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Tick-Borne Pathogens in Ticks Feeding on Migratory Passerines in Western Part of Estonia

Julia Geller,[1,2] Lidia Nazarova,[1,2] Olga Katargina,[1] Agu Leivits,[3] Lilian Järvekülg,[2] and Irina Golovljova[1]

Abstract

During southward migration in the years 2006–2009, 178 migratory passerines of 24 bird species infested with ticks were captured at bird stations in Western Estonia. In total, 249 nymphal ticks were removed and analyzed individually for the presence of *Borrelia burgdorferi* sensu lato (s.l.), tick-borne encephalitis virus (TBEV), and *Anaplasma phagocytophilum*. The majority of ticks were collected from *Acrocephalus* (58%), *Turdus* (13%), *Sylvia* (8%), and *Parus* (6%) bird species. Tick-borne pathogens were detected in nymphs removed from *Acrocephalus*, *Turdus*, and *Parus* bird species. TBEV of the European subtype was detected in 1 *I. ricinus* nymph removed from *A. palustris*. *B. burgdorferi* s.l. DNA was found in 11 ticks (4.4%) collected from *Turdus* and *Parus* species. Bird-associated *B. garinii* and *B. valaisiana* were detected in *I. ricinus* nymphs removed from *T. merula*. Rodent-associated *B. afzelii* was detected in 3 *I. ricinus* nymphs from 2 *P. major* birds. One of the *B. afzelii*-positive nymphs was infected with a mix of 2 *B. afzelii* strains, whereas 1 of these strains was also detected in another nymph feeding on the same great tit. The sharing of the same *B. afzelii* strain by 2 nymphs indicates a possible transmission of *B. afzelii* by co-feeding on a bird. *A. phagocytophilum* DNA was detected in 1 *I. ricinus* nymph feeding on a *T. iliacus*. The results of the study confirm the possible role of migratory birds in the dispersal of ticks infected with tick-borne pathogens along the southward migration route via Estonia.

Key Words: Tick-borne pathogen—TBEV—Borrelia—Reservoir—tick—Ixodidae—Bird.

Introduction

Migratory birds may play an important role in global dispersal, transport, and dissemination of ticks and tick-borne pathogens as they migrate within and between continents (Georgopoulou and Tsiouris 2008). Birds can carry ticks infected by such tick-borne pathogens as tick-borne encephalitis virus (TBEV) (Waldenstrom et al. 2007), *Anaplasma phagocytophilum* (Alekseev et al. 2001, Bjoersdorff et al. 2001, Daniels et al. 2002, Franke et al. 2010a) and *Borrelia burgdorferi* sensu lato (s.l.) (Olsen et al. 1995, Gylfe et al. 2000, Hanincova et al. 2003b, Poupon et al. 2006, Franke et al. 2010a).

Ixodes ricinus and *I. persulcatus* are the most important vectors for tick-borne pathogens in Europe and Asia, respectively. Estonia is located in a unique area where both tick species co-circulate. Our previous studies showed that all three known TBEV subtypes (Suss 2011), Western TBEV (W-TBEV), Far-Eastern TBEV (FE-TBEV), and Siberian TBEV (S-TBEV), co-circulate in Estonia (Golovljova et al. 2004). The prevalence of *B. burgdorferi* s.l. in the tick population varied from 3.8% to 23% (mean 9.7%) in different regions of Estonia (Geller, unpublished). *A. phagocytophilum* was found in questing *I. ricinus* ticks with a prevalence of 1.7% for mainland Estonia and 2.6% for Saaremaa island (Katargina et al. 2012).

Estonia is situated in the breeding area of long-distance migrating passerines, such as *Acrocephalus* spp., that might be potentially important in the dispersal of ticks and tick-borne pathogens along its southward migration route from Estonia, Fennoscandia, and southeastern Russia to Africa (A. Leivits, personal communication). Short-distance migrants of *Turdus* species, which are known to be reservoirs (Hanincova et al. 2003b, Humair et al. 1998) for pathogenic *B. garinii* and potentially pathogenic *B. valaisiana,* also breed in Estonia, Finland, and southeastern Russia. In this study, we investigated the prevalence of TBEV, *B. burgdorferi* s.l., and *A. phagocytophilum* in ticks harbored by passerines during southward migration. This is the first study regarding the role of birds in the dispersal of these pathogens in Estonia.

