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Original article

Tick-borne pathogens in ticks collected from breeding and migratory birds in Switzerland

Elena Lommano[a], Charles Dvořák[b], Laurent Vallotton[c,d], Lukas Jenni[e], Lise Gern[a,*]

[a] Institute of Biology, Laboratory of Eco-Epidemiology of Parasites, Emile-Argand 11, 2000 Neuchâtel, Switzerland
[b] Rue Louis Ruchonnet 14, 1337 Vallorbe, Switzerland
[c] Department of Mammalogy and Ornithology, Natural History Museum of Geneva, Geneva, Switzerland
[d] Jaman's Group of Faunal Studies, Lausanne, Switzerland
[e] Swiss Ornithological Institute, Seerose 1, 6204 Sempach, Switzerland

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ABSTRACT

From 2007 to 2010, 4558 migrating and breeding birds of 71 species were caught and examined for ticks in Switzerland. A total of 1205 specimens were collected; all were *Ixodes ricinus* ticks except one *Ixodes frontalis* female, which was found on a common chaffinch (*Fringilla coelebs*) for the first time in Switzerland. Each tick was analysed individually for the presence of *Borrelia* spp., *Rickettsia* spp., *Anaplasma phagocytophilum* and tick-borne encephalitis virus (TBEV). Altogether, 11.4% of birds (22 species) were infested by ticks and 39.8% of them (15 species) were carrying infected ticks. Bird species belonging to the genus *Turdus* were the most frequently infested with ticks and they were also carrying the most frequently infected ticks. Each tick-borne pathogen for which we tested was identified within the sample of bird-feeding ticks: *Borrelia* spp. (19.5%) and *Rickettsia helvetica* (10.5%) were predominantly detected whereas *A. phagocytophilum* (2%), *Rickettsia monacensis* (0.4%) and TBEV (0.2%) were only sporadically detected. Among *Borrelia* infections, *B. garinii* and *B. valaisiana* were largely predominant followed by *B. afzelii*, *B. bavariensis*, *B. miyamotoi* and *B. burgdorferi* ss. Interestingly, *Candidatus* Neoehrlichia mikurensis was identified in a few ticks (3.3%), mainly from chaffinches.

Our study emphasizes the role of birds in the natural cycle of tick-borne pathogens that are of human medical and veterinary relevance in Europe. According to infection detected in larvae feeding on birds we implicate the common blackbird (*Turdus merula*) and the tree pipit (*Anthus trivialis*) as reservoir hosts for *Borrelia* spp., *Rickettsia* spp. and *A. phagocytophilum*.

Introduction

Wild birds play a significant role in the ecology and circulation of tick-borne pathogens. They can disperse infected tick vectors over long distances and across geographical barriers (Olsen et al., 1995; Poupon et al., 2006) but they can also act as reservoir hosts in natural foci of disease (Humair et al., 1998). In Europe, passerine birds are hosts of immature *Ixodes ricinus* ticks, which are vectors of a wide range of zoonotic pathogens. Therefore they can transport and disseminate infected ticks along their migration routes. Migratory birds have been found to harbour *I. ricinus* ticks infected by tick-borne encephalitis virus (TBEV) (Ernek et al., 1968; Waldenström et al., 2007), *A. phagocytophilum* (Alekseev et al., 2001b; Paulauskas et al., 2009; Hildebrandt et al., 2010), *Borrelia* spp. (Olsen et al., 1995; Gylfe et al., 1999; Poupon et al., 2006; Dubska et al., 2009), *Rickettsia* spp. (Elfving et al., 2010; Movila et al., 2011) and *Candidatus* Neoehrlichia mikurensis (Spitalská et al., 2006). All these pathogens have been described in questing ticks in Switzerland where they represent a threat for human health (Lommano et al., 2012a).

The introduction of infected ticks by birds into new areas can allow emergence of endemic foci of disease if biotic and abiotic conditions are favourable for the maintenance of the pathogen in ticks and vertebrate hosts. In Switzerland, birds have been suspected to play a role in the emergence of new TBE foci (Lommano et al., 2012b). In fact, at the beginning of 2000, new TBE endemic areas have been identified in the Western part of the country outside a perimeter where all clinical cases had been reported during the 30 previous years (Lommano et al., 2012b). Genotyping of TBEV strains detected in questing *I. ricinus* ticks revealed that they were closely

* Corresponding author. Tel.: +41 32 718 3000; fax: +41 32 718 3001.
E-mail address: lise.gern@unine.ch (L. Gern).

