Anaplasma in ticks feeding on migrating birds and questing ticks in Lithuania and Norway

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Introduction

Human granulocytic anaplasmosis due to Anaplasma phagocytophilum has been reported in Europe, including in Norway. Ixodes ricinus has been identified as a/the vector of this rickettsial agent in Europe [1]. In Europe, in addition to ruminants, small rodents have been shown to harbor A. phagocytophilum and were suggested as potential reservoirs. Migrating birds have also been considered to be important in the dispersal of A. phagocytophilum infected I. ricinus in Europe and in the distribution of granulocytic anaplasmosis [2,3]. Our previous studies conducted in Norway and Lithuania did not show any presence of A. phagocytophilum in small rodents. The involvement of birds in the ecology and in the epidemiology of tick-borne diseases in Lithuania and Norway has not yet been studied. To define the role of migrating birds as reservoirs and disseminators of Anaplasma we analysed the immature stage of ticks feeding on different passerine bird species, and questing ticks in some areas of Lithuania and Norway.

Material and Methods

Sample collection

During the spring-summer of 2005-2007, migrating passerine birds were captured at ornithological stations in Lithuania (Ventes Ragas Ornithological Station, situated on the eastern coast of Curonian Lagoon) and in southern Norway (Jomfruland and Lista). In Lithuania 41 passerine birds representing 15 species were caught; in southern Norway, 153 birds of 26 species were caught (Table 1). A total of 816 (152 larvae and 664 nymphs) I. ricinus ticks were collected from birds and an additional 410 ticks were collected from the vegetation in the birds' sampling places.

Table 1. Anaplasma in Ixodes ricinus ticks feeding on migrating birds in Lithuania and Norway

Birds species	No. of examined birds	No. of ticks collected on birds	Infestation rate: no. ticks/infest. birds	No. of Anaplasma infected ticks/no. tested ticks			
				by PCR with primers Ehr521/Ehr747		by TaqMan RT-PCR with ApMSP primers	
				Larvae	Nymphs	Larvae	Nymphs
LITHUANIA (Ventes ragas: 55°34' N, 21°20'E)							
Sylvia atricapilla	2	5	2.5	1/2	0/3	0/2	0/3
Sylvia communis	2	5	2.5	2/2	0/3	0/2	0/3
Sylvia curruca	1	1	1		0/1		0/1
Troglodytes troglodytes	4	4	1		1/4		0/4
Phylloscopus collybita	1	1	1		0/1		0/1
Phylloscopus sibilatrix	2	2	1		0/2		0/2
Phylloscopus trochilus	3	3	1		0/3		0/3
Erithacus rubecula	10	13	1.3	0/3	3/10	0/3	0/10
Phoenicurus phoenicurus	3	3	1		0/3		0/3
Parus major	3	3	1		0/3		0/3
Acrocephalus palustris	3	3	1	0/2	0/1	0/2	0/1
Acrocephalus scirpaceus	1	1	1		1/1		0/1
Luscinia luscinia	4	10	2.5	3/8	0/2	0/8	0/2
Sturnus vulgaris	1	1	1		0/1		0/1
Regulus regulus	1	1	1		0/1		0/1
Total	41	56	1.3	6/17	5/39	0/17	0/39
NORWAY							
Jomfruland: 58°52' N, 09°36'E							
Carduelis cabaret	1	4	4		0/4		0/4
Carduelis cannabina	2	2	1		0/2		0/2

Carduelis chloris	2	4	2		3/4		0/4
Turdus merula	33	367	11.1	9/39	87/250	8/39	25/250
Turdus philomelos	2	3	1.5		2/3		2/3
Erithacus rubecula	9	14	1.5	0/4	6/10	0/4	2/10
Sylvia atricapilla	1	1	1	0/1		0/1	
Sylvia borin	1	4	4		1/4		1/4
Sylvia communis	1	1	1	0/1		0/1	
Sylvia curruca	1	2	2		1/2		0/2
Hippolais icterina	2	19	9.5	0/10	0/3	0/10	0/3
Fringilla coelebs	5	22	4.4	4/6	3/16	1/6	2/16
Carpodacus erythrinus	1	1	1		0/1		0/1
Sturnus vulgaris	3	6	2		3/6		1/6
Phylloscopus collybita	2	2	1	0/2		0/2	
Phoenicurus phoenicurus	2	3	1.5		0/3		0/3
Total	68	455	6.7	13/63	106/308	9/63	33/308
Lista: 58°07' N, 06°40'E							
Turdus iliacus	2	2	1		0/2		0/2
Turdus merula	31	130	4.2	1/7	24/123	0/7	2/123
Turdus pilaris	5	24	4.8	1/1	11/23	0/1	0/23
Turdus philomelos	10	27	2.7	4/5	1/22	1/5	0/22
Erithacus rubecula	7	10	1.4	0/3	1/7	0/3	0/7
Lullula arborea	1	4	4	0/2	0/2	0/2	0/2
Oenanthe oenanthe	1	1	1		0/1		0/1
Saxicola rubetra	1	3	3		1/3		0/3
Prunella modularis	8	19	2.4	0/1	0/18	0/1	0/18
Anthus pratensis	1	1	1		1/1		0/1
Phylloscopus trochilus	3	3	1	0/1	0/2	0/1	0/2
Carduelis	2	3	1.5	0/1	1/2	0/1	0/2

