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INFESTATION WITH *IXODES RICINUS* TICKS ON MIGRATING PASSERINE BIRDS IN LITHUANIA AND NORWAY

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Ticks are known to carry several pathogenic agents of human diseases. To define the role of migrating birds as host and disseminators of ticks in Lithuania and Norway we analysed immature stage of ticks feeding on different passerine bird species. During April-May of 2006-2007 and August-September of 2008, migrating passerine birds were captured at ornithological stations in southern Norway and in Lithuania respectively. In Norway were investigated 152 passerine birds representing 26 species, in Lithuania - 36 birds of 14 species. A total of 668 immature stages of *I. ricinus* ticks were collected. The most infested bird species in Norway were Starling (*Sturnus vulgaris),* Icterine Warblers (*Hippolais icterina)* and Blackbird (*Turdus merula)* and in Lithuania Reed Warbler (*Acrocephalus scirpaceus)* and Trush Nightingale *(Luscinia luscinia).* In Norway the total bird infestation rate was 4.1, but in Lithuania birds were 2.4 times less infested. The infestation with nymphal stages of *I. ricinus* was more frequent than the larval. The results of the study support previous observations that migratory birds play an important role in the dispersal of *I. ricinus* ticks.

Key words: passerine migrating birds, infestation, *Ixodes ricinus* ticks

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INTRODUCTION

Birds are increasingly considered important in the global dispersal of tick-borne pathogens. Birds play an important role not only in maintaining pathogens, but also through their migration by spreading ticks within and between continents. The migratory routes of birds in Europe are diverse, with both North-South and West-East directions. Migrating birds have used different stopover sites along their routes. At these sites, where birds feed and rest, ticks and other ecto-parasites may attach and later detach along the migration routes or in the breeding areas (Olsen et al., 1995). New foci of tick-borne

diseases may be created in this way (Mehl et al., 1984; Olsen et al., 1995).

In Lithuania and Norway studies on the infestation of birds with ticks and their role in spreading of tick-borne pathogens are scarce. Mehl et al. (1984) study has shown that most infested migratory passerines with *Ixodes ricinus* ticks in Norway were *Turdus* spp., *Erithacus rubecula*, *Phoenicurus phoenicurus*, *Prunella modularis*, *Anthus trivalis* and *Luscinia svecica*. It is difficult to determine the place of origin of the ticks that are transported to Norway with migratory birds. They could originated from a very large area, because the species of birds have different overwintering areas, different migratory routes, and migrate during different periods during spring and autumn. The dominant direction of migration during spring in Northern Europe is from southwest towards northeast, and the opposite direction during autumn.

In Lithuania are two main migratory routes (Patapavièius 1998; 2006). The birds migrate from southwest towards northeast during the spring. This route passes through inland area, 200 km from the Baltic Sea. During the autumn the birds migrates from northeast towards southwest through Baltic Sea coastal area. On this route two bird ringing stations are located: Ventes Ragas Ornithological Station, situated on the eastern coast of Curonian Lagoon, and Neringa Birds Ringing Station located in the Curonian Spit which is a narrow land of 97 km length between the Baltic Sea and Curonian Lagoon. A huge amount of birds from North Europe migrate via these sites. Some few reports from Norway about birds ringed in Lithuania and some in Lithuania about birds ringed in Norway during 1979-2003 are available (Patapavièius 1990; 1998). Our previous study in genetic diversity of *I. ricinus* ticks in Lithuania and Norway indicated that genetic variation of *I. ricinus* ticks were highest in those Lithuanian and Norwegian populations which are situated on the main migratory birds routes (Paulauskas et al., 2006) Birds could play an important role for the long-range migration of *I. ricinus* from Eastern and Central Europe to Scandinavian countries, affect the tick population dynamics and structure and transporting infected ticks. It is also possible that migratory birds are dispersing infected *I. ricinus* in new areas, where they could raise a public health risk. In Norway the first case of tick-borne encephalitis has been diagnosed in1997 (Skarpaas et al., 2006). During the 1997-2007, 27 serologically confirmed cases were reported. All cases were acquired within a limited area along the southern coast and in municipally of Trom¸y (Skarpaas et al., 2006, Süss, 2008). As described by some authors (Ogden et al., 2005, 2008) ticks are capable of surviving through the moult to quest and attach to humans or animals (and possibly infect them with tick-borne pathogens) even in areas that are currently thought too cold to sustain reproducing. Thus it is possible, that ticks feeding on birds introduced into new locations could expanding the northern range of reproducing *I. ricinus* populations (Madhav et al., 2004), particularly due to climate change and global warming (Ogden et al., 2006; Paulauskas et al., 2008, Gray et al.,2009).

