

Spatial associations among density of cattle, abundance of wild canids, and seroprevalence to *Neospora caninum* in a population of beef calves

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Objective—To determine the epidemiologic plausibility of a sylvatic transmission cycle for *Neospora caninum* between wild canids and beef cattle.

Design—Spatial analysis study.

Animals—1,009 weaned beef steers from 94 beef herds in Texas.

Procedure—Calves were grouped on the basis of seroprevalence for *N caninum* and ecologic region in Texas. The Moran's *I* test was used to evaluate spatial interdependence for adjusted seroprevalence by ecologic region. Cattle density (Number of cattle/259 km² [Number of cattle/100 mile²] of each ecologic region) and abundance indices for gray foxes and coyotes (Number of animals/161 spotlight-transect [census] km [Number of animals/100 census miles] of each ecologic region) were used as covariates in spatial regression models, with adjusted seroprevalence as the outcome variable. A geographic information system (GIS) that used similar covariate information for each county was used to validate spatial regression models.

Results—Spatial interdependence was not detected for ecologic regions. Three spatial regression models were tested. Each model contained a variable for cattle density for the ecologic regions. Results for the 3 models revealed that seroprevalence was associated with cattle density and abundances of gray foxes, coyotes, or both. Abundances of gray foxes and coyotes were collinear. Results of a GIS-generated model validated these spatial models.

Conclusions and Clinical Relevance—In Texas, beef cattle are at increased risk of exposure to *N caninum* as a result of the abundance of wild canids and the density of beef cattle. It is plausible that a sylvatic transmission cycle for neosporosis exists. (*J Am Vet Med Assoc* 2000;217:1361–1365)

Slightly more than a decade ago, *Neospora caninum*, an apicomplexan protozoan, was found to be a cause of abortions in cattle.¹ Since that report, other

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details concerning neosporosis in cattle have been documented (calves born with neurologic deficits, vertical transmission without clinical signs of infection).² Exposure to neosporosis was found to decrease milk production in dairy cows³ and to impair weight gain after weaning in beef calves,^a both of which cause substantial economic losses to the cattle industry.^{4,5}

Most epidemiologic studies conducted on neosporosis in cattle indicate that vertical transmission is the major mode of transmission within a herd.² However, questions still persist concerning the route of entry of *N caninum* into a herd, and considerable effort has been devoted to elucidating the life cycle of this organism. For example, studies conducted in controlled laboratory settings have documented that dogs are capable of producing infectious *N caninum* oocysts,^{6,8} and seroepidemiologic studies have documented that *N caninum* may elicit an antibody response in wild canids⁹⁻¹¹; however, to our knowledge, oocysts of *N caninum* have not been detected in canids other than those used in controlled laboratory settings. Reports^{12,13} also have established that deer, a natural prey of wild canids, are susceptible to natural infections attributable to *N caninum*. We are unaware of any study in which investigators have evaluated a sylvatic transmission cycle for neosporosis by use of analytic methods.

Recently, studies that involved a heterogeneous population of beef cattle in Texas have identified management-related risk factors for exposure of beef calves to *N caninum*.^a Analysis of these risk factors suggests vertical and horizontal exposure to *Neospora* organisms. Ecologic factors (eg, cattle density) significantly influenced the modeling process. Therefore, we evaluated this population of beef cattle by use of a spatial analysis. Geographic units of interest were 10 ecologic regions in Texas, and the outcome variable was seroprevalence of beef calves to *N caninum*. Our primary interest was to determine the epidemiologic plausibility that wild canids are involved in a sylvatic transmission cycle of neosporosis to cattle. Our specific objectives were to measure the degree of spatial correlation for adjusted seroprevalence to *N caninum* by ecologic region; to evaluate a spatial regression model for prediction of seroprevalence to *N caninum* for each ecologic region, using covariates of cattle density and abundances of gray foxes (*Urocyon cinereoargenteus*), coyotes (*Canis latrans*), or both; and to validate the spatial regression model by use of a geographic infor-

mation system (GIS)-generated model for seroprevalence, using the same spatial covariates but applied for each county.

Materials and Methods

Study population—Calves involved in the 1998 to 1999 Texas A&M University Ranch-to-Rail Program^a at 2 feedlots in Texas were included in the study. Calf consignments were from each ecologic region in the state. Consignment to 1 of the 2 feedlots was made on the basis of the consignors' preference. Ranchers were not aware of this study prior to arrival of their calves at the feedlots, thus preventing selection bias for this study.