chloris							
Sylvia atricapilla	2	3	1.5		0/3		0/3
Sylvia communis	2	2	1		0/2		0/2
Sturnus vulgaris	3	57	19	6/21	14/18	0/21	3/18
Fringilla coelebs	4	14	3.5	0/10	0/14	0/10	0/14
Coccotraustes coccotraustes	1	1	1		0/1		0/1
Luscinia svecica	1	1	1	0	0/1		0/1
Total	85	305	3.6	12/52	54/245	1/52	5/245

Detection of A. phagocytophilum DNA

The DNA from ticks was isolated as described Stańczak et al. [4].

The first screening PCR for Anaplasma was carried out with primers Ehr521/Ehr747 [4], which amplify a 247 bp sequence of 16S rRNA gene. For confirmation, A. phagocytophilum specific PCR was primed with ApMSP2f/r, complemented with a TaqMan probe ApMSP2p-FAM [5]. This was used to amplify a 77-bp fragment in the msp2 gene, highly conserved regions of this pathogen. RT-PCR was performed by using TaqMan Master Mix in a quantitative thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Negative and positive controls were included in all runs.

Results and Discussion

All ticks collected from birds in Lithuania and Norway were identified as I. ricinus. In Norway the most infested with I. ricinus were Turdus merula and Sturnus vulgaris; in Lithuania the most infested were Erithacus rubecula and Luscinia luscinia. According to a previous study, the most important and most heavily infested hosts for I.ricinus in Norway were Turdus spp., E. rubecula, Phoenicurus phoenicurus and L. svecica.

In the present study, the most tick-infested birds were captured in Jomfruland (with an average of 6.7 ticks per infested bird), followed by Lista (3.6 ticks per bird), and the less infested birds were captured in Ventes Ragas (Lithuania) with an average of 1.3 ticks per bird (Table 1).

In Ventes Ragas, according to Ehr521/747 primers, Anaplasma DNA was detected in six out of 17 (35.3%) larvae and in five of the 39 (12.8%) nymphs (Table 1). However, using more specific primers, ApMSP2f/r, for detection of A. phagocytophilum, none of the examined ticks from the birds captured in Ventes Ragas was found to be infected. Moreover no Anaplasma DNA was detected in any of the 53 I. ricinus collected from vegetation in that place. In Norway, according to Ehr521/747 primers, 16 of the captured bird species carried infected ticks. In Jomfruland, the overall infection prevalence was 20.6% and 34.4% in feeding larvae and in nymphs, respectively. In Lista, 23% of feeding larval and 22% of nymphal ticks were positive according to PCR results. However, according to ApMSP2f/r primers, the overall prevalence of A. phagocytophilum infection in these two sites was lower and only 10 of the bird species were determined to harbour infected ticks (Table1). In Jomfruland, 14.3% of larvae and 10.7% of nymphs, and in Lista, 1.9% of larvae and 2% of nymphs, collected from birds were positive according to RT-PCR results.

The bird species that carried the highest number of ticks infected with A. phagocytophilum were Turdus spp., S. vulgaris and Fringilla coelebs.

The prevalence of A. phagocytophilum in questing ticks collected from vegetation in Jomfruland was 10.4% (23 out of 222), and in Lista it was 2.3% (3 out of 129).

Our data suggest that birds are important in the dispersal of Anaplasma infection in Lithuania and Norway.

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References

1. Parola P. Tick-borne rickettsial diseases: emerging risks in Europe. Comp Immunol Microbiol Infect Dis 2004; 27: 297–304.

2. Bjöersdorff A, Bergström S, Massung R, Haemig P, Olsen B. Ehrlichia-infected ticks on migrating birds. Emerg Infect Dis 2001; 7 (5): 877–879.

3. Stuen S. Anaplasma phagocytophilum– the most widespread tick-borne infection in animals in Europe. Vet Res Comm 2007; 31 (suppl 1): 79–84.

4. Stańczak J, Racewicz M, Kubica-Biernat B et al. Prevalence of Borrelia burgdorferi sensu lato in Ixodes ricinus ticks (Acari, Ixodidae) in different Polish woodlands. Ann Agric Environ Med 1999; 6: 127–132.

5. Courtney JW, Kostelnik LM, Zeidner NS, Massung RF. Multiplex real-time PCR for detection of Anaplasma phagocytophilum and Borrelia burgdorferi. J Clin Microbiol 2004; 42: 3164–3168.