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Source: Journal of Wildlife Diseases, 42(4) : 739-749

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-42.4.739

Journal of Wildlife Diseases, 42(4), 2006, pp. 739–749

DEMOGRAPHIC FEATURES OF *BARTONELLA* INFECTIONS IN RICHARDSON'S GROUND SQUIRRELS *(SPERMOPHILUS RICHARDSONII)*

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ABSTRACT: The epidemiology of *Bartonella* infections in Richardson's ground squirrels (*Spermophilus richardsonii*) was studied at multiple sites in Saskatchewan, Canada, from 2002 to 2004. The overall prevalence of *Bartonella* infection was 48%. Juvenile squirrels were significantly more likely to be infected with *Bartonella* than were adults (58% and 37%, respectively), and juvenile animals also were significantly more likely to have high levels of bacteremia compared to adult animals. Prevalence of *Bartonella* infection appeared to decrease with age; only 24% of animals known to be ≥2 yr old were infected with *Bartonella*. Prevalence of infection was lowest in May (27%) and highest in late summer and early autumn (71%). The prevalence of fleas also varied seasonally, and animals were more likely to have fleas in the late summer and early autumn than in early summer. We found no relationship between *Bartonella* prevalence and host density or flea prevalence.

Key words: *Bartonella*, epidemiology, Richardson's ground squirrel, rodent, *Spermophilus richardsonii*.

INTRODUCTION

The genus *Bartonella* is comprised of gram-negative vector-borne bacteria that parasitize mammalian red blood cells. Currently, there are twenty recognized species of *Bartonella* (Dehio, 2004), and rodents are reservoirs for at least four *Bartonella* species known to cause human and animal disease. *Bartonella grahamii*, first detected in bank voles (*Clethrionomys glareolus*) in the UK (Birtles et al., 1995), was subsequently found in a human patient with neuroretinitis (Kerkhoff, 1999). Rats (*Rattus norvegicus*) have been implicated as reservoirs for *B. elizabethae* (Ellis et al., 1999), which has been detected in one human patient with endocarditis (Daly et al., 1993) and is recognized as a potential pathogen of dogs (Mexas et al., 2002). White-footed mice (*Peromyscus leucopus*) are likely reservoirs for *B. vinsonii* subsp. *arupensis*, which was found in a human patient with fever and bacteremia (Welch et al., 1999). *Bartonella washoensis*, first found to be responsible for cardiac disease in a human patient, was subsequently detected in a California ground squirrel (*Spermophilus beecheyi*) (Kosoy et al., 2003) and identified as a cause of endocarditis in a dog (Chomel et al., 2003).

Evidence that rodent-associated *Bartonella* can cause human disease has led to numerous cross-sectional surveys, which have found high prevalence of *Bartonella* in a variety of rodents at many locations (Birtles et al., 1994; Kosoy et al., 1997). Although useful for identifying potential rodent reservoirs of *Bartonella*, cross-sectional studies do not explain how these bacteria are maintained and transmitted among their rodent hosts.

Longitudinal and multiple cross-sectional studies at the same sites allow us to determine the dynamics of infectious agents in their reservoir hosts, which may aid in understanding when and why these

TABLE 1. Description of trapping sites with dates trapped and number of blood samples collected (and tested for *Bartonella*) from Richardson's ground squirrels at each site.

