

## *Borrelia burgdorferi* Sensu Lato in Siberian Chipmunks (*Tamias sibiricus*) Introduced in Suburban Forests in France

GWENAËL VOURC'H,<sup>1</sup> JULIE MARMET,<sup>2</sup> MICHELLE CHASSAGNE,<sup>1</sup>  
SÉVERINE BORD,<sup>1</sup> AND JEAN-LOUIS CHAPUIS<sup>2</sup>

### ABSTRACT

Numerous vertebrate reservoirs have been described for *Borrelia burgdorferi* sensu lato (sl), which includes the etiological agents of Lyme Borreliosis (LB). The Siberian chipmunk (*Tamias sibiricus*) is a rodent originating from Asia, where it is suspected to be a *B. burgdorferi* reservoir. It has been intentionally released into the wild in Europe since the 1970s, but has not yet been subject to any study regarding its association with the LB agent. In this paper we studied Siberian chipmunk infestation with the LB vector (*Ixodes ricinus*) and infection prevalence by LB spirochetes in a suburban introduced population. We compared these findings with known competent reservoir hosts, the bank vole (*Myodes [clethrionomys] glareolus*) and wood mouse (*Apodemus sylvaticus*). All Siberian chipmunks were infested with larvae and larval abundance was higher in this species (mean number of larvae [95% Confidence Interval]: 73.5 [46.0, 117.2]) than in the two other rodent species (bank voles: 4.4 [3.0, 6.3] and wood mice: 10.2 [4.9, 21.2]). Significant factors affecting abundance of larvae were host species and sampling season. Nymphs were most prevalent on chipmunks (86.2%, mean: 5.1 [3.3, 8.0]), one vole carried only two nymphs, and none of the mice had any nymphs. Nymph abundance in chipmunks was affected by sampling season and sex. Furthermore, the infection prevalence of *B. burgdorferi* sl in the Siberian chipmunk was the highest (33.3%) and predominantly of *B. afzelii*. The infection prevalence was 14.1% in bank voles, but no wood mouse was found to be infected. Our results suggest that the Siberian chipmunk may be an important reservoir host for LB. Key Words: *Tamias sibiricus*—reservoir hosts—*Borrelia*—Lyme disease—*Ixodes*—Tick(s).

### INTRODUCTION

LYME BORRELIOSIS (LB) is caused by *Borrelia burgdorferi* sensu lato (sl), which is transmitted by hard ticks, mainly *Ixodes ricinus* in Europe. Numerous vertebrate species have been described as reservoir hosts (Kurtenbach et al. 2006). As their competence varies, reservoir host diversity and abundance in an ecosystem affect LB risk (LoGiudice et al. 2003).

The Siberian chipmunk (*Tamias sibiricus*), a sciurid originating from Asia, has been sold in

European pet shops since the 1960s and has been intentionally released into the wild since the 1970s. European populations have been identified in Germany, Italy, Belgium, the Netherlands, Switzerland, and France with some of them numbering several thousands (Chapuis 2005). The reservoir role of its North American sibling species, the Eastern chipmunk (*Tamias striatus*), is known (Slajchert et al. 1997). *B. burgdorferi* has been reportedly isolated from Siberian chipmunks in China (Wan et al. 1999) but to our knowledge no study has

<sup>1</sup>National Institute for Agricultural Research (INRA), UR346 Animal Epidemiology, F-63122 Saint Genès Champanelle, France.

<sup>2</sup>National Museum of Natural History, Ecology and Management of Biodiversity Department, UMR 5173 MNHN-CNRS-P6, F-75005 Paris, France.

been published on the association between the Siberian chipmunk and LB vector or agent in Europe.

The aim of this paper was to test whether the Siberian chipmunk could be a reservoir host for LB. Infestation by *I. ricinus* and infection prevalence by *B. burgdorferi* sl were studied in a suburban forest with an established population (Chapuis 2005) and compared to known competent reservoir hosts: bank voles (*Myodes [clethrionomys] glareolus*) and wood mice (*Apodemus sylvaticus*) captured at the same site.

