A B S T R A C T

A survey for antibodies against agents of plague, tularemia, and Rocky Mountain spotted fever (RMSF), and against Sin Nombre hantavirus (SNV), *Bartonella henselae* and *B. clarridgeiae* was conducted in the summer of 1995 using serum from rural dogs and cats living in the vicinity of four public parks in southeastern Alberta and southwestern Saskatchewan. Antibodies to all pathogens were detected in all survey areas. Overall prevalence rates were 0.075 for *Yersinia pestis*, 0.089 for *Francisella tularensis*, 0.025 for *Rickettsia rickettsii* (dogs only), and 0.029, 0.178 and 0.186 for SNV, *B. henselae* and *B. clarridgeiae*, respectively (cats only). This serological survey of rural dogs and cats was more sensitive and efficient than previous surveys based on collection and culture of rodents and ectoparasites. All six pathogens appear endemic to the region. Surveillance for plague, tularemia, RMSF and SNV, and management of associated public risks should be done in endemic regions.

A B R É G É

Durant l'été 1995, nous avons mené une enquête sur la présence d'anticorps spécifiques des agents de la peste, de la tularémie, de la fièvre des montagnes rocheuses (RMSF) et du Sin Nombre virus (SNV), *Bartonella henselae* et *B. clarridgeiae* en utilisant des sérums provenant de chiens et de chats ruraux vivant à proximité de quatre parcs publics du sud-est de l'Alberta et du sud-ouest de la Saskatchewan. Des anticorps contre tous les agents pathogènes étudiés étaient présents dans toutes les localités ciblées par l'étude. Les taux de prévalence globaux étaient de 0,075 pour *Yersinia pestis*, 0,089 pour *Francisella tularensis*, 0,025 pour *Rickettsia rickettsii* (chiens seulement) et de 0,029, 0,178 et 0,186, respectivement, pour SNV, *B. henselae* et *B. clarridgeiae* (chats seulement). Cette enquête sérologique visant des chiens et des chats s'est avérée plus sensible et plus efficace que les enquêtes précédentes, fondées sur la collecte et/ou la culture d'ectoparasites et de rongeurs. Les six pathogènes susmentionnés semblent être endémiques dans les régions étudiées. Il faudrait effectuer dans ces régions une épidémiologie des agents de la peste, de la tularémie, de la RMSF et du SNV et gérer les risques auxquels la population pourrait être exposée.

A Serological Survey of Rural Dogs and Cats on the Southwestern Canadian Prairie for Zoonotic Pathogens

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The agents of several important zoonotic diseases persist among populations of wild rodents and lagomorphs (rabbits, hares and picas) and cause sporadic disease in humans. Surveys for three such diseases – plague, Rocky Mountain spotted fever (RMSF) and tularemia – were carried out in western Canada from 1938 to 1946, and the agents of all three diseases were detected on the southwestern Canadian prairie, including one fatal case of plague in a person in southeastern Alberta in 1939.¹⁻³ Since that time, very little has been published about the occurrence of these disease-causing agents on the Canadian prairies. In 1993, a new virus of the deer mouse (*Peromyscus maniculatus*), now called Sin Nombre virus (SNV), was discovered as a cause of acute, often fatal pneumonia in people in the southwestern United States (Hantavirus Pulmonary Syndrome).⁴ Subsequently, this virus was detected in deer mice in southern Alberta and Saskatchewan.⁵

In the early 1970s, experimental and field studies showed that antibodies in the

sera of free-ranging dogs and cats could serve as a practical index of the prevalence of plague in local wild rodent populations, which was helpful in predicting epidemics in wildlife and increased risk of transmission to people.⁶⁻⁹ The study described here was undertaken to assess the presence and prevalence of *Yersinia pestis*, *Rickettsia rickettsii*, *Francisella tularensis*, and SNV, and also of *Bartonella henselae* (the cause of Cat Scratch Disease) and *B. clarridgeiae* (a related zoonotic bacterium) by detection of antibodies in the blood of rural dogs and cats in the area surrounding four public nature parks in southwestern Saskatchewan and southeastern Alberta.