Site	Description	Latitude/ Longitude	Dates	Number of samples tested
1	Horse pasture, mildly to moderately grazed; rural	52°1′N, 106°36′W	2002 May, June, August; 2003 April–September; 2004 April–September	258
2	Sheep pasture, heavily grazed; within city	52°8′N, 106°37′W	2002 May, July; 2003 April–August	89
3	Grassy edge of road, adjacent to cultivated field; within city	52°8′N, 106°36′W	2003 May–July; 2004 April–August	59
4	Disturbed site (parking area) within natural area; rural	51°58′N, 106°48′W	2003 May–September; 2004 April–August	88
5	Grassy edge of unused lot; within city	52°11′N, 106°40′W	2003 May–August	20
6	Cattle pasture, heavily grazed; rural with outbuildings	52°3′N, 106°30′W	2002 May	11
7	Alfalfa field; rural	51°59′N, 106°40′W	2002 September	4
8	Cattle pasture, moderately to heavily grazed; rural	51°57′N, 107°6′W	2002 June	5
9	Cattle pasture, heavily grazed; rural	52°12′N, 107°7′W	2002 June	5
10	Cattle pasture, moderately to heavily grazed; rural	52°13′N, 107°0′W	2002 May, June	11
12	Alfalfa field; within city	52°9′N, 106°37′W	2004 June–September	35

agents "emerge" to infect humans. There have been three longitudinal studies of *Bartonella* in wild rodents that have provided valuable information about the dynamics of *Bartonella* infections. Kosoy et al. (2004) described the demographic and temporal patterns of *Bartonella* infections in cotton rats (*Sigmodon hispidus*) in the USA. Fichet-Calvet et al. (2000) studied *Bartonella* in fat sand rats (*Psammomys obesus*) in Tunisia, and Birtles et al. (2001) studied the dynamics of *Bartonella* infections in 12 rodent hosts in the UK. Such studies, in a variety of rodent hosts, at a variety of locations, are needed to determine if any general conclusions can be made about the dynamics of *Bartonella* infections in wild rodents.

In a previous study, we found that 49% of Richardson's ground squirrels (*Spermophilus richardsonii*), which are semi-colonial rodents that are common on the Canadian prairie, were infected with *Bartonella* sp. (Jardine et al., 2005). Here we present temporal, age, and sex-specific demographics of *Bartonella* infections in Richardson's ground squirrels.

MATERIALS AND METHODS

Study design

We conducted this study as a repeated cross-sectional study of 11 Richardson's ground squirrel populations. We did recapture some individuals and accounted for repeat captures of individual squirrels in our analysis.

Trapping and processing

Procedures for trapping and handling rodents were approved by the animal care committee at the University of Saskatchewan (University Committee on Animal Care and Supply Protocol 2020028). Richardson's ground squirrels were trapped live at up to 11 sites around Saskatoon, Saskatchewan, from April to September in 2002, 2003, and 2004, using unbaited burrow traps (Wobeser and Leighton, 1979) placed in burrow entrances where squirrels were seen. Sites were chosen based on known presence of Richardson's ground squirrels and their proximity to the laboratory. Sites used in the study varied from year to year because of changes in land use and study design (Table 1). In 2002, sites were

trapped for only a portion of 1 day for 1 or 2 mo in the summer. In 2003 and 2004, sites were trapped from approximately 1 hr after sunrise until 1 hr after midday, for 2 to 3 days each month, starting in April or May.

In 2002, Richardson's ground squirrels were anesthetized using an intramuscular injection of 0.4 mg/kg medetomidine hydrochloride (Domitor 1 mg/ml; Novartis Animal Health Canada, Inc., Ontario, Canada) and 15 mg/kg ketamine hydrochloride (Vetalar 100 mg/ml; Vetrepharm Canada Inc., Ontario, Canada) prior to blood sample collection, and they were euthanized using an overdose of halothane (MTC Pharmaceuticals, Ontario, Canada). In 2003 and 2004, animals were anesthetized using isoflurane (IsoFlo, Abbott Laboratories, Ltd., Saint-Laurent, Quebec, Canada) and released at the point of capture after sample collection. We sampled individual squirrels only once per monthly trapping session; however, multiple samples were collected from individuals caught in different trapping sessions. Appropriate precautions (Mills et al., 1995) were taken by workers to avoid exposure to zoonotic agents.

Numbered metal ear tags (#1005-1; National Band and Tag Co., Kentucky, USA) were placed in both ears for subsequent identification, and sex, age (adult or juvenile), and mass were recorded for each animal. Blood was collected by cardiac puncture from animals that were to be euthanized and from the medial saphenous vein of animals that were to be released. In 2003 and 2004, we examined and brushed each animal for 3 min and recorded the presence or absence of fleas and ticks.

