RECORDS OF IXODID TICKS ON WILD BIRDS IN BULGARIA

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ABSTRACT

Background: Ixodid ticks are widely studied due to their epidemiological and epizootic importance. It is of great value to know vertebrate host preferences of ticks in order to understand their ecological relationships and distribution. Earlier studies report birds as important hosts of ixodid ticks and a total of 136 ticks species have been frequently found in birds worldwide. However, only few studies related to host preferences of ticks among wild birds in Bulgaria are conducted till now.

Aim: To obtain new data on the host preferences of ixodid ticks among wild birds at three locations in Bulgaria.

Material and methods: The study was conducted between 2010 – 2020 in three sites in Bulgaria – Biological Experimental Station Kalimok (Silistra region), near Brodilovo village (Burgas region), and the city of Sofia (in a city park). The birds were caught by mist nets and inspected for ticks. Ticks found were removed by fine tweezers or a special removal tool for ectoparasites. Tick identification was based on external morphological characteristics.

Results: A total of 2419 individual birds belonging to 78 species were examined. Of these, 70 individual birds (16 species) carried ticks, thus representing 2.9% infestation rate. A total of 262 ticks were collected and identified as: *Ixodes ricinus*, *Hyalomma marginatum*, *Haemaphysalis punctata*, *Hae. concinna*, *Hae. sulcata* and two specimens could only be identified to the genus *Haemaphysalis*. *Ixodes ricinus* was the most commonly encountered tick (224/262), predominantly nymphs, followed by *Hyalomma marginatum* (27/262). The highest prevalence of tick infestation was observed among the Eurasian blackbird, *Turdus merula* (28.6%), followed by the common nightingale, *Luscinia megarhynchos* (12.9%) and the common chaffinch, *Fringilla coelebs* (11.3%). These three bird species harboured the majority of ticks on their heads – some single individuals were infested by 10 up to 25 ticks per bird.

Conclusion: *Ixodes ricinus* was found as the predominant tick species in this study. Nymphs were the most frequently observed stage. Infesting both birds and humans, this tick species has the potential to spread local tick-borne infections.

Keywords: *Ixodes ricinus*, host preference, infestation rate

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INTRODUCTION

Ticks are considered as the second most important vector group after mosquitoes for transmitting pathogens to humans (1). Studies, focused on hosts of *Ixodidae* (hard ticks), reveal that birds play an important role in their life cycle and distribution (2, 3). According to existing knowledge, 136 tick species have been frequently found on birds worldwide (4). Tick prevalence seems to depend mostly on bird`s foraging behaviour (5, 6). As far as we know, there are just two published studies on ticks infesting birds in Bulgaria from the early 70s of the 20th century (7, 8). The aim of this study was to obtain new data on the host preferences of

ixodid ticks in wild birds in Bulgaria, to assess their infestation rate and to contribute to the knowledge of tick population dynamics.

MATERIALS AND METHODS

SITE DESCRIPTION

The study took place between 2010 – 2020 at three sites in Bulgaria:

1. Biological Experimental Station Kalimok at the Institute of Biodiversity and Ecosystem Research (Bulgarian Academy of Sciences) located in north-eastern Bulgaria (44°00'42.4"N, 26°26'17.1"E), Silistra region, 2 km south of the Danube river, with an altitude of 15 m. This site is part of the Protected area Kalimok-Brashlen under Natura 2000, it has a flat-hilly relief and a temperate-continental climate (9).
2. Brodilovo, a village in south-eastern Bulgaria (42°05'10.4"N 27°51'28.0"E), 56 m above sea level, within the Strandzha Natural Park. The location has hilly and low mountain relief and continental Mediterranean climate (9).
3. Borisova gradina, which is the oldest park in Sofia (42°40'36.4"N, 23°20'51.2"E). Sofia city is situated in a valley in western Bulgaria at 595 m above sea level. The relief is flat and the climate is temperate continental (9).

BIRD CAPTURE AND TICKS COLLECTION

Birds were caught with mist nets measuring 12 m/2.5 m and with a mesh size of 16 x 16 mm. Mist nets were set in deciduous forest and reed bed areas in Kalimok, amidst small shrubs and deciduous trees in the Strandzha mountain, and mixed forest in the park "Borisova gradina".