[1]National Institute for Health Development, Tallinn, Estonia.
[2]Tallinn University of Technology, Department of Sciences, Tallinn, Estonia.
[3]Environmental Board, Nature Conservation Department, Tallinn, Estonia.

Materials and Methods

Collection of ticks from migratory birds

Birds were captured by ornithologists in Kabli (58.01431N 24.44942 E) and Pulgoja (58.09947N 24.4844E) bird stations situated on the East coast of the Gulf of Riga. Birds were trapped during the fall migration periods in August–October from 2006 to 2009. The birds were identified, examined, and released back into the wild as quickly as possible to minimize stress. Only birds from which ticks had been removed were included in the study. Ticks were collected from the bird's head using tweezers and placed in 70% ethanol. Samples were sent to the laboratory within 1 month after ticks' collection and processed immediately. The tick species were identified morphologically by use of a stereomicroscope. Ticks were washed in sterile water and homogenized in 300 μL phosphate-buffered saline (PBS) solution with TissueLyzer (Retsch, Haan, Germany). Two hundred microliters of suspensions were used for nucleic acid extraction. Due to small numbers, larvae collected from birds were not included in the study.

Nucleic acid isolation

Nucleic acids were extracted by the guanidinium thiocyanate–phenol–chloroform method using the TriPure isolation system (Roche Diagnostics, Lewes, UK) according to the manufacturer's recommendations. Sterile water was used as a negative control for every preparation set. DNA was stored at -20°C and RNA at -70°C until further use.

To avoid any possible contamination, all steps were performed in separate rooms with sterile techniques, and addition of the sample DNA to the PCR mix was performed in a laminar flow cabinet. All primers and probes used in the current study are presented in Table 1.

Detection of tick-borne encephalitis virus RNA

TBEV real-time reverse transcriptase PCR detection in ticks was carried out with F-TBE1 and R-TBE1 primers and TBE-probe-WT probe as described (Schwaiger and Cassinotti 2003) with some modifications. Forward primer concentration of 3 μM, reverse primer concentration of 0.3 μM, and probe concentration of 0.2 μM were used, and the reverse transcription step was performed at 42°C. The 7900 Real Time PCR system (Applied Biosystems) was used for all PCR reactions and fluorescent detections.

Samples positive for TBEV RNA by real-time PCR were amplified and sequenced for the partial E gene, as described earlier (Skarpaas et al. 2006) with outer primers 283F1 and 827R1 used for the cDNA synthesis and inner primers 349F2 and 814R2 for the second round of PCR amplification.

Detection of B. burgdorferi *s.l. DNA*

All samples were screened for the presence of *B. burgdorferi* s.l. by amplification of the 5S–23S rRNA intergenic spacer (IGS) region as described earlier (Postic et al. 1994, Rar et al. 2005), with modified cycling conditions. In the first-round PCR, the annealing step at 58°C was increased to 1 min and the extension step at 72°C was increased to 2 min. In the nested PCR, cycling conditions were performed as described (Postic et al. 1994).

The amplified products were separated by electrophoresis on a 1% agarose gel, stained with ethidium bromide and visualized under ultraviolet (UV) light.

Detection of A. phagocytophilum *DNA*

Ticks were screened for the presence of *A. phagocytophilum* by real-time PCR with ApMSP2f and ApMSP2r primers and ApMSP2 probe, as described previously (Courtney et al. 2004).

DNA sequencing and data analysis

Sequencing was performed by the Estonian Biocentre (Tartu, Estonia), where PCR products were sent. Assembling, editing, and analysis of collected sequences were performed using BioEdit Sequences Alignment Editor v.7.0.9.0.

Table 1. Primers and Probes Used for PCR, RT-PCR, and Real-Time PCR of Tick-Borne Pathogen Nucleic Acid Detection

