Assessing the association between the geographic distribution of deer ticks and seropositivity rates to various tick-transmitted disease organisms in dogs

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Objective—To determine whether the geographic distribution of deer ticks (*Ixodes scapularis*) was associated with the distribution of dogs seropositive for various tick-transmitted disease organisms (ie, *Borrelia burgdorferi*, *Rickettsia rickettsii*, the human granulocytic ehrlichiosis [HGE] agent, *Ehrlichia canis*, and *Bartonella vinsonii* subsp *berkhoffii*).

Design—Serologic survey.

Sample Population—Serum samples from 277 dogs in animal shelters and veterinary hospitals in Rhode Island.

Results—Overall, 143 (52%) dogs were seropositive for *B burgdorferi*, 59 (21.3%) were seropositive for *R rickettsii*, 40 (14.4%) were seropositive for the HGE agent, 8 (2.9%) were seropositive for *E canis*, and 6 (2.2%) were seropositive for *B vinsonii*. Regression analysis indicated that the natural logarithm of nymphal deer tick abundance was correlated with rate of seropositivity to the HGE agent and to *B burgdorferi* but not to rate of seropositivity to *R rickettsii*, *E canis*, or *B vinsonii*. Percentages of samples seropositive for *B burgdorferi*, *R rickettsii*, the HGE agent, and *E canis* were significantly higher for samples from the southwestern part of the state where ticks in general and deer ticks in particular are abundant than for samples from the northern and eastern portions of the state, where ticks are relatively rare.

Conclusions and Clinical Relevance—Results suggested that all 5 disease agents are in Rhode Island and pose a risk to dogs and humans. Knowledge concerning tick distributions may be useful in predicting the pattern of disease associated with particular tick species and may aid diagnostic, prevention, and control efforts. (*J Am Vet Med Assoc* 2001;218:1092–1097)

Lyme disease, **human granulocytic ehrlichiosis (HGE)**, **Rocky Mountain spotted fever (RMSF)**, and canine ehrlichiosis are all tick-borne diseases with potentially serious outcomes in dogs and humans.[1-10] Additionally, bartonellosis is suspected of being tick borne, as tick exposure has been identified as a risk factor for *Bartonella vinsonii* antibodies in dogs.[11] Lyme disease, caused by infection with the spirochete *Borrelia burgdorferi* sensu lato, and granulocytic ehrlichiosis, caused by *Ehrlichia phagocytophila*, are thought to be transmitted by ticks in the *Ixodes ricinus* complex; in the northeastern and upper midwestern United States, the black-legged or deer tick, *Ixodes scapularis*, serves as the principal vector of these agents.[12-13] The American dog tick, *Dermacentor variabilis*, and the wood tick, *D andersoni*, are largely responsible for transmitting the agent of RMSF, *Rickettsia rickettsii*, in the eastern and western United States, respectively.[14] Brown dog ticks, *Rhipicephalus sanguineus*, are considered the principal vector of *Ehrlichia canis*, now 1 of 3 agents known to cause canine ehrlichiosis,[15] and the lone star tick, *Amblyomma americanum*, is the suspected vector of *B vinsonii*.[11] Recently, a newly described subspecies, *B vinsonii arupensis*, was isolated from a human and from rodents naturally infected with *B burgdorferi* and *Babesia microti*, agents typically associated with black-legged ticks.[16]

In the United States, tick-borne diseases have emerged as public health threats at a rate of about 1 per decade ever since the first, Texas cattle fever, was documented by Smith and Kilbourne in 1893.[17-18] As the public health threat from ticks increases, it becomes

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imperative to identify and understand factors that influence the spatial and temporal patterns of disease. A pathogen's association with a specific vector species is 1 such critical factor. If such vector associations with disease-causing agents are known, it may be possible to predict the expected pattern of disease on the basis of ecological information related to particular tick species. In this way, delineating the distribution of vector ticks would aid disease diagnosis, prevention, and control activities.

Ticks in the *I ricinus* complex, and particularly *I scapularis* in the northern United States, are known to transmit an array of zoonotic organisms to humans and domestic animals.[13,19-20] Several diseases, including Lyme disease, HGE, and human babesiosis, have emerged principally within the distribution of *I scapularis*. To test whether this tick should be implicated in the emergence of additional tick-borne pathogens, we examined the possible overlap between the geographic distribution of deer ticks (*I scapularis*) and that of dogs seropositive for *B burgdorferi*, *Rickettsia rickettsii*, the HGE agent, *E canis*, and *B vinsonii* subsp *berkhoffii*. In particular, we determined antibody prevalence to these antigens among Rhode Island dogs from regions of the state where *I scapularis* are abundant or relatively rare.