All birds were ringed, measured, weighed, identified to species level and, if possible, sexed and aged (10). Bird taxonomy followed the Digital Checklist of Birds of the World issued in 2019 (11). Birds' heads, necks, and ear openings were examined carefully for ticks. All ticks were collected with the help of fine tweezers or a special removal tool for ectoparasites. Ticks were placed in 1.5 ml tubes prefilled with 1 ml 95% ethanol, or stored in a refrigerator at 4^0C. Ticks were separated by instars and identified to species based on external morphological characteristics (12) under a Leica EZ4 stereomicroscope 4.4:1 zoom. The prevalence of tick infestation (infestation rate) was calculated as the percentage of the number of birds infested with ticks divided by the number of all examined birds. The relative infestation intensity was calculated as the mean number of ticks per individual host (for each avian species, the number of ticks of each stage was divided by the total number of birds examined).

RESULTS

A total of 2419 bird individuals of 78 species, belonging to 32 families and 11 orders, were caught and examined for ticks. Overall, 2.9% (70/2419) bird individuals of 16 species were found infested. We found 262 ticks belonging to *Ixodes ricinus*, *Hyalomma marginatum*, *Haemaphysalis punctata*, *Haemaphysalis concinna*, *Haemaphysalis sulcata*. Two *Haemaphysalis* specimens had a damaged capitulum and could not be identified to the species level. The most commonly encountered tick species comprising 82.9% (58/70) of all infestations, was *I. ricinus* (n = 224; 8 larvae, 206 nymphs, and 10 female adults). Out of all tick-infested birds, 5.7% carried *Hy. marginatum,* the second most prevalent tick species (27 nymphs). The relative intensity of each of the two most abundant tick species in the avian host species is shown in Table 1.

Some other tick species which were less frequently found in our study, were *Haemaphysalis punctata* (one female collected from an European robin, *Erithacus rubecula*), *Haemaphysalis concinna* (2 nymphs collected from an Eurasian blackbird, *Turdus merula*), *Haemaphysalis sulcata* (6 nymphs; 3 removed from a Savi's warbler, *Locustella luscinioides*, one from a Great reed-warbler, *Acrocephalus arundinaceus*, one from a thrush nightingale, *Luscinia luscinia* and, one from an Eurasian blackbird). Two ticks, identified as *Haemaphysalis*

spp., were collected from a great reed-warbler and a common reed-warbler, *Acrocephalus scirpaceus*.

Out of the 16 bird species infested, 8 were represented by at least 50 host individuals. Of these, the highest prevalence of tick infestation was found among three passerine species: the Eurasian blackbird – 28.6% (18/63), followed by the common nightingale – 12.9% (20/155) and the common chaffinch – 11.3% (8/71). The latter bird species had the highest burden of *Ixodes ricinus* – some single individuals were infested by 10 up to 25 ticks per bird. The infestation rate on the other infested bird species ranged from 0.5% in the common reed-warbler to 3.6% in the marsh warbler (*Acrocephalus palustris*).

Table 1. *Bird species infested with ticks. The relative infestation intensity was calculated as an average number of ticks per bird species.*

Host species	Family	Total no. infested/ examined	Relative infestation intensity				
			I. ricinus			*Hy. marginatum*	
			Larvae	Nymphs	Adults		Nymphs
Accipiter nisus (Linnaeus, 1758) Eurasian sparrowhawk	*Accipitridae*	1/3	—	0.33 (n=1)	0.33 (n=1)		—
Acrocephalus arundinaceus (Temminck & Schlegel, 1847) Great reed-warbler	*Acrocephalidae*	3/239	—	0.004 (n=1)	—		—
Acrocephalus palustris (Bechstein, 1798) Marsh warbler	*Acrocephalidae*	3/83	—	0.01 (n=1)	—		0.02 (n=2)
Acrocephalus scirpaceus (Hermann, 1804) Common reed-warbler	*Acrocephalidae*	1/199	—	—	—		—
Coccothraustes coccothraustes (Linnaeus, 1758) Hawfinch	*Fringillidae*	2/16	—	0.06 (n=1)	0.06 (n=1)		—
Fringilla coelebs (Linnaeus, 1758) Common chaffinch	*Fringillidae*	8/71	0.04 (n=3)	0.3 (n=21)	—		—
Locustella luscinioides (Savi, 1824) Savi's warbler	*Locustellidae*	1/36	—	—	—		—
Erithacus rubecula (Linnaeus, 1758) European robin	*Muscicapidae*	3/45	—	0.09 (n=4)	—		—

