The prevalence of *Borrelia burgdorferi* sensu lato in *Ixodes persulcatus* and *I. ricinus* ticks in the zone of their sympatry

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Abstract. A total of 7210 unfed adult *Ixodes persulcatus* Schulze, 1930 and *I. ricinus* (L., 1758) ticks were collected from the vegetation by flagging in 35 study sites located in the zone of their sympatry (mainly in Leningrad region, Russia). *Borrelia* infection in ticks was estimated by the dark-field microscopic analysis of gut contents in standard vital preparations at a magnification of $\times 600$. No correlation was revealed between the series of parameters characterising the abundance of each tick species ($\tau = -0.13$) and between the series of these parameters and the prevalence of *Borrelia* in each vector. It is concluded that in the broad zone of *I. persulcatus* and *I. ricinus* sympatry, the presence and proportion of one vector in the ecosystem does not have any significant effect on the extensity of infection and on the epizootic and epidemic significance of the other vector. Each tick species has its independent (of the other species) and relatively original functional role in the focal ecosystem.

As is known from numerous publications, the ixodid ticks *Ixodes persulcatus* Schulze, 1930 and *I. ricinus* (L., 1758) are the principal vectors and long-term reservoirs of *Borrelia burgdorferi* sensu lato spirochetes in Eurasia. The latter cause human infections of the Lyme disease group, with the geographic range of infection closely corresponding to the distribution pattern of the principal vectors (Korenberg et al. 1987, Korenberg 1994). *I. persulcatus* has a vast range in Eurasia, which is located mostly in Russia; west of the Russian border, the spread of this species is limited. Conversely, *I. ricinus* range covers all the countries of western, central, and southern Europe and, as a narrow band, stretches out to northern Africa and the Near East. Its eastern part is in Russia, where it occupies a vast territory expanding from the western state boundary approximately to the middle reaches of the Volga River. Thus, the ranges overlap in eastern Europe, forming a broad zone in which these tick species are generally distributed sympatrically. This zone covers the entire European part of the *I. persulcatus* range, which is characterised by southern-taiga and mid-taiga plant formations spreading to this zone from the east, combined with deciduous and mixed forests of the European type (Filippova 1977, 1999, Korenberg 1979, Korenberg and Kovalevskii 1981).

Within this zone, however, both tick species do not necessarily occur simultaneously in all forests, and there are regions with the prevalence of allopatric or almost allopatric populations of only one species (Zolotov et al. 1974). Different forest stands jointly inhabited by *I. persulcatus* and *I. ricinus* populations differ from each other in the quantitative ratio of these ticks (Zolotov 1989, Korenberg et al. 1991a), which is explained by their landscape and biotopic preferences. *I. persulcatus*

ticks occur more often in upland moraine landscapes and lowland glacial-lake landscapes with southern-taiga and mid-taiga dark coniferous forests or secondary forests replacing them, whereas *I. ricinus* ticks prefer lowland moraine and glacial-lake landscapes with open nemoral broadleaved-mixed forests of the European type (Zolotov et al. 1969, 1974, Tokarevich et al. 1975, Filippova 1999). Ticks of both species have similar spectra of hosts and often jointly feed on the same animal. Nevertheless, the total duration and pattern of seasonal activity as well as some other biological characteristics of each species have certain specific features (Ul'yanova et al. 1969, Zolotov et al. 1969, 1974, Sukhomlinova 1977, Zolotov 1989, Filippova 1999). Assessing the role of cohabiting *I. persulcatus* and *I. ricinus* ticks as reservoirs of various microorganisms, it is important to take into account their complete reproductive isolation (Balashov et al. 1998a, b).

It was shown that both species under conditions of sympatry are vectors of *B. burgdorferi* sensu lato (Korenberg et al. 1988, 1991b). Significant seasonal and annual changes in the proportion of infected ticks are not typical for foci in this zone (Kovalevskii et al. 1993, Korenberg and Kovalevskii 1996). Ticks with a high spirochete content are much less abundant than weakly infected ticks, irrespective of their species (Kovalevskii and Korenberg 1995). However, infection rate and the abundance of infected ticks are generally higher in *I. persulcatus* than in *I. ricinus* (Korenberg et al. 1991a, b, Kovalevskii et al. 1993). On the whole, as in the case of tick-borne encephalitis virus (Vasilenko et al. 1990), *I. persulcatus* is a more effective vector of *Borrelia* than *I. ricinus* (Korenberg et al. 1991a, b, Korenberg 1993, 1994, 1995, Alekseev et al. 1998).

