Peculiarities of behaviour of taiga (*Ixodes persulcatus*) and sheep (*Ixodes ricinus*) ticks (Acarina: Ixodidae) determined by different methods

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Abstract. A comparison of the behavioural peculiarities of *Ixodes persulcatus* Schulze, 1930 (north-western population, Russia) and *Ixodes ricinus* (L., 1758) from western Russia and Denmark was determined by using two methods. Method 1 involved a sojourn of ticks on vertical plastic sticks and showed that the questing behaviour of *I. ricinus* nymphs was dependent on temperature and relative humidity (RH). A significantly greater number of nymphs quested at 22°C and 100% RH than at 18°C. When the humidity was reduced to 30% all of the nymphs departed. In the second method, the activity of ticks on an inclined “ticksdrome” was estimated. The activity of *I. ricinus* adults from the Danish population was 1.2 times greater than that of ticks from Russia. Females of the species studied and specimens from all study areas were more active than all other stages of development. The locomotor activity of both adult and immature *I. ricinus* that were infected with *Borrelia burgdorferi sensu lato* was suppressed when compared with uninfected specimens. The locomotor activity of *I. persulcatus* females infected by *Borrelia* with exoskeleton anomalies was 1.3 times greater (P<0.05) than that of infected ticks without anomalies. Our data showed that infected females with exoskeleton anomalies could crawl faster on a human and reach uncovered parts of the body that are vulnerable for attachment and feeding. A study of locomotor activity and questing behaviour may be useful for comparing the risk for different tick species and populations to transmit tick-borne pathogens.

Successful transmission of pathogens to humans depends on the behaviour of the vectors inclusive of their activity, mobility, and ability to quest for prey and attack hosts. Ixodid ticks of different species and populations within species differ in their abilities to transmit pathogens and for successful maintenance of disease foci. For example, the ticks *Ixodes persulcatus* Schulze, 1930, are more effective vectors of pathogens than *Ixodes ricinus* (L., 1758) (Alekseev and Dubinina 1994, Kovalevskii and Korenberg 1995). Methods used to estimate tick behaviour vary and are generally directed to ascertain locomotor activity, which implies an orientation to physical parameters of the environment such as relative humidity, temperature and light and behaviour during questing for a host.

The predominant behaviour of *I. ricinus* is the tendency of the tick to ascend vegetation during the day and descend at night (McLeod 1935, Lees and Milne 1951, Arthur 1962). A general survey of unfed *I. persulcatus* was given by Babenko (1985) and Babenko et al. (1985). However the work of Okulova (1978) may be the most interesting example of the orientation behaviour of unfed ticks as related to the physical parameters. Okulova (1978) has presented data on the vertical and horizontal movements of questing taiga ticks, which were studied in a forest environment under naturally occurring temperature and relative humidity. Adults climbed up the aconite and fern stems to a maximum height of 80 cm when the temperature 23.3°C. According to the observations of Filippova (1977) some of *I. persulcatus* specimens in Primorye (Russian Far East) climb to a height of 2 m. Browning (1976) studied tick questing behaviour, by placing ticks of the genus *Rhipicephalus* on vertical sticks. Lefcort and Durden (1996) also used sticks to study the behaviour of *Ixodes scapularis* Say, 1821 nymphs and adults, and compared specimens that were infected in the laboratory by *Borrelia burgdorferi* Say, 1821 nymphs and adults, and compared specimens that were infected in the laboratory by *Borrelia burgdorferi* sensu stricto to uninfected ticks. In addition, *I. persulcatus* adults infected with *Borrelia burgdorferi* sensu lato were less active than those that were uninfected (Alekseev and Dubinina 1994, Alekseev 1996, Alekseev et al. 1996).

Our research was conducted to compare the behaviour of the different stages of *I. ricinus* that belonged to two different European populations of this species in North Zealand (Denmark) and Curonian Spit (Kaliningrad region, Russia). In addition, the locomotor characteristics of larvae and nymphs of *I. ricinus* and *I. persulcatus* versus adults were compared.

The main purpose of the study was to determine differences in the behaviour of two species of ticks that belong to the different geographic regions. We also
tested a hypothesis that the locomotor activity of ticks changes when the vector is under the influence of Borrelia infection. In addition, we wanted to determine the effects of infection on uninfected I. persulcatus and on the specimens that had abnormal exoskeletons.

MATERIALS AND METHODS

Two species of ticks and three allopatric populations were used in the experiments. Ixodes persulcatus ticks were collected from April to July, 1996 in the vicinity of St. Petersburg (Russia) and I. ricinus were collected from August to September, 1997 and 1998, on the Curonian Spit of the Kaliningrad region of the Russian enclave and in October, 1997, in Grib Skov (North Zealand, 80 km from Copenhagen, Denmark). All ticks in Russia were collected by flagging, placed into wet gauze bandage, transferred to the laboratory, and sorted using a stereomicroscope to determine their stage, sex and exoskeleton structure. Adults collected from the heavy metal polluted area of St. Petersburg had crater-like depressions, a crumpled female scutum, and different kinds of female scutum dents on their exoskeleton, and sometimes, undeveloped palps (Alekseev and Dubinina 1993).