Luscinia luscinia (Linnaeus, 1758) Thrush nightingale	*Muscicapidae*	3/46	—	0.02 (n=1)	0.04 (n=2)		—
Luscinia megarhynchos (Brehm, 1831) Common nightingale	*Muscicapidae*	20/155	0.02 (n=3)	0.21 (n=34)	0.04 (n=6)		0.2 (n=24)
Parus major (Linnaeus, 1758) Great tit	*Paridae*	2/79	0.01 (n=1)	0.03 (n=2)	—		—
Phylloscopus collybita (Viellot, 1817) Common chiffchaff	*Phylloscopidae*	1/48	—	0.02 (n=1)	—		—
Sylvia communis (Latham, 1787) Common whitethroat	*Sylviidae*	1/43	—	—	—		0.02 (n=1)
Sylvia curruca (Linnaeus, 1758) Lesser whitethroat	*Sylviidae*	2/116	—	0.02 (n=2)	—		—
Troglodytes troglodytes (Linnaeus, 1758) Northern wren	*Troglodytidae*	1/7	—	0.14 (n=1)	—		—
Turdus merula (Linnaeus, 1758) Eurasian blackbird	*Turdidae*	18/63	0.02 (n=1)	2.15 (n=136)	—		—
Total (16 species)		70/2419					

DISCUSSION

In this study, we report for the first time *Ixodes ricinus* as the predominant species infesting wild birds in Bulgaria, contrary to earlier studies conducted in the 1970s, which reported a lack or very low prevalence of *I. ricinus* among wild birds (7, 8).

The bird species, preferably inhabiting forest undergrowth and foraging on the ground had the highest infestation rate in our study, in accordance with earlier studies from other European countries (13, 14, 15). *Ixodes ricinus* nymphs predominated among the life stages (92%). It is most likely due to the lower larvae activity in the months April and May (16) when the major part of this study was carried out. The small number of adult ticks in our study is in accordance with the suggestion that they rarely infest birds (17).

Ixodes ricinus has been identified as the primary vector of Lyme disease in Europe (18), which is the most widespread tick-borne disease among humans in Bulgaria. As stated in the Annual Analyses of Acute Infectious Diseases in Bulgaria (19) during the 2010 – 2019 period, the average number of Lyme disease cases was 452, ranging from 290 (2016) to 599 (2018). In relation to the epidemiological importance of *I. ricinus*, this study provides material for future molecular screening of the collected ticks for presence of pathogens like *Borrelia* spp., *Babesia* spp., *Anaplasma, Rickettsia/Coxiella*, and tick-borne encephalitis virus (15).

With reference to others ticks species, we found only nymphs of *Hy. marginatum* infesting three species of birds. *Haemaphysalis* ticks have been frequently found in previous studies in Bulgaria but they are rarely presented in this survey.

Nevertheless, we found *Hae. sulcata* and *Hae. concinna* that have not been previously reported as avian ectoparasites in Bulgaria (20).

As a limitation of our study, we can point out that our data did not allow a comparison of tick and host species composition between the three study sites since our sampling was not done at a regular basis and in a comparable way. Future studies, based on a data-driven, long-term approach are needed to examine the complex associations between ticks, birds and multiple environmental factors.

CONCLUSIONS

Ixodes ricinus was found as the predominant species in this study and nymphal instars were the most frequently observed stage. Infesting both birds and humans, this tick species has the potential to spread widely tick-borne infections. Bird species of the families *Turdidae, Muscicapidae* and *Fringillidae* are frequently infested by hard ticks and may have a significant role as disseminators and reservoirs of tick-borne pathogens to humans.

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