## MATERIALS AND METHODS

The study took place 22 km southeast of Paris (48°39'N and 2°29'E) in Sénart forest (3200 ha), which is dominated by oaks (*Quercus* spp) and common hornbeam (*Carpinus betulus*). In 2005, rodents were captured with a non-standardized protocol. Siberian chipmunks were live-trapped using 40 baited Sherman<sup>®</sup> traps over one day in April and 20 traps over three to five days twice in July and August, and once at the end of September (356 trap-days). Small rodents were captured over three consecutive nights using 210 baited INRA<sup>®</sup> traps in March, in June, and in September (1890 trap-nights). In addition, some were occasionally trapped from March to November in Sherman<sup>®</sup> traps that were set as part of another study on chipmunk population dynamics.

The animals were terminated by cervical dislocation. Ear biopsies were thereafter obtained and stored in 90% ethanol. Rodents were frozen until further examination in the laboratory. They were weighed, sexed, and aged (juvenile or adult). Ticks were collected in the laboratory using the stereomicroscope and identified under the microscope (Cotty 1985).

Ear biopsy DNA extraction was performed using a NucleoSpin<sup>®</sup> Tissue kit (Macherey-Nagel, Düren, Germany). Detection and identification of *B. burgdorferi* sl species were performed by PCR-RFLP on the rrf (5S)—rrl (23S) intergenic spacer adapted from Postic et al. (1994) using [5'-CTGCCGAGTTCGCGGGAG-AG-3'] and [5'-AAGCTCCTAGGCATTCACC-ATA-3'] primers. Positive PCR products were also sequenced (Genoscreen, Lille, France).

Mean tick counts were reported and Wald 95% confident intervals were obtained using the GENMOD procedure (SAS 8.1 Institute Inc., Cary, NC) in an intercept-only model with negative binomial distribution. The number of *I. ricinus* larvae per individual was modeled using a negative binomial distribution and stepwise backward elimination for variable selection (PROC GENMOD, link = log, SAS). For each significant factor, the antilog of regression coefficient estimates (RC) were used to assess the relative change in larval abundance for one level compared to a reference. A similar analysis was performed with *I. ricinus* nymphal abundance as the response variable. Risk factors assessed included species, sex, age, sampling season (March–April, May–July, and August–November; questing larvae and nymphs usually having their activity peak between May and July at this site, J-L Chapuis, unpublished), and weight (terciles within species and age). The correlation between larval and nymphal abundances in chipmunks was tested using Spearman's rank correlation. The association between *B. burgdorferi* sl infection and the same risk factors was quantified as Odds Ratio (OR) using a logistic regression with backward elimination (PROC LOGISTIC, SAS).

## RESULTS AND DISCUSSION

In total, 33 Siberian chipmunks, 64 bank voles, and 31 wood mice were trapped and tested for *B. burgdorferi* DNA. All animals were examined for ticks except for 14 individual animals that were excluded from the tick infestation analyses. Among the animals, 2629 *I. ricinus* larvae and 151 nymphs were collected (Table 1). Other larval and nymphal tick species found were *I. acuminatus*, *I. trianguliceps*, and *Dermacentor* sp. In addition, seven adult *I. acuminatus* females (one on a chipmunk, one on a mouse, and five on voles) and one adult *I. trianguliceps* female (on a vole) were collected. Lastly, 67 *Ixodes* sp could not be identified (65 larvae, one nymph, and one adult).

Some individuals were highly infested: three chipmunks had over 280 larvae and 15 nymphs. The percentage of animals with ticks and the mean number of larvae and nymphs per host

TABLE 1. TICK INFESTATION OF SIBERIAN CHIPMUNKS (*TAMIAS SIBIRICUS*), BANK VOLES (*MYODES GLAREOLUS*), AND WOOD MICE (*APODEMUS SYLVATICUS*)

