Ticks and Tick-borne Diseases 10 (2019) 704–708

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Hyalomma rufipes on an untraveled horse: Is this the first evidence of *Hyalomma* nymphs successfully moulting in the United Kingdom?

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ARTICLE INFO

Keywords:
Hyalomma
Crimean-Congo Haemorrhagic fever virus
Migratory birds
Public health
Importation
Rickettsia aeschlimannii

ABSTRACT

During September 2018, a tick was submitted to Public Health England's Tick Surveillance Scheme for identification. The tick was sent from a veterinarian who removed it from a horse in Dorset, England, with no history of overseas travel. The tick was identified as a male *Hyalomma rufipes* using morphological and molecular methods and then tested for a range of tick-borne pathogens including; Alkhurma virus, *Anaplasma*, *Babesia*, Bhanja virus, Crimean-Congo Haemorrhagic fever virus, *Rickettsia* and *Theileria*. The tick tested positive for *Rickettsia aeschlimannii*, a spotted fever group rickettsia linked to a number of human cases in Africa and Europe. This is the first time *H. rufipes* has been reported in the United Kingdom (UK), and the lack of travel by the horse (or any in-contact horses) suggests that this could also be the first evidence of successful moulting of a *Hyalomma* nymph in the UK. It is postulated that the tick was imported into the UK on a migratory bird as an engorged nymph which was able to complete its moult to the adult stage and find a host. This highlights that passive tick surveillance remains an important method for the detection of unusual species that may present a threat to public health in the UK. Horses are important hosts of *Hyalomma* sp. adults in their native range, therefore, further surveillance studies should be conducted to check horses for ticks in the months following spring bird migration; when imported nymphs may have had time to drop off their avian host and moult to adults. The potential human and animal health risks of such events occurring more regularly are discussed.

1. Introduction

Public Health England's (PHE) Tick Surveillance Scheme (TSS) detects rare or imported tick species found in the United Kingdom (UK) (Hansford et al., 2018). The TSS is one of the strategies used by PHE to assess the risk of tick-borne diseases to UK public health and its success is largely due to the high numbers of individuals across the country submitting ticks for identification. Some of the key tick vectors of public health importance that the scheme aims to detect include those of the *Hyalomma* genus. Ticks within this genus are responsible for the transmission of Crimean-Congo Haemorrhagic fever virus (CCHFV), a potentially deadly virus which is absent from the UK due to the current lack of established *Hyalomma* populations. The UK climate is thought to be a major limiting factor for the survival of *Hyalomma*, but confirmed regular importation of these ticks on migratory birds (Jameson et al., 2012), sporadic reports on travelling dogs (Hansford et al., 2016), and the ability of arthropod vectors to adapt to changing environmental conditions, makes monitoring of importations and detection of new foci important.

Until recently, *Hyalomma rufipes* was considered a subspecies of *H. marginatum*, but is now recognised as a valid species (Apanaskevich and Horak, 2008). It is a two-host tick which is widespread across Africa, and also found in Greece, Northern China, Russia, Turkey, Iraq, Syria, Pakistan, Egypt, Yemen and Oman (Chitimia-Dobler et al., 2016). Unfed *H. rufipes* adults collected from vegetation in South Africa have been found infected with CCHFV, and a number of laboratory studies provide evidence of its role in the transmission of the pathogen (Gargili et al., 2017; Hornok and Horváth, 2012). The virus causes severe disease in

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https://doi.org/10.1016/j.ttbdis.2019.03.003
Received 7 January 2019; Received in revised form 5 March 2019; Accepted 8 March 2019

humans, being fatal in 3–50% of cases, and the first autochthonous cases in western Europe were reported in September 2016 in Spain (De Liberato et al., 2018). Along with CCHFV, *H. rufipes* is also reported to be a potential vector of Alkhurma Haemorrhagic Fever virus (AHFV) (Hoffman et al., 2018), *Anaplasma marginale* (Nijhof et al., 2007), *Babesia occultans*, *Rickettsia aeschlimannii*, *Rickettsia conorii* (Chitimia-Dobler et al., 2016) and *Theileria annulata* (Jongejan et al., 1983). Immature stages of *H. rufipes* feed on smaller mammals such as hares, hedgehogs and birds, remaining attached to the host for a period of 12–26 days. Adults feed on larger hosts such as cattle, sheep, horses, goats, camels, deer and wild boar and are also known to bite humans (Capek et al., 2014). Records of this tick species Europe have been associated with importation via migratory birds in France, Greece, Hungary, Italy, and Norway (Hagman et al., 2014; Hasle et al., 2009; Hoffman et al., 2018; Hornok et al., 2016; Vial et al., 2016). Occasional reports in recent years of adult *H. rufipes* on wild boar in Spain (Ruiz-Fons et al., 2006), cattle in Hungary (Hornok and Horváth, 2012), and horses in the Netherlands (Nijhof et al., 2007) and Germany (Chitimia-Dobler et al., 2016) are thought to be a result of fully-fed nymphs imported on migratory birds which have successfully moulted and found a larger host to feed on. This species is known to be able to successfully moult from engorged nymphs to adults under continental climatic conditions in Europe (Hornok et al., 2016), but none of these importation events are thought to have resulted in established populations of *H. rufipes* (Chitimia-Dobler et al., 2016; Estrada-Peña et al., 2012).

Another *Hyalomma* species, *H. marginatum,* is regularly imported into the UK as nymphs on migratory birds during spring, but it is unclear if nymphs can successfully moult in the environment due to temperature limitations during the summer months (Jameson et al., 2012). The lack of records received by the TSS suggests that few *Hyalomma* survive following importation (Cull et al., 2018). To date, only five *Hyalomma* ticks have been received by the TSS including a male *H. marginatum* on a horse in 2009 (Jameson and Medlock, 2009), three *H. lusitanicum* on two dogs and a human in 2016 (Hansford et al., 2016), 2017 and 2018, and a male *H. truncatum* on a human in 2017. All records have been linked to overseas travel to *Hyalomma*-endemic regions. To the best of our knowledge, no confirmed records of *H. rufipes* have been previously reported in the UK.

2. Methods

The TSS generates a tick record by identifying the species, capturing the date of collection and mapping the location the tick was acquired (Cull et al., 2018). When unusual or rare species are encountered, appropriate morphological keys and molecular methods are employed to determine species. On 4th September 2018, a veterinarian removed a tick from a horse in Dorset, southern England, and submitted the sample to the TSS. Morphology of the specimen was investigated (Apanaskevich and Horak, 2008; Walker et al., 2003) and provisional species identification was confirmed with molecular methods targeting the COI DNA region gene (Folmer et al., 1994) as described by de Marco et al. (2017). It was confirmed that the infested horse had never travelled outside the country, and neither had any other in-contact horses. To extract RNA for pathogen analysis, the tick was placed in a 2 ml homogenization tube containing stainless steel beads (1. 4 mm diameter) and suspended in 350 µl of RLT buffer (taken from a Qiagen RNeasy kit). Four homogenization cycles of 5 s at 5500 rpm with 30 s intervals were carried out in a Precellys homogenizer. Total RNA was purified from the homogenate using the RNeasy mini kit in accordance with the manufacturer's instructions. RNA was eluted in a total of 80 µl water and stored at − 80 °C prior to use as template for qPCR / qRT-PCR assays. The tick was tested using qPCRs for AHFV targeting the E gene (Table 1), *Anaplasma* targeting the 16S rRNA gene (Courtney et al., 2004