METHODS

Community awareness

In May 1995, letters were sent to all farmsteads in each survey area and to local physicians, health centres, hospitals, veterinarians, police, mayors and municipal offices. The letters explained that a survey of diseases of wild rodents was being conducted and asked for endorsement and cooperation. A total of 2,242 such letters were mailed. Copies of the letter addressed to farmsteads also were given to animal owners at the time they were approached in person to permit their animals to be included in the survey.

Survey locations

The survey was carried out in four large areas centred around public parks: Dinosaur (DP) and Writing-On-Stone (WS) Provincial Parks in SE Alberta, and Saskatchewan Landing Provincial Park (SL) and Grasslands National Park (GL) in SW Saskatchewan (Figure 1). All are located in the short-grass prairie ecological

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region. Habitat includes arid grassland plains transected irregularly by river valleys with intermittent or continuous water flow and associated moist and dry riverside plant communities. Farmsteads closest to each park were visited first, and the area of the survey was expanded irregularly around each park until the target number of 60 serum samples from each species was obtained. The location of each participating farmstead was recorded.

Capture and sampling

Sample collection in each survey area required 8 to 17 working days, and was carried out on 17 May to 2 June (SL), 9 to 22 June (GL), 30 June to 12 July (DP), and 21 to 28 July (WS), 1995. Field personnel were vaccinated against rabies prior to the start of sample collection.

The owner of each farmstead was asked, in person, for permission to take a blood sample from dogs, cats or both on the premises, and a permission form was signed by the owner. Dogs were restrained gently, a light gauze muzzle was placed around the snout, and 10cc of blood was taken from the cephalic vein with a 20 or 21 gauge needle and a 12 cc syringe. Cats were either restrained manually or caught in wire box traps (Tomahawk Live Trap, Tomahawk, WI, 54487 USA) baited with sardines. Cats were weighed and anaesthetized with an intramuscular injection of acepromazine (0.05 mg/kg) (Atravet®, Ayerst Laboratories, Montreal, Quebec) and ketamine (10 mg/kg) (Vetalar®, Vetrepharm Canada Inc., London, Ontario) given together. If anaesthesia was not sufficient with this dose, a second dose of one half of the original dose was administered, and this was repeated once, when necessary, to a maximum total of one additional full dose. Eye drops (Eylube®, Sabex Inc., Boucherville, Quebec) were administered to the anaesthetized cats and 10 mL of blood was taken from the jugular vein with a 20 or 21 gauge needle and a 12 cc syringe. Cats were placed in a protected area to recover from anaesthesia, and recovery was monitored and confirmed.

Blood was placed in sealed glass tubes and kept at approximately 4°C in a cooler until evening, when the serum was separated from clotted elements by gentle centrifuga-

Figure 1. Survey Areas. The four survey areas around public parks in south-eastern Alberta and southwestern Saskatchewan are shown in black. DP - Dinosaur Provincial Park, WS - Writing-On-Stone Provincial Park, SL - Saskatchewan Landing Provincial Park, GL - Grasslands National Park.

tion, divided into aliquots and frozen in liquid nitrogen. Samples were transferred to a freezer at -70°C for storage and were shipped to analytical laboratories on dry ice.

Serological analyses

Serum antibody reactivity to *Francisella tularensis*, and *Yersinia pestis* antigens was measured in both dogs and cats; reactivity to *Rickettsia rickettsii* was measured only in dog sera and to Sin Nombre hantavirus, *Bartonella henselae* and *B. clarridgeiae* only in cat sera.

