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First record of hard tick species, *Hyalomma marginatum marginatum* and *H. marginatum rufipes* (Acari: Ixodidae), as probable vectors of Crimean-Congo hemorrhagic fever virus, from the spur-thighed tortoise, *Testudo graeca* (Reptilia: Testudinidae), SE Iran

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Ticks (Acari: Ixodidae) are hematophagous arthropods that can transmit various pathogens to animal hosts and humans causing detrimental infectious diseases including Crimean-Congo hemorrhagic fever (CCHF) (Farhadpour *et al.* 2016). They can readily be identified by their morphological traits. They are generally divided into three families of Nuttalliellidae, a small monotypic family present in South Africa, Argasidae (soft) and Ixodidae (hard) ticks. The latter has a worldwide distribution and are more frequent than soft ticks in temperate regions (Service 2008). Most ticks have a wide spectrum of animal hosts. Only a few species prefer a specific host species for their blood feeding activities (Estrada-Peña *et al.* 2004). Ticks usually feed on a variety of Mammalia, Aves, and Reptilia comprising tortoises (Service 2008). Hard ticks transmit a wide range of pathogens such as viruses, rickettsia, bacteria and protozoa to birds and mammals including man. Most of the diseases caused by these pathogens are carried out through tick bites. In most tick vectors, the transovarial transmission of certain pathogens could occur (Service 2008).

The genus of hard tick, *Hyalomma*, consists of 25 species (Nava *et al*. 2009). *Hyalomma marginatum* is a two-host tick species. It locates its suitable vertebrate host during the larval period and then following complete blood sucking and development to nymph stage, it drops off the same individual host. After ecdysis into adult stage, it engorges on second dissimilar hosts. Finally, female ticks oviposit on the ground (Estrada-Peña *et al*. 2004; Service 2008).

Most hard tick species have negligible host specificities for blood feeding on specific animal groups and only a few ticks are actively host-specific vectors. Different species from *Dermacentor* and *Rhipicephalus* tick genera basically target mammals. Nymph stages of some species in *Amblyomma* genus were, however, isolated most often from migratory birds (Estrada-Peña *et al*. 2004; Service 2008).

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Hyalomma marginatum is found in most parts of Iran. It is active from spring to autumn and frequently seen in small focal populations. Cattle and horses are its specific hosts but it is also found on other domestic animals. Nymphs mostly feed on birds and small mammals. This tick is the main vector of Crimean-Congo hemorrhagic fever (CCHF) virus and transmits various *Theileria* species to cattle (Hosseini-Chegeni *et al*. 2013).

The eastern spur-thighed tortoise, *Testudo graeca* Linnaeus, 1758 is a complex of possibly about 20 distinct conspecific species with a patchy distribution spanning three continents (Široký *et al*. 2007). This species has a worldwide distribution from Eurasia to Africa and countries of the Mediterranean basin and the Black Sea including Spain, Egypt, Ukraine, and Iran (Fakoorziba *et al*. 2012). Its etymology is due to the presence of prominent tubercles on its medial thighs. It has radiated from the Transcaucasia region (Široký *et al*. 2007).

Testudo graeca is native to the Iranian plateau. There are three subspecies of this species in Iran whose present distribution is predominantly restricted by precipitation (Javanbakht *et al.* 2017). Their leathery yellow-brown shell has patches of black rings. They are primarily herbivorous and undergo hibernation in the wild.

The main aim of this study was to describe the species identity and likely infection with CCHF virus RNA among hard ticks recovered on native wild tortoises in a highland region of southeast Iran. To the best of authors' knowledge, there has been no previous record of these ticks on this chelonian species from this part of the country so far.

The Dahdivan village lies in Sardouieh District of Jiroft County, Kerman province of Iran. It has a mountainous cold climate. Its altitude above sea level reaches > 2800 m a.s.l. lying at 57° 27' E, 29° 21' N (Fig. 1). The collected tortoises were carefully inspected for ticks. Hard ticks were randomly captured from two individual tortoises. All the ticks were manually removed with a curved sharply pointed tip forceps and placed in vials containing 75% ethanol. Attachment sites were noted and vials were accordingly labeled. The first species of tick was found on a peridomestic tortoise in August 2018. It was identified to the species level using a common taxonomic key (Estrada-Peña *et al*. 2004). The ambient temperature and relative humidity at this collection site were 27 °C and 26%, respectively. The second wild tortoise was discovered under a thorny bush, *Astragalus adscendens*, in hibernation in November 2018. This semi-buried reptile was removed from overlying soil and vegetation, and two individual ticks, one attached to the underneath of its shell and the other penetrated the thigh of the left hind limb, were removed. The ambient temperature was 2 °C following torrential rain and relative humidity of air was registered as 48% at this site. All of the captured ticks were transferred to the Medical Entomology Laboratory and stored at −20 °C until the time of dispatch to the Institute of Pasteur, Tehran, Iran, for virus RNA assay. Reverse transcription polymerase chain reaction (RT-PCR) was implemented to detect CCHF virus RNA.