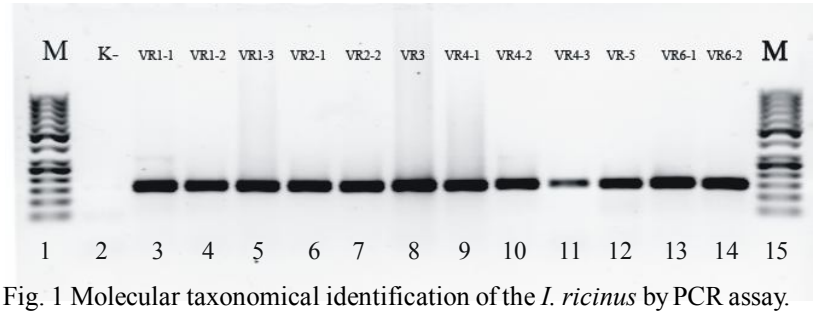

Fig. 1 Molecular taxonomical identification of the *I. ricinus* by PCR assay. Lines 1 and 15 – 50 bp marker; Line 2 –negative control; Lines 2-13 –positive results: amplified 150 bp specific fragment for *I. ricinus*; Line 14 – positive control of *I. ricinus* (150 bp)

The aims of the present study was to define the role of birds in dispersing

Table 1. Passerine migrating birds infestation with ticks in Norway

Birds species	No of examined birds	No of collected ticks from birds		Infestation rate (No ticks/ Infested birds)	Infestation rate with larvae	Infestation rate with nymphs
		Larvae	Nymphs			
Jomfruland: 58°52?N, 09°36Œ						
Carduelis cabaret	1		4	4		4
Carduelis cannabina	2		2	1		1
Carduelis chloris	2		4	2		2
Turdus merula	33	39	223	7,9	1,2	6,8
Turdus philomelos	2		3	1,5		1,5
Erithacus rubecula	9	3	11	1,6	0,3	1,2
Sylvia atricapilla	1	1		1	1	
Sylvia borin	1		4	4		4
Sylvia communis	1	1		1	1	
Sylvia curruca	1		2	2		2
Hippolais icterina	1	8	2	10	8	2
Fringilla coelebs	5	6	15	4,2	1,2	3,0
Carpodacus erythrinus	1		1	1		1
Sturnus vulgaris	3		6	2		2
Phylloscopus collybita	2	2		1	1	
Phoenicurus phoenicurus	2		3	1,5		1,5
Total	**67**	**60**	**280**	**5**	**0,9**	**4,2**
Lista: 58°07?N, 06°40Œ						
Turdus iliacus	2		2	1		1
Turdus merula	31	8	111	3,8	0,3	3,6
Turdus pilaris	5	1	18	3,8	0,2	3,6
Turdus philomelos	11	5	23	2,5	0,45	2,1
Erithacus rubecula	7	3	7	1,4	0,4	1
Lullula arborea	1	2	2	4	2	2
Oenanthe oenanthe	1		1	1		1
Saxicola rubetra	1		3	3		3
Prunella modularis	6	1	18	3,2	0,17	3
Anthus pratensis	1		1	1		1
Phylloscopus trochilus	3	1	2	1	0,3	0,6
Carduelis chloris	2	1	2	1,5	0,5	1
Sylvia atricapilla	2		3	1,5		
Sylvia communis	3	1	3	1,3	0,3	1
Sturnus vulgaris	3	16	15	10,3	5,3	5
Fringilla coelebs	4	10	4	3,5	2,5	1
Coccotraustes coccotraustes	1		3	3		3
Luscinia svecica	1		1	1		1
Total	**85**	**49**	**219**	**3,2**	**0,6**	**2,6**

ticks and study the infestation in migrating passerine birds captured in ornithological stations in Lithuania and Norway.

MATERIAL AND METHODS

Sample collection

During April-May of 2006-2007 and August-September of 2008, migrating passerine birds were captured at Jomfruland and Lista ornithological stations in southern Norway and Ventes Ragas Ornithological Station in Lithuania.

In Norway 152 passerine birds representing 26 species were captured (Table 1), in Lithuania 36 birds representing 14 species (Table 2). Birds were carefully examined and ticks removed by using sterile tweezers. The ticks were placed in sterile tubes and dispatched to the laboratory for identification using appropriate taxonomic keys (Филиппова, 1977; Hillyard, 1996) and for molecular assays. A total of 668 (117 larvae and 551 nymphs) of ticks were collected from birds. No adult stages were found.

Molecular identification of ticks

To confirm taxonomic identification of immature stages of *I. ricinus* ticks we performed taxonomical identification by molecular methods. The DNA from ticks was extracted as described Stañczak et al. (1999). Oligonucleotide primers Ixri-F: 5. GGAAAT CCC GTC GCA CG 3. and Ixri-R: 5. CAA ACG CGC CAA CGA AC 3 designed by A. Jenkins (A/S Telelab, Skien, Norway) on the basis of data on available genomes in GenBank (Accession N D88863; Fukunaga et al., 2000) were used in PCR reaction. These primers amplify a 150 bp segment of the 5.8 S rRNA gene, which is specific for *I. ricinus* (Fukunaga et al. 2000) (Figure 1).

