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Investigation of Crimean Congo Hemorrhagic Fever Virus in Ticks (Acari: Ixodidae) Infesting on Hedgehogs in Turkey

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Abstract – Southern white-breasted hedgehogs, *Erinaceus concolor*, are synanthropic and very common wild mammals sharing the same habitats with humans, especially in rural areas. Hedgehogs may be infested by ticks and contribute to circulation of various tick-borne disease agents. In the present study, we investigated ixodid ticks infesting on hedgehogs killed by cars on the highways in the Tokat province for the presence of Crimean Congo hemorrhagic fever virus (CCHFV) using real-time RT-PCR. A total of 54 ixodid ticks were collected from 52 hedgehogs and they were identified as *Hyalomma aegyptium* (9.26%), *Hyalomma marginatum* (9.26%), *Hyalomma scupence* (3.7%) and *Rhipicephalus turanicus* (77.8%), using morphological keys. According to real-time RT-PCR tests, CCHFV was detected in one of five *H. aegyptium* samples. The results shown that *H. aegyptium* may be contribute transmission of the CCHFV to humans in the urban areas. In addition, CCHFV in the *H. aegyptium* were documented for the first time in this study.

Keywords – Ticks, Hedgehogs, CCHF, Turkey, *Hyalomma aegyptium*

1. Introduction

As the mandatory blood feeding arthropods, ticks may cause several important diseases, such as Lyme disease, relapsing fever, Crimean Congo hemorrhagic fever, human babesiosis, ehrlichiosis, boutonneuse fever, tularemia, and encephalitis [1]. Crimean Congo hemorrhagic fever (CCHF) has caused numerous fatal cases between 2002 and 2012 in Turkey. In the meantime, 6396 confirmed CCHF cases and 322 deaths were recorded in Turkey by the Republic of Turkey Ministry of Health [2].

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The CCHF virus belongs to Bunyaviridae and causes a fatal hemorrhagic disease in humans with the mortality rate of 3% and 30% depending on the location [3]. Principal responsibility of transmission of the virus to humans is belong to member of genus *Hyalomma* ticks, especially *Hyalomma marginatum* in Turkey [3], but also, the virus may transferred by direct contact with the blood or tissues of CCHF infected humans and livestock [4]. The virus has been commonly demonstrated among small mammals such as hares and hedgehogs [5]. The main tick vectors of the CCHF are the species of the genus *Hyalomma* [6], nevertheless, other ixodid tick species belong to the genera *Ixodes*, *Dermacentor*, *Haemaphysalis* and *Rhipicephalus* can contribute to CCHFV transmission [7]. So far, the virus isolated from several ixodid tick species such as *Haemaphysalis concinna*, *Hyalomma anatolicum*, *Hyalomma marginatum*, *Hyalomma scupence*, *Rhipicephalus bursa*, *Rhipicephalus turanicus*, and *Ixodes ricinus* in Turkey [8, 9].

Hedgehogs are synanthropic and very common wild mammals sharing the same habitats with humans, especially in rural areas [10]. It has been confirmed that they may harbor several pathogenic organisms, such as *Borrelia burgdorferi* sensu lato [11], *Anaplasma phagocytophilum* [12], *Salmonella tilene, Yersinia pseudotuberculosis* and *Mycobacterium marinum* as well as several fungal and viral disease agents [13]. Southern white-breasted hedgehog, *Erinaceus concolor*, is widespread all over the Turkey, especially urban and suburban areas. Although the role of hedgehogs as potential reservoirs for the pathogens is shown in previous studies [11-15], there is no information about their role in the transmission of tick-borne diseases such as CCHF in Turkey, especially in the Kelkit Valley region where the CCHF is endemic. In the present investigation, tick species infesting on hedgehogs and their potential for harboring CCHFV in the Tokat province in the Kelkit Valley Region were studied.

2. Materials and Methods

The study area

Tokat province is located in the Lower Kelkit Valley area in Turkey (Fig. 1). The vegetation and climatic characteristics of this region is described by Karaer et al. [16]. This region is a CCHF endemic region where approximately 24 tick species have been collected from humans, so far [17].

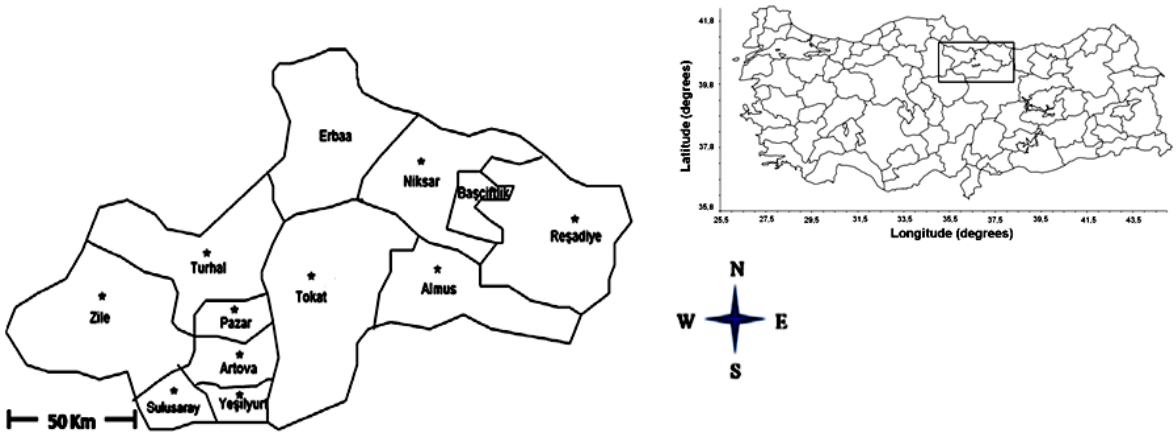

Figure 1. Map of study area, Tokat province.

