cattle population divided by county land area), county beef cattle density (county beef cattle population divided by county land area), county dairy cattle density (county dairy cattle population divided by county land area), and county forest density (number of trees divided by county land area), were derived because primary data were unavailable. All environmental exposure variables were analyzed as binary variables. For continuous data, above and below median values were used to create binary data. Because of limitations on location accuracy and to account for a possible home range of dogs included in the study, a buffer of 1,000 m was used. For example, for the variable streams, exposed locations were within 1,000 m of a stream or other natural water feature; unexposed locations were those > 1,000 m from such geographic features.

Logistic regression[c] was used to estimate the risk (odds) of being an exposed case versus an unexposed case (**odds ratio [OR]**). The association between leptospirosis and exposure status for all environmental variables was screened by use of a liberal *P* value (0.2). The association between leptospirosis and environmental factors was then modelled by use of stepwise logistic regression[c] and variables significantly ($P < 0.2$) associated with leptospirosis in bivariable analysis. For stepwise analysis, a value of $P < 0.05$ was required for the variable to be retained in the model, and a value of $P < 0.10$ was required for a variable to be removed from the model. The final model was assessed by use of classification tables.[c] Potential confounding by host factors was assessed by refitting the final model and forcing age, sex, or breed into the model. A change in estimated coefficients for the environmental variables of > 10% was considered to represent confounding, in which case the confounding variables were retained in the model and adjusted ORs were estimated.

Results

A total of 36 case and 138 control dogs were identified with Indiana addresses. Study dogs were located in most areas of Indiana and generally reflected the distribution of the human population of the state **(Figure 1)**.

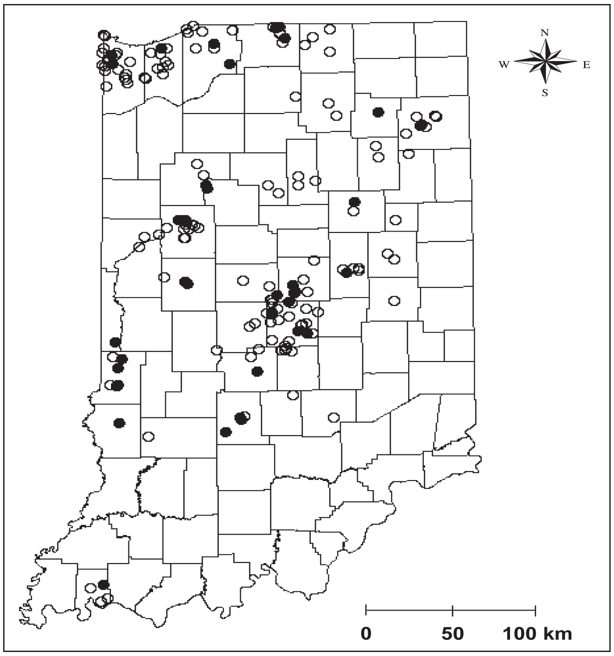

Figure 1—Locations of dogs in Indiana evaluated at a veterinary teaching hospital from 1997 through 2002 with leptospirosis (all serovars, closed circles) or that were seronegative for *Leptospira interrogans* serovars bratislava, canicola, hardjo, icterohaemorrhagiae, and pomona and *Leptospira kirschneri* serovar grippotyphosa as determined by microscopic agglutination test (open circles).

For 19 of the 30 case dogs in which an infecting *Leptospira* serovar could be identified (4-fold increase in MAT titers of paired sera or an MAT titer ≥ 1:800 detected in a single serum sample), leptospirosis was associated with *L kirschneri* serovar grippotyphosa infection.

Significant ($P < 0.2$) associations were found between leptospirosis status and 4 environmental factors including being located within 1,000 m of a rural area, an area urbanized between 1990 and 2000, an area frequently flooded, and within a county in which the density of dairy cattle was greater than the median density of dairy cattle in the state of Indiana (**Table 1**). Significant ($P < 0.2$) associations were found between leptospirosis associated with serovar grippotyphosa and 7 environmental factors including being located within 1,000 m of a rural area, a school with a recreational facility, an area urbanized between 1990 and 2000, an area frequently flooded, an area with poor drainage, and within a county in which the density of dairy cattle or the density of forest trees was greater than the median density of dairy cattle or forest trees in the state of Indiana (**Table 2**).