Primer or probe name	*Sequences of oligonucleotide probes and primers (5′→3′)*	*References*
TBEV		
283F1	GAGAYCAGAGTGAYCGAGGCTGG	Skarpaas et al. 2006
827R1	AGGTGGTACTTGGTTCCMTCAAGT	
349F2	GTCAAGGCGKCTTGTGAGGCAA	
814R2	TTCCCTCAATGTGTGCCACAGG	
F-TBE1	GGGCGGTTCTTGTTCTCC	Schwaiger and Cassinotti 2003
R-TBE1	ACACATCACCTCCTTGTCAGACT	
TBE-Probe-WT	TGAGCCACCATCACCCAGACACA	
B. burgdorferi s.l.		
NC1	CCTGTTATCATTCCGAACACAG	Rar et al. 2005
NC2	TACTCCATTCGGTAATCTTGGG	
NC3	CTGCGAGTTCGCGGGAGA	Postic et al. 1994
NC4	TCCTAGGCATTCACCATA	
A. phagocytophilum		
ApMSP2f	ATGGAAGGTAGTGTTGGTTATGGTATT	Courtney et al. 2004
ApMSP2r	TTGGTCTTGAAGCGCTCGTA	
ApMSP2 (probe)	FAM-TGGTGCCAGGGTTGAGCTTGAGATTG-TAMRA	

TBEV, tick-borne encephalitis virus.

Table 2. Birds Captured in Estonia During the Summer—Autumn Migration Period and Their Infestation with *I. ricinus* Ticks

Bird species		*No. of birds*	*% of all birds*	*No. of ticks collected*
Great reed-warbler	*Acrocephalus arundinaceus*	1	*0.6*	1
Blyth's reed-warbler	*A. dumetorum*	9	*5.1*	11
Marsh warbler	*A. palustris*	61	*34.3*	99
Sedge warbler	*A. schoenobaenus*	7	*3.9*	6[a]
Eurasian reed-warbler	*A. scirpaceus*	25	*14.0*	28
Tree pipit	*Anthus trivialis*	2	*1.1*	7
Common rosefinch	*Carpodacus erythrinus*	1	*0.6*	4
Eurasian treecreeper	*Certhia familiaris*	2	*1.1*	2
Reed bunting	*Emberiza schoeniclus*	1	*0.6*	1
European robin	*Erithacus rubecula*	2	*1.1*	1
Red-backed shrike	*Lanius collurio*	1	*0.6*	1
Common grasshopper-warbler	*Locustella naevia*	1	*0.6*	1
Blue tit	*Parus caeruleus*	2	*1.1*	2
Great tit	*P. major*	14	*7.9*	14
Common redstart	*Phoenicurus phoenicurus*	1	*0.6*	1
Common chiffchaff	*Phylloscopus collybita*	10	*5.6*	10
Willow warbler	*Ph. trochilus*	4	*2.2*	5
Goldcrest	*Regulus regulus*	1	*0.6*	1
Garden warbler	*Sylvia borin*	1	*0.6*	1
Whitethroat	*S. communis*	14	*7.9*	18
Winter wren	*Troglodytes troglodytes*	2	*1.1*	2
Redwing	*Turdus iliacus*	2	*1.1*	2
Song thrush	*T. philomeros*	2	*1.1*	2
Common blackbird	*T. merula*	12	*6.7*	29
Total		178		249

[a]One *I. persulcatus* nymph is included.

Results

Examination of birds and ticks

In total, 178 birds of 24 bird species infested with ticks were examined in the study. All birds were juvenile passerines (order *Passeriformes*). Only 1 bird (great tit) was identified by ornithologists as an adult (>1 year old). The most common birds were the marsh warbler (*A. palustris*), the Eurasian reed-warbler (*A. scirpaceus*), and the great tit (*P. major*), comprising 34.3%, 14%, and 7.9% of all birds, respectively. All details concerning the captured birds, their species and the number of ticks removed from them are presented in Table 2.

Overall, 249 nymphs were removed from the examined birds, of which 248 (99.6 %) were morphologically identified as *I. ricinus* and 1 (0.4%) as *I. persulcatus* (removed from *A. schoenobaenus*, sedge warbler). The majority of ticks (58%) were collected from *Acrocephalus*, *Turdus* (13%), *Sylvia* (8%), and *Parus* (6%) bird species.

Tick-borne pathogens detected in ticks

All ticks were investigated for the presence of TBEV, *B. burgdorferi* s.l., and *A. phagocytophilum.* Tick-borne pathogens were detected in nymphs removed from *Acrocephalus*, *Turdus*, and *Parus* bird species. Overall, 13 ticks (5.2%) removed from 7 birds were infected by tick-borne pathogens (Table 3).

TBEV detection

TBEV RNA was detected only in 1 tick (0.4% of all examined ticks) removed from a marsh warbler. Another 4 nymphs attached to the same bird were negative for TBEV. Phylogenetic analysis of partial E gene showed that it belonged to the TBEV-Eu subtype and is closely related to the strain Korppoo-259 from Finland (Jaaskelainen et al. 2010).

