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RESEARCH ARTICLE

A massive infestation of the long-legged buzzard, *Buteo rufinus* (Cretzschmar), by *Hyalomma marginatum* Koch (Acari: Ixodidae) ticks in Türkiye

Ayşe SARI[1], Emine Hesna KANDIR[2,3], Bilal DİK[4], Adem KESKİN[1,5]

[1]*Department of Biology, Faculty of Sciences and Arts, Tokat Gaziosmanpaşa University, Tokat, Türkiye*
[2]*Department of Wild Animal Diseases and Ecology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye*
[3]*Wildlife Rescue Rehabilitation, Training, Practice and Research Center (AKUREM), Afyon Kocatepe University, Afyonkarahisar, Türkiye*
[4]*Department of Parasitology, Faculty of Veterinary Medicine, Selçuk University, Konya, Türkiye*
[5]Corresponding author: adem.keskin@gop.edu.tr

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ABSTRACT: Ticks are obligate blood-sucking ectoparasites of vertebrate animals, including mammals, birds, reptiles, and even amphibians. As a suitable host, birds may carry and spread ticks and serve as reservoirs for some tick-borne pathogens. The present study reports an impressive tick infestation on a long-legged buzzard, *Buteo rufinus* (Cretzschmar), in Türkiye. One hundred fifty-nine engorged nymphal ticks were removed with tweezers from a *B. rufinus* in the Wildlife Rescue Rehabilitation, Training, Practice, and Research Center (AKUREM), Afyon Kocatepe University, Afyonkarahisar province, Türkiye. All ticks were morphologically identified as the *Hyalomma marginatum* group. For accurate species identification, a molecular study on randomly selected two engorged nymphs was performed through Polymerase Chain Reaction (PCR) amplification of a ~460 bp fragment of the mitochondrial 16S rRNA gene. Comparing our mitochondrial 16S rRNA sequences with those from the NCBI Genbank database showed that our ticks have a significant genetic similarity over 99% with *Hyalomma marginatum* Koch. Further, the extracted tick DNAs were also screened for the presence of *Rickettsia*, *Borrelia* and *Bartonella* bacteria targeting the rickettsial citrate synthase (*gltA*, ~750 bp), flagellin B (*flaB*, ~659 bp), NADH dehydrogenase gamma subunit (*nuoG*, ~346) genes, respectively; but samples were negative for these bacteria. To our knowledge, this is the first report of *H. marginatum* infesting *B. rufinus* in Türkiye and the first observation of the massive infestation of *H. marginatum* on *B. rufinus*.

Keywords: Biodiversity, fauna, birds, parasites, vectors, wild animals.
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INTRODUCTION

Ticks (Ixodidae) are ectoparasites that have to suck blood throughout all developmental stages in terrestrial vertebrates, mainly mammals and birds. Today, there are a thousand tick species in three living families: Ixodidae (778 species), Argasidae (221 species) and Nuttalliellidae (1 species). In addition, the extinct families Deinocrotonidae (2 species) and Khimairidae (1 species) were described based on fossil materials from Burmese amber in Northern Myanmar (Dantas-Torres, 2018; Chitimia-Dobler et al., 2022; Guglielmone et al., 2023). Due to its geographical location and climatic characteristics, Türkiye has vegetation, habitats and rich wildlife, allowing a suitable habitat for various tick species. In studies conducted in the country, more than 50 tick species belonging to the Ixodidae and Argasidae families have been reported (Bursalı et al., 2012; Keskin et al., 2014; Keskin and Selçuk, 2021).

Ticks serve as a reservoir of various pathogenic organisms and play an important role in transmitting many pathogens to their hosts, animals, and humans (Jongejan and Uilenberg, 2004; Stafford, 2007; Dantas-Torres et al., 2012). In addition, many ticks may infest a single host animal, cause anaemia and weight loss in animals, and even cause death if they can suck blood excessively (Uilenberg, 1992).

Türkiye has a rich bird fauna, but ectoparasites of birds are unfortunately poorly studied. Although various studies on ticks infesting birds, mainly passerines, have been recently conducted (Keskin et al., 2014; Keskin and Erciyas-Yavuz, 2016, 2019); there is still limited information about the ticks infesting many bird species in Türkiye. In previous reports, more than twenty tick species belonging to the genera *Amblyomma*, *Argas*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Ornithodoros* and *Rhipicephalus* infested on birds have been reported in Türkiye (Bursali et al., 2012; Keskin et al., 2014; Keskin and Erciyas-Yavuz, 2016, 2019; Eren and Açıcı, 2021). One of the tick species infested birds in Türkiye is *Hyalomma marginatum*. The tick species is the primary vector of the Crimean-Congo Hemorrhagic Fever Virus (CCHFV), which is endemic in Africa, southern Europe, the Middle East and Asian countries (Ergönül, 2009). It has high ecological plasticity and can adapt to regions where low or moderate humidity and a long dry season. All active stages of the tick typically live in the steppe, savannah and scrubland hill and valley biotypes in North Africa and Western Asia (Santos-Silva and Vatansever, 2017).

