A longitudinal study of the prevalence of borreliae in ticks in the urban locality of Brno - Pisárky, Czech Republic

A. Žákovská, K. Vostal, and H. Martiníková

Department of Animal Physiology and Immunology, Institute of Experimental Biology, Faculty of Sciences, Masaryk University, Brno, Czech Republic

Received 4 April 2008; Accepted 1 August 2008

Ticks in the genus *Ixodes* are the primary vectors of *Borrelia burgdorferi* sensu lato, the causative agent of Lyme disease. European genospecies from the *Borrelia burgdorferi* sensu lato complex that are associated with human Lyme disease (*B. afzelii*, *B. garinii* and *B. burgdorferi* sensu stricto) (Wilske 2005, Shapiro 2008) are vectored by *Ixodes ricinus*, which is prevalent in Europe (Vassallo and Perez-Eid 2002). The presence of *B. burgdorferi* in *I. ricinus* has been reported in the Czech Republic (Kmety et al. 1987, Hubálek et al. 1990, Hubálek et al. 1991, 2003, Hubálek et al. 2006) at a frequency of up to 43% (approximate mean of 10%) of ticks in particular localities. The aim of this study was to evaluate potential effects of the infection rate and the frequency of the infected individual developmental stages of *I. ricinus* on Lyme borreliosis incidence, based on seven years of data. Pisárky Park was chosen for this long-term project because it is a popular recreational area for inhabitants of Brno city, Czech Republic.

*Ixodes ricinus* ticks were collected in an area of 150×300 m in the Pisárky site by flagging for 1 h at a time in regular intervals of 14 days over the period of seven years (from September 1996 until October 2002 in period of April-October). Pisárky Park, which is situated in the city of Brno (49° 11'N, 16° 34'E), 2 km from the center of town, was chosen for our research because the park is a typical mixed-forest with a large variety of trees, shrubs, and native plants. A more detailed description of the site can be found in our previous study (Žákovská et al. 2002).

The density of the *I. ricinus* population was determined by dragging a white flannel cloth over the vegetation for one hour at a time. All ticks from the 78 samples (from 1996 to 2002) were placed in tubes and stored in cool (5° C) and humid (R.H. 90%) conditions until they were examined for the presence of spirochetes. The ticks were dissected and their midgut contents were individually triturated on a slide in a drop of saline solution. Each sample was examined for the presence of spirochetes by dark-field microscopy (DFM) at 200× magnification.

Between 2001 and 2002, *Borrelia* DNA was examined in each sample by PCR. First, the DNA from the sample was isolated using a DNA isolation kit (Malamíte, v.o.s., the Czech Republic). The one-tube-nested PCR method was used for the amplification of DNA, based on a specific sequence of the flagellin gene of *B. burgdorferi* sensu lato (Picken et al. 1996). Specific primers for the flagellin gene of *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* were used. The final amplified product was 276 bp long and was separated by electrophoresis (3% agarose gel with ethidium bromide in a concentration of 5 µg/l ml) and visualized by UV (312 nm) illumination. The system of controls (positive, negative, and internal) was used for better accuracy. The sensitivity of this method was 30 borreliae per reaction, corresponding to 120 bacteria per sample.

The normality of data was verified using the Kolmogorov- Smirnov test. It was found that the series do not rise from normal distribution. The Kruskal–Wallis test was applied for verifying a hypothesis that all distributions are identical. The Two-Choice Wilcoxon test was used to verify the hypothesis that two independent random choices arose from the same distribution. The Binomic test was applied for those cases where we needed to establish whether the ratios obtained independently in two series are significantly different from a statistical point of view. We used the Spearman Correlation Coefficient, which does not require two-dimensional data normality.

From a total of 2,813 *I. ricinus* (316 larvae, 1,893 nymphs, 300 males, 304 females) collected over 78 samples between 1996 and 2002 in the Pisárky Park, 164 samples were positive for the presence of B.b.s.l. In the individual collections, the number of infected samples ranged between 0-11, with a mean value of 2.1 (Table 1). In the seven years of our research in this locality, the mean positive rate was