Seroprevalence of this population of cattle has been reported.^a Briefly, samples were obtained from 1,009 beef calves from 92 consignments at time of arrival at 2 feedlots in Texas during a 4-day period in October 1998. Serum samples were tested for detection of antibodies to *N caninum*, using an agglutination assay.¹⁴ Number of calves per consignment ranged from 1 to 116. Overall, 131 of 1,009 (12.98%) calves were seropositive for *N caninum*, and 54 (58.7%) of the consignments had ≥ 1 seropositive calf. One consignments originated from 2 ranches; thus, the total number of ranches was 94. All ranches were located in Texas.

Seroprevalence for each ecologic region was determined by determining the number of seropositive calves and total number of calves from ranches that were located in a given ecologic region. The number of seropositive calves for a given region was divided by the total number of calves from that same region. Boundaries of each of the 10 ecologic regions in Texas were determined, using a map provided by the Texas Parks and Wildlife Department (Fig 1).^b

Covariate data—Density of cattle for each county and ecologic region were determined, using information found in the 1997 USDA Agricultural Census.^c Density of cattle for each county was derived by calculating the number of female cattle giving birth during 1997 in each county and dividing that value by the number of pasture units for each county in 1997. Each pasture unit constituted 259 km² (100 mile²). The number of pasture units per county was calculated by dividing the number of acres of pasture per county by 64,000. Density of cattle in each ecologic region was calculated by determining the number of female cattle that had given birth during 1997 in a region, using census information for each county in a region, and dividing that value by the number of pasture units (259 km²) in each region. Similarly, number of pasture units in each ecologic region was determined by calculating the number of acres of pasture in each region, using census information for each county in that region, and dividing that value by 64,000. Data from the 1997 Agricultural Census was selected for use, because it represented the most reliable estimates of cattle density during the time frame of the study.

Abundance indices for gray foxes and coyotes were provided by the Texas Parks and Wildlife Department.^{d,e} Departmental wildlife biologists counted the number of fur-bearing animals observed along spotlight-transect (census) routes in all 10 ecologic regions, and the number of census kilometers (census miles) was calculated. These values are used as indices of changes in the abundance of fur-bearing animals among years. Indices are determined on an ecologic region-by-county basis (Fig 1). We used the mean of these abundance indices for gray foxes and coyotes for 2 consecutive years (1998 and 1999) in our analyses. An abundance index for wild canids was calculated by adding the abundance index for gray foxes with that for coyotes. The 2-year period was used, because it is similar to the time frame when the cattle in our study were born.

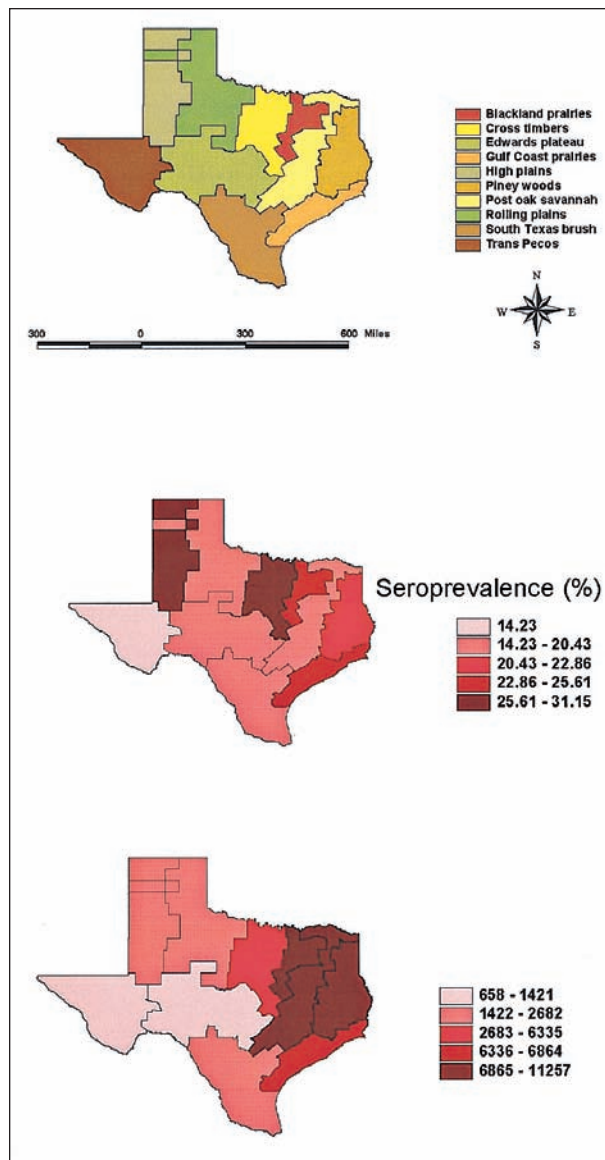


Figure 1—Top—Ecologic regions in Texas on the basis of counties in each region. Center—Adjusted seroprevalence for *Neospora caninum* in beef calves by ecologic region in Texas. Results represent percentage of seropositive calves per ecologic region. To convert miles to kilometers, multiply value by 8/5. Bottom—Density of cattle in each ecologic region in Texas. Results represent No. of cows/259 km² of pasture/ecologic region (No. of cows/100 miles² of pasture/ecologic region).