In the present study, we reported the massive infestation of *Hyalomma marginatum* Koch on the long-legged buzzard, *Buteo rufinus* (Cretzschmar), in Türkiye.

MATERIALS AND METHODS

Collection and morphological identification of ticks

One hundred fifty-nine engorged nymphal ticks were removed with tweezers from a *B. rufinus* in the Wildlife Rescue Rehabilitation, Training, Practice, and Research Center (AKUREM), Afyon Kocatepe University, Afyonkarahisar province, Türkiye. Ticks were placed in glass tubes containing 70% ethanol and sent to the Parasitology Research Laboratory, Department of Biology, Tokat Gaziosmanpaşa University, Tokat province of Türkiye. Ticks were morphologically identified using keys by Apanaskevich and Horak (2008) and Estrada-Peña et al. (2017).

A molecular study was performed on randomly selected two engorged nymphs for accurate species identification.

DNA isolation and Polymerase Chain Reaction (PCR)

DNA isolation of ticks was performed by a commercial DNA extraction kit (PureLink™ Genomic DNA Mini Kit, Invitrogen, Carlsbad, CA, USA) with minor modifications, according to the manufacturer's protocol. Briefly, ticks were rinsed in absolute ethanol and dried in a 1.5 ml plastic tube before DNA isolation. After the samples were dried entirely, the tick was mechanically crushed in 180 µl of Digestion Buffer, and 40 µl of Proteinase K was added and incubated overnight at 55 °C. The samples were centrifuged at max speed for 3 minutes, and the supernatant was transferred to a clean tube. 20 µl of RNase A was added to the lysate, mixed well by brief vortexing, and incubated at room temperature for two minutes. 200 µl of Lysis/Binding buffer was added, and then 200 µl of 96% ethanol was added to the lysate. The lysate was transferred to a spin column and centrifuged at 10000 rpm for one minute. The collection tube was discarded, and the spin column was placed in a clean collection tube. 500µl of Wash Buffer 1 was added and centrifuged at 10000 rpm for one minute. The collection tube was discarded, the spin column was placed in a clean collection tube, and 500 µl of Wash Buffer 2 was added and centrifuged at 14000 rpm for three minutes. The column was placed in a sterile 1.5 ml microcentrifuge tube, 50 µl of Elution Buffer was added, and the isolation process was completed by centrifuging at 14000 rpm for 90 seconds. DNA concentration was evaluated using a spectrophotometer (Multiskan Go, Thermo Scientific, Vantaa, Finland) at 260/280 wavelength, and ticks' DNAs were stored at -20 °C until the PCR.

For the molecular identification of ticks, DNAs of randomly selected two engorged nymphs were screened by Polymerase Chain Reaction (PCR) (Bio-Rad T100™ Thermal Cycler, Hercules, CA, USA) amplification of a ~460 bp fragment of the mitochondrial 16S rRNA gene using primers set 16S+1 and 16S-1. The extracted tick DNAs were also examined for the presence of *Rickettsia*, *Borrelia* and *Bartonella* bacteria by PCR using specific primers targeting the rickettsial citrate synthase (*gltA*, ~750 bp), flagellin B (*flaB*, ~658 bp), NADH dehydrogenase gamma subunit (*nuoG*, ~346 bp) genes, respectively. The primer sequences are shown in Table 1.

PCR conditions for all assays were as follows; denaturation at 94 °C for 5 min, then 35 cycles of 40 s at 94 °C, 60 s at 51 °C, and 60 s at 72 °C, followed by 10 min at 72 °C. The PCR reaction mixture (50 µl) contained 25 µl Dream Taq™ PCR Master Mix 2x (Thermo Fisher Scientific, Vilnius, Lithuania), 2 µl forward primer, 2 µl reverse primer, 2 µl of DNA template and 19 µl molecular grade water.

PCR products were verified by electrophoresis in a 1% agarose gel, pre-stained with ethidium bromide and visualized using a gel documentation system (UVP, Upland, CA, USA). Double distilled water was used as a negative control. Purified DNAs were sequenced by Macrogen Inc. (Amsterdam, The Netherlands). For editing raw sequences and generating consensus sequences, BioEdit Sequence Alignment Editor (version 7.0.1.5) was used (Hall, 1999).

RESULTS

In the present study, we collected 159 engorged nymphal ticks from a *B. rufinus* (Fig. 1) in the AKUREM, Afyon Kocatepe University, Afyonkarahisar province. All ticks belonged to the *Hyalomma marginatum* complex. Due to a lack of reliable external characters for differentiating the immature stages of *H. marginatum* complex, the morphological identification of these ticks is currently not possible; therefore, we performed a molecular study on randomly selected two engorged nymphs.