The tick deformations (Alekseev and Dubinina 1993), caused most probably by the action of heavy metal ions (Alekseev and Dubinina 1996a), were called anomalies. All ticks collected in Russia were sorted and placed in selected humidity-gradient glass tubes (Alekseev and Dubinina 1996b). In the tubes the ticks were able to select a location that had their desired relative humidity that ranged from 98% near the wet bottom to 45-50% near the cotton plug. In Denmark the ticks were also collected by flagging, but they were removed from the flag by a small vacuum suction device and then placed into 4 cm in diameter plastic Petri dishes. No more than 20-25 larvae or nymphs and 10-12 adults were put into each dish. Dishes with ticks were maintained in plastic boxes with a 1×1 cm piece of damp paper in darkness for a minimum 2 wk before they were used in experiments. After completion of the behavioural experiments most of ticks were screened for natural infection with B. burgdorferi s.l. by darkfield microscopy and/or IFA analysis using polyclonal antibodies and fluorescent microscopy.

Two methods were used for estimating tick behaviour. The first method represented a modification of using sticks described by Browning (1976). A thick piece of polyethylene foam was put into a 10×25×45 cm plastic box and 3×3 mm, 25 cm long plastic straws were pushed vertically into it. The polyethylene foam was either kept dry or immersed in water up to a height of 3 cm from the sticks’ bases. Experiments were carried out at temperatures of 18, 22 and 24°C. Nymphs and larvae were maintained in darkness under 100% humidity until the beginning of the experiment. One group of ticks was illuminated for 1 h directly before the beginning of the experiment and another group was tested just after being kept in darkness. The ticks were placed on the plastic sticks 2-3 cm above the surface of the dry foam or the water level. Experimental conditions permitted nymphs either to stay in place or to move either up or down the stick. The percentage of ticks remaining on the sticks was determined and an average rate of climb was calculated after 4 h from the beginning of the experiment.
The second method consisted of studying tick activity on an inclined (45°) surface of a ticksdrome (Fig. 1). This device (Alekseev 1996, Alekseev et al. 1996) was made of a white (3 cm wide, 22 cm long) tight calico strip with a dry upper half and periodically moistened lower part (Fig. 1). Tracks, which mirrored the route followed by the ticks, was made by pen on transparency and permitted the measurement of the speed, height and other parameters involved in tick locomotor activity during 3 min of testing. Examples of the types of tick movement is shown in Fig. 2. A locomotor activity index (LAI) was calculated for each specimen using the modified equation (1) published by Alekseev (1996):

\[
LAI = \frac{s}{s'} + \frac{h}{h'} + \frac{wa}{m} - \frac{m}{m'} - \frac{f}{f'}
\]  

(1)

\[s - \text{speed (cm/min)}; s' = 1 \text{ cm/min}; h - \text{height of tick movement (cm)}; h' = 1 \text{ cm}; wa - \text{portion of the upward path relative to the total path}; wb - \text{portion of the path above the humidity gradient}; m - \text{meander, the number of turns to the length of the path}; m' - 1 \text{ number of turn to the length of the path}; f - \text{number of falls from or on the ticksdrome surface}; f' = 1 \text{ fall from or on the ticksdrome surface.}

Mean locomotor activity of the group (LAI') of the different stages and sexes of the normal and anomalous uninfected and infected ticks was calculated using another equation (2):

\[
LAI' = K_1 \left( \frac{S}{\sigma} \right)^2 + \left( \frac{H}{\sigma} \right)^2 + \left( \frac{WA}{\sigma} \right)^2 + \left( \frac{WB}{\sigma} \right)^2 - K_2 \left( \frac{M}{\sigma} \right)^2 - 20
\]  

(2)

\[S/\sigma - \text{mean value of speed divided by standard deviation}; H/\sigma - \text{same for height etc (see equation 1); K}_1 = 2; K_2 = 0.5; K_3 = 10.\]

Experiments were carried out at 21-22°C, 38-40% RH, and an illumination of about 500 lx.

A total of 2,070 nymphs were used in the experiments for determining the questing activity of I. ricinus and a total of 612 specimens were used to compare the activity of Borrelia-infected and uninfected larvae, nymphs and adults of I. ricinus obtained in Denmark and Russia (152 larvae, 187 nymphs and 273 adults). We used 1,091 specimens for comparing the activity of different stages of development of uninfected I. ricinus and I. persulcatus (Fig. 4). We compared 74 females of Borrelia-infected I. persulcatus with (20) and without (54) anomalies of their exoskeleton. Statistical analysis of the differences in the questing behaviour of ticks under different conditions and in the activity of the infected and uninfected specimens of different populations of I. ricinus was done using the t-test (Wilkinson 1990).