Statistical analysis—Multiple statistical software programs were used to conduct our spatial analyses.¹⁴ Regional seroprevalence were adjusted for normality, using the Freeman-Tukey method.¹⁵ Data, including adjusted seroprevalence, density of cattle, and abundance indices of gray foxes, coyotes, and wild canids, were entered in a lattice format for each ecologic region.^{14,j}

The Morans *I* test,¹⁶ a spatial measure of autocorrelation, was used to determine the extent of spatial interdependence (clustering) for seroprevalence among ecologic regions. A second-order contiguity matrix between contiguous ecologic regions was constructed for use in the computation of the Morans *I* test and subsequent spatial regression analyses.^{17,h} To adjust for irregularities in the variance function caused by varying sample sizes among ecologic regions, a weight-adjustment method for each ecologic region was used.¹⁷ We

computed the inverse of the total number of calves from which samples were obtained in each region and applied these values to the spatial regression analyses.⁸

Spatial regression analyses were conducted for each ecologic region, using the adjusted seroprevalence proportions per region as the dependent variable.⁸ Multivariate spatial regression models were constructed to evaluate the effects of cattle density and abundances of gray foxes, coyotes, and wild canids. A simultaneous autoregressive covariance matrix structure with a second-order contiguity matrix with adjusted weights for varying sample size among regions was used for all regression models.¹⁷ The χ^2 likelihood ratio statistic and the model-based *P* value were used to determine significance of a covariate in the model.⁸ Values were considered significant at values of $P \leq 0.05$. Adjusted spatial residual values were visually examined to detect values that were outliers.¹⁸ The Morans *I* test was used to evaluate changes in spatial autocorrelation following application of each regression model.

Validation of the spatial regression models was performed, using GIS map overlays.¹⁴ Data for all 254 counties in Texas (density of cattle, abundance indices for gray foxes, coyotes, and wild canids) were used in a lattice format to conduct map queries concerning covariates for each county. Counties located in similar ecologic regions had similar abundance indices for gray foxes, coyotes, and wild canids, whereas each county was assigned its own specific cattle density. The GIS-generated map overlays were optimized by use of a kernel-smoothed estimate of nonadjusted prevalence rates for each ranch.¹⁸ Location of each ranch was defined as the longitude and latitude of the nearest town.¹⁴ Kernel smoothing was accomplished by calculating the bivariate density of nonadjusted prevalence rates for each ranch and an area of 91 km² (35 miles²). Use of an area of 91 km² allowed visual detection of effects across or near county lines.

After optimization of the GIS-generated model, overall seroprevalence was tabulated for the geographic area that fit the model covariates. In addition, overall seroprevalence was tabulated for counties adjacent to the modeled area and for the remainder of the state. A 3 × 2 contingency table was constructed, consisting of the number of seropositive and seronegative calves for each of the geographic areas considered. A χ^2 test of trend was used to evaluate the GIS model.^k

Results

Frequency distribution of adjusted seroprevalences and covariates for cattle (Fig 1) and wild canids (Fig 2) used in the spatial analyses of ecologic regions were determined. The Morans *I* statistic for adjusted seroprevalence of ecologic regions was not significant. Conditional multivariate spatial regression analyses produced 3 models that fulfilled our specifications. Cattle density had a significant positive effect in each of the 3 multivariate models. Variable estimates (\pm SE) were determined for spatial regression coefficients of gray foxes (0.81 ± 0.13 ; $P < 0.001$), coyotes (1.39 ± 0.38 ; $P = 0.01$), and wild canids (0.54 ± 0.10 ; $P = 0.002$). Abundance indices for gray foxes and coyotes did not fit into any model simultaneously because of collinearity. Spatial residuals for the 3 models were examined, with substantial improvement towards normality being evident in residuals of all ecologic regions except the piney woods and rolling plains for at least 1 of the 3 models. The Morans *I* statistic was not significant for any of the 3 regression models.