Collection and morphological identification of ticks

In the present study, 52 hedgehogs killed on the highways were collected and surveyed for ticks (Fig. 2). A total of 54 ixodid ticks were collected from hedgehogs and identified to species using taxonomic keys by Filippova [18] and Estrada-Peña et al. [19]. Ticks were placed in DNA/RNA stabilization solution (Roche, USA) and stored at −86 ^{0}C until viral RNA isolation.

Figure 2. Collection of hedgehogs and their ticks.

Viral RNA isolation and Real-time reverse transcription PCR (RRT-PCR)

To determine the presence of CCHFV in tick collected from hedgehogs, High Pure Viral RNA isolation kit (Roche, USA) was used for the viral RNA from each of 54 tick samples. 9 µl of viral RNA from each tick was used in cDNA synthesis using Transcriptor High Fidelity cDNA synthesis kit (Roche, USA) according to the manufacturer's procedure. cDNA synthesis and real time RT-PCR were performed as described by Tekin et al. [8].

The RNA samples from the CCHFV positive ticks and ddH_2O were used as positive and negative control respectively.

3. Results

Tick species infesting hedgehogs

A total of 54 adults ticks were collected from hedgehogs. *Hyalomma aegyptium* (5), *H. marginatum* (5), *H. scupence* (2) and *R. turanicus* (42) were found on the hedgehog samples (Table 1). *Rhipicephalus turanicus* ticks (%77.8) were the most prevalent tick species present on hedgehogs. Prevalence of *H. aegyptium*, *H. marginatum* and *H. scupence* on the hedgehogs was 9.26%, 9.26% and 3.7% respectively.

Table 1. Tick species collected from hedgehogs in the Tokat province.

Dates (DMY)	Locality	Hedgehogs	Ticks
11.03.2009	Taslıciflik	1	1
18.05.2009	Artova	1	1
02.06.2009	Taslıciflik	1	35
25.06.2009	Erbaa	4	2
25.06.2009	Taslıciflik	1	0
26.06.2009	Niksar	3	1
29.06.2009	Pazar	4	0
03.07.2009	Resadiye	5	1
06.07.2009	Artova, Yesilyurt, Camlıbel	3	1
10.07.2009	Pazar, Turhal	2	1
13.07.2009	Erbaa	3	2
24.07.2009	Erbaa	3	1
27.07.2009	Turhal, Zile	3	2
31.07.2009	Resadiye	4	2
03.08.2009	Sulusaray	3	1
05.08.2009	Erbaa	5	1
07.08.2009	Turhal	3	1
10.08.2009	Erbaa	3	1
Total		52	54

Presence of CCHFV in ticks

Viral RNA samples isolated from tick samples collected from hedgehogs were tested for the presence of CCHFV using real time RT-PCR. According to results of real time RT-PCR tests, one *H. aegyptium* tick (%1.85) out of 54 ticks is CCHFV positive (Fig. 3).

4. Discussion

In the present work, variety of tick species infesting hedgehogs and their potential for harboring CCHFV were studied. Four ixodid tick species, *H. aegyptium*, *H. marginatum*, *H. scupence* and *R. turanicus* were collected from hedgehogs in the Tokat province. *Rhipicephalus turanicus* were the most prevalent ticks species found on hedgehogs. Although, the presence of CCHFV and many other tick-borne pathogens reported in *R. turanicus* [20], no CCHFV detected in the *R. turanicus* ticks tested in this study.