RESULTS

Estimation of questing behaviour

Only 10% of the Ixodes ricinus nymphs (Danish) that were held at 20°C and constant illumination continued questing when the RH was 30% but 50% of these nymphs continued questing when the humidity was 100%. Only one-tenth of the initial number of nymphs continued questing in dry conditions, whereas more than 50% of the nymphs placed above the water layer continued questing (Fig. 3). The difference was statistically significant (P<0.001). Temperature influenced the length of time that the ticks were questing (Fig. 4). The number of the ticks remaining on the sticks maintained at 22°C was 2-3 times greater than at 18°C (A: P<0.01; B: P<0.001). Before the experiments one group of ticks was exposed to 1 h of illumination. Another group was transferred from darkness to light immediately. During the experiments, a decrease in the number of ticks on sticks was observed. Decrease was different in the tested groups. However, 4 h later, this difference was not statistically significant and the number of ticks on the sticks depended on temperature alone.
Active behaviour

The low level of natural infection in the larvae and nymphs made comparisons of infected tick activity for these stages difficult. Nevertheless, the LAI of infected larvae from Russia were 2.35-2.51 times (P<0.05) and those from Denmark 11.2 times (P<0.001) less than the LAI of uninfected larvae (Table 1). Nymphs infected with borreliae collected in Russia also had a lower LAI when compared with uninfected ticks. The differences in LAI were found to be very small but significant (1.44 and 1.88, P<0.01). No infected specimens were found among nymphs that were collected in Denmark, in October 1997, but the LAI of uninfected nymphs from the Danish population was approximately 2 times more than that of the uninfected nymphs from the Russian population. Comparison of the LAI of Borrelia-infected nymphs from the Russian population with uninfected specimens from Denmark showed differences of 2.6-4 times. The LAI of Borrelia-infected adults collected in Denmark was also lower (6.53 times) than the LAI of uninfected ticks. Activity indexes of infected adults, collected in Russia, were lower than that of uninfected specimens. The differences were not great (1.36 and 1.25), but statistically significant (P<0.05). In all experiments, the locomotor activity index of all I. ricinus stages was lower among infected specimens than among uninfected ticks. This difference was found to be greater among ticks that had been collected in Denmark. Also, uninfected nymphs and larvae collected in Denmark had greater LAI than those from Russia (LAI of larvae was 1.2 times, LAI of nymphs was 1.8 times greater).

The use of the inclined ticksdrome gave us an opportunity to compare the speed, ascent height, meandering and the entire activity of different tick stages that belonged to two allopatric populations of I. ricinus (Fig. 5A, B) and I. persulcatus (Fig. 5C). We confirmed that different stages of ticks moved differently.

Nymphs of both populations of I. ricinus were more active than larvae. Females moved more quickly and were more active than males. The highest index of meandering occurred in larvae. We noted that nymphs collected in Denmark in comparison with adults climbed to a lower height (1.1 times), but that the nymphs collected in Russia (both I. ricinus and I. persulcatus) climbed to a height only ½ that of adults. The LAI was larger in all phases of development for ticks of the Danish population of I. ricinus when compared with I. ricinus ticks collected in Russia. The activity parameters of I. persulcatus ticks were greater than that of I. ricinus. The LAI was twice that of the Russian I. ricinus index and one and a half times greater than the LAI of Danish I. ricinus (Fig. 5C). The presence of borreliae in I. ricinus tick suppressed the locomotor activity of all stages of their development (Table 1).

The same phenomenon had been previously observed for the normal adult I. persulcatus ticks by Alekseev and Dubinina (1994) and Alekseev (1996), but the presence of B. burgdorferi s.l. in the specimens with exoskeleton anomalies appeared to make I. persulcatus-infected specimens more active when compared with infected specimens having no anomalies (Fig. 6). Under the same meandering indexes, the entire locomotor activity indexes (LAI’), the speed of movement and the height of climbing above the humidity gradient were appreciably greater in the infected specimens with exoskeleton anomalies. The difference was statistically significant (P<0.05).

DISCUSSION

These studies on the questing and locomotor activity of ticks suggest the following conclusions. The questing behaviour of nymphs appeared to be dependent on the relative humidity and air temperature. The optimal conditions for questing I. ricinus nymphs above the water surface was 22°C. The inclined ticksdrome successfully determined the locomotor activity of ticks and demonstrated the differences in the behaviour of the different species and stages of Borrelia-infected and uninfected specimens.