Detection of B. burgdorferi *s.l.*

B. burgdorferi s.l. DNA was detected in 11 ticks (4.4% of all examined ticks) removed from 5 birds of 2 species (common

Table 3. Tick-Borne Pathogens in Ticks Removed from Birds

Bird species	*Infested tick species*	*No. ticks infected/no. ticks on bird*	*Infectious agent*
Marsh warbler *(A. palustris)*	*I. ricinus*	1N/4N	TBEV-Eu
Great tit *(P. major)*	*I. ricinus*	2N/2N	*B. afzelii*
Great tit *(P. major)*	*I. ricinus*	1N/1N	*B. afzelii*
Common blackbird *(T. merula)*	*I. ricinus*	4N/5N	*B. garinii*
Common blackbird *(T. merula)*	*I. ricinus*	3N/3N	*B. garinii*
Common blackbird *(T. merula)*	*I. ricinus*	1N/1N	*B. valaisiana*
Redwing *(T. iliacus)*	*I. ricinus*	1N/1N	*A. phagocytophilum*

TBEV, tick-borne encephalitis virus.

blackbird and great tit). *B. garinii* was detected in 7 *I. ricinus* nymphs removed from 2 common blackbirds. One common blackbird carried 1 *B.valaisiana*–positive nymph. Analysis of the 5S–23S IGS showed that the sequences were identical to those of *B. garinii* 20047 (Postic et al. 1994) and NE11 (Gern et al. 2010), and to the sequence of *B. valaisiana* genotype I-214 (Derdakova et al. 2003). Three *I. ricinus* nymph samples from 2 birds of *P. major* (great tit) were identified as *B. afzelii*. Two *I. ricinus* nymphs recovered from the same great tit were infected by 2 different types of *B. afzelii*. One nymph had a mixture of 2 sequences (*B. afzelii* strain PGau and *B. afzelii* strain Tom1503), and the sequence obtained from another nymph was identical only to *B. afzelii* strain PGau.

Despite the large number of ticks (145/249, 58%) removed from *Acrocephalus* species none of them were infected with *B. burgdorferi* s.l.

Detection of A. phagocytophilum

Only 1 *I. ricinus* nymph (0.4% of all examined ticks), removed from a redwing (*T. iliacus*) was positive for *A. phagocytophilum* by real-time PCR analysis with a cycle threshold (Ct) value of 25.3.

Discussion

Thousands of passerines migrate southward every autumn from their places of breeding in Estonia, Finland, and northwestern Russia to their wintering areas located in Southeast Asia, Europe, and Africa. The present study confirms the potential role of migratory passerines in the distribution of tick-borne pathogens by harboring infected ticks, as we found a TBEV-infected nymph harbored by *Acrocephalus* species, and ticks infected by *Borrelia* and *A. phagocytophilum* that were removed from *Turdus* species.

Birds carrying TBEV infected ticks may contribute to the introduction of TBEV and thereby to the emergence of new TBE foci (Lommano et al. 2012). Data from Sweden showed 0.5% TBEV prevalence in bird-feeding ticks removed from the tree pipit (*Anthus trivialis*), the European robin (*Erithacus rubecula*), the common redstart (*Phoenicurus phoenicurus*), and the song thrush (*T. philomeros*) (Waldenstrom et al. 2007). The prevalence of TBEV in ticks from birds in the current study was the similar (0.4%). A TBEV-positive *I. ricinus* nymph was removed from a marsh warbler (*A. palustris*), indicating a potential implication of *Acrocephalus* species in the dispersal of TBEV-infected nymphs from breeding areas in Estonia, Finland, and northwestern Russia along the route to the wintering areas in Central and South Africa. Although there were 4 nymphs attached to the same bird, only 1 of them was TBEV positive, indicating that transmission by co-feeding did not occur on this host. The close sequence relationship to the TBEV strain reported from Finland (Jaaskelainen et al. 2010) could be explained by the marsh warbler migration route, which is directed southward from their breeding areas in South Finland through Estonia to South Africa.