Testing for IgG antibodies to Sin Nombre virus was conducted at the Canadian Science Centre for Human and Animal Health, Winnipeg, Manitoba by enzyme-linked immunosorbent assay (ELISA) according to a standardized protocol.¹⁰ Briefly, serum samples were diluted in 5% skim milk in 0.01 M phosphate buffered saline with 0.5% Tween-20. Four-fold dilutions from 1:100 to 1:64,000 were made in microtitre plates. Samples were tested against a recombinant nucleocapsid

antigen and a recombinant control antigen. A peroxidase-labelled affinity-purified antibody to cat IgG (H and L) (Kirkegaard & Perry Laboratories Inc, Gaithersburg, MD) was used to detect bound immunoglobulin. Adjusted optical densities (OD) for each dilution were calculated by subtracting the OD₄₁₀ of the control antigen from the OD₄₁₀ of the Sin Nombre antigen. Titres were assigned on the basis of an adjusted OD value exceeding 0.20 for each dilution. Cats with titres ≥ 400 were categorized as serologically positive.

Antibody titres to *Y. pestis* F1 were measured by passive hemagglutination (PHA) followed by passive hemagglutination inhibition (PHI) to confirm positive titres,¹¹ and titres to *F. tularensis* were measured by microagglutination (MA)¹² at the Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins. Animals with PHA titres ≥ 16 and confirmed by PHI were considered serologically positive for *Y. pestis*, and animals with MA titres ≥ 128

TABLE I
Serological Prevalence of Zoonotic Pathogens in Rural Dogs and Cats Near Four Public Parks in the Southern Canadian Prairie, Summer 1995

Location	Pathogens					
	<i>Y. Pestis</i>	<i>Francisella tularensis</i>	<i>Sin Nombre virus</i>	<i>Rickettsia rickettsii</i>	<i>Bartonella henselae</i>	<i>Bartonella clarridgeiae</i>
Saskatchewan						
Landing						
Dog	0.133 (60)*	0.167 (60)	NA	0.033 (60)	NA	NA
Cat	0.050 (62)	0 (62)	0.016 (62)	NA	0.210 (62)	0.129 (62)
Combined	0.090 (122)	0.082 (122)				
Grasslands						
Dog	0.067 (60)	0.133 (60)	NA	0.017 (60)	NA	NA
Cat	0.017 (60)	0.050 (60)	0.083 (60)	NA	0.333 (60)	0.333 (60)
Combined	0.042 (120)	0.092 (120)				
Dinosaur						
Dog	0.117 (60)	0.183 (60)	NA	0.017 (60)	NA	NA
Cat	0.117 (60)	0.050 (60)	0.017 (60)	NA	0.083 (60)	0.083 (60)
Combined	0.117 (120)	0.117 (120)				
Writing-On-Stone						
Dog	0.067 (60)	0.083 (60)	NA	0.033 (60)	NA	NA
Cat	0.033 (60)	0.050 (60)	0 (60)	NA	0.083 (60)	0.200 (60)
Combined	0.050 (120)	0.067 (120)				
All Sites						
Dog	0.096 (240)	0.142 (240)	NA	0.025 (240)	NA	NA
Cat	0.054 (242)	0.037 (242)	0.029(242)	NA	0.178 (242)	0.186 (242)
Combined	0.075 (482)	0.089 (482)				

* Prevalence with sample size (n) in parentheses

were considered serologically positive for *F. tularensis*.

Antibody titres to *R. rickettsii* and to both species of *Bartonella* were measured by indirect immunofluorescence at the Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta.^{13,14} Briefly, 2-fold dilutions of serum were incubated on slides that had each of the antigens fixed to its surface. Following thorough washing, anti-species IgG conjugated to fluorescein isothiocyanate was incubated, followed by another thorough washing. Slides were observed for evidence of apple-green fluorescence with a microscope using ultraviolet illumination. The highest dilution of serum that gave a strong fluorescence was considered the endpoint antibody titre of the serum. Cats with titres ≥ 64 were considered serologically positive in each assay.

