SHORT COMMUNICATION

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# Migratory birds, ticks, and *Bartonella*

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*Bartonella* spp. infections are considered to be vector-borne zoonoses; ticks are suspected vectors of bartonellae. Migratory birds can disperse ticks infected with zoonotic pathogens such as *Rickettsia* and tick-borne encephalitis virus and possibly also *Bartonella*. Thus, in the present study 386 tick specimens collected in spring 2009 from migratory birds on the Mediterranean islands Capri and Antikythera were screened for *Bartonella* spp. RNA. One or more ticks were found on 2.7% of the birds. Most ticks were *Hyalomma rufipes* nymphs and larvae with mean infestation rates of 1.7 nymphs and 0.6 larvae per infested bird. *Bartonella* spp. RNA was not detected in any of the tick specimens.

Keywords: Bartonella; *migratory birds*; *ticks*; *zoonosis*; *emerging infection*

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*Bartonella* infections are widespread in wild and domesticated mammals and several new species have been described during the last decades. Thirteen *Bartonella* species and subspecies have, so far, been associated with human disease, e.g. catch-scratch disease, Carrión's disease, and trench fever (1). The bartonelloses are considered to be vector-borne zoonoses and for some *Bartonella* species the vector is known: *B. henselae* – fleas, *B. quintana* – human louse, and *B. bacilliformis* – phlebotomine sandflies (2). *Bartonella* spp. have been detected – based almost exclusively on polymerase chain reaction (PCR) – in several tick species including *Ixodes ricinus, I. scapularis, I. persulcatus, Dermacentor reticulatus, Rhipicephalus sanguineus,* and *Carios kelleyi* (2). Although Cotté et al. (3) experimentally demonstrated that *I. ricinus* can transmit *B. henselae* during a blood meal, replicating bacteria have rarely been reported in ticks and there is little support for the hypothesis that ticks are natural competent vectors of *Bartonella* bacteria (1).

There is a growing body of evidence showing that migratory birds are important in the dispersal of ticks infected with important human pathogens, e.g. tick-borne encephalitis virus and *Rickettsia* spp. (4, 5). Possibly this may also be true for *Bartonella* spp.

In order to investigate the potential presence of *Bartonella* spp. in ticks infesting birds, a total of 386 ticks was removed from migratory birds net-captured on two Mediterranean islands and later screened for the presence of *Bartonella* spp. RNA.

## Materials and methods

A total of 7,453 springtime migratory birds were captured in mist nets at Capri bird observatory in Italy ($n = 4{,}924$) and at Antikythera bird observatory in Greece ($n = 2{,}529$) between April 2nd 2009 and May 18th 2009. Each captured bird was identified to species and the ears, throat, nape, and abdomen of each bird were checked for ticks. Any tick observed was removed with forceps and individually submerged in Eppendorf tubes filled with RNAlater buffer (QIAGEN) and frozen at $-20^{\circ}$C.

The dorsal and ventral areas of each tick were photographed with a DinoLite USB-microscope. The pictures were carefully analyzed in order to determine stage and species of the tick. The morphological





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species identification of immature ticks is difficult, especially with regard to the genus *Hyalomma* (6). Therefore, to confirm the identifications based on tick morphology a molecular approach was chosen for 10 larval and nymphal tick specimens, identified morphologically as *Hyalomma* sp. and considered to be representative for the whole *Hyalomma* sample. Available sequences of the different genes of *Hyalomma* species were compared in the GeneBank, and the mitochondrial 12S rDNA was identified as an appropriate target gene.

The ticks were homogenized using a QIAGEN TissueLyzer (QIAGEN) and RNA extraction was performed in a QIAGEN M48 BioRobot using the MagAttract® RNA Tissue Mini M48 kit. Random hexamer primers and Illustra™ Ready-to-GO RT-PCR beads kit (GE Healthcare, UK) were used for cDNA synthesis. The cDNA was then used for analyzes of *Bartonella* spp. and tick identification.

A total of 386 tick specimens were analyzed for potential presence of *Bartonella* spp. RNA using a quantitative real-time PCR (q-PCR) targeting the citrate synthase gene (*glt*A) (7). The reaction was adjusted by using 5 µl cDNA template (instead of 1 µl). Negative and positive controls were included in each step, i.e. RNA extraction, cDNA synthesis, and q-PCR.