All euthanized animals were necropsied, and tissues were held frozen at −70 C or in 10% neutral buffered formalin as appropriate for subsequent examination. Blood samples were placed in liquid nitrogen in the field and then transferred to a −70 C freezer for storage.

Population density estimates

We did not estimate squirrel density in 2002. Direct counts are considered to be reliable for estimating squirrel population density because squirrels are "easily spotted, rather sedentary, and colonial, and their activity correlates well with weather conditions" (Zegers, 1982). We estimated squirrel population density twice per year in 2003 and 2004 based on: 1) the number of animals trapped in early May when all adults were aboveground and juveniles had not yet emerged from their natal burrows; and 2) on the number of animals trapped in June when all juveniles had emerged from their natal burrows and adults were still aboveground. Ten squirrels that were caught prior to and after, but not during, the census period were included in the estimates. For the sites where the trapped area was a portion of a larger colony, we estimated the effective trapping area by adding an area equal to half the mean of the maximum distance moved by squirrels caught in multiple sessions (Wilson and Anderson, 1985). For sites where traps were set over the entire colony, we used the area of the colony with no buffer.

Bartonella isolation

Published procedures for isolating *Bartonella* from blood (Kosoy et al., 1997) were used. Briefly, 0.15 ml of whole blood was plated on commercial sheep blood agar (BBC Columbia Agar; Becton Dickinson Company, Sparks, Maryland, USA) and on ATCC GC Agar medium prepared at our institution, and plates were incubated at 37 C in an aerobic atmosphere with 7% carbon dioxide for up to 30 days. The plates were checked twice weekly for growth, and isolated colonies were subcultured to confirm purity. Bacterial colonies were tentatively identified as *Bartonella* based on morphology and standard biochemical tests (Welch and Slater, 1999). All morphologically distinct colony types from each sample were identified individually. The level of bacteremia was classified as low if there were fewer than ten colony-forming units (CFU) and high if there were ten or more CFU on the original plate. This cutoff point was selected as a midpoint in a bimodal distribution of the data.

Bartonella polymerase chain reaction

DNA was extracted using a standard phenol-chloroform extraction from bacterial colonies that were tentatively identified as *Bartonella*. The extracted DNA was stored in TE buffer at −70 C prior to polymerase chain reaction (PCR) amplification.

Two oligonucleotides (BhCs781.p and BhCs1137.n), specific for the citrate synthase (*gltA*) gene of *Bartonella*, were used as PCR primers, resulting in a 379 base pair product (Norman et al., 1995). The PCR mixture consisted of 2 μl of sample DNA, 35.25 μl of sterile ultrapure water, 5 μl of 10X PCR buffer, 3 μl of (25 mM) $MgCl_2$, 0.5 μl (25 mM) dNTPs, 2 μl (20 pmol) of each primer, and 0.25 μl (5 U/μl) Taq polymerase, resulting in a total volume of 50 μl. PCR amplifications were carried out in a PTC200 DNA-Engine (MJ Research, Watertown, Mas-

sachusetts, USA). The mixture was incubated at 94 C for 2 min, then amplified for 40 cycles at 94 C for 30 sec, 50 C for 60 sec, and 72 C for 60 sec, then held at 72 C for 5 min. To visualize the PCR amplicons, PCR products were separated by electrophoresis in 1.25% agarose gel with ethidium bromide staining, according to standard methods.