Data archive

All survey data were deposited in the National Wildlife Health Database of the Canadian Cooperative Wildlife Health Centre, Saskatoon.

Statistical methods

The sample size (60 samples per species per area) used in this survey was chosen in order to have 95% confidence that, for each

of the four survey areas, at least one serologically positive animal of each species would be included in the sample if the prevalence in the population was 5% or greater.¹⁵

RESULTS

A total of 240 dog sera and 242 cat sera were collected. One or more animals serologically positive for each pathogen was present in each of the four survey areas with the exception of the WS area, in which no cat positive for SNV was found. Results for each area and for the total survey are in Table I. Overall, the serological prevalence in dogs and cats of *Yersinia pestis* was 0.075 and of *Francisella tularensis* was 0.089. The prevalence of antibodies to *Rickettsia rickettsii* in dogs was 0.025. The prevalence in cats of antibodies to SNV was 0.029, to *Bartonella henselae* was 0.178 and to *B. clarridgeiae* was 0.187. Thirty-one cats were serologically positive to both species of *Bartonella* (13% of all cats and 54% of cats positive to either of the two *Bartonella* species).

DISCUSSION

Serological testing of rural domestic dogs and cats was a sensitive and practical means of estimating the presence of the

zoonotic pathogens included in this survey, particularly in comparison to previous surveys based on direct detection of these pathogens in rodents and their ectoparasites. Surveys of plague in the same general region from 1939 to 1946 collected 20,212 ground squirrels (*Spermophilus* sp.) and 62,369 of their fleas, made 2,239 isolation attempts by inoculation into guinea pigs of pooled sample homogenates and detected *Y. pestis* in only 40 samples for an overall frequency of detection of 0.018 (1.8%).³ In contrast, this serological survey required only 2.5 months of field work by two people, relatively inexpensive serological procedures, and detected antibodies to *Y. pestis* in about 10% of 240 dogs. The current survey also appears to be the first to report serology of cats as an approach to surveillance of SNV.

Plague was detected in all survey areas, with an overall prevalence of 9.6% in dogs and 5.4% in cats. Oral exposure of dogs to *Y. pestis* seldom results in clinical disease, but it evokes an antibody response that becomes detectable 1-2 weeks after exposure, peaks at 3-4 weeks, and is no longer detectable 4-8 months after exposure.^{7,8,16} Thus, the serologically positive dogs sampled in this survey had been exposed to *Y. pestis* sometime during the preceding 4-8 months. While it is possible that some

dogs included in the survey had visited other regions of North America during the previous year, the vast majority had not left the survey area. The lower serological prevalence in cats is partially explained by the fact that many cats exposed to *Y. pestis* die of plague, and thus do not survive to become serologically positive. Barnes (1982) reported serological prevalence of 15.9% in dogs and 9.3% in cats in 7,037 samples collected in the western United States between 1976 and 1980.⁶

In much of the western United States, including areas of Montana and North Dakota adjacent to the survey areas, *Y. pestis* is endemic in ecological associations of wild rodents and their fleas in arid habitat.^{6,17-19} Results from the current and from previous surveys in the southwestern Canadian prairie indicate that plague is endemic in this region also.³

The prevalence of antibodies to tularemia was 14.2% in dogs, 3.7% in cats and 8.9% overall. Tularemia has been detected in ticks, mammals and a Franklin's gull (*Larus pipixican*) in the general survey area previously.^{3,20-22} The serological method used in this survey does not distinguish among the two biovars of *Francisella tularensis*, but it is most likely that Type A is predominant in the survey areas.^{19,23} Aquatic habitat is sparse, and the region is dominated by arid terrestrial habitats inhabited by lagomorph species (cottontail rabbits - *Sylvilagus nuttallii*, white-tailed jack rabbit - *Lepus townsendii* and, to a lesser extent, snowshoe hare - *Lepus americanus*) and tick genera (*Dermacentor*, *Haemophysalis*) capable of maintaining *F. tularensis*. The difference in prevalence between dogs and cats may reflect differences in attachment rate of vector ticks or differences in rates of predation on lagomorph prey species.