According to BLAST comparisons with the NCBI GenBank database, our sequences (OQ975264-OQ975265) obtained in nymphal *Hyalomma* were 99.78% similar to *Hyalomma marginatum* isolate Hymr1 (KT391060) from Israel, 99.55% *Hyalomma marginatum* isolate HM-Z384US 2017 (MW172439) from Italy, and 99.56% *Hyalomma marginatum* isolate 1 (OL347853) from Türkiye.

For the presence of *Rickettsia*, *Borrelia* and *Bartonella* bacteria, the tick DNAs obtained from two *Hyalomma* specimens were also screened by PCR; but both two samples were negative for these bacteria.

This is the first report *of H. marginatum* infesting *B. rufinus* in Türkiye and the first case of the massive infestation of *H. marginatum* on *B. rufinus*.

DISCUSSION

Wild birds play a significant role in the dispersal of both ticks and various tick-borne diseases such as *Anaplasma phagocytophilum*, *Borrelia* spp., *Rickettsia* spp., *Babesia* spp., and *Neoehrlichia mikurensis* from one region to another region (Hoogstraal et al., 1961, 1963; Dubska et al., 2009; Movilla et al., 2012; Capek et al., 2014; Leblebicioglu et al., 2014; Morozov et al., 2022).

Table 1. The nucleotide sequences of primers, target genes and product size were used for the PCR gene amplification.

Target organism	Target gene	Nucleotide sequence (5' → 3')	Product size (~bp)	References
Ticks	16S rDNA	CTGCTCAATGATTTTTTAAATTGCTGTGG CCGGTCTGAACTCAGATCAAGT	460	Black and Piesman (1994)
Rickettsia	*gltA*	CCTATGGCTATTATGCTTGC ATTGCAAAAAGTACAGTGAACA	750	Roux et al. (1997)
Borrelia	*flaB*	ACATATTCAGATGCAGACAGAGGT GCAATCATAGCCATTGCAGATTGT	658	Barbour et al. (1996)
Bartonella	*nuoG*	GGCGTGATTGTTCTCGTTA CACGACCACGGCTATCAAT	346	Colborn et al. (2010)

Figure 1. The long-legged buzzard, *Buteo rufinus,* presents massive infestation by nymphs of *Hyalomma marginatum* ticks.

Buteo rufinus is a medium-sized and wide-winged predator bird seen in almost every region of our country; they are much more common in the Inner Aegean, Central Anatolia and Eastern Anatolia of Türkiye. The small and medium-sized mammals, birds and reptiles are main foods of *B. rufinus*. The European *B. rufinus* are mainly migratory, but others reside and breed in the Balkans, southern Greece and Türkiye. Also, stragglers, juveniles and subadults migrate to the north and west of the breeding range before autumn (Forsman, 1999).

Like the other birds, *B. rufinus* is a suitable host for the many ectoparasite species. Early studies conducted in Türkiye shown that many parasite species, including ticks [*Haemaphysalis parva* (Neumann), *Hyalomma* spp. and *Rhipicephalus sanguineus* (Latreille)] (Orkun et al., 2014, 2017) and lice [*Colpocephalum nanum* Piaget, *Craspedorrhynchus platystomus* (Burmeister), *Degeeriella fulva* (Giebel), *Kurodaia fulvofasciata* (Piaget) and *Laemobothrion maximum* (Scopoli)], could be infested on *B. rufinus* (Dik and Ozkayhan, 2007; Dik and Kandir, 2021; Dik et al., 2022).

In the present study, we reported *H. marginatum* ticks on *B. rufinus* in Türkiye for the first time. *Hyalomma marginatum* is a two-host tick completed one generation per year in nature. Its immatures mainly feed on wild small mammals and ground-feeding birds, but adults prefer to feed on artiodactyls (Guglielmone et al., 2014). In Türkiye, *H. marginatum* is one of the most common tick species on domestic animals, including cattle, goats, sheep, donkeys and horses. The tick species is responsible for the majority of tick infestations on humans (Bursali et al., 2011; Karaer et al., 2011; Keskin et al., 2015; Karasartova et al., 2018).

In the present study, we also investigated the presence of some pathogenic bacteria, such as *Rickettsia*, *Borrelia* and *Bartonella*, in two tick specimens collected from *B. rufinus*, but these ticks were negative for these bacteria.

Our examination findings suggest that further studies should be conducted to reveal the ectoparasite fauna of the native and migratory populations of *B. rufinus*.

Authors' contributions

Ayşe Sarı: Investigation, resources, writing-original draft. **Emine Hesna Kandır:** Conceptualization, investigation, resources, supervision, visualisation, writing – review & editing. **Bilal Dik:** Conceptualization, investigation, resources, supervision, visualisation, writing – review & editing. **Adem Keskin:** Conceptualization, investigation, resources, supervision, visualisation, writing – original draft, writing – review & editing.

Statement of ethics approval

Not applicable.

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Conflict of interest

The authors declared that there is no conflict of interest.

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