Data analysis

We examined the associations between various demographic factors and *Bartonella* prevalence, flea prevalence, and prevalence of a high level of bacteremia using multilevel mixed models, which allowed us to account for clustering of individual animals within a site using random intercepts for the site and to account for repeated measures within individual animals over time (MLwiN version 2.0, Centre for Multilevel Modelling, London, UK). A binomial distribution and logit link function were used to model all outcomes. Factors examined for association with *Bartonella* prevalence included year, month, age, sex, population density, and flea prevalence. Factors examined for association with flea prevalence and prevalence of high level bacteremia were year, month, age, and sex. For each outcome, all factors with $P<0.25$ on their own (univariate models) were combined and backward elimination was used to identify a final (multivariate) model. All factors where $P<0.05$ were considered statistically significant.

Monthly prevalence estimates and 95% confidence intervals for *Bartonella*, fleas, and high level of bacteremia were calculated using MLwiN models, taking into account clustering of individual animals within site and year (Dohoo et al., 2003).

RESULTS

Seasonal activity of Richardson's ground squirrels

The proportion of adults and juveniles making up the trapped population varied throughout the summer (Fig. 1). Primarily adult animals were trapped in April and May, and juveniles only began to enter traps after emerging from their natal burrows at the end of May. The proportion of trapped animals that were adult declined over the summer, with adults representing only 4% of animals trapped in August and September. The sex ratio of adult ground squirrels in April was four females/male, which is typical for adults of this species (Michener and Michener, 1977). Juveniles did not show the same skewed sex ratio: 102 of 222 (46%) juveniles trapped in June were males. The proportion of juvenile males in the trapped population increased over the summer to 67% (18 of 27 animals) by September.

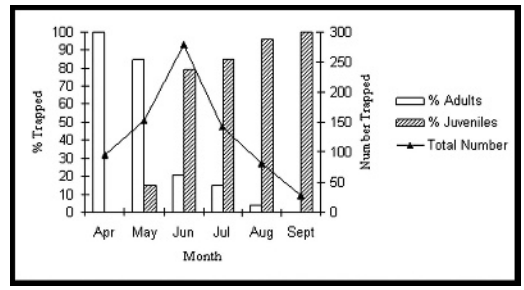

FIGURE 1. The percentage of adult and juvenile Richardson's ground squirrels in the trapped population in each monthly trapping session (bars) and the total number of squirrels caught in each monthly trapping session (line), for all years and sites combined.

Bartonella infections

Of 781 blood samples collected from Richardson's ground squirrels captured at ten sites in 2002, 2003, and 2004, 585 (75%) were submitted for *Bartonella* culture. The overall prevalence of *Bartonella* infection, based on culture and confirmed by PCR, was 48%. This estimate of prevalence includes multiple samples from individual squirrels captured in more than one monthly trapping session. Our estimate of prevalence remains the same (48% of 368) if we include only results from first capture samples; however, our estimate of *Bartonella* prevalence increases to 57% if we count an animal as positive if it was positive at any of the times it was sampled. The likelihood of detecting *Bartonella* infections in individual animals increased with the number of times it was sampled. Of 92 animals sampled twice, 68% were *Bartonella* positive at least once and 74% of 46 animals sampled on three or more occasions were positive for *Bartonella* at least once.

TABLE 2. Estimated number of Richardson's ground squirrels per hectare in May and June from study sites trapped in 2003 and 2004.

	2003		2004	
Site	May	June	May	June
1	13	46	20	60
2	—	50	—	—
3	5	14	9	35
4	20	45	38	31
5	—	40	—	—

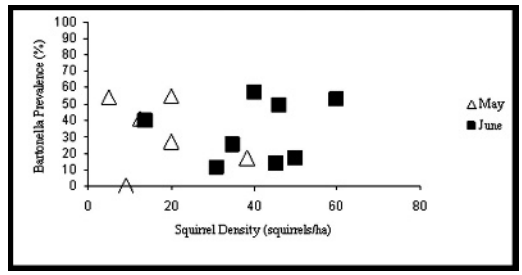

FIGURE 2. Prevalence of *Bartonella* infections in Richardson's ground squirrels in May and June, at sites where population density could be estimated, in 2003 and 2004, as a function of ground squirrel population density at the same sites and months.