The GIS-generated model was optimized for counties in Texas that had $\geq 4,240$ cattle/259 km² and that

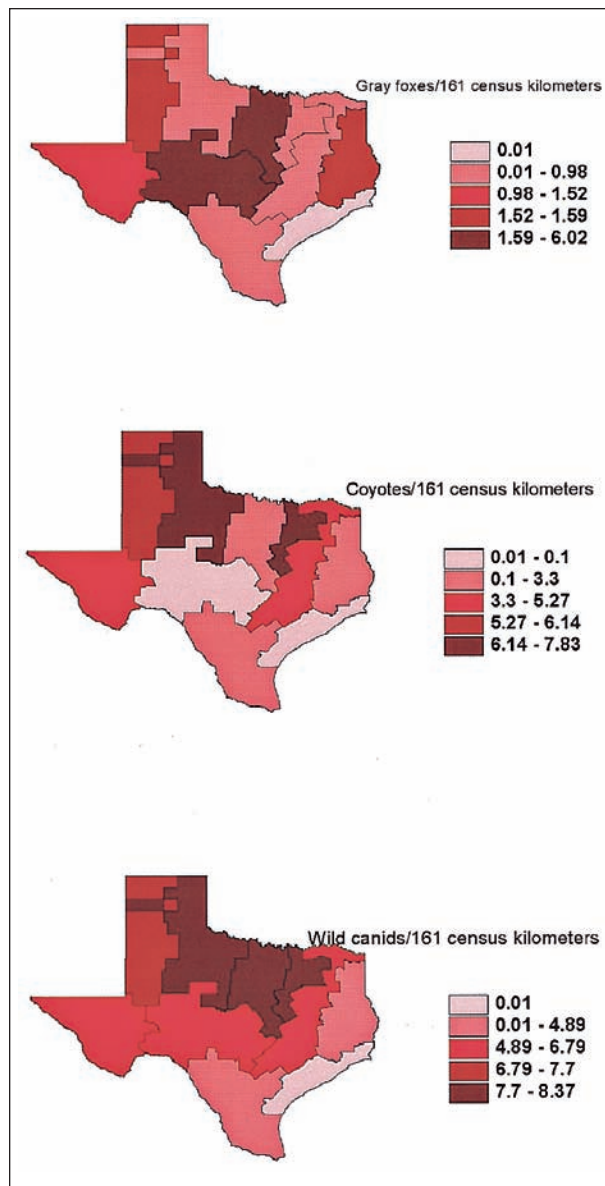


Figure 2—Top—Abundance of gray foxes in each ecologic region in Texas. Results represent No. of gray foxes/161 spotlight-transect (census) km (No. of gray foxes/100 census miles) of ecologic region. Center—Abundance of coyotes in each ecologic region in Texas. Results represent No. of coyotes/161 census km (No. of coyotes/100 census miles) of ecologic region. Bottom—Abundance of wild canids in each ecologic region in Texas. Abundance of wild canids was determined by adding the abundances of gray foxes and coyotes. Results represent No. of wild canids/161 census km (No. of wild canids/100 census miles) of ecologic region.

also had ≥ 4.98 gray foxes/161 census km or ≥ 5.27 coyotes/161 census km. Counties that fit the covariate query with the overlay of kernel-smoothed estimates of nonadjusted consignment prevalences, based on ranch location and an area of 91 km², were identified (Fig 3). Overall seroprevalence for counties identified in the map query was 17.4% (45/259) and represented 31 consignments. Overall seroprevalence for counties adjacent to the counties identified in the map query was 12.8% (25/196) and represented 28 consignments. Overall seroprevalence for the remainder of the state

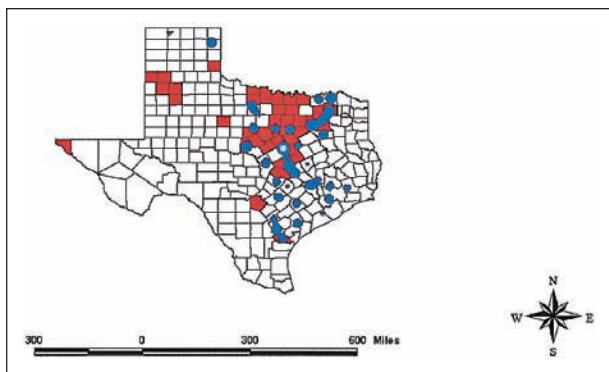


Figure 3—Geographic information system-generated model of the spatial relationship of nonadjusted seroprevalence for *N caninum* in beef calves with cattle density and abundances of gray foxes or coyotes. Blue areas represent the kernel-smoothed density of seroprevalence in calves determined on the basis of ranch location and a radius of 91 km² (35 miles²). Red areas represent counties that had $\geq 4,240$ cattle/259 km² and that also had ≥ 4.98 gray foxes/161 census km or ≥ 5.27 coyotes/161 census km.

was 11% (61/554) and represented 35 consignments. Results of the Mantel-Haenszel χ^2 test of trend for the 3-layer model was 6.051 ($P = 0.01$).