Materials and Methods

Sample collection—Serum samples used in the study were obtained between November 1997 and May 1998 from 277 dogs examined at veterinary hospitals (n = 220; 79.4%) or held at animal shelters (57; 20.6%) in Rhode Island (**Fig 1**).

Samples were obtained from dogs residing in the 10-town deer tick-abundant region of Rhode Island, as well as from 10 towns where deer tick abundance is low.[21] Veterinarians enrolled dogs in the study if they resided in 1 of the 20 towns, even if the clinic was not located in 1 of these towns. Only animal shelters located in the 20-town study area were invited to participate. The town of residence of shelter-held dogs was defined as the town in which the shelter was located.

Towns included in this study were designated as either deer tick-abundant or -rare on the basis of a nymphal deer tick density map of Rhode Island.[21] There were 10 towns where deer ticks are considered abundant (10 to > 50 nymphal ticks/h of sampling). An equal number of towns with low deer tick levels (< 10 nymphal ticks/h) was selected on the basis of veterinarian willingness to participate and attempts to include as broad a geographic distribution statewide as possible. This resulted in a convenience sample rather than a random sample for our study. In particular, the northwestern corner of Rhode Island was undersampled owing to a lack of veterinarians willing to participate.

Animal shelters were contacted to participate in this study on the basis of their location in 1 of the 20 towns in the study area. All animal shelters known to us in these towns were invited to participate. When shelter personnel agreed to participate, a date was set for study personnel to travel to the shelter to obtain blood samples from all dogs available, except for extremely aggressive dogs. Veterinary hospitals were solicited to participate in the study by obtaining listings of veterinary hospitals from local telephone books and telephoning veterinarians working in those hospitals. Each veterinarian who agreed to participate was asked to collect a blood sample from each of the first 20 dogs seen by that veterinarian that met the study criteria. These criteria were that dogs were brought to the veterinary hospital for routine physical examination and vaccination or for elective surgery,

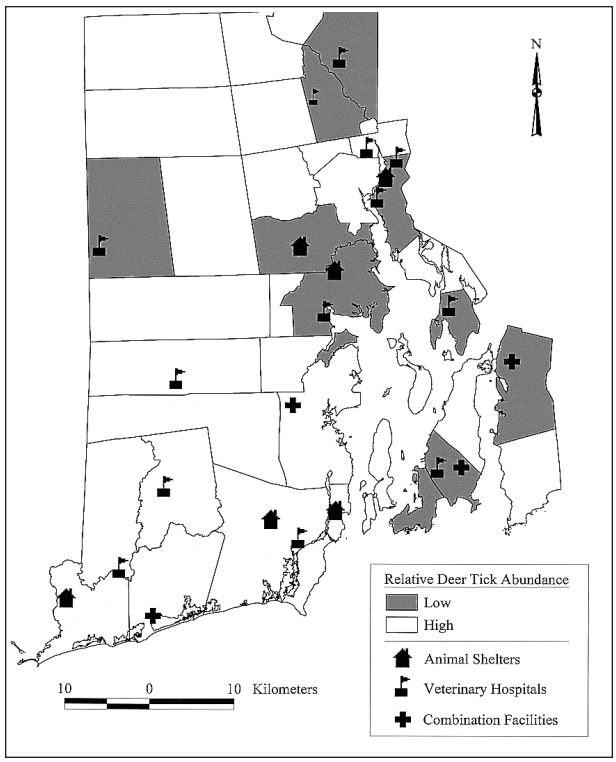

Figure 1—Relative deer tick abundance (low, < 10 nymphal ticks/h of sampling; high, ≥ 10 nymphal ticks/h of sampling) and location of Rhode Island towns involved in a study to determine whether the geographic distribution of deer ticks (*Ixodes scapularis*) was associated with the distribution of dogs seropositive for various tick-transmitted disease organisms.

dogs must not have been vaccinated against Lyme disease, and dogs must reside in one of the 20 towns in the study. Samples were obtained from dogs in all Rhode Island counties.