According to Filippova (1999), the period of *I. persulcatus* and *I. ricinus* sympatry is 8,000-10,000 years, which is sufficient for producing approximately 2,000 generations of these ticks; it was proposed that sympatry of vectors should have an effect on micro-evolutionary processes in the groups of pathogens biologically associated with them. Some authors claimed that *I. persulcatus* ticks in the zone of sympatry significantly enhance *Borrelia* circulation among *I. ricinus* (Alekseev 1993a, b, Alekseev et al. 1993). In this context, we considered it useful to present our previously unpublished data on *Borrelia* infection in these ticks, which were obtained in different areas of the zone of their sympatry between 1986 and 1992.

MATERIALS AND METHODS

Unfed adult *I. persulcatus* and *I. ricinus* ticks were collected from the vegetation by flagging during the period of their seasonal activity peak (the second half of May and the first half of June), and their number was recalculated per flag-hour. This work was performed in forest biotopes at 35 study sites located in different administrative districts of Leningrad and Novgorod regions (the north-western part of Russia), in which the distribution of these ticks is generally characterised by a well-manifested patchwork pattern (Pomerantsev 1935, Khodakovskii 1948, Olenev 1954, Ul'yanova et al. 1969, Zolotov et al. 1974, Tokarevich et al. 1975, Sukhomlinova 1977, Zolotov 1989). At most of the sites from which representative samples were taken (Table 1), ticks were collected for 20 to 64 flag-hours; at sites No. 2, 10 and 11, long-term studies were performed, and ticks were collected for 387, 252 and 214 flag-hours, respectively; and only at sites No. 6 and 8, the amount of work was less than 20 flag-hours.

Two members of the *B. burgdorferi* complex, *B. garinii* and *B. afzelii*, circulate in this zone with approximately equal frequencies (Postic et al. 1997, Alekseev et al. 1998, Dubinina and Alekseev 1999).

Borrelia infection in ticks was assessed by the dark-field microscopic analysis of tick gut contents in standard vital preparations at a magnification of $\times 600$. A total of 7210 ticks (4636 *I. persulcatus* and 2574 *I. ricinus*) were examined. In 1431 ticks, concentration of spirochetes was individually estimated by counting bacterial cells in 250 microscopic fields and expressed as the average number of cells per 100 microscopic fields. Values of the latter parameter were ranked using grades of pathogen content in individual ticks, which were statistically justified previously (Kovalevskii et al. 1991): low content, 10 or less bacterial cells; moderate, 10.1-50 cells; high and very high content, 50.1 or more bacterial cells per 100 microscopic fields.

Calculated mean values and percentages were processed statistically using a confidence interval based on double error of mean or sampling error. Significance of differences between mean values ($P = 0.95$) was determined using Student's *t*-test. Correlation coefficient τ was determined using Kendall's rank correlation analysis.

RESULTS

Concrete parameters of tick abundance and the prevalence of *Borrelia* infection at 18 study sites, for which generally representative data were obtained, are shown in Table 1. These sites, located between 57°59'-60°54'N and 28°09'-34°42'E, differed both in the abundance of *I. persulcatus* and *I. ricinus* ticks (which ranged from 0.9 to 18.0 and from 0.1 to 3.4 ticks per flag-hour, respectively) and in the ratio of these species in collections. Such a result provided evidence that the distribution of these ticks in the zone of sympatry is distinctly mosaic, with their ratio differing significantly (up to the total absence of one of the two species) even at sites located close to one another and having similar geographic co-ordinates (e.g., sites No. 1 and 18, or 2 and 10). At sites where both *I. persulcatus* and *I. ricinus* were present (sites No. 2-11), the correlation between the series of parameters characterising the abundance of each tick species was virtually absent ($\tau = -0.13$).