Host species	N <sup>a</sup>	<i>Ixodes ricinus</i>			<i>Ixodes acuminatus</i>			<i>Ixodes trianguliceps</i>			<i>Dermacentor</i>		
		n <sup>b</sup>	P <sup>c</sup>	M <sup>d</sup> (CI)	n	P	M (CI)	n	P	M (CI)	n	P	M (CI)
Larvae													
<i>T. sibiricus</i>	29	2130	100.0	73.5 (46.0, 117.2)	102	44.8	3.5 (1.6, 7.8)	0	0.0	—	127	79.3	4.4 (2.9)
<i>M. glareolus</i>	63	274	73.0	4.4 (3.0, 6.3)	167	41.3	2.6 (1.5, 4.8)	0	0.0	—	80	30.1	1.3 (0.7)
<i>A. sylvaticus</i>	22	225	68.2	10.2 (4.9, 21.2)	6	18.2	0.3 (0.1, 0.8)	0	0.0	—	10	27.3	0.5 (0.2)
Nymphs													
<i>T. sibiricus</i>	29	149	86.2	5.1 (3.3, 8.0)	1	3.5	0.0 <sup>e</sup>	1	3.5	0.0 <sup>e</sup>	0	0.0	—
<i>M. glareolus</i>	63	2	1.6	0.0 (0.0, 0.4)	38	28.6	0.6 (0.4, 1.0)	4	4.8	0.1 (0.0, 0.2)	30	15.9	0.5 (0.2)
<i>A. sylvaticus</i>	22	0	0.0	—	2	9.1	0.1 (0.1, 0.4)	0	0.0	—	0	0.0	—

<sup>a</sup>Number of examined hosts.  
<sup>b</sup>Number of ticks.  
<sup>c</sup>Percentage of hosts with ticks.  
<sup>d</sup>Mean number of ticks per host [Wald 95% Confidence Interval]  
<sup>e</sup>Confidence Interval not computable due to insufficient counts.

were higher in the Siberian chipmunk than in the two other species in this study (Table 1) or in other studies (e.g., Humair et al. 1999). They were also higher than reported counts on Siberian chipmunks in China (Ai et al. 1991). Similar counts have been found in red squirrels (*Sciurus vulgaris*) (Humair and Gern 1998), probably because of similarities in the behavior of these vertebrate species. Compared to Eastern chipmunks, Siberian chipmunks carried higher burden of both tick stages (Slajchert et al. 1997) or similar nymphal burden (Schmidt et al. 1999). In Siberian chipmunks, larval and nymphal abundances were highly correlated ( $r = 0.77$ ,  $p$ -value < 0.001). Since all chipmunks carried larvae, co-feeding transmission of spirochetes could potentially occur on those carrying infected nymphs (Kurtenbach et al. 2006).

Significant factors affecting larval abundance were species and sampling season. Chipmunks carried more larvae than voles (RC [Wald 95% Confidence Interval] = 22.6 [12.5, 40.9] or mice (RC = 10.1 [5.0, 20.7]). Mice had a higher larval burden than voles (RC = 2.2 [1.2, 4.2]). Animals sampled in March–April and May–July had more larvae than those sampled in August–November (RC = 4.9 [2.3, 10.4] and RC = 5.8 [3.4, 9.9], respectively). As shown in Table 2, the difference between species was most noticeable in May–July, which includes the questing larvae peak activity at the site. Because of the small sample size, the variation within species over time should be investigated with a more comprehensive study.

*I. ricinus* nymphal burden in small rodents was very low, as one vole had only two

TABLE 2. INFESTATION BY *IXODES RICINUS* OF SIBERIAN CHIPMUNKS (*TAMIAS SIBIRICUS*), BANK VOLES (*MYODES GLAREOLUS*), AND WOOD MICE (*APODEMUS SYLVATICUS*) BY SPECIES AND SEASON OF SAMPLING (2 NYMPHS FOUND ON A BANK VOLE IN JULY ARE NOT SHOWN)

Host species	March–April		May–July		Aug.–Nov.	
	N hosts <sup>a</sup>	M <sup>b</sup> (CI)	N hosts	M (CI)	N hosts	M (CI)
Larvae						
<i>T. sibiricus</i>	5	3.0 (1.8, 5.1)	10	162.9 (100.2, 264.8)	14	34.7 (21.6, 55.9)
<i>M. glareolus</i>	10	8.8 (4.1, 18.8)	24	6.7 (4.2, 10.6)	29	0.9 (0.6, 1.4)
<i>A. sylvaticus</i>	3	13.7 (11.8, 15.8)	11	14.8 (5.2, 42.1)	8	2.6 (0.8, 9.2)
Nymphs						
<i>T. sibiricus</i>	5	1.0 (0.6, 1.9)	10	11.3 (7.0, 18.2)	14	2.2 (1.3, 3.7)