Two hard tick specimens, *H. m. rufipes*, were captured on the first tortoise and then identified to species level. The second case involved a species of *H. m. marginatum* and another *H. aegyptium* (Fig. 2). Both of these tick species associated with the second tortoise were in hibernation. After acclimation to above 15 °C, they became active and hibernation thus terminated. *Hyalomma m. marginatum* was captured from beneath the shell adjacent to the right hind leg, while *H. aegyptium* was caught from behind thigh of the left hind limb (Figs. 3, 4). In this study, a single wild lizard, *Trachylepis vittata* (Olivier), being sympatric with *T. graeca*, undergoing hibernation was also captured which was devoid of any tick species. All of the collected tick species from the spur-thighed tortoise were subjected to RT-PCR for the detection of CCHF virus RNA, but none were positive.

Only four specimens representing three species in one genus of *Hyalomma* hard ticks were identified for the first time on the Eastern spur-thighed tortoises, *T. graeca*, from a highland region in southeast Iran. Most of the valid literature on ectoparasitic Acari of tortoises have been published in the last few decades which indicates that research on chelonian ticks has largely been neglected.

Both ticks and tortoises are primarily terrestrial animals whose natural history and evolutionary life styles have preceded many of those of higher animal taxa including birds and mammals. The tortoise tick, *H. aegyptium* (L.), for instance, is the most frequently reported vector to be associated with the Palearctic tortoise, *T. graeca*. It may thus be asked: has there been any adaptation between these two species of tick and tortoise which could have led to their major co-evolutionary association in nature? Which ecological (edaphic) factors were involved in this crucial parasitism? What significant evolutionary selection pressure was in action for this association to arise? These and many other inquiries remain unchallenged and lots of research is required to fulfill the wide gaps in our knowledge on this associative way of life among chelonians. A total of 10 tick species including three argasid (soft) and seven ixodid (hard) ones were named from the tortoise genus of *Testudo* (Barnard and Durden 2000). The potential risks of exotic tick-infested tortoises were revealed after their illegal importation into Italy. There was a preponderance for hard ticks to attach mostly (≈ 90%) to *T. graeca* on their hind limbs (Brianti *et al*. 2010).

Figure 1. Habitat of the Eastern spur-thighed tortoise, *Testudo graeca*, infested with *Hyalomma* ticks in SE Iran.

The first report on the presence of ticks from the eastern spur-thighed tortoise, *T. graeca*, was published in 2002 from Iran (Nabian and Mirsalimi 2002). In a recent study, the tick species of *H. aegyptium* was found on the front leg of *T. graeca* from west Iran (Razmjo *et al*. 2013). In the current investigation on this tortoise species, the hard ticks of *H. m. marginatum* and *H. m. rufipes* were caught on the ventral side of shell in close vicinity to rear legs and *H. aegyptium* from the inner part of left hind limb thigh. In another study from Kurdistan province of northwest Iran, only 11 specimens (0.9%) of *H. aegyptium* were collected from two tortoises (Banafshi *et al*. 2018). In an investigation from the adjacent Urmia province of NW Iran, less than half of all 32 collected tortoises (43.75%) were infested with ticks, *H. aegyptium*. A total of 117 hard ticks were captured from *T. graeca* tortoise in this study (Tavassoli *et al*. 2007). An investigation conducted in the Balkan countries of Bulgaria, Romania and Croatia on three species of tortoises: *T. marginata*, *T. graeca* and *T. hermanni* revealed the presence of four hard tick species of *H. aegyptium*, *H. sulcata* and *H. inermis* as well as the brown dog tick, *Rhipicephalus sanguineus*, on these chelonians (Široký *et al*. 2006). The last tick species was experimentally shown to be capable of transovarial and transstadial transmission of visceral leishmaniasis agent, *Leishmania infantum*, in an endemically infected dog (Dabaghmanesh *et al*. 2016). In another study from the Middle East, 27 out of 38 (71%) captured tortoises were infected with the Crimean-Congo hemorrhagic fever virus RNA, and 74 out of 245 (30.2%) hard tick species of *H. aegyptium* removed from these tortoises were identified to be infected with CCHF virus RNA (Široký et al. 2014). In a more recent study from Algeria, of 56 *H. aegyptium* ticks collected on *T. graeca*, 16 (28.6%) infected ticks were identified to be CCHF virus RNA positive (Kautman *et al*. 2016). No ticks were positive for CCHF virus RNA following RT-PCR assay in the present study. These studies reveal that *T. graeca* tortoise is not the sole host for *H. aegyptium*, although most captured ticks from this chelonian belonged to this species of tick. It also indicates that in case of a thorough study on reptiles of this region, the likelihood of collecting more tick species exists.