Blood samples obtained by veterinarians were collected into serum separator tubes and refrigerated at veterinary hospitals for no more than 7 days. Samples were transported to the laboratory in coolers with ice packs where they were centrifuged for 10 minutes at 5,000 × g, and serum was removed from the serum separator tube and aliquoted into storage tubes. Blood samples from animal shelters were collected into serum separator tubes, transported to the laboratory in coolers with ice packs, centrifuged for 10 minutes at 5,000 × g, and aliquoted into storage tubes on the day the samples were collected. Serum samples were stored at –70 C until analyzed at the end of the 7-month collection period. Information on physical appearance of the dog, town of residence, sex, breed, and approximate age was obtained for each dog from which a serum sample was obtained. One hundred twenty-nine (46.6%) samples were collected from dogs that resided in deer tick-abundant regions of Rhode Island and 148 (53.4%) samples were collected from dogs residing in towns where deer tick abundance was low. Twenty-two (17%) of the samples collected from the deer tick-abundant region were from animal shelters, whereas 38 (25%) samples collected from areas with low deer tick abundance were from animal shelters.

Serologic assays—An **indirect fluorescent antibody (IFA)** test incorporating *E phagocytophila* group antigens[6] was used to determine whether dogs were seropositive for the HGE agent. Briefly, HL60 cells infected with the USG3 strain of the HGE agent[a] were applied to 12-well microscope slides. Serum samples were diluted 1:32 and applied to the slides, and slides were incubated at 37 C for 30 minutes.

Slides were then washed in phosphate-buffered saline solution for 20 minutes and air-dried. **Fluorescein isothiocyanate (FITC)**-labeled goat anti-dog IgG[b] diluted 1:20 was applied to each well, and slides were incubated, washed, mounted with a 1:9 phosphate-buffered saline solution-glycerol mixture, and examined at 400× by means of epifluorescence microscopy.[c] Each slide contained appropriate positive and negative controls, including wells that did not contain HL60 cells, wells with uninfected HL60 cells, and wells with infected HL60 cells. Serial dilutions were performed on samples that yielded positive results to determine antibody titer. Titer was expressed as the reciprocal of the highest dilution that still yielded a positive result. Dogs with titer ≥ 64 were considered seropositive.

A similar IFA test was used to determine whether dogs were seropositive for *R rickettsii*, except that the antigen[d] was applied to wells on the slides by use of a capillary tube technique.[22] Dogs with titer ≥ 64 were considered seropositive.[22]

Indirect fluorescent antibody tests were also used to determine whether dogs were seropositive for *E canis* (Florida) and *B vinsonii* subsp *berkhoffii* (93-C0-1). Thirty-well polytef-coated slides were used,[11,23] and serial 2-fold dilutions of serum samples were reacted with FITC-labeled anti-dog IgG conjugates.[e,f] Titer was determined as the highest dilution for which brightly staining organisms could be detected by means of fluorescence microscopy using exciter and barrier filters. For both tests, dogs with titers ≥ 128 were considered seropositive.

An ELISA similar to that described previously[24] was used to determine whether dogs were seropositive for *B burgdorferi*. Briefly, serum and control samples were diluted 1:100 in phosphate-buffered saline solution with 10% rabbit serum and added to wells coated with antigen (*B burgdorferi* strain B31). Wells were incubated for 1 hour at room temperature (approx 20 C) and washed 4 times with distilled water containing 0.05% Tween 20. Horseradish peroxidase-labeled goat anti-dog IgG (heavy and light chain specific)[b] was diluted 1:2,000 and added to each well, and wells were again incubated and washed. Developing solution[b] was added, and the reaction was stopped with 1*N* sulfuric acid. **Optical density (OD)** was determined by use of an ELISA plate reader[g] at 405 nm. Dogs for which OD was ≥ 0.9402 were considered seropositive. This value was equal to 3 SD greater than the mean OD for 8 negative control samples.

Statistical analyses—For each disease organism, Pearson χ^2 tests were used to determine whether sex, age, **American Kennel Club (AKC)** group classification of breed, season during which blood samples were collected (autumn [Nov 10–Dec 21] vs winter [Dec 22–Mar 21] vs spring [Mar 22–May 29]), or relative deer tick abundance (low vs high) was associated with whether dogs were seropositive for that particular organism (yes vs no). Values for deer tick abundance[21] in each of the 20 towns represented in the study were log transformed (ln [n + 1]), and regression analysis was performed to determine whether deer tick abundance correlated with seropositivity rate. All analyses were performed with standard software.[h] Values of $P < 0.05$ were considered significant.