The estimated density of ground squirrels on areas trapped in 2003 and 2004 ranged from five to 38 animals/ha (average 18/ha) in May and from 14 to 60 animals/ha in June (average 40/ha) (Table 2). Squirrel density was not associated with *Bartonella* prevalence ($P=0.72$, Fig. 2).

The overall prevalence of *Bartonella* infections in ground squirrels in both 2002 and 2003 was 52% (66 and 322 animals tested, respectively). In 2004, the overall prevalence was 40% (197 animals tested). In our final model, after adjusting for age and month, squirrels were significantly less likely to be infected with *Bartonella* in 2004 compared with 2002 and 2003 (Table 3).

Bartonella prevalence was lowest in May and increased during the summer to a peak in September (Fig. 3). In our final model, after adjusting for age and year, infection with *Bartonella* was significantly more likely in April, July, August, and September compared with June (Table 3).

Fifty-eight percent of juvenile Richardson's ground squirrels sampled were infected with *Bartonella* compared to 37% of adults (299 and 286 tested, respectively). In our final model, after adjusting for year and month, juvenile squirrels were significantly more likely to be infected with *Bartonella* compared to adult squirrels (Table 3). In a small subset of animals of known age, we found that 58% of 24 animals known to be 1 year of age were infected with *Bartonella*, whereas, only 24% of 25 animals known to be ≥2 years of age animals were infected with *Bartonella*.

More males (58%) than females (43%) were positive for *Bartonella* (203 and 382 animals tested, respectively). Although males were significantly more likely to have *Bartonella* infections in our univariate model ($P=0.001$), after adjusting for age, year, and month, we found that there was no significant difference in the prevalence of *Bartonella* infections between males and females ($P=0.14$). Therefore, sex was not included as a factor in our final model.

Flea prevalence was included as a factor in building our final model ($P=0.1$ in univariate model); however, because month and flea prevalence varied collinearly, we could not include them both in the same model. No relationship was found between the prevalence of *Bartonella* infections and flea prevalence after adjusting for age and year ($P=0.22$, Fig. 4), so flea prevalence was excluded in our final model. Month, as discussed previously, remained significant after adjusting for age and year, and was included in our final model (Table 3).

Ectoparasites

Fleas were found on 82% of 465 animals examined. Ticks were detected on only 8% of 455 animals examined. Due to the small number of ticks detected, we considered only fleas for further analysis.

TABLE 3. Site-adjusted final model showing multivariate associations between demographic features that were significant in the final model (year, month of trapping session, and age) and *Bartonella* infections in Richardson's ground squirrels in 2002, 2003, and 2004.

Variable	Odds ratio	95% confidence interval		*P* value
		Lower	Upper	
Year				0.001
2002	3.5	1.6	7.5	0.001
2003	2.1	1.3	3.3	0.002
2004	Reference category			
Month				0.001
April	2.9	1.4	5.9	0.003
May	1.0	0.5	1.8	0.98
June	Reference category			
July	1.8	1.0	3.0	0.04
August	2.3	1.2	4.6	0.01
September	2.8	1.0	7.7	0.04
Age				
Juvenile	2.5	1.5	4.4	0.001
Adult	Reference category			

Fleas were found on 81% of female and 84% of male animals examined (286 and 179 animals examined, respectively). There was no significant difference in flea prevalence on male and female ground squirrels ($P=0.29$).

Fleas were found on 82% of 208 animals examined in 2003 and 83% of 257 animals examined in 2004, and there was no significant difference in prevalence of fleas between years after adjusting for age and month ($P=0.53$); therefore, year was not included in our final model. The prevalence of fleas on ground squirrels varied seasonally, decreasing from 87% in April to 74% in June and then increasing to 99% by August–September (Fig. 5). We combined August and September for the analysis due to the small sample size in September. The prevalence of fleas on Richardson's ground squirrels was significantly higher in July and August–September than in June in our final model (after adjusting for age) (Table 4).