Being located within 1,000 m of an area urbanized between 1990 and 2000 was the only variable that was retained following stepwise regression for both leptospirosis (OR 8.09; 95% **confidence interval [CI]**, 2.22 to 29.44; $P = 0.0015$) and leptospirois associated with serovar grippotyphosa (OR 15.46; 95% CI, 3.86 to 61.91; $P = 0.0001$) models. Confounding of the association between leptospirosis and urbanization by age and sex and leptospirosis associated with serovar grippotyphosa and urbanization by sex was found. By use of these final models, the disease status of 81.6% and 89.8% of dogs, respectively, were correctly classified (**Table 3**).

Table 1—Association (odds ratio [OR]) between a diagnosis of leptospirosis (all serovars) and location within 1,000 m of various environmental factors (exposed) for dogs with (cases; n = 36) or seronegative for (controls; 138) leptospirosis evaluated at a veterinary teaching hospital from 1997 through 2002.

	Cases		Controls				
Environmental factor	**Exposed**	**Unexposed**	**Exposed**	**Unexposed**	**OR**	**95% CI**	***P* value**
Streams	31	5	113	25	1.37	0.49, 3.88	0.55
Rural areas	16	20	34	104	2.45	1.14, 5.25	0.02*
Recreational facility	9	27	49	89	1.65	0.72, 3.79	0.24
School with recreational facility	16	20	74	64	1.45	0.69, 3.02	0.33
Urbanized areas, 1990–2000	7	29	4	127	8.09	2.22, 29.4	< 0.01*
Wetlands	35	1	129	9	2.44	0.32, 18.8	0.40
Areas frequently flooded	4	31	32	107	0.43	0.15, 1.29	0.14*
Areas with poor drainage	23	13	98	40	0.72	0.33, 1.57	0.41
Farmland	27	9	99	39	1.18	0.51, 2.74	0.95
Annual rainfall > 40 inches	25	11	109	29	1.65	0.73, 3.75	0.23
County hog population > median	8	28	33	105	0.91	0.38, 2.19	0.83
County cattle density > median	20	16	67	71	1.32	0.63, 2.77	0.45
County beef cattle density > median	22	14	70	68	1.53	0.72, 3.23	0.27
County dairy cattle density > median	13	23	73	65	0.50	0.24, 1.07	0.08*
County forest density > median	14	18	57	60	0.82	0.37, 1.80	0.62

*Significantly ($P < 0.20$) associated with a diagnosis of leptospirosis.
CI = Confidence interval.

Table 2—Association (OR) between a diagnosis of leptospirosis caused by *Leptospira kirschneri* serovar grippotyphosa and location within 1,000 m of various environmental factors (exposed) for dogs with (cases; n = 19) or seronegative for (controls; 138) leptospirosis evaluated at a veterinary teaching hospital from 1997 through 2002.

	Cases		Controls				
Environmental factor	**Exposed**	**Unexposed**	**Exposed**	**Unexposed**	**OR**	**95% CI**	***P* value**
Streams	16	3	113	25	1.18	0.32, 4.33	0.80
Rural areas	8	11	34	104	2.22	0.83, 5.96	0.11*
Recreational facility	15	4	49	89	2.06	0.65, 6.56	0.22
School with recreational facility	13	6	74	64	2.51	0.9, 6.97	0.08*
Urbanized areas, 1990–2000	6	13	4	127	15.46	3.86, 61.9	< 0.01*
Wetlands	18	1	129	9	1.26	0.15, 10.3	0.83
Areas frequently flooded	1	18	32	107	0.19	0.03, 1.40	0.10*
Areas with poor drainage	10	9	98	40	0.45	0.17, 1.19	0.11*
Farmland	13	6	99	39	0.85	0.30, 2.40	0.76
Annual rainfall > 40 inches	5	14	109	29	1.34	0.45, 4.03	0.60
County hog population > median	2	17	33	105	0.37	0.08, 1.70	0.20
County cattle density > median	10	9	67	71	1.18	0.45, 3.07	0.74
County beef cattle density > median	11	8	70	68	1.34	0.51, 3.51	0.56
County dairy cattle density > median	7	12	73	65	0.52	0.19, 1.40	0.19*
County forest density > median	5	11	57	60	0.48	0.16, 1.46	0.20*

*Significantly ($P < 0.20$) associated with a diagnosis of leptospirosis caused by *L kirschneri* serovar grippotyphosa.
See Table 1 for remainder of key.