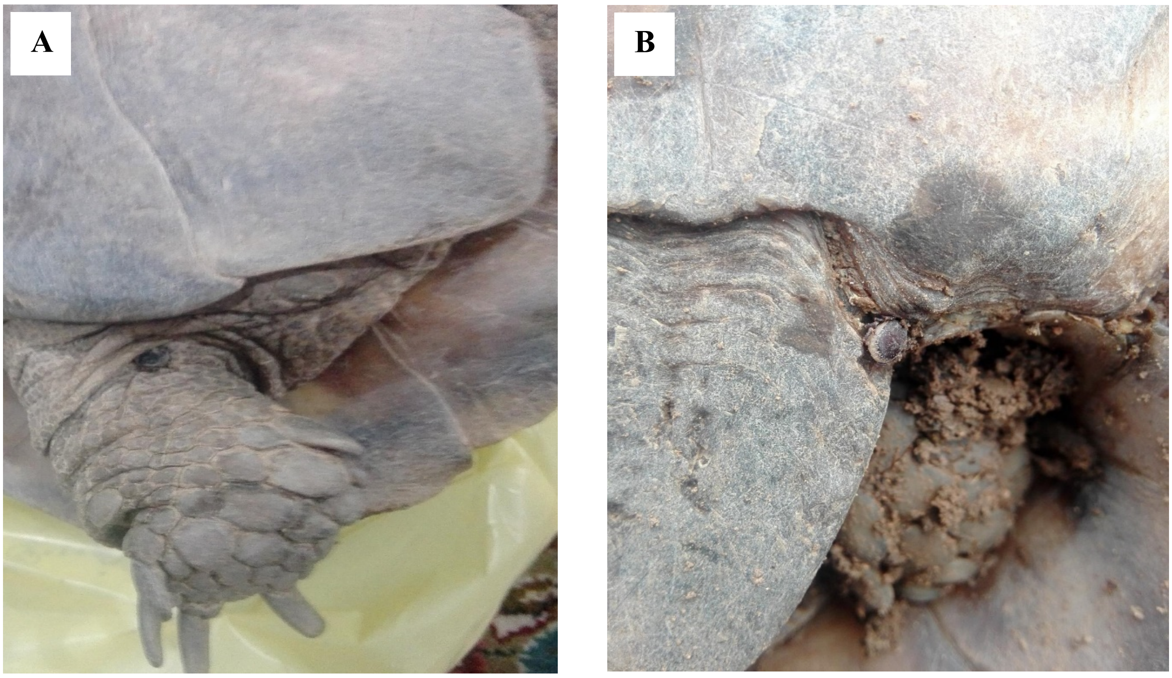

Figure 2. **A.** An individual of *Hyalomma aegyptium* hard tick partly embedded on the thigh of the left hind limb of *Testudo graeca* tortoise; **B.** A specimen of *Hyalomma marginatum marginatum* attached to the right hind limb adjacent to the ventral side of the shell.

Figure 3. The dorsal habitus of *Hyalomma marginatum rufipes* caught from *Testudo graeca* in SE Iran.

It can be concluded that the presence of wild reptiles often infested with hard ticks as reservoirs of various as-yet-undetected pathogens could pose a potential threat to domestic animals and humans in the relevant regions.