For the molecular identification of the 10 selected ticks, standard PCR amplifications were carried out in 25 µl reaction mixtures containing 5 µl of the cDNA, 1.65 mM $MgCl_2$, 0.2 mM of the four dNTPs, 10 pM of each primer (T1B122S and T2A12S), 1 UTaq polymerase enzyme (Promega), and 1 µl Yellow SubTM (GENEO Bioproducts, Hamburg, Germany). The reaction mixture was overlaid by a drop of fine neutral mineral oil (ICN) and placed on a heating block of a programmable thermocycler (Biometra, Westburg). After a denaturation step of 4 min at 94°C, each of the 40 cycles consisted of 30 s at 92°C, 45 s at 58°C, and 60 s at 72°C before a final elongation step of 8 min at 72°C (8).

The PCR products were cloned prior to sequencing. For this, a TOPO TA Cloning® Kit was used (Invitrogen™). The clones thus obtained were sequenced by the VIB (Flemish Institute for Biotechnology) Genetic Service Facility at the University of Antwerp, using the ABI PRISM® BigDyeTM Terminator cycle sequencing kit and a capillary DNA sequencer (Applied Biosystems 3730 DNA Analyzer).

## Results

One or more ticks were found on 2.7% of the birds, with means of 1.7 nymphs and 0.6 larvae, respectively, per infested bird (Table 1). The majority of the 386 ticks found were nymphs and larvae of *Hyalomma* ($n = 367$; Table 2). Sequencing data for the 10 *Hyalomma* ticks revealed that nine were *H. rufipes* and one was *H. marginatum*. These findings supported the diagnoses based on tick morphology. In total, 119 (30.8%) ticks were larvae and 250 (68.1%) were nymphs, i.e. 98.9% of the ticks were larvae or nymphs. In the present study only one adult tick, a female *I. ricinus*, was found.

*Bartonella* spp. RNA was not detected in any of the 386 ticks analyzed.

## Discussion

None of the 386 ticks collected from birds captured on Antikythera, Greece and Capri, Italy during April–May 2009 was positive for RNA of *Bartonella*. Adult ticks rarely infest small and medium-sized birds and, accordingly, 98.9% of the ticks in the present study were larvae and nymphs.

We could not find any published report on *Bartonella* infections in ticks collected from migratory birds. However, in agreement with the present results, Monks et al. (9) screened ticks collected from free-living and captive birds with suspected avian tick-related syndrome for the presence of zoonotic pathogens. All 161 ticks were negative for *Bartonella* DNA (9). Furthermore, 64 *Carios capensis* ticks from a brown pelican (*Pelecanus occidentalis*) rookery in South Carolina, US, were also negative for *Bartonella* DNA (10). Wild birds may be important hosts of several blood-feeding arthropods, including ticks, potentially infected with clinically important pathogens (4, 5). However, these published investigations on *Bartonella* and the present one do not support the notion of a geographic spread of *Bartonella* in ticks infesting migratory birds.

The proportion of ticks positive for *Bartonella* spp. DNA in other studies varies from very low, i.e. 0.43% in questing *Amblyomma americanum* in the southern United States (11) and 1.2% in *I. ricinus* ticks collected in the Czech Republic (12) to much higher in *I. ricinus* ticks collected from roe deer (*Capreolus capreolus*) in The Netherlands, where 60% of the ticks were positive for *Bartonella* spp. DNA (13). All 167 *I. ricinus* ticks collected by flagging vegetation in central Sweden were negative for *Bartonella* DNA (14). This could possibly be explained by the fact that 95% of the ticks were host-seeking larvae that had never taken a blood meal, and host-seeking nymphs that had previously (as larvae) taken one blood-meal.

In conclusion, the results of the present study provided no support to the hypothesis that ticks infesting spring-time migratory birds may be infected with *Bartonella* bacteria. To our knowledge, this is the first published report about the potential presence of *Bartonella* bacteria in ticks carried by migratory birds.