<table>
<thead>
<tr>
<th>Number of collections</th>
<th>Mean</th>
<th>IS -95%</th>
<th>IS +95%</th>
<th>Median</th>
<th>Total</th>
<th>Min.</th>
<th>Max.</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>78</td>
<td>2.1</td>
<td>1.5</td>
<td>2.7</td>
<td>1</td>
<td>164</td>
<td>0</td>
<td>11</td>
<td>2.63</td>
</tr>
</tbody>
</table>
5.8%, and the highest positive rate in ticks obtained in one single collection was 35.7% in October, 1999 (Table 2). Generally, the level of tick infectivity during one year can be divided into three periods: March-April, May-August, and September-November, with a significant increase in the autumn months (Figure 1). The level of infection had also changed over the years, culminating in 1997 (9.6%), and 1998 (13.2%) (Figure 2). In terms of the single developmental stages, the infestation with borreliae prevailed in adults (females 6.9%, males 10.3%). The nymphs showed a value of 5.6%, and the least afflicted were larval stages (1.6%). The total infectivity of larvae was statistically lower than in the other growth stages, and the positive rate of males was significantly higher. The infectivity of nymphs and females was not different statistically (Figure 3).

Our long-term study was focused on the presence of pathogenic borreliae in their most natural vector - *Ixodes ricinus*. Pisárky Park is a busy recreational reserve popular with walkers and joggers, situated in the outer suburb of the city of Brno. Between 1996-2000, the *Ixodes ricinus* ticks were examined by dark-field microscopy. Between 2001-2002, the PCR Method (single-step, one-tube-nested) was used, which is generally considered to be the most sensitive. These methods were used for detection of borreliae by other Czech authors in the past (Hubálek et al. 1998, Bašt a et al. 1999). The results of our research can be compared with other similar long-term studies: (Peťko et al. 1996, Hubálek et al. 1998, 2003).

An average number of 36 ticks was collected in a 1-h interval (Žákovská et al. 2002). Peťko et al. (1996) recorded an average of 32-41 ticks. The number of ticks collected over a period of seven years (1996-2002) was fairly constant (Žákovská et al. 2002).

Rauter and Hartung (2005) claim that the highest infection rates in Europe are found in the central regions (namely in Austria, Czech Republic, Germany, Switzerland, Slovakia, and Slovenia). The infection rates from the Pisárky site appear to be lower (5.8%) in comparison to some other studies undertaken in Czech Republic and Slovakia. These studies reveal an average infectivity as follows: 20.4% (Hubálek et al. 1998); 16.8% (Hubálek et al. 2003); 14.1% (Peťko et al. 1996). These are generally higher in comparison to the results of our study that is based on sample collections performed irregularly in periods of increased tick activity. Collections in the park reserves in the capital city of Prague recorded positive rates of 5% (Bašt a et al. 1999) similar to those from the Brno region of Prague. We can only speculate that our lower infectivity is a result of the long-term study summarizing regular collections over seven years in 9-month periods. Similarly, all authors mentioned above reported higher positive rates in adults than in nymphs. In our opinion, however, the nymphs are the main vectors due to their greater abundance.

The positivity of ticks in Pisárky also varied throughout the year. Depending on the activity of ticks in relation to time of year (Žákovská et al. 2002), the year can be divided into three time-zones that differ statistically: March-April, May-August, and September-November. The autumn period showed the highest rates of infectivity. It is interesting that the activity culminated in summer, but the infectivity

<table>
<thead>
<tr>
<th>Number of collections</th>
<th>Mean</th>
<th>IS -95%</th>
<th>IS +95%</th>
<th>Median</th>
<th>Min.</th>
<th>Max.</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>78</td>
<td>5.8</td>
<td>4.9</td>
<td>6.7</td>
<td>3.6</td>
<td>0</td>
<td>35.7</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Table 2. Descriptive statistics of tick infectivity.

Figure 1. Tick infectivity in particular collection months.
peaked in the autumn months.

Our study also reveals that infectivity in the Pisárky site has changed over the years and also within one year. The highest tick infectivity occurred in 1997 and 1998. Peťko et al. 1996 had noticed that the Forest Park in Košice showed a cyclically higher occurrence of borreliae in ticks in the five years of observation. With respect to the duration of our study we cannot confirm such trends in the Pisárky forest.

The prevalence of *Borrelia* infection in ticks is one of the most essential components of risk assessment for Lyme borreliosis. The prevalence of all developmental stages of *I. ricinus* in Pisárky Park in Brno was confirmed in our longitudinal study. Nymphs were established as the main vector stage infecting humans. The outcome of our research reveals that the risk of Lyme borreliosis can be potentially present on a larger regional scale, rather than just in the immediate area of Brno city.

Acknowledgments

We thank Mgr. Julie Šebková for editing modifications. This work was partially supported by MSM: 0021622415.

REFERENCES CITED