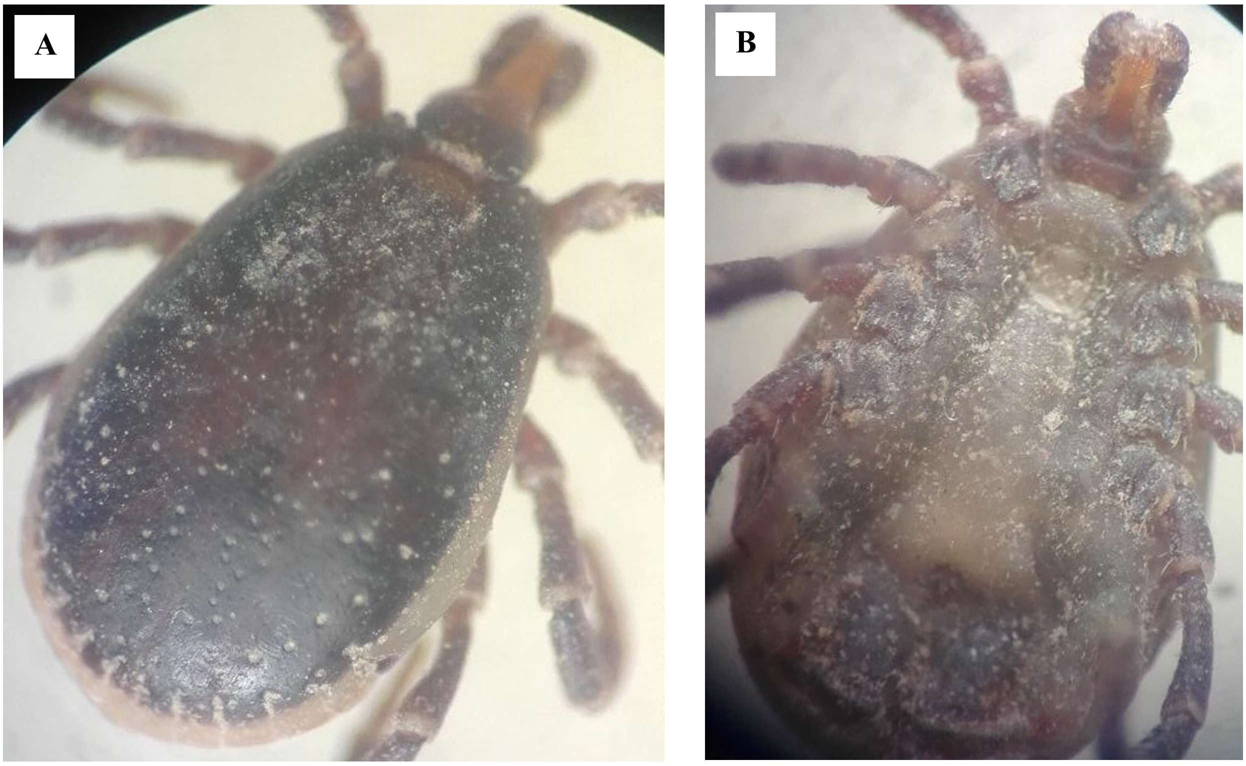

Figure 4. The dorsal (A) and ventral (B) views of *Hyalomma marginatum* tick.

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REFERENCES

Barnard, S. & Durden, L. (2000) Order: parasitiformes, superfamily: Ixodoidea (ticks). *In*: Barnard S.M., Durden, L.A. (Eds.), *A veterinary guide to the parasites of reptiles. Vol. 2, Arhropods (excluding mites)*. Krieger Publishing Company, Florida (FL), pp. 57–189.

Banafshi, O., Hanafi-Bojd, A.A., Karimi, M., Faghihi, F., Beik-Mohammadi. M., Gholami, S., Javaherizadeh, S., Edalat, H., Vatandoost, H. & Telmadarraiy, Z. (2018) Tick ectoparasites of animals in borderline of Iran-Iraq and their role on disease transmission. *Journal of Arthropod-Borne Diseases*, 12(3): 252–261.

Brianti, E., Dantas-Torres, F., Giannetto, S., Risitano, A., Brucato, G., Gaglio, G. & Otranto, D. (2010) Risk for the introduction of exotic ticks and pathogens into Italy through the illegal importation of tortoises, *Testudo graeca*. *Medical and Veterinary Entomology*, 24(3): 336–339.