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related to Swiss strains previously identified in the North and the East of the country, two regions distant from the new TBE endemic area (Lommano et al., 2012b; Burri et al., 2011a).

Implication of birds in the maintenance of tick-borne pathogen enzootic cycles has been widely demonstrated for *Borrelia* spp. in Europe (Humair et al., 1998; Hanincová et al., 2003; Poupon et al., 2006; Michalik et al., 2008), in North America (Levine et al., 1991; Hamer et al., 2011) as well as in Asia (Nakao et al., 1994). In Europe, the reservoir competence of pheasants (*Phasianus colchicus*), common blackbirds (*Turdus merula*), song thrushes (*Turdus philomelos*) and puffins (*Fratercula arctica*) for *B. garinii* and *B. valaisiana* was clearly demonstrated (Humair et al., 1998; Kurtenbach et al., 1998; Gylfe et al., 1999; Taragel'ova et al., 2008). However, the role of wild birds in the circulation and transmission of *Rickettsia* spp., *A. phagocytophilum* and TBEV is still discussed. They are suspected to be reservoir hosts for *A. phagocytophilum* (Daniels et al., 2002) and *Rickettsia* spp. (Spitalská et al., 2011; Elfving et al., 2010) but their exact role is still unclear. Concerning TBEV, the avian contribution in the maintenance cycle of TBEV was early suspected (Ernek et al., 1968; Hoogstraal, 1972) but could never be clearly demonstrated.

A reservoir host is defined by its capacity to infect ticks feeding on it (Kahl et al., 2002). One method that helps to identify reservoirs in nature is the comparison of infection rate of questing larvae and nymphs with larvae fed on the suspected host in the same habitat. A higher infection rate in larvae feeding on tested host is a strong indication that the host is reservoir, particularly in case the pathogen is not transmitted transovarially from the female to the eggs. The goal of the present study was to evaluate the role of avian hosts in the circulation and dissemination of tick-borne pathogens like *A. phagocytophilum*, TBEV, *Candidatus* N. mikurensis as well as pathogens belonging to the genus *Borrelia* (*B. burgdorferi* sensu lato complex, *B. miyamotoi*) and the genus *Rickettsia*. All these pathogens, except *Rickettsia* spp., are rarely transmitted transovarially meaning that high infection rate in larvae from birds would suggest that birds act as reservoirs.

Materials and methods

Bird trapping and tick sampling

Birds were captured using Japanese mist nets during the breeding period and fall migration. Breeding birds were captured in 2008, 2009 and 2010 at two sylvatic locations situated between 600 and 650 m above sea level: Bois de l'Hôpital (Canton of Neuchâtel, 47°01.00′N, 6°56.00′E) and Agiez (Canton of Vaud, 46°43.23′N, 6°29.90′E). Both sites are mixed and deciduous forests and were subject to previous studies concerning prevalence of pathogens in questing ticks (Jouda et al., 2004; Lommano et al., 2012a,b; Morán Cadenas et al., 2007). At one site (Agiez), TBEV is known to occur in free-living ticks (Lommano et al., 2012b). All birds were carefully examined for ticks, especially around the eyes and the beak. Permits only allowed banding of some *Turdus* spp. and *Erithacus rubecula*. An injured hawfinch (*Coccothraustes coccothraustes*) found 200 m from Bois de l'Hôpital and carrying ticks, was included in the study.

From August to October 2007, 2008 and 2009, birds migrating southwestward (fall migration) were caught and banded by ornithologists during regular ringing work at the Col de Jaman (VD), a Pre-Alpine pass situated at 1512 m above sea level (46°26.95′N, 6°58.41′E), an altitude in Switzerland that is too high for establishment of *I. ricinus* population (unpublished data). All attached ticks were removed with forceps, identified to stage and species (Cotty, 1985) and stored at −20 °C until further analysis, using a separate vial for each bird. Engorgement status was recorded for most ticks. Ticks collected in 2007 were stored in RNA*later*® solution (Qiagen, Hombrechtikon, Switzerland) at 4 °C for one week and then at −20 °C until nucleic acid extraction. All birds were released immediately after tick removal.