In Europe the prevalence of *B. burgdorferi* s.l spirochetes in *I. ricinus* nymphs feeding on bird ticks varies considerably. The *B. burgdorferi* s.l. prevalence found in this study is similar to that reported from the Lista Bird Observatory in southern Norway, where *B. burgdorferi* s.l was detected in 5.4% and 7.5% of *I. ricinus* nymphs removed from birds in the spring and autumn, respectively (Kjelland et al. 2010). Later studies from the southern Norwegian coast reported 13.6% prevalence of *Borrelia* spp. in bird-feeding nymphs (Hasle et al. 2011); the study was, however, performed during the spring migration period. A large-scale study from southeastern Sweden showed a 19.3% prevalence of *B. burgdorferi* s.l. in nymphs collected from birds (Comstedt et al. 2006). In contrast, data from Central Europe showed higher *B. burgdorferi* s.l. prevalence in bird-feeding ticks. In Thuringia, Germany, *B. burgdorferi* s.l. was detected in 35% of nymphs removed from birds (Kipp et al. 2006). Similar results were reported from Czech Republic (Dubska et al. 2009) and Switzerland (Poupon et al. 2006), where *Borrelia* spp. prevalence in bird-derived nymphs was 31.4%, and 34.6%, respectively.

Common blackbirds and thrushes serve as main reservoirs for *B. garinii* and *B. valaisiana* in Europe (Humair et al. 1998, Hanincova et al. 2003b, Taragel'ova et al. 2008). The results of our study also support the notion that birds of *Turdus* spp. may play an essential role in distribution of *B. burgdorferi* s.l. by ticks in Europe, as 8 of 11 ticks infected with *Borrelia* were removed from *T. merula*. Furthermore, sequences of *B. garinii* and *B. valaisiana* detected in our study were identical to those of *B. garinii* 20047 (Postic et al. 1994) and NE11 (Gern et al. 2010), and to sequences of *B. valaisiana* genotype I-214 (Derdakova et al. 2003), which are widely spread in Europe.

There are several studies reporting the presence of *B. afzelii* in bird-feeding ticks (Humair et al. 1998, Comstedt et al. 2006, Kipp et al. 2006, Poupon et al. 2006, Taragel'ova et al. 2008, Franke et al. 2010b), although *B. afzelii* is regarded to be a rodent-associated genospecies in Europe (Humair et al. 1999, Hanincova et al. 2003a). It has been previously shown that birds do not serve as adequate reservoirs for *B. afzelii,* and furthermore, that the uptake of avian blood initiates the elimination of *B. afzelii* in the tick (Kurtenbach et al. 2002). Because the *B. afzelii* DNA, detected in the current study, was recovered from nymphs feeding on great tits, we suggest that *B. afzelii* infection in these ticks was acquired at the larval stage from a rodent host. However detection of a mix of *B. afzelii* strains PGau and Tom1503 in 1 nymph and detection of strain PGau in another nymph removed from the same great tit may indicate transmission of *B. afzelii* PGau strain through co-feeding on a bird. It is theoretically possible that the nymph acquired a mix of both strains of *B. afzelii* from a rodent during the larval stage (Hu et al. 1997, Humair et al. 1999), but transmission through co-feeding would be a much more likely event than that 2 ticks independently acquired the same strain of *B. afzelii* and accidentally entered the same host.

The low prevalence (0.4%) of *A. phagocytophilum* in bird-feeding ticks in the present study corresponds to the data from other studies (Franke et al. 2010a, Palomar et al. 2012). It is also in correspondence with our previous studies that showed 0.9% prevalence of *A. phagocytophilum* in *I. ricinus* ticks collected from the vegetation in the area near Kabli and Pulgoja bird stations (Katargina et al. 2012). Because this microorganism was detected in an *I. ricinus* nymph feeding on *T. iliacus*, short-distance migrants of *Turdus* species might be considered as potential dispersers of this tick-borne pathogen from Fennoscandia to Central Europe.

Conclusions

This is the first study on the detection of tick-borne pathogens in ticks removed from passerines during southward

migration from Estonia. These data support the notion that migratory birds may be essential in the long-distance distribution and introduction to new foci of TBEV, *B. burgdorferi* s.l., and *A. phagocytophilum* by transporting infected ticks while migrating from Fennoscandia and Russia to South Africa and Central Europe. Further large-scale studies are needed to reveal the significance of certain avian species in the maintenance and competence as reservoirs for different tick-borne pathogens in Europe.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Irina Golovljova Department of Virology
National Institute for Health Development
Hiiu 42
11619 Tallinn
Estonia

E-mail: irina.golovljova@tai.ee