<sup>a</sup>Number of examined hosts.  
<sup>b</sup>Mean number of ticks per host (Wald 95% Confidence Interval)

nymphs and none of the mice had any. Therefore the nymphal burden analysis was performed only with chipmunks. Significant factors were sampling season and sex. Animals sampled in May–July had more nymphs (Table 2) than those sampled in March–April and August–November (RC = 8.8 [2.9, 26.6] and RC = 5.2 [2.8, 9.7] respectively). The nymphal burden in chipmunks was higher in females than in males (RC = 2.1 [1.1, 3.7]). Higher tick burdens in rodent males are commonly reported and linked to their higher activity rates and larger home ranges (Hughes and Randolph 2001). However, Schmidt et al. (1999) reported that sex had little effect on infestation of Eastern chipmunks.

In the Siberian chipmunk, 11 of the 33 tested animals (33.3%) carried *B. burgdorferi* sl DNA. Among them, nine were infected with *B. afzelii* (a small rodent associated species, Kurtenbach et al. 2006), one with *B. burgdorferi* sensu stricto (ss) (the dominant species infecting red squirrels; Humair and Gern 1998), and one coinfecting with both species. Sato et al. (1996) detected no infection on eight Siberian chipmunks sampled in far eastern Russia. In the bank vole, nine out of 64 (14.1%) were positive, all with *B. afzelii*. All 31 wood mice were negative and thus excluded from analysis. The only significant factor was species: chipmunks were significantly more infected than voles (OR = 3.7 [1.3, 10.3]). Elsewhere in Europe infection prevalence rates reaching 18–28% have been reported in bank voles (Hanincová et al. 2003, Humair et al. 1999) and red squirrels (Humair and Gern 1998). Although the absence of infection in mice is surprising, studies have shown that wood mice commonly have lower infection rates than bank voles due to stronger immune response against *B. burgdorferi* sl (Humair et al. 1999, Kurtenbach et al. 1994).

The high *I. ricinus* abundance and *B. burgdorferi* sl infection prevalence found in Siberian chipmunks indicates that in French suburban forests this species has an important degree of contact with the tick vector and can become infected with LB agents. However, other factors should still be examined in order to assess reservoir competence and importance, in particular the infectivity to the vector and the capacity of the Siberian chipmunk to sustain *B.*

*burgdorferi* sl infection long enough to serve as a source of infection for uninfected ticks (Humair et al. 1999, Lane et al. 2005). If its reservoir role is demonstrated, their presence in suburban forests may greatly increase LB risk in humans because Siberian chipmunks live in suburban habitats, are unafraid of humans, and have densities reaching over 10 individuals per hectare (Chapuis 2005).

## ACKNOWLEDGMENTS

We are grateful to the Région Ile-de-France, the Conseil Général des Hauts-de-Seine, and the Office National des Forêts for funding. We thank Marina Roussel and Benoît Pisanu for their help with the small rodent sampling, Jean-Pierre Girardeau and Valérie Poux for their help with the molecular biology analyses, and Dr. Postic (Pasteur Institute, Paris) and Dr. Vayssier-Taussat (UMR BIPAR, Maisons-Alfort) for providing *Borrelia burgdorferi* sl reference strains. Finally, we thank Maria Diuk-Wasser and Heidi Brown as well the anonymous referees for their useful comments on the manuscript.