Fleas were found on 90% of 184 adult animals and 78% of 281 juvenile animals. In our final model, after adjusting for month, adult animals were significantly more likely to have fleas than juvenile animals (Table 4).

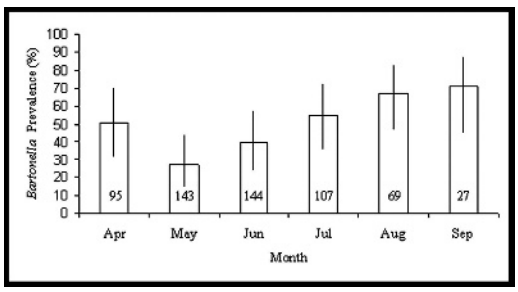

FIGURE 3. Monthly prevalence of *Bartonella* in Richardson's ground squirrels for all years combined. Error bars represent 95% confidence intervals. Sample size is shown inside column.

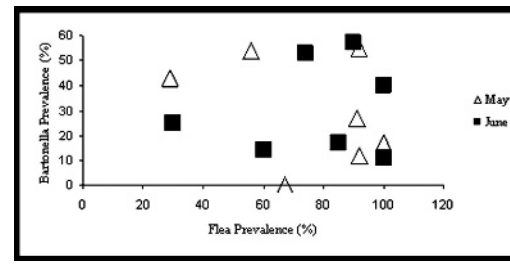

FIGURE 4. Prevalence of *Bartonella* infections in Richardson's ground squirrels in May and June at sites where population density estimates could be made in 2003 and 2004, as a function of flea prevalence at same sites and same months.

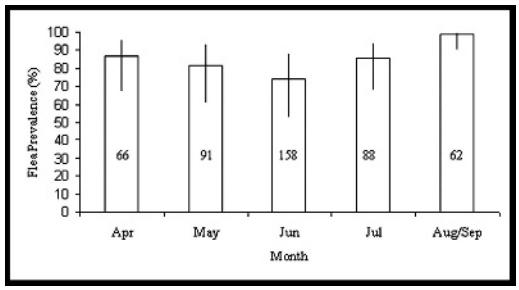

FIGURE 5. Monthly prevalence of fleas on Richardson's ground squirrels for all years combined. Error bars represent 95% confidence intervals. Sample size is shown inside column.

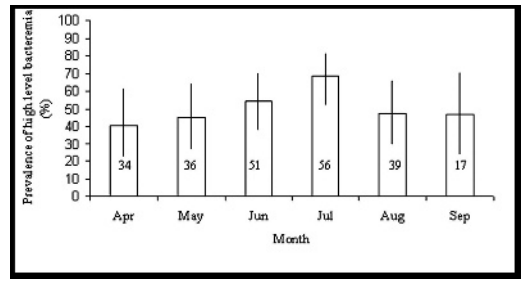

FIGURE 6. Monthly prevalence of a high level of bacteremia in *Bartonella*-positive Richardson's ground squirrels for all years combined. Error bars represent 95% confidence intervals. Sample size is shown inside column.

Bartonella bacteremia

The level of bacteremia (high or low) was determined for 233 animals infected with *Bartonella*. Overall, 46% of animals had a low level of bacteremia and 54% had high levels of bacteremia.

In 2003, the majority of animals, from which *Bartonella* was isolated, had high levels of bacteremia (57% of 167 animals tested), while 47% of animals in 2004 had high levels of bacteremia (66 animals tested). The difference between years was not significant after adjusting for age and month ($P=0.1$) and was not included in our final model. The proportion of *Bartonella*-positive animals with a high level of bacteremia varied throughout the summer, increasing from 40% in April to 69% in July and then decreasing to 47% in September (Fig. 6), but there was no difference in the monthly proportions of animals with high levels of bacteremia after adjusting for age and year ($P=0.36$). Therefore month was not included in the final model.