The comparison of activity of I. persulcatus and of I. ricinus that were collected in the north-western and western Russia (Fig. 5B, C) demonstrated that the entire locomotor activity (LAI) of I. persulcatus nymphs was 4 times and that of adults approximately 2 times as great as that of I. ricinus. Females of the populations used in this study were more active than other stages of development. It is likely that the greater activity and “aggressiveness” of I. persulcatus ticks particularly the females, made them more competent as vectors of the agents of borreliosis when compared with I. ricinus. We also noted that nymphal activity in the Danish population of I. ricinus (Fig. 5A) was comparable with that of the adults. These data corresponded to the greater role of nymphs as vectors of borreliosis in Denmark reported by Jensen (1998), which was also correlated by a greater abundance of nymphs in comparison with adults.

We showed that the activity of infected I. ricinus larvae, nymphs and adults was less than that of uninfected ticks (Table 1). Most of the I. ricinus were infected by Borrelia afzelii (Alekseev et al. 1998), whereas I. scapularis in North America are reported to be infected with B. burgdorferi s.s. (Lefcort and Durden 1996). This difference could be the cause that they demonstrated a stimulating effect of B. burgdorferi s.s. on the activity of I. scapularis nymphs. Borreliae depressed the nymphal activity of I. ricinus and I. persulcatus (Table 1, Fig. 5A, B), but this does not exclude the possibility of their participation in borreliosis transmission to humans.
Fig. 5. Locomotor activity of different stages of uninfected *Ixodes persulcatus* and *Ixodes ricinus* ticks. Abscissa – parameters: 1 – speed, cm/min; 2 – height, cm; 3 – meander, number of turns to the length of the path; 4 – locomotor activity index (LAI). Ordinate – values of parameters. **A** – *I. ricinus* from Grib Scov, North Zealand, Denmark (49 larvae, 51 nymphs, 31 adults); **B** – *I. ricinus* from Curonian Spit, Kaliningrad region, Russia (88 larvae, 277 nymphs, 276 adults); **C** – *I. persulcatus* from St. Petersburg vicinity, Russia (94 nymphs, 225 adults).

Table 1. Differences in the locomotor activity of *Borrelia*-infected and uninfected *Ixodes ricinus* ticks, collected in Denmark (1997) and Russia (1997, 1998). These tests were conducted using the ticksdrome.

<table>
<thead>
<tr>
<th>Tick characteristic</th>
<th>Stage</th>
<th>Place of collection</th>
<th>Infection</th>
<th>No. of tests</th>
<th>Locomotor activity index (LAI) values *</th>
<th>t-test, P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1997</td>
<td>1998</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Larvae</td>
<td>Russia</td>
<td>uninfected</td>
<td>33</td>
<td>66</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>infected</td>
<td>2</td>
<td>1</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Denmark</td>
<td>uninfected</td>
<td>49</td>
<td>–</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>infected</td>
<td>1</td>
<td>–</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Nymphs</td>
<td>Russia</td>
<td>uninfected</td>
<td>43</td>
<td>72</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>infected</td>
<td>15</td>
<td>6</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Denmark</td>
<td>uninfected</td>
<td>51</td>
<td>–</td>
<td>7.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>infected</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>Russia</td>
<td>uninfected</td>
<td>82</td>
<td>114</td>
<td>9.7 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>infected</td>
<td>20</td>
<td>25</td>
<td>7.1 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Denmark</td>
<td>uninfected</td>
<td>31</td>
<td>–</td>
<td>11.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>infected</td>
<td>1</td>
<td>–</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* Means ± S.D.

Alekseev and Dubinina (1997) demonstrated that adults having exoskeleton anomalies and *Borrelia* infection moved more actively than ticks with normal morphology and with infection (Fig. 6). This phenomenon appeared to be associated with the consequences of the anthropogenic pressure and with environmental pollution, which caused the development of an *I. persulcatus* population with changed morphology (Alekseev and Dubinina 1993) and metabolism. Those data do not mean that these specimens became more active under the influence of *B. burgdorferi* s.l. but, however, demonstrated that a lower suppression of activity was caused by the pathogen. Nevertheless, the data indicated that a greater danger of human contact...
Fig. 6. Comparison of activity parameters of *Borrelia*-infected normal (N) and abnormal (P) *Ixodes persulcatus* females. Abscissa – parameters of locomotor activity; ordinate – values of parameters. Female P – specimens with exoskeleton abnormalities or pathology (20 ticks); Female N – specimens without them (54 ticks). Error bars, S.D.

REFERENCES


JENSEN P.M. 1998: Seasonal and geographical variations in the abundance of *Ixodes ricinus* and Lyme borreliosis transmission in Denmark. PhD Dissertation. The Royal Veterinary and Agricultural University, Copenhagen, 208 pp.


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