Discussion

To our knowledge, this is the first attempt to perform a spatial analysis of covariates for association with seroprevalence for *N caninum* in beef cattle for each ecologic region in Texas. We report seroprevalence data for each ecologic region of Texas, a state with diverse ecologic regions that has the largest population of beef cattle in the United States. It has been suggested in much of the literature that vertical infection is the primary means by which neosporosis was maintained in cattle. Recently, however, it became apparent that dogs are a definitive host for *N caninum*.^{6,8} Furthermore, seroepidemiologic analysis of data supports the hypothesis that dogs are associated with cattle in production systems with confirmed neosporosis.^{19,25} The findings reported here provide epidemiologic evidence that supports the hypothesis of a sylvatic transmission cycle between wild canids and beef cattle.

An important consideration in our analysis was the use of data derived on the basis of ecologic regions and the link to data derived on the basis of political boundaries (ie, counties). Borders of ecologic regions do not typically correspond with political boundaries and would pose a source of error with analytic methods that simultaneously consider data for each county. We consider this type of error to be minimal in this study, because the abundance indices for gray foxes and coyotes were generated through surveillance of ecologic regions from data compiled for each county in that region, thus providing a consistent framework that allowed linkage of such data and minimized the possibility of error.

Another important consideration in the study reported here was the identification of a research population that is representative of the population of beef cattle in Texas. The Texas A&M University Ranch-to-Rail Program provides such a population. Evident in this population is heterogeneity of genetic potential, management style, and geographic origin.

We did not find evidence of spatial autocorrelation for seroprevalence to *N caninum* in beef calves for ecologic regions before or after spatial regression analyses. Density of cattle was found to be important in these analyses. Density of cattle previously was identified as a risk factor for exposure of these calves to *N caninum* at the ranch of origin.⁸ Ranches with a high density of cattle are more likely to use supplemental feeding practices, which may increase the risk of horizontal transmission through fecal contamination of feed and water sources by a definitive host in regions with high estimates for the abundance of wild canids.

Evidence of collinearity between abundance indices of gray foxes and coyotes was identified when it was found that abundance of gray foxes did not fit when inserted into the model simultaneously with density of cattle and abundance of coyotes. The 3-layer GIS model and the regression model constructed by use of cattle density and the abundance index for wild canids yielded significant results, providing proof of collinearity between abundances of gray foxes and coyotes. Examination of spatial residual values suggested that differing patterns of risk for the wild canids were evident among ecologic regions. On the basis of these findings, we consider coyotes and gray foxes to be risk factors.

Results of the GIS-generated model validated the spatial regression models. This validation was performed on the basis of county. Construction of a 3-layer GIS model clearly supported the hypothesis that substantial horizontal exposure of beef cattle in Texas to *N caninum* may be attributable to wild canids.

^aBarling KS. The study of *Neospora caninum* in a Texas beef cattle population. PhD Dissertation, Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, Tex, 2000.

^bTexas Parks and Wildlife Department, Austin, Tex.

^cUSDA. 1997 Agriculture census of agriculture. Washington, DC: National Agriculture Statistical Service, 1999.

^dSmall game research and surveys: fur-bearing animal regulations—evaluation of annual fur harvest. Austin, Tex: Texas Parks and Wildlife Department, 1998.

^eSmall game research and surveys: fur-bearing animal regulations—evaluation of annual fur harvest. Austin, Tex: Texas Parks and Wildlife Department, 1999.

^fS-PLUS 2000P, MathSoft Inc, Seattle, Wash.

^gS+ spatial stats, version 1.5, MathSoft Inc, Seattle, Wash.

^hS-PLUS for Arcview, MathSoft Inc, Seattle, Wash.

ⁱTexas almanac characterization tool, version 1.5, Texas A&M University System, Texas Agricultural Experiment Station, Blackland Research Center Report No. 99-03, 1999.

^jArcview GIS, version 3.1, Environmental Systems Research Institute Inc, Redlands, Calif.

^kSAS, version 6.12, SAS Institute Inc, Cary, NC.

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