Statistically significant data on *Borrelia* prevalence in ticks were obtained for 17 sites (No. 2-18) in the case of *I. persulcatus* and only five sites (No. 1-3, 8, and 10) in the case of *I. ricinus*. Coefficients of correlation between the series of parameters characterising the abundance of ticks and their infection by *Borrelia* were $\tau = 0.01$ for *I. persulcatus* and $\tau = -0.27$ for *I. ricinus*, providing evidence that these parameters actually do not correlate. The correlation between indices of *Borrelia* prevalence in ticks of each species was also weak ($\tau = 0.21$). This coefficient was possible to calculate from the data on only four sites (No. 2, 3, 8, and 10), as indices of infection in *I. ricinus* at all the remaining sites lacked statistical significance because of insufficient number of ticks studied.

All the data characterising the prevalence of *Borrelia* in both tick species under conditions of natural differences in their quantitative ratio, calculated for 35 study sites, are shown in Table 2. In the forests where *I. persulcatus* accounted for 50-100% of collected ticks, parameters of infection in this species do not differ statistically ($t = 0.9$ -1.1). Under conditions of actual sympatry of the two species, parameters of infection in *I. ricinus* ticks from the forests with significantly different proportions of *I. persulcatus* also do not differ ($t = 0.1$ -1.0). In any situation, they were slightly lower than or equal to those in *I. persulcatus* ticks; moreover, the corresponding series of parameters demonstrated a slight negative correlation ($\tau = -0.53$). In the absence of *I. persulcatus* ticks, the prevalence of infection in *I. ricinus* was $12.0 \pm 5.2\%$; when the former were completely dominant, this parameter was $15.7 \pm 6.0\%$. These values do not differ statistically ($t = 0.9$).

Table 1. Abundance of adult *Ixodes persulcatus* and *I. ricinus* ticks and prevalence of *Borrelia burgdorferi* sensu lato in them in different study sites of Leningrad region.

No. of study site	Nearest large settlement	Latitude and longitude	Percent of <i>I. persulcatus</i> in collection	Mean number of ticks per flag-hour		<i>Borrelia</i> prevalence, %	
				<i>I. persulcatus</i>	<i>I. ricinus</i>	<i>I. persulcatus</i>	<i>I. ricinus</i>
1	Belich'e	60°54' N 30°04' E	0	-	1.5	-	12.5 ± 5.5
2	Kirsino-East	59°40' N 31°09' E	20.9	0.9	3.4	18.2 ± 4.5	21.0 ± 1.7
3	Plotichno	60°54' N 34°42' E	72.5	2.9	1.1	35.0 ± 9.5	21.9 ± 14.6
4	Borschevo	58°49' N 30°21' E	86.9	2.0	0.3	36.6 ± 7.8	5.3 ± 10.3
5	Rosson'	59°36' N 28°09' E	90.2	4.5	0.5	39.2 ± 0.7	5.0 ± 9.7
6	Zaostrov'e*	57°59' N 31°20' E	91.4	18.0	1.7	27.8 ± 9.4	28.5 ± 34.1
7	Lyuban'	59°21' N 31°17' E	94.9	3.7	0.2	38.1 ± 9.5	0 (3)**
8	Ermolino*	58°31' N 31°16' E	95.2	7.9	0.4	36.5 ± 8.2	37.5 ± 34.2
9	Semrino	59°40' N 30°26' E	96.3	2.6	0.1	38.0 ± 8.8	+ (1)**
10	Kirsino-West	59°40' N 31°08' E	97.6	12.1	0.3	28.8 ± 2.4	16.7 ± 8.4
11	Mga	59°44' N 31°04' E	99.3	14.6	0.1	46.8 ± 3.4	0 (4)**
12	Maksimovka	59°12' N 29°09' E	100	2.1	-	47.8 ± 8.6	-
13	Kirishi	59°28' N 32°00' E	100	3.2	-	33.3 ± 9.1	-
14	Shugozero	59°56' N 34°12' E	100	4.8	-	19.8 ± 7.6	-
15	Lipnaya Gorka	59°35' N 33°12' E	100	5.4	-	21.8 ± 8.2	-
16	Savozero	60°53' N 34°03' E	100	6.3	-	36.6 ± 8.3	-
17	Pupyshevo	59°52' N 31°48' E	100	10.0	-	8.6 ± 4.7	-
18	Lisii Nos	60°02' N 30°03' E	100	12.0	-	46.7 ± 10.4	-

* Settlements in Novgorod region

** In parentheses – number of ticks studied

Table 2. Prevalence of *Borrelia burgdorferi* sensu lato in ticks, calculated for all study sites with different percent of *Ixodes persulcatus* in collection.