Overall, 2.5% of dogs in the survey were serologically positive for *Rickettsia rickettsii*. Surveys from 1939 to 1946 examined 49,201 ticks (*Dermacentor andersoni*) from southern Alberta and 2,007 from southern Saskatchewan, and detected *R. rickettsii* in 10 pooled tick samples from Alberta (0.02%). This rickettsia is maintained in nature in a wide range of ecological associations among several genera of ticks and their small mammal hosts. The usual vec-

tor for human infections in western North America is *Dermacentor andersoni*, and this tick also is probably a major source of exposure for dogs in the survey areas.^{3,19,21}

The serological prevalence of SNV antibody titres ≥ 400 in cats ranged from 0% to 8.3% and was 2.9% overall. If a titre ≥ 100 is used instead as the criterion for serological positivity, the overall prevalence was 5.8% and at least one positive cat was found in each sample area. There are no data available upon which to establish the titre which indicates with certainty that a cat has been exposed to SNV. SNV itself has been detected in deer mice (*Peromyscus maniculatus*) adjacent to two of the survey areas (Health Canada, unpublished data). The prevalence of antibodies reported in deer mice outside of epidemic foci has been in the range of 10% to 15% in surveys carried out in the United States since 1993.²⁴ Thus, a survey based on deer mice might be more sensitive than one based on domestic cats. However, the risk of infection for people conducting surveys of deer mice is substantial, and these risks are reduced or eliminated when samples are taken from cats instead.²⁵

The serological prevalence for *Bartonella henselae* ranged from 8.3% to 33% and was 17.8% overall; that for *B. clarridgeiae* was similar, with the same range and an overall prevalence of 18.6%. Cats and their fleas are thought to be the primary hosts and vectors of *B. henselae*, but infection and reservoirs in other species also may exist since, in general, species of *Bartonella* have a wide host range. Prevalence of infection in domestic cats has been correlated with climate (highest in warm, moist environments), which, in turn, has been related to the population density of fleas.^{26,27} Results of the current survey are consistent with these correlations. The occurrence and prevalence observed in this survey do not suggest that people in the survey areas are at any increased risk of infection from these *Bartonella* species compared to people elsewhere.

The results of this survey document that the agents of plague, tularemia, Rocky Mountain spotted fever and the Sin Nombre virus all were present in the ecosystem of the southern Canadian prairie in 1994-95. These are diseases with mod-

erate to high human case-fatality ratios (plague: 50-60% untreated, 18% actual; tularemia: 5-7% untreated; spotted fever: 23-70% untreated, 3-5% treated; Hantavirus pulmonary syndrome: 43% actual).^{6,19,24} Residents and visitors to this region who undertake outdoor activities risk exposure to these diseases, either through direct contact with infected animals or through bites of vector ticks and fleas. In particular, public parks that attract visitors to outdoor activities in natural habitat must be fully aware of the health hazards presented by these endemic diseases, and should adopt policies and procedures to reduce the likelihood that employees or visitors will be exposed.

A variety of feasible management strategies to reduce the likelihood of vector-borne infections by delivering insecticides and acaricides to small mammals in high risk areas, such as camping and picnic areas, have been used and evaluated.⁶ Guidelines for minimizing aerosol exposure to hantavirus also are available.²⁵ Public health authorities would be well advised to undertake surveillance of these disease-causing agents in endemic areas in order to develop an understanding of their dynamics geographically and over time. Plague, in particular, creates periodic epidemics among susceptible rodents, such as ground squirrels and prairie dogs, and the incidence of human plague has been found to be highest in years of such epidemics.^{6,19} Serological surveys of free-ranging domestic carnivores offer a sensitive and low-cost approach to such public health surveillance.

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