## REFERENCES

- Ai, C, Qiu, G, Shi, Z, Wu, X, Liu, X, and Zhao, J. Host associations and seasonal abundance of immature *Ixodes persulcatus* (Acari: Ixodidae) in a Lyme-disease-endemic area in China. *Exp Appl Acarol* 1991; 12:251–257.
- Chapuis, J-L. [Distribution in France of a naturalized pet, the Siberian Chipmunk (*Tamias sibiricus*)] [in French]. *Rev Ecol* 2005; 60:239–253.
- Cotty, A. [Identification key for Ixodidae and Amblyomidae in Switzerland] [in French]. Université de Neuchâtel, Neuchâtel, Switzerland 1985.
- Hanincová, K, Schäfer, SM, Etti, S, Sewell, H-S, Taragelová, V, Ziak, D, Labuda, M, and Kurtenbach, K. Association of *Borrelia afzelii* with rodents in Europe. *Parasitology* 2003; 126:11–20.
- Hughes, VL, and Randolph, SE. Testosterone depresses innate and acquired resistance to ticks in natural rodent hosts: a force for aggregated distributions of parasites. *J Parasitol* 2001; 87:49–54.
- Humair, PF, and Gern, L. Relationship between *Borrelia burgdorferi* sensu lato species, red squirrels (*Sciurus vulgaris*) and *Ixodes ricinus* in enzootic areas in Switzerland. *Acta Trop* 1998; 69:213–227.
- Humair, PF, Rais, O, and Gern, L. Transmission of *Borrelia afzelii* from *Apodemus* mice and *Clethrionomys* voles

- to *Ixodes ricinus* ticks: differential transmission pattern and overwintering maintenance. *Parasitology* 1999; 118:33–42.
- Kurtenbach, K, Dizij, A, Seitz, HM, Margos, G, Moter, SE, Kramer, MD, Wallich, R, Schaible, UE, and Simon, MM. Differential immune responses to *Borrelia burgdorferi* in European wild rodent species influence spirochete transmission to *Ixodes ricinus* L. (Acari: Ixodidae). *Infect Immun* 1994; 62:5344–5352.
- Kurtenbach, K, Hanincová, K, Tsao, JI, Margos, G, Fish, D, and Ogden, NH. Fundamental processes in the evolutionary ecology of Lyme borreliosis. *Nat Rev Microbiol* 2006; 4:660–669.
- Lane, RS, Mun, J, Eisen, RJ, and Eisen, L. Western gray squirrel (Rodentia: Sciuridae): A primary reservoir host of *Borrelia burgdorferi* in Californian oak woodlands? *J Med Entomol* 2005; 42:388–396.
- LoGiudice, K, Ostfeld, RS, Schmidt, KA, and Keesing, F. The ecology of infectious disease: Effects of host diversity and community composition on Lyme disease risk. *Proc Natl Acad Sci USA* 2003; 100:567–571.
- Postic, D, Assous, MV, Grimont, PA, and Baranton, G. Diversity of *Borrelia burgdorferi* sensu lato evidenced by restriction fragment length polymorphism of rrf (5S)-rrl (23S) intergenic spacer amplicons. *Int J Syst Bacteriol* 1994; 44:743–752.
- Sato, Y, Miyamoto, K, Iwaki, A, Masuzawa, T, Yanagihara, Y, Korenberg, EI, Gorelova, NB, Volkov, VI, Ivanov, LI, and Liberova, RN. Prevalence of Lyme disease spirochetes in *Ixodes persulcatus* and wild rodents in far eastern Russia. *Appl Environ Microbiol* 1996; 62:3887–3889.
- Schmidt, KA, Ostfeld, RS, and Schaubert, EM. Infestation of *Peromyscus leucopus* and *Tamias striatus* by *Ixodes scapularis* (Acari : Ixodidae) in relation to the abundance of hosts and parasites. *J Med Entomol* 1999; 36:749–757.
- Slajchert, T, Kitron, UD, Jones, CJ, and Mannelli, A. Role of the eastern chipmunk (*Tamias striatus*) in the epizootiology of Lyme borreliosis in northwestern Illinois, USA. *J Wildl Dis* 1997; 33:40–46.
- Wan, K, Zhang, Z, Wang, H, and Hou, X. [Preliminary investigation on reservoir hosts of *Borrelia burgdorferi* in China] [in Chinese] Wei Sheng Yan Jiu [Journal of Hygiene Research] 1999; 28:7–9.

Address reprint requests to:

Gwenaël Vourc'h

INRA, UR346 Animal Epidemiology

F-63122 Saint Genès Champanelle

France

E-mail: gvourch@clermont.inra.fr.