Percent of <i>I. persulcatus</i> in collection	Number of ticks studied		Prevalence in ticks	
	<i>I. persulcatus</i>	<i>I. ricinus</i>	<i>I. persulcatus</i>	<i>I. ricinus</i>
0	-	158	-	12.0 ± 5.2
0.1 - 25.0	291	2219	18.2 ± 4.5	21.0 ± 1.7
25.1 - 50.0	N O D A T A			
50.1 - 75.0	141	51	31.9 ± 7.9	21.6 ± 11.5
75.1 - 99.9	2923	146	34.5 ± 1.7	15.7 ± 6.0
100	1281	-	33.8 ± 2.6	-

Among infected *I. ricinus* ticks, the frequency of individuals with a low *Borrelia* content was higher throughout the study area (50-68%), whereas that of ticks with a high spirochete content was 12-16%, irrespective of the proportion of *I. persulcatus* ticks in catches: even in the absence of the latter, this frequency was about 16%.

DISCUSSION

The data presented above are in good agreement with the results of previous studies showing that *Borrelia burgdorferi* s.l. in natural foci is more closely associated with *I. persulcatus* than with *I. ricinus* ticks (Korenberg et al. 1991a, b, Korenberg 1993, 1994, 1995, Kovalevskii et al. 1993, Alekseev et al. 1998). There is no doubt that *I. persulcatus* ticks are more efficient as hosts and vectors of these spirochetes than *I. ricinus* (Korenberg et al. 1991a, b, Kovalevskii and Korenberg 1995). At the same time, the opinion (Alekseev 1993b, Alekseev et al. 1993) that in the zone of sympatry, in the presence of *I. persulcatus*, the prevalence of infection in *I. ricinus* ticks strongly increases (by a factor of 9), which makes them more efficient vectors of *Borrelia* and markedly contributes to their role in the epidemic and epizootic processes, was not proven. In the same measure, there are no facts confirming the possibility of an inverse process, i.e., a decrease in the prevalence of infection in *I. persulcatus* as a result of receiving spirochetes from *I. ricinus*. Such conclusions were drawn by the authors (Alekseev 1993a, b, Alekseev et al. 1993) who analysed mainly nonrepresentative data on 36 administrative districts of Moscow region. In this context, it is demonstrative that in the

only administrative district from which more than 10 ticks of each species were analysed by these authors, the prevalence of infection in *I. ricinus* (32.2 ± 10.2) proved to be even higher than in *I. persulcatus* (20.0 ± 16.0). The similar level of *Borrelia* prevalence in *I. ricinus* may be also in the zone of its allopatry, e.g., beyond the north-western boundary of *I. persulcatus* range, in the vicinity of Helsinki (Junttila et al. 1999) or in some provinces of southern Sweden (Gustafson et al. 1995). Differences in the prevalence of infection in vectors under similar biotic conditions, both in allopatric and sympatric populations, appear to be determined primarily by the complex of abiotic factors affecting the rate of spirochete reproduction. The data described above allow the conclusion that in the broad zone of *I. persulcatus* and *I. ricinus* sympatry, the presence and proportion of one species in the ecosystem do not exert any significant influence on the extensity of infection and on the epizootic and epidemic significance of the other species. Therefore, each of the two tick species has its independent and relatively original functional role in the focal ecosystem, which contributes to the stability of this ecosystem under conditions of sympatry. The functional roles of *I. persulcatus* and *I. ricinus* ticks are apparently determined by their specific ecological features rather than by adaptation of *Borrelia* genospecies to a certain vector species, as markedly diverse variants of mixed *Borrelia* infection are often observed in tick vectors (Korenberg 1999).

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