The majority of *Bartonella*-positive adult animals had low levels of bacteremia (57% of 80 animals), while the majority of *Bartonella*-positive juvenile animals had high levels of bacteremia (60% of 153 animals). Juvenile animals were significantly more likely to have high levels of bacteremia compared with adult animals after adjusting for month and year. The only significant factor in our final model was age; juvenile animals were twice as likely as adult animals to have high level bacteremia (95% confidence interval 1.2 to 3.5; $P=0.01$).

TABLE 4. Site-adjusted final model showing multivariate associations between demographic features that were significant in the final model (month of trapping session and age) and prevalence of fleas on Richardson's ground squirrels in 2003 and 2004.

Variable	Odds ratio	95% confidence interval		*P* value
		Lower	Upper	
Month				0.007
April	0.9	0.2	3.6	0.89
May	0.9	0.3	2.2	0.75
June	Reference category			
July	2.7	1.2	6.0	0.01
August–September	39.9	3.5	460.0	0.003
Age				
Adult	5.0	1.8	14.2	0.003
Juvenile	Reference category			

Males and females infected with *Bartonella* were equally likely to have high levels of bacteremia (54% of 136 females and 54% of 97 males; $P=0.9$).

DISCUSSION

The overall prevalence of *Bartonella* infection in Richardson's ground squirrels in this study was 48%. Many studies have reported similar, or even higher, prevalence of *Bartonella* in wild rodents in a variety of geographical locations (Kosoy et al., 1997; Fichet-Calvet, 2000; Ying et al., 2002; Holmberg et al., 2003).

We found no relationship between host density and *Bartonella* prevalence in this study. In contrast, Fichet-Calvet et al. (2000) found an inverse relationship between prevalence of infection with *Bartonella* and host abundance. Ideally, to investigate whether *Bartonella* prevalence is related to host density, the prevalence of *Bartonella* should be determined for multiple independent sites with different host densities. We attempted to do this, but our sample size was very small, and our estimates were not all independent (we used the same sites within and among years). Therefore, we cannot rule out that there was a relationship that we were unable to detect. An alternative approach for investigating the relationship between host abundance and *Bartonella* prevalence would be to experimentally reduce or increase host density.

The temporal patterns seen in this study are similar to those reported in other longitudinal studies of *Bartonella* (Fichet-Calvet et al., 2000; Kosoy et al., 2004), with high levels of infection occurring in late summer and autumn. A significant proportion of adult squirrels (51%) were infected with *Bartonella* during the first trapping session in April. Because Richardson's ground squirrels hibernate over winter, this suggests that infections are maintained either in the squirrels or ectoparasites over the winter. We found that the prevalence of *Bartonella* decreased in the early summer as reported by Kosoy et al. (2004). In our study, the prevalence was lowest in May, which corresponds to the first few weeks of life for juvenile squirrels before they emerge from their natal burrows. Once juveniles emerge in late May, the prevalence of *Bartonella* increased for the remainder of the summer, with the majority of juvenile animals developing *Bartonella* bacteremia after emerging from their natal burrows. Not only was the prevalence of *Bartonella* infections highest in the late summer, but the prevalence of fleas was also greatest at this time, making the late summer the period of greatest risk for transmission of the bacteria among squirrels and potentially to other species, including humans.

Juvenile animals were significantly more likely to be infected with *Bartonella* than were adult animals (58% and 37%, respectively), and were more likely to have high levels of bacteremia. Although we did not know the exact age of all adult ground squirrels, only 24% of animals that were known to be $\geq$2 years old were infected with *Bartonella*. Kosoy et al. (2004) also reported that the prevalence of *Bartonella* infections decreased with age and Kosoy et al. (2004) and Fichet-Calvet et al. (2000) found that juvenile animals had significantly higher bacterial loads than adult animals.

Decreasing prevalence with age combined with lower levels of bacteremia in older animals suggest that animals are able to clear infections through acquired immunity (Kosoy et al., 2004). In experimental infections, antibodies play an essential role in terminating *B. grahamii* infections in laboratory mice (Koesling et al., 2001). We did not measure antibodies in this study. Kosoy et al. (2004) found few animals with antibodies (15%) in a longitudinal field study, and the majority of animals with antibodies had very low titers, suggesting that antibody production is not a feature of *Bartonella* infections in, at least some, species of wild rodents in North America.