Table 3—Association between leptospirosis (n = 36) and leptospirosis caused by *Leptospira kirschneri* serovar grippotyphosa (19) and being located within 1,000 m of an area urbanized between 1990 and 2000 for dogs with or seronegative for (138) leptospirosis evaluated at a veterinary teaching hospital from 1997 through 2002.

Model	Coefficient	SE	OR	95% CI	*P* value
All *Leptospira* serovars					
Intercept	–3.4425	1.5877	NA	NA	0.0301
Urbanization	3.2566	0.9265	25.96	4.22, 159.6	0.0004
L kirschneri serovar grippotyphosa					
Intercept	–2.8962	0.8857	NA	NA	0.0011
Urbanization	2.4385	0.7373	11.46	2.70, 48.60	0.0009

Age- and sex-adjusted and sex-adjusted estimates for leptospirosis and leptospirosis caused by *L kirschneri* serovar grippotyphosa, respectively, are depicted. SE = Standard error of the regression coefficient. NA = Not applicable.

See Table 1 for remainder of key.

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Discussion

The risk of a dog having a diagnosis of leptospirosis was significantly and substantially associated with a dog being located in an area that had been rural in 1990, but was classified as urban in 2000. The appearance of new pathogens in populations may be caused by several factors, including cross-species transfer, spatial diffusion, pathogenic evolution, and changes in the host-environment relationship.[23] Areas characterized by low residential density and a landscape of recently reforested deciduous forest are strongly associated with the risk of another spirochete disease, Lyme disease.[24,25] Peridomestic transmission of this tick-borne infection has been detected,[24,26] and human behavior, including how humans move in their environment, their outdoor activities, and individual protection responses, is a strong predictor of disease risk. Reforestation in areas previously used for agriculture results in more favorable conditions for ticks and reservoir hosts, whereas the trend towards residential preferences in well-shaded surburban and rural areas exacerbates the interactions between ticks and humans.[27] An unintended consequence of real estate development has been an increasing occurrence of Lyme disease.[23] Results of the study reported here suggest that disease transmission patterns similar to Lyme disease may be occurring with respect to exposure of dogs to leptospires and this reemerging disease[7] in dog populations. Urbanized areas contain a high proportion of ecotone habitats (residential interface between ornamental areas and woods and wooded corridors), in which direct or indirect contact between humans, their pets, and wildlife is facilitated.[25] Increasing contact between dogs and wildlife, which may serve as reservoir hosts of many serovars including grippotyphosa and bratislava, could be one explanation of the resurgence of leptospirosis in dogs. Harkin and Gartrell[6] and Bolin[3] suggest that housing developments that encroach on areas previously inhabited only by wildlife leads to greater exposure of dogs to *Leptospira* serovars that have wildlife species as their maintenance hosts. Supporting this hypothesis is the observation made by Rentko et al[11] that the prevalence of leptospirosis caused by serovar grippotyphosa diagnosed at Tufts University Veterinary Teaching Hospital increased when the hospital was relocated from an urban environment to a rural location in central Massachusetts. Raccoons have been suggested as the source of infection for cases of leptospirosis caused by serovar grippotyphosa in Georgia.[12] Being located within a county with a forest density greater than the median density of forest trees in the state of Indiana was not associated with the risk of leptospirosis in the study reported here. However, this variable does not adequately describe small, periurban areas of forest. Ecotone information on the scale necessary to further investigate this association needs to be specifically collected in field studies. Similarly, statewide spatial information on wildlife populations was unavailable for analysis in the study reported here. Studies at foci of exposure of dogs to leptospires incorporating wildlife data may provide stronger evidence of causality. In addition to urbanization and forest density, being within a county with greater than the median dairy cattle density and within 1,000 m of a school with a recreational facility, an area with poor drainage, and an area frequently flooded were associated ($P < 0.20$) with leptospirosis on bivariate analysis. Leptospires shed in the urine of infected animals can survive for long periods in surface water, and susceptible hosts are infected through contact with contaminated water.[4] Flooding has been associated with outbreaks of leptospirosis in humans and animals, and leptospirosis is an occupational hazard for rice- and sugarcane-field workers and a recreational hazard for swimmers and campers and during sporting events.[4] Gardening, the presence of dogs around the home, and walking through ponds or stagnant water have been identified as risk factors for leptospirosis in humans,[28] and rainfall may be useful in predicting the number of cases of leptospirosis in dogs[8,15] and cattle.[29,30] However, in the present study, areas with poor drainage and areas frequently flooded were apparently protective for leptospirosis. Random error, misclassification, and confounding bias may have been responsible for this unexpected finding. Classification of areas with poor drainage and areas frequently flooded was determined on the basis of long-term, historical data.[19,20] Short-term exposure of case dogs to areas with poor drainage or frequently flooded may not have been captured by the use of long-term data; therefore, exposure status may have been misclassified. Confounding, for example, by age, breed, or sex of dogs may be another explanation for the apparent protective effect of these factors on bivariate analysis. Confounding was only assessed in this study for those factors included in the final model.