RESULTS AND DISCUSSION

All ticks collected from birds in Lithuania and Norway were identified as *I. ricinus*. The most infested bird species in Norway were Starling (*Sturnus vulgaris),* Icterine Warblers (*Hippolais icterina)* and Blackbird (*Turdus merula)* (Table 1), in Lithuania – Reed Warbler (*Acrocephalus scirpaceus)* and Trush Nightingale *(Luscinia luscinia)* (Table 2). The difference between the most infested bird (infestation rate 10.3, Starling *S. vulgaris*) in Norway and in Lithuania (infestation rate 3, Reed Warbler *A.scirpaceus*) was 3.4 times.

The average of the infested birds on Jomfruland was 5 ticks per birds followed by Lista 3.2 ticks per bird and the Ventes Ragas with 1.7 ticks per bird. The total infestation rate in Norway was 4.1. The Lithuanian birds were 2.4 times less infested compared with Norway.

The highest *I.ricinus* larva stage infesting rate was found on Icterine Warblers (*H. icterina)* (8 larvae per bird) and the highest nymph stage infesting rate was in Blackbird (*T. merula)* (6.8 nymphs per bird). The highest larvae per bird rate (0.9) was found in Jomfruland followed by Lista (0.6 larvae per bird) and in Ventes Ragas (0.2 larvae per bird) (Figure 3). The infestation with nymphal stage was more frequent. In Jomfruland the infestation rate with nymphs was 4.2 (82.4% of all ticks), in Lista 2.6 nymphs per bird (81.3% of all), and in Ventes Ragas 1.4 nymphs per bird (82.4% of all ticks).

According to Olsen et al. (1995) approximately 100 million birds migrate every spring into or through Scandinavian countries importing a large number of potentially infected ticks. We found that infesting rate during the spring migration in different localities of Norway ranged from 3.2 to 5 ticks per bird. According to these, a huge amount of ticks are carried into Norway every spring. These data support previous observations that migratory birds are an important component in the dispersal of ticks (Gylfe et al., 2000; Kurtenbach et al., 2002; Hanincova et al., 2003; Comstedt et al., 2006).

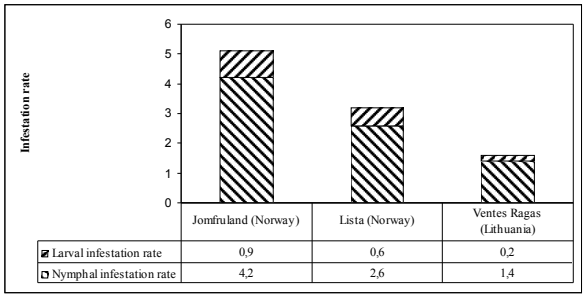

Fig. 2 Larval and nymphal infestation rates of passerine migratory birds in Norway and Lithuania

In contrast to Norway were birds were captured in spring and had a higher infestation rate, the birds captured in Lithuania in autumn had lover prevalence

Table 2. Passerine migrating birds infestation with ticks in Lithuania

Birds species	No of examined birds	No of collected ticks from birds		Infestation rate (No ticks/ Infested birds)	Infestation rate with larvae	Infestation rate with nymphs
		Larvae	Nymphs			
LITHUANIA (Ventes ragas: 55°34?N, 21°20?E)						
Sylvia atricapilla	1		1	1		1
Sylvia communis	2		2	1		2
Phylloscopus trochilus	1		2	2		2
Erithacus rubecula	9	4	14	2	0,4	1,6
Parus major	5		5	1		1
Acrocephalus palustris	1		1	1		1
Acrocephalus scirpaceus	1	1	2	3	1	2
Luscinia luscinia	6	3	11	2,3	0,5	1,8
Fringilla coelebs	1		1	1		1
Acrocephalus arundinaceus	1		1	1		1
Acrocephalus schoenobaenus	2		4	2		2
Lanius collurio	1		1	1		1
Parus montanus	3		3	1		1
Turdus Pilaris	2		4	2		2
Total	**36**	**8**	**52**	**1,7**	**0,2**	**1,4**

of tick infestation. The lower prevalence of ticks on migrating birds in the autumn is probably due to the northern location of the breeding grounds where the tick density is low and the fact that the ticks are less active at this time of the year (Olsen et al., 1995).

The results of our study support the hypothesis that *I.ricinus* has migrated into new areas by host-mediated dispersal, primarily on avian hosts. Birds may play an important role for the long-range migration of *I. ricinus* from Eastern and Central Europe to Scandinavian countries; affect the tick population dynamics, the structure and the transport of infected ticks.

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