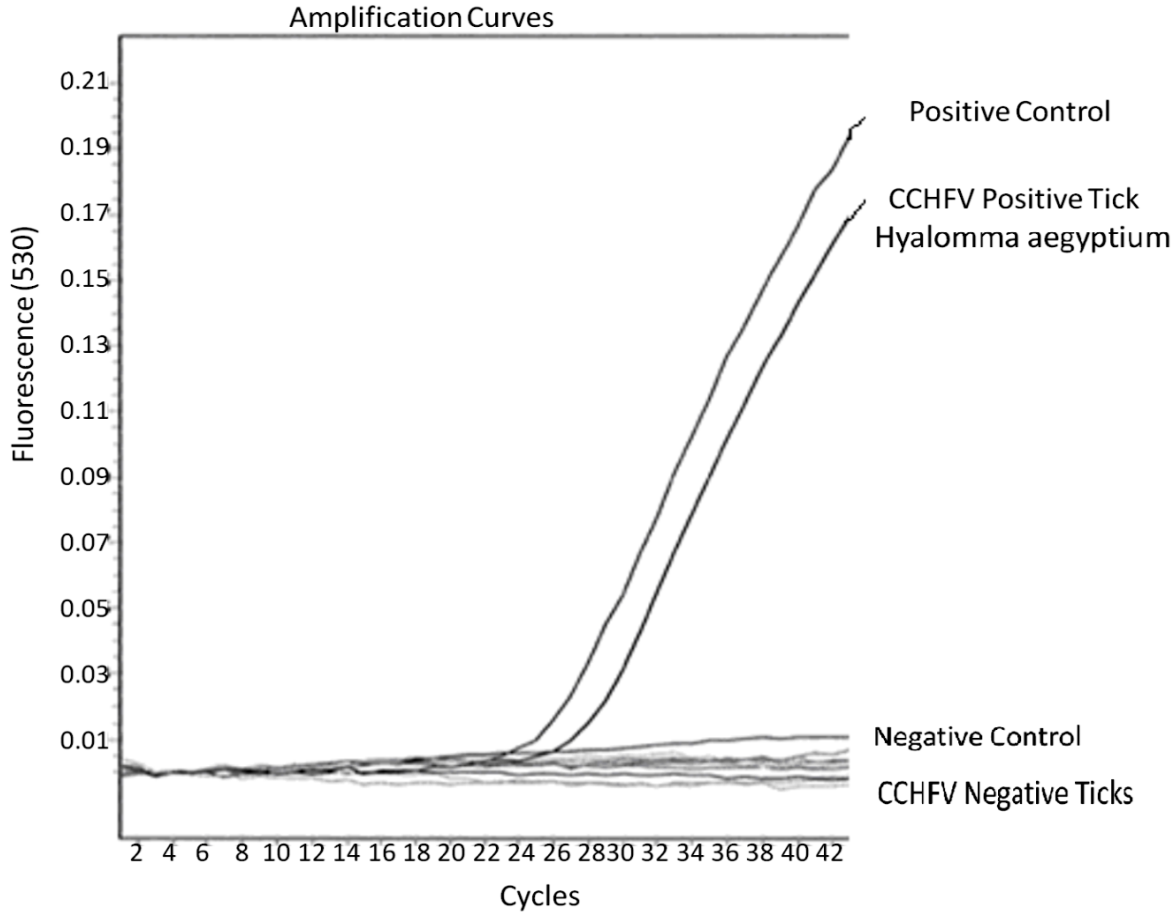

Figure 3. Determination of CCHFV in ticks by RRT-PCR. A *H. aegyptium* tick was CCHFV positive, while other ticks were negative by using CCHFV specific primers and probe.

The other ticks collected in hedgehogs were *H. marginatum* and *H. scupence* which may also play an important role of transmission of CCHF [8, 9, 12, 21]. Moreover, *H. scupence* ticks are the important vectors of several pathogenic protozoan such as *Theileria annulata* and *Theileria equi* [22, 23]. Although CCHF was not detected in *H. marginatum* and *H. scupence* in this study, the presence of CCHF [12, 21] and several pathogenic organisms such as *Rickettsia* and *Theileria* in these ticks have been documented [22, 24, 25]. Therefore, it is suggested that *H. marginatum* and *H. scupence* may play an important role in the transmission of several tick-borne diseases to humans.

The last tick species collected from hedgehogs was *H. aegyptium*. The tick has three-host life cycle and tortoises are the main hosts of this tick in the Mediterranean region and the Middle East [26, 27]. *Hyalomma aegyptium* can infest on hedgehogs, lizards, partridges, hares and mice [26] and humans, especially immature stages [28, 29] in Turkey. It is believed that *H. aegyptium* are not responsible for any human disease, although it frequently bites humans [30]. However, *H. aegyptium* is a vector of *Hemolivia mauritanica* which is a heteroxenous haemogregarine apicomplexan in tortoises [27]. In addition, *Theileria hirci*, a parasitic protozoan that causes malignant theileriosis in sheep and goats [31], and several bacteria such as *Borrelia turcica* [32], *Rickettsia aeschlimannii* [25, 33] and *Rickettsia africae* [25] and Q fever agent, *Coxiella burnetii* [34] have been detected in *H. aegyptium*. In addition to other tick-borne pathogens reported in earlier studies, the presence of CCHFV in *H. aegyptium* was demonstrated first time in this study, indicating its role in the transmission of CCHF to humans in Turkey. Since hedgehogs can be co-infested with various tick species [35], they may support non-viremic transmission of pathogens. It is possible that CCHFV may be transmitted from infected to uninfected ticks during cofeeding on the same host. However, further studies needed to confirm the role of *H. aegyptium* for the transmission of CCHF to humans.

In summary, our results suggest that various tick species may infest on hedgehogs and they may harbor and transmit tick-borne pathogens such as CCHFV. Therefore, hedgehog populations sharing the same habitat with humans in especially urban and suburban areas may be monitored to prevent transmission of tick-borne zoonotic diseases.

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