Alternatively, a low prevalence of *Bartonella* in older animals might indicate that animals infected with *Bartonella* are less likely to survive. Boulouis et al. (2001) found no difference in survival of experimentally infected and noninfected rodents, and Fichet-Calvet et al. (2000) reported no differences in the weight of *Bartonella* infected and noninfected animals in the field. However, there may be species differences in effects on rodent hosts, and it also is possible that the effects of the bacteria are subtle; for example, *Bartonella* may cause chronic disease, which may affect survival only later in life. Because the majority of Richardson's ground squirrels are infected at some point in their life (74% sampled three or more times in this study were positive for *Bartonella* at least once), it is difficult to compare survival rates of infected and noninfected animals. Determination of the "cost" of *Bartonella* infections to their hosts remains an area for future research.

Ectoparasites have been implicated as potential vectors for *Bartonella* in rodents (Bown et al., 2003; Durden et al., 2004). Ticks were found on only 8% of Richardson's ground squirrels in this study, and Hilton and Mahrt (1971) found that only 2% of Richardson's ground squirrels sampled in Alberta, Canada, were infested with ticks. Because ticks occur uncommonly on Richardson's ground squirrels, it is unlikely that they would be important vectors of *Bartonella* for this species. We found fleas on more than 80% of animals examined. Although we did not find a significant relationship between the occurrence of fleas on animals and *Bartonella* prevalence in this study, it is interesting that, based on monthly prevalence levels, these two factors appear to vary in parallel (Figs. 3, 5). High flea prevalence in all months combined with relatively small sample sizes make it difficult to detect any relationship that might be present.

We found that infections with *Bartonella* were less common in ground squirrels in 2004 than in 2003 or 2002. Although it is beyond the scope of this study to determine why fewer ground squirrels were infected with *Bartonella* in 2004, it should be noted that the summer of 2004 was the coolest and wettest of the three years included in this study. It would be interesting to determine if our findings are consistent and if they are related to how ectoparasites fare in different climatic conditions. Although we found no difference in the prevalence of fleas on squirrels in 2003 and 2004, we did not measure the intensity of flea infestations. Ryckman (1971) found that, during the summer months, the number of fleas on California ground squirrels was lower in experimental areas where natural precipitation was supplemented with water sprinklers. It is possible that the number of fleas per host, not just the presence of fleas on a host, is an important factor influencing the transmission of the bacteria. Separating the relationships among climatic conditions, ectoparasites, and *Bartonella* prevalence will require long-term studies.

We did not sequence the isolates that were found in this study, and some of the demographic and temporal features that we report may be a consequence of Richardson's ground squirrels acquiring and losing infections with different genotypes of *Bartonella*. The purpose of this paper was to present the demographic and temporal features of *Bartonella* infections in Richardson's ground squirrels in a general sense. Future research will be required to determine the mechanism for the findings in this study.

Description and understanding of the ecology of zoonotic pathogens in their reservoir hosts are essential for predicting and preventing disease occurrence in humans. We are only beginning to unravel the complex relationships between *Bartonella* spp. hosts and vectors.

ACKNOWLEDGMENTS

We thank all landowners for giving us permission to conduct this study on their

property and E. Garde and B. Macbeth for assistance with trapping. We thank M. Chirino-Trejo for culturing our samples and G. Appleyard for assistance with PCR. Funding for this project was provided by Western College of Veterinary Medicine (WCVM) Dean's Medical Research Council grant, WCVM Wildlife Health Fund, and the Animal Determinants of Emerging Diseases Research Group (Michael Smith Foundation for Health Research). C. Jardine was supported by a WCVM interprovincial graduate student fellowship.

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Received for publication 4 November 2005.