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*Table 1.* Bird species infested with ticks during springtime migration

| Scientific name | Common name | No. birds | No. ticks | No. (%) birds infested | Mean infestation rate (No. ticks/ No. infested bird) | Mean no. larvae/infested bird | Mean no. nymphs/infested bird |
|---|---|---|---|---|---|---|---|
| *Acrocephalus schoenobaenus* | Sedge warbler | 250 | 16 | 6 (2.4) | 2.7 | 0.8 | 1.7 |
| *Acrocephalus scirpaceus* | European reed warbler | 9 | 4 | 1 (11) | 4.0 | 0.0 | 4.0 |
| *Anthus trivialis* | Tree pipit | 208 | 11 | 7 (3.4) | 1.6 | 0.1 | 1.3 |
| *Erithacus rubecula* | European robin | 52 | 5 | 2 (3.8) | 2.5 | 0.0 | 2.5 |
| *Ficedula albicollis* | Collared flycatcher | 29 | 3 | 1 (3.4) | 3.0 | 0.0 | 3.0 |
| *Ficedula hypoleuca* | Pied flycatcher | 1032 | 56 | 37 (3.6) | 1.5 | 0.5 | 1.0 |
| *Hippolais icterina* | Icterine warbler | 292 | 4 | 4 (1.4) | 1.0 | 0.8 | 0.3 |
| *Hippolais pallida* | Eastern olivaceous warbler | 24 | 1 | 1 (4.2) | 1.0 | 0.0 | 1.0 |
| *Lanius senator* | Woodchat shrike | 53 | 28 | 7 (13) | 4.0 | 0.9 | 3.1 |
| *Luscinia megarhynchos* | Nightingale | 118 | 15 | 6 (5.1) | 2.5 | 2.3 | 0.2 |
| *Motacilla flava* | Yellow wagtail | 6 | 9 | 1 (17) | 9.0 | 4.0 | 5.0 |
| *Muscicapa striata* | Spotted flycatcher | 572 | 2 | 2 (0.4) | 1.0 | 0.5 | 0.5 |
| *Oenanthe oenanthe* | Wheatear | 4 | 2 | 2 (50) | 1.0 | 0.0 | 1.0 |
| *Oriolus oriolus* | Eurasian golden-oriole | 148 | 12 | 7 (4.7) | 1.7 | 0.3 | 1.4 |
| *Phoenicurus phoenicurus* | Common redstart | 176 | 20 | 12 (6.8) | 1.7 | 0.2 | 1.5 |
| *Phylloscopus sibilatrix* | Wood warbler | 543 | 18 | 16 (2.9) | 1.1 | 0.5 | 0.6 |
| *Phylloscopus trochilus* | Willow warbler | 464 | 3 | 3 (0.7) | 1.0 | 0.3 | 0.7 |
| *Saxicola rubetra* | Whinchat | 745 | 80 | 38 (5.1) | 2.2 | 0.9 | 1.2 |
| *Sylvia borin* | Garden warbler | 1005 | 6 | 4 (0.4) | 1.5 | 1.0 | 0.3 |
| *Sylvia communis* | Common whitethroat | 863 | 85 | 42 (4.9) | 2.1 | 0.4 | 1.6 |
| *Turdus philomelos* | Song thrush | 6 | 5 | 1 (17) | 5.0 | 0.0 | 5.0 |
| Other species | | 854 | 1* | 0 | 0 | 0.0 | 0.0 |
| | Total | 7,453 | 386 | 200 (2.7) | 1.9 | 0.6 | 1.7 |

*One tick was found on an unidentified bird species.

*Table 2.* Genus and stage of ticks

| Tick genus | No. ticks | No. larvae (%) | No. nymphs (%) | No. adults | Unidentifiable |
|---|---|---|---|---|---|
| *Hyalomma* | 369 | 117 (32%) | 250 (68%) | – | 2 |
| *Ixodes* | 7 | 1 | 5 | 1 | – |
| *Amblyomma* | 2 | – | 2 | – | – |
| *Haemaphysalis* | 2 | – | 2 | – | – |
| Unidentifiable | 6 | 1 | 4 | – | 1 |
| Total | 386 | 119 (30.8%) | 263 (68.1%) | 1 | 3 |

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