Although urbanization was the most important environmental factor influencing leptospirosis occurrence, estimates were not precise. Leptospirosis was

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diagnosed in the 36 case dogs included in this study during a period of 6 years. To obtain more dogs with a diagnosis of leptospirosis and thus increase the precision of estimates, further studies need to be multicentered. Because of variability in environmental factors and consistency of geographic data, such studies should be based at institutions located within the same geographic region. Even so, it may be difficult to obtain high study power to detect important associations between environmental factors and leptospirosis.

In the analysis, the spatial exposure factors for each dog included were considered best identified by the home address of each owner. Certain case dogs may have been infected by *Leptospira* serovars > 1,000 m from their home, and certain control dogs may have spent a substantial amount of time away from their home address. In dogs, the incubation period for leptospirosis varies between 3 and 20 days[3]; therefore, this is the relevant period of exposure that must be considered. In humans, Lyme disease is mostly acquired in the peridomestic environment.[24,26] Although the routes of transmission are different between Lyme disease (tick-borne) and leptospirosis (exposure to urine-contaminated water), peridomestic infection supports the simplifying assumption that a dog's home address represents a dog's environmental exposure in the preceding 3 weeks. In a study to identify and locate residential environmental risk factors for Lyme disease in Baltimore County, Md, eliminating case patients who were exposed to the disease away from their residences did not affect the spatial pattern of the disease map produced.[31] As an additional correction for potential misclassification of exposure status in the study reported here, environmental risk factors were buffered by 1,000 m to take into account short-distance movement by dogs (eg, roaming or supervised visits to a nearby park) and also any inaccuracies in geocoding. Finally, this was a retrospective, exploratory analysis. As with any GIS analysis to determine causal relations in which estimates or approximations are derived to support or refute hypotheses, conclusions should be confirmed by field epidemiologic studies.[32]

Geographic information systems are increasingly being used to measure environmental factors for disease. Perhaps the most common GIS-based process applied to environmental and disease data is overlay analysis.[26] A common approach is to identify disease cases and controls and estimate environmental exposure to a range of factors (including demography, vegetation, land use, geology, hydrography, topography, and health services) on the basis of point locations of individuals or geographic centers of units included in the study.[26,32] Data can be analyzed, usually through the use of logistic regression, to generate risk models.[31] Another approach, particularly useful when polygons (eg, prevalence of disease by zip code areas) are the unit of study, is to use raster coverages in a GIS and measure correlations between exposure and disease.[26] Combining a GIS with epidemiologic methods can be used to rapidly identify risk factors for zoonotic disease over large areas.[31] Combining epidemiologic methods (identification of factors associated with disease) with GIS methods (determination of where factors occur) provides a powerful approach to study the distribution of diseases that are influenced by a multitude of environmental factors.[31]

Identification of environmental risk factors for leptospirosis permits better advice to be given to owners of dogs at risk of exposure. If geographic foci presenting an increased risk can be identified, these areas can be avoided, owners can be encouraged to vaccinate their dogs, and awareness of clinical signs associated with leptospirosis can be increased. Dogs could also act as sentinels for human disease in such environments.

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[a]Purdue University Animal Disease Diagnostic Laboratory, West Lafayette, Ind.

[b]ArcView, version 3.3, Environmental Systems Research Institute Inc, Redlands, Calif.

[c]SPSS for Windows, version 10.1.0, SPSS Inc, Chicago, Ill.

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