Dabaghmanesh, T., Asgari, Q., Moemenbellah-Fard, M.D., Soltani, A. & Azizi, K. (2016) Natural transovarial and transstadial transmission of *Leishmania infantum* by naive *Rhipicephalus* *sanguineus* ticks blood feeding on an endemically infected dog in Shiraz, south of Iran. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 110(7): 408–413.

Estrada-Peña, A., Bouattour, A., Camicas, J. & Walker, A. (2004) *Ticks of domestic animals in the Mediterranean region*. University of Zaragoza, 122 pp.

Farhadpour, F., Telmadarraiy, Z., Chinikar, S., Akbarzadeh, K., Moemenbellah-Fard, M.D., Faghihi, F., Fakoorziba, M.R., Jalali, T., Mostafavi, E., Shahhosseini, N. & Mohammadian, M. (2016) Molecular detection of Crimean-Congo haemorrhagic fever virus in ticks collected from infested livestock populations in a new endemic area, South of Iran. *Tropical Medicine & International Health*, 21(3): 340–347.

Hosseini-Chegeni, A., Hosseini, R., Tavakoli, M., Telmadarraiy, Z. & Abdigoudarzi, M. (2013) The Iranian *Hyalomma* (Acari: Ixodidae) with a key to the identification of male species. *Persian Journal of Acarology*, 2(3): 503–529.

Javanbakht, H., Ihlow, F., Jablonski, D., Široký, P., Fritz, U., Rödder, D., Sharifi, M. & Mikulíček, P. (2017) Genetic diversity and Quaternary range dynamics in Iranian and Transcaucasian tortoises. *Biological Journal of the Linnean Society*, 121(3): 627–640.

Nabian, S. & Mirsalimi, S. (2002) First report of presence of *Hyalomma aegyptium* tick from *Testudo graeca* turtle in Iran. *Journal of Veterinary Research*, 57(3): 61–63.

Kautman, M., Tiar, G., Papa, A. & Široký, P. (2016) AP92-like Crimean-Congo hemorrhagic fever virus in *Hyalomma aegyptium* ticks, Algeria. *Emerging Infectious Diseases*, 22(2): 354–356.

Nava, S., Guglielmone, A.A. & Mangold, A.J. (2009) An overview of systematics and evolution of ticks. *Frontiers in Biosciences*, 14(8): 2857–2877.

Razmjo, M., Bahrami, A., Hosseini, E. & Bahrami, A.M. (2013) The first report of lizard and turtle ticks from Ilam, Western Province of Iran. *Advanced Biomedical Research*, 4(3): 118–122.

Service, M. (2008) *Medical entomology for students*. Cambridge University Press, 303 pp.

Fakoorziba, M.R., Golmohammadi, P., Moradzadeh, R., Moemenbellah-Fard, M.D., Azizi, K., Davari, B., Alipour, H., Ahmadnia, S. & Chinikar, S. (2012) Reverse transcription PCR-based detection of Crimean-Congo hemorrhagic fever virus isolated from ticks of domestic ruminants in Kurdistan province of Iran. *Vector-Borne and Zoonotic Diseases*, 12(9): 794–799.

Široký, P., Bělohlávek, T., Papoušek, I., Jandzik, D., Mikulíček, P., Kubelová, M. & Zdražilová-Dubská, L. (2014) Hidden threat of tortoise ticks: high prevalence of Crimean-Congo haemorrhagic fever virus in ticks *Hyalomma aegyptium* in the Middle East. *Parasites and Vectors*, 7(1): 101.

Široký, P., Fritz, U., Türkozan, O., Wink, M., Lehmann, J., Mazanaeva, L., Auer, M., Kami, H. & Hundsdörfer, A. (2007) Phenotypic plasticity leads to incongruence between morphology-based taxonomy and genetic differentiation in western Palaearctic tortoises (*Testudo graeca* complex; Testudines, Testudinidae). *Amphibia-Reptilia*, 28(1): 97–121.

Široký, P., Petrželková, K.J., Kamler, M., Mihalca, A.D. & Modrý, D. (2006) *Hyalomma aegyptium* as dominant tick in tortoises of the genus *Testudo* in Balkan countries, with notes on its host preferences. *Experimental and Applied Acarology*, 40(3–4): 279–290.

Tavassoli, E., Rahimi-Asiabi, N. & Tavassoli M. (2007) *Hyalomma aegyptium* on spur-thighed tortoise (*Testudo graeca*) in Urmia Region West Azerbaijan, Iran. *Iranian Journal of Parasitology*, 2(2): 40–47.