Nucleic acid extraction

All feeding ticks were individually disrupted and homogenized with a mixer mill MM 300 (Retsch, Arlesheim, Switzerland) during 5 min in tubes containing 50 μl Tris–EDTA buffer (pH = 8) and a 3-mm ball. Ticks collected in 2007 and stored in RNA*later*® solution (Qiagen) were first dried on a paper towel. Lysis was performed by adding 1.5 ml of lysis buffer (bioMérieux, France) in each tube and incubated during 30 min. Total nucleic acid extraction was performed using the NucliSENS® easyMAG™ (bioMérieux, France) and the protocol provided by the manufacturer. Negative controls, consisting of reagents without tick, were included in each extraction session. A total of 120 μl of eluted nucleic acid was directly divided into 5 μl and 10 μl aliquots ready for further amplifications and stored at −20 °C. The remaining eluted nucleic acid was divided into two aliquots and stored at −20 °C (DNA) and −80 °C (RNA).

Borrelia spp.

A real-time PCR was used to amplify and detect a 132 bp fragment of the *flagellin* gene of *Borrelia* spp. (Schwaiger et al., 2001), as described in Herrmann and Gern (2010, 2012). The real-time PCR mixture (25 μl) consisted of 0.4 μM of each primer (FlaF1A and FlaR1), 0.2 μM of TaqMan probe, 0.025 U of KAPATaq™ Hotstart (Kapabiosystems by Labgene Scientific, Switzerland), 10× buffer (provided by the manufacturer), 200 μM dNTPs, 500 μM $MgCl_2$ and 5 μl of template DNA. Isolate of *B. burgdorferi* ss (B31), *B. garinii* (NE11), *B. afzelii* (NE632) and *B. valaisiana* (VS116) were used as positive controls. Each positive sample in real-time PCR was analysed by PCR and RLB in order to identify *B. burgdorferi* sl genospecies as previously described (Herrmann and Gern, 2010, 2012). Amplification of the intergenic spacer region between 5S and 23S rRNA genes was performed (Alekseev et al., 2001a). The PCR reaction mixture (25 μl) consisted of each primer (23S-Bor and B5S-Bor), 0.75 U of Taq polymerase (Qiagen, Hombrechtikon, Switzerland), dNTPs, 10× buffer (provided by the manufacturer) and 5 μl of template DNA. Isolate of *B. burgdorferi* ss (B31), *B. garinii* (NE11), *B. afzelii* (NE632), *B. lusitaniae* (PotiB1, PotiB2) and *B. valaisiana* (VS116) were used as positive controls. A touchdown program (Burri et al., 2007) was used. For *Borrelia* identification by RLB, PCR products were hybridized to 15 genospecies-specific probes (Gern et al., 2010).

Rickettsia spp.

For the detection and identification of *Rickettsia* spp., a PCR targeting a 345 bp fragment of the 23S–5S rRNA internal spacer (Jado et al., 2006) coupled with RLB hybridization was used, as described in Lommano et al. (2012a). Each PCR mixture (25 μl) consisted of 0.5 μM of each primer (RCK/23–5F and RCK/23–5R), 200 μM dNTPs, 1.5 U of Taq polymerase (Qiagen), 10× buffer (provided by the manufacturer) and 5 μl of template DNA. DNA of *R. conorii*, kindly provided by Simona Casati (Instituto di Microbiologia, Ticino, Switzerland) and Olivier Péter (Institut Central des Hôpitaux du Valais, Sion, Switzerland) was used as positive control. RLB was performed with modifications in the temperatures of hybridization (48 °C) and washing steps (52 °C) (Burri et al., 2011b, modified from Jado et al., 2006). Probes for RLB are described in Lommano et al. (2012a). To identify the *Rickettsia* species of some samples, citrate synthase gene (*gltA*) was amplified (Bernasconi et al., 2002) and sequenced.