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Probable overwintering of adult *Hyalomma rufipes* in Central Europe

Hyalomma spp. ticks (Acari: Ixodidae) represent a public health threat because of their prominent role in the transmission of Crimean-Congo haemorrhagic fever (CCHF) virus. Moreover, these ticks can transmit other medically important arboviruses such as Thogoto, Dhori, West Nile, and Bhanja viruses; human pathogenic bacteria like *Rickettsia conorii*, *R. aeschlimannii*, and *Anaplasma marginale*; and protozoans like *Theileria annulata* and *Babesia caballi* (Hubálek and Rudolf, 2011). Many records of larval and nymphal *H. marginatum* ticks on birds migrating from North Africa and Southern Europe into Central and Northern Europe have been published. On the other hand, adult ticks in the *Hyalomma marginatum* complex are rarely found in Central Europe (Hubálek et al., 2020), usually removed from cattle, horses, humans, and even vegetation, which might indicate that nymphs were able to molt into the adult stage in the summer or autumn of the same year of collection. In the Czech Republic, a male *Hyalomma rufipes* was recorded for the first time in October 2019 near the village of Sedlec in the South Moravian region (Fig. 1A), climatically the warmest part of the country (Hubálek et al., 2020). Importantly, there are no records regarding overwintering of this species in Central Europe, while recent data from Germany indicate possible overwintering of adult *Hyalomma* spp. ticks (ProMED-mail: Archive Number: 20190625.6536558).

On April 26, 2020, we received one live specimen of an adult tick morphologically resembling *Hyalomma* spp. (Fig. 1B), a non-native tick species for the Czech Republic. The tick was found at the same locality as another tick *H. rufipes* adult in 2019 and was again removed from the hind limb (close to the anal region, under the horse's tail) by a horse owner. We performed the morphological identification using a stereomicroscope. The tick was determined to be a male *H. rufipes* Koch, 1844, based on the morphology of the spiracular plate and the setae around it, which differentiate this species from the closely related *H. marginatum* Koch, 1844 (Apanaskevich and Horak, 2008). Morphological identification was subsequently complemented by molecular identification *via* amplification of a 710 bp fragment of the mitochondrial cytochrome c oxidase subunit I gene (*COI*) using universal barcoding primers LCO1490 and HCO2198 (Folmer et al., 1994). Sequence analysis confirmed the morphological identification of the tick as *H. rufipes*.

In order to discover if additional specimens of this species are present or possibly established at the locality near Sedlec (Fig. 1C), three independent field trips were performed in May 2020. Host-seeking ticks were sampled by flagging vegetation with white flannel cloths (0.9×0.6 m). No additional *Hyalomma* spp. ticks were present at the locality after extensive tick surveillance.

The adult male *H. rufipes* collected in April 2020 was subjected to molecular screening for the presence of flaviviruses (Scaramozzino et al., 2001), bunyaviruses (Kuno et al., 1996), phleboviruses (Matsuno et al., 2015), CCHF virus (Burt et al., 1998), and medically important *Rickettsia* spp. (Regnery et al., 1991). All procedures were done using standard, published protocols. All PCRs were negative for examined arboviruses as well as for *Rickettsia* spp.

To examine how temperature accumulates in the year in the locality of collection (Sedlec), we downloaded the daily temperature values for Sedlec for late 2019 and the beginning of 2020 from official weather records (http://a.la-a.la/chart/cl.php?probe=11359170). Fig. 1D shows that the sum of accumulated temperature in winter above a threshold of 6 °C (a threshold commonly used to denote an activation temperature for *H. marginatum*) is about 400 °C in Sedlec. However, the accumulated temperature since the beginning of February to the tick collection date is slightly below than 200 °C. This second interval has been chosen because it is the period between the early arrival of birds into the southern Czech Republic and the collection of the *H. rufipes* specimen in Sedlec. Considering that the molting time for immature *H. rufipes* has been calculated to require about 400 °C of accumulated temperature (Knight et al., 1978; Chen et al., 2012), immature ticks introduced in spring or summer could molt in the long period between autumn and the following spring in Sedlec. However, the sum of temperature would not allow the molting of immature ticks if they were introduced in the spring of the same year, considering the date of early bird flights and the collection date of the adult *H. rufipes* specimen. We cannot exclude an alternative working hypothesis on the arrival of *Hyalomma rufipes* nymphs with early arrival of migrating birds (*e.g.*, *Upupa epops*, *Luscinia svecica*, *Saxicola torquata*, or *Acrocephalus schoenobaenus*) and the molting of nymphs into adults during April 2020. However, taking into account local weather conditions, this hypothesis seems unlikely, because accumulated temperature is about half of the reported as necessary for nymphal-adult molt.

These results open interesting questions regarding the numerous findings of single *Hyalomma* spp. adults in northern European localities. The recent findings are far from the historically known range of these ticks, but never in high enough numbers to be considered stable populations (reviewed by Hubálek et al., 2020). This may be a restriction owed to the high relative humidity in the area (Hoogstraal, 1956; Chen et al., 2012). However, it is necessary to consider that the number of introduced ticks must be large enough for adults to have a chance to mate on large ungulates or livestock and produce a robust and sustainable population. It seems that winter temperatures are no longer a limiting factor for these ticks, especially with milder winters expected in Central Europe due to climate change. Moreover, recent data from Germany and Sweden also indicate possible overwintering of *Hyalomma* spp. ticks in Central and in Northern Europe, respectively (Chitimia--Dobler et al., 2019; Grandi et al., 2020).