Results

Overall, 143 (52%) dogs were seropositive for *B burgdorferi*, 59 (21.3%) were seropositive for *R rickettsii*, 40 (14.4%) were seropositive for the HGE agent, 8 (2.9%) were seropositive for *E canis*, and 6 (2.2%) were seropositive for *B vinsonii*. Of the 148 dogs residing in areas with low deer tick abundance, 56 (38%) were seropositive for *B burgdorferi*, 24 (16%) were seropositive for *R rickettsii*, 9 (6%) were seropositive for the HGE agent, 1 (0.7%) was seropositive for *E canis*, and 3 (2%) were seropositive for *B vinsonii*. Of the 129 dogs residing in areas with high deer tick abundance, 87 (67%) were seropositive for *B burgdorferi*, 35 (27%) were seropositive for *R rickettsii*, 31 (24%) were seropositive for the HGE agent, 7 (6%) were seropositive for *E canis*, and 3 (2%) were seropositive for *B vinsonii*. Relative deer tick abundance (high vs low) was significantly associated with whether dogs were seropositive (yes vs no) for *B burgdorferi* ($P < 0.001$), *R rickettsii* ($P = 0.027$), the HGE agent ($P < 0.001$), and *E canis* ($P = 0.027$) but not for *B vinsonii*.

One hundred six (38.3%) dogs were seronegative for all 5 agents, 110 (39.7%) were seropositive for only 1 agent, 41 (14.8%) were seropositive for 2 agents, 16 (5.8%) were seropositive for 3 agents, and 4 (1.4%) were seropositive for 4 agents (**Table 1**).

Regression analysis indicated that deer tick abundance was correlated with seropositivity rates for *B burgdorferi* ($R^2 = 0.467$; $P < 0.001$) and the HGE agent ($R^2 = 0.529$; $P < 0.001$) but not with seropositivity rates for *R rickettsii* ($R^2 = 0.034$; $P = 0.439$), *E canis* ($R^2 = 0.089$; $P = 0.210$), or *B vinsonii* ($R^2 = 0.003$; $P = 0.831$).

Of the 254 dogs for which age was known, 44 (17.3%) were < 1 year old, 77 (30.3%) were between 1 and 3 years old, 48 (18.9%) were between 4 and 6 years old, and 85 (33.5%) were > 6 years old. Pearson χ^2 analysis indicated that age was significantly associated with whether dogs were seropositive for the HGE agent ($P = 0.037$), with dogs ≥ 4 years old significantly ($P = 0.032$) more likely to be seropositive (26/133; 19.6%) than dogs < 4 years old (12/121; 9.9%). Age was not associated with whether dogs were seropositive for *B burgdorferi*, *R rickettsii*, *B vinsonii*, or *E canis*.

Sex was reported for 261 dogs; 134 (51.3%) were male, and 127 (48.7%) were female. Sex was significantly ($P = 0.026$) associated with whether dogs were seropositive for *E canis*, with females more likely to be seropositive (5/126; 4.0%) than males (0/134; 0%). However, sex was not associated with whether dogs were seropositive for *B burgdorferi*, *R rickettsii*, the HGE agent, or *B vinsonii*.

Overall, 52 (18.8%) of the serum samples were collected during autumn, 143 (51.6%) were collected

Table 1—Results of serologic testing of 277 dogs in Rhode Island for antibodies to various tick-transmitted disease organisms

Organisms for which test results were positive	No. seropositive (%)
None of the disease organisms tested	106 (38.3)
HGE agent only	4 (1.4)
Borrelia burgdorferi only	84 (30.3)
Rickettsia rickettsii only	20 (7.2)
Bartonella vinsonii only	1 (0.4)
Ehrlichia canis only	1 (0.4)
HGE agent and *B burgdorferi*	17 (6.1)
B burgdorferi and *R rickettsii*	22 (7.9)
HGE agent and *B vinsonii*	1 (0.4)
B burgdorferi and *B vinsonii*	1 (0.4)
HGE agent, *B burgdorferi,* and *R rickettsii*	10 (3.6)
HGE agent, *B burgdorferi,* and *B vinsonii*	2 (0.7)
HGE agent, *R rickettsii,* and *E canis*	1 (0.4)
HGE agent, *B burgdorferi,* and *E canis*	1 (0.4)
R rickettsii, B burgdorferi, and *B vinsonii*	1 (0.4)
R rickettsii, B burgdorferi, and *E canis*	1 (0.4)
HGE agent, *B burgdorferi, R rickettsii,* and *E canis*	4 (1.4)

HGE = Human granulocytic ehrlichiosis.

Table 2—Results of serologic testing of 152 dogs classified on the basis of the American Kennel Club group classification for antibodies to various tick-transmitted disease organisms

Group classification	No. tested	No. (%) of seropositive dogs				
		Borrelia burgdorferi	The HGE agent	*Rickettsia rickettsii*	*Bartonella vinsonii*	*Ehrlichia canis*
Herding	20	8 (40)	1 (5)	4 (20)	0 (0)	0 (0)
Hound	10	6 (60)	1 (10)	2 (20)	0 (0)	1 (10)
Nonsporting	13	7 (54)	0 (0)	2 (15)	0 (0)	0 (0)
Sporting	68	46 (68)	19 (28)	15 (22)	3 (4)	5 (7)
Terrier	12	5 (42)	0 (0)	4 (33)	0 (0)	0 (0)
Toy	9	2 (22)	0 (0)	3 (33)	0 (0)	0 (0)
Working	20	8 (40)	0 (0)	2 (10)	0 (0)	0 (0)

during winter, and 82 (29.6%) were collected during spring. Season during which samples were collected was not significantly associated with whether dogs were seropositive for any of the organisms.

When dogs were classified according to the AKC group classification of breeds, 68 (24.6%) dogs were considered sporting-group dogs, 10 (3.6%) were considered hound-group dogs, 20 (7.2%) were considered working-group dogs, 20 (7.2%) were considered herding-group dogs, 12 (4.3%) were considered terrier-group dogs, 9 (3.3%) were considered toy-group dogs, and 13 (4.7%) were considered nonsporting-group dogs. The remaining 125 (45.1%) dogs were of mixed breeding, unknown breeding, or a breed not recognized by the AKC. Breed group classification was significantly associated with whether dogs were seropositive for *B burgdorferi* ($P = 0.046$) and for the HGE agent ($P = 0.002$; **Table 2**) but not with whether dogs were seropositive for *R rickettsii* ($P = 0.857$), *B vinsonii* ($P = 0.892$), or *E canis* ($P = 0.294$).

Discussion

Results of the present study indicated that the geographic distribution of deer ticks was predictive of canine seropositivity to *B burgdorferi* and the agent of HGE. This is an important finding, because vector associations with the HGE agent have yet to be definitively established. Our results demonstrate an association between the distribution of seropositive dogs and deer ticks, a probable natural vector of the HGE agent. Results also indicated that substantial percentages of dogs in Rhode Island have been exposed to *B burgdorferi*, the HGE agent, *R rickettsii*, *E canis*, and *B vinsonii*. Unfortunately, travel histories of these dogs were not obtained, so it is not possible to say that exposure to infected ticks was restricted to the location of residence; therefore, we could not determine whether they were exposed to these agents while living in Rhode Island or while traveling to other areas. However, because human acquisition of *B burgdorferi* in the Northeast most commonly occurs in the environment surrounding the home,[25] it is likely that dogs acquire *B burgdorferi* similarly. In addition, because antibodies to *R rickettsii* and to related spotted fever group rickettsiae crossreact, we could not determine whether seropositive dogs had been exposed to *R rickettsii* or to 1 of the related spotted fever group rickettsiae.

The sampling scheme was chosen to reduce geographic and other biases. Serum samples were obtained from dogs residing in animal shelters and from dogs examined at veterinary hospitals to ensure that dogs that lived indoors and dogs that lived outdoors were included, along with dogs that had various levels of risk of exposure to deer ticks. Dogs known to have received the *B burgdorferi* bacterin were excluded, because vaccination can interfere with interpretation of results of serologic testing. Although only a few veterinary hospitals said they could not participate, because they routinely vaccinated all dogs, including only unvaccinated dogs may have introduced a selection bias. In addition, vaccination status of dogs residing in animal shelters was not known. However, it seems likely that most of these dogs were not vaccinated, because they were residing at an animal shelter. χ^2 Analysis indicated that the percentage of dogs seropositive for *B burgdorferi* that resided in animal shelters was not significantly ($P = 0.198$) different from the percentage of *B burgdorferi* seropositive dogs examined at veterinary hospitals.