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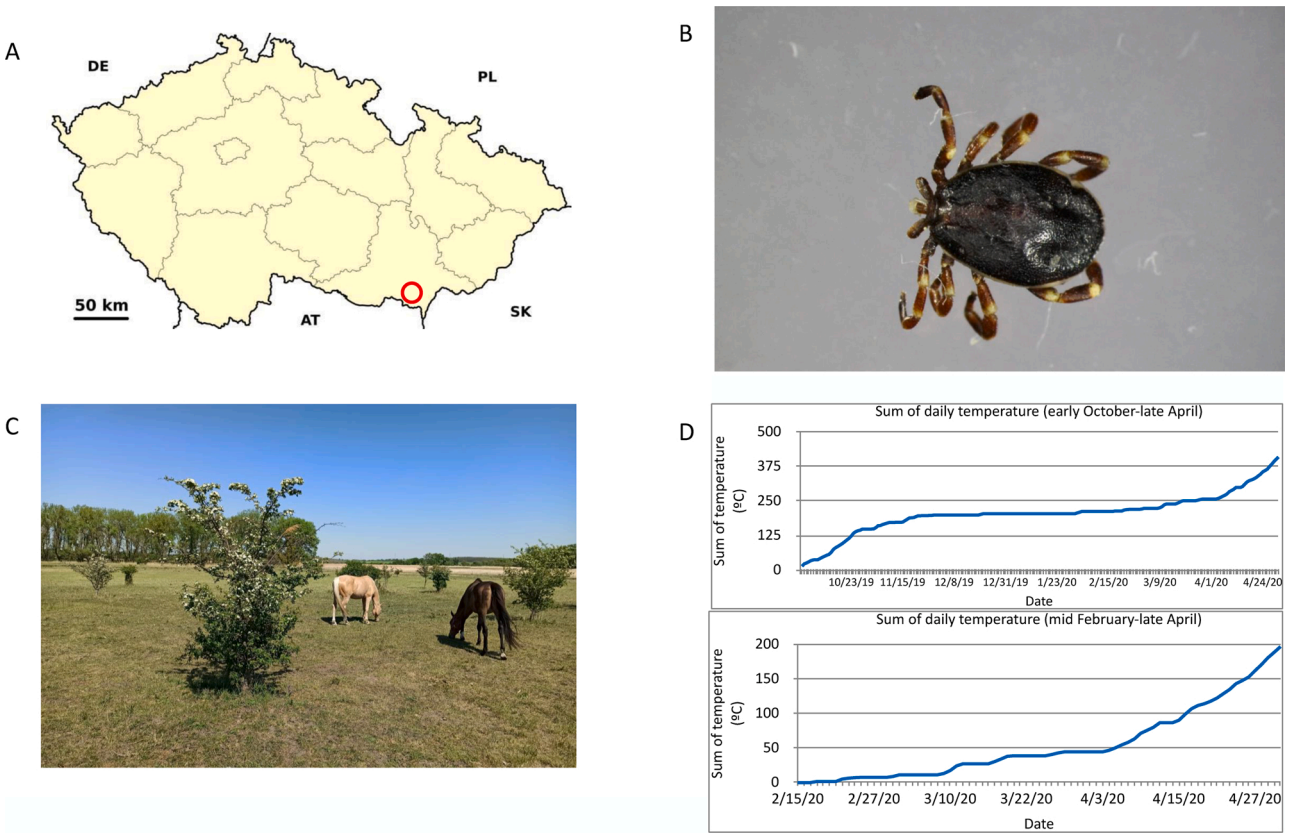

Fig. 1. A. Map of the Czech Republic and the Sedlec study site (red circle). DE – Germany, AT – Austria, SK – Slovakia, PL – Poland. **B.** Adult, male *Hyalomma rufipes* collected at the Sedlec site on April 26, 2020. **C.** Photograph of the landscape at the study site. **D.** Comparison of accumulated temperature in the locality of collection of *Hyalomma rufipes* in the Czech Republic (Sedlec) in the period late October-late April (hypothesized period of introduction, molting and overwintering of the collected specimen) and in the period early February-late April. The later includes the accumulated temperature from the arrival of the first migratory birds from Africa to the Czech Republic until the date of collection, to show that it is insufficient temperature for nymphal-adult molting of *H. rufipes*.

1. Conclusion

Taking into account the weather factors, the biology of *Hyalomma* spp. ticks, and data on bird arrivals, we can assume that the adult *Hyalomma rufipes* specimen found at the end of April 2020 in the Czech Republic, might have molted during the autumn-spring period and overwintered in an adult stage. This finding could portend future establishment of *Hyalomma* spp. ticks in Central Europe, potentially contributing to transmission of CCHF virus and other tick-borne pathogens in this region.

Author's contributions

IR, JV, RK, SŠ collected ticks in the field. ZH and AEP identified tick (s). SŠ, JV, RK, KP, JM performed laboratory analyses and interpreted results. IR, ZH and AEP coordinated the study and drafted the manuscript. All co-authors revised the final version and contributed significantly to the manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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