For the present study, serum samples were collected between November and May and not during the summer when tick activity is highest. The assays used in this study detect IgG, and it typically takes 2 to 4 weeks after infection for detectable IgG concentrations to develop. However, titers may remain high for extended periods after infection, particularly if infection persists. The geometric mean titer for antibodies to *B burgdorferi* in dogs typically is highest during the winter.[26] Moreover, Magnarelli et al[27] found there was little, if any, change in *B burgdorferi* antibody titer during the 2 months after blood samples are collected in dogs treated with antibiotics. Breitschwerdt et al[28] observed sustained increases in antibody titers for at least 3 months after *R rickettsii* infection and concluded that the timing of convalescent sample collection was not critical for detecting antibodies to the spotted fever group rickettsiae. They also found that tetracycline treatment did not significantly alter antibody responses. Bakken et al[29] determined that antibody titers remained high for at least 1 year in human patients with HGE. Rodgers et al[30] did not find any seasonal variation in seroreactivity to *E equi*, *R rickettsii*, or *B burgdorferi*. Thus, timing of sample collection in the present study likely did not have a substantial effect on seropositivity rates.

Although information on health of the dogs included in the present study was not routinely reported, some veterinarians commented on the general

health of these dogs, and some dogs, including some that were seropositive for multiple organisms, were reportedly healthy. Lyme disease may be subclinical in humans[31] and dogs,[32] and in a study of 17 dogs with granulocytic ehrlichiosis, severe secondary infection was not reported.[5]

Coinfection with multiple tick-transmitted pathogens is receiving closer scrutiny, because it may lead to prolonged severe illness.[33,34] Although results of the present study do not indicate whether any of these dogs were coinfected, they do demonstrate that a substantial percentage of these dogs were exposed to more than 1 of these disease organisms. Because at least 2 of these organisms are transmitted by the same tick species, and coinfection of ticks has been documented,[35,36] it is very likely that some of these dogs were exposed to more than 1 organism by the bite of a single tick.

Previous studies have found overall rate of seropositivity to *B burgdorferi* in dogs to be 4.3% in Maine,[37] 20.3% in Massachusetts,[38] 49.2% in Westchester County, New York,[39] and 53% in Wisconsin.[40] The seropositivity rate of 52% in the present study was consistent with results for New York and Wisconsin. However, the seropositivity rate of 38% in the portion of Rhode Island where deer ticks were relatively rare was higher than that previously reported for other areas where deer ticks are not abundant. In the present study, travel histories were not obtained, so it is possible that dogs residing in areas of low relative deer tick abundance were exposed to the organism when traveling in areas where deer ticks are abundant. Regardless, these data indicate that the risk of exposure to *B burgdorferi* may be high even when deer tick abundance is low.

Results of regression analysis of the *B burgdorferi* seropositivity rate in the present study supported previous research showing a positive relationship between seropositivity rates for dogs and numbers of deer ticks on deer[41] and vector tick distribution surveys.[36] The present study also found a positive relationship between deer tick abundance and seropositivity rate for the HGE agent among dogs in Rhode Island. As expected, similar relationships were not detected for *R rickettsii*, *E canis*, and *B vinsonii*. The first 2 of these agents are transmitted principally by *D variabilis* and *R sanguineus*, respectively. The natural vector for *B vinsonii* in Rhode Island has not been determined.

[a]Aquila Biopharmaceuticals, Worcester, Mass.
[b]Kirkegaard and Perry Laboratories Inc, Gaithersburg, Md.
[c]E-600, Nikon, Yokohama, Japan.
[d]Supplied by Dr James Olsen, Centers for Disease Control and Prevention, Atlanta, Ga.
[e]Cappel, Durham, NC.
[f]Organon Teknika, West Chester, Pa.
[g]Spectra MAX 340, Molecular Devices, Sunnyvale, Calif.
[h]SAS version 6.12, SAS Institute Inc, Cary, NC.

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Correction: Public Veterinary Medicine: Public Health—Rabies surveillance in the United States during 1999

In "Rabies surveillance in the United States during 1999" (*JAVMA*, 2000;217:1799-1811), the date of death for the human with rabies in Georgia in October 2000 (Table 2, p 1809) should be changed from "13 Oct 00" to "10 Oct 00" and the rabies virus variant should changed from "Unknown" to "Bat, Tb." In the first paragraph in the Addendum (p 1810), the sentence reading "On October 13, 2000 . . . ." should be changed to "On October 10, 2000, the Georgia Department of Human Resources announced that rabies had been diagnosed as the cause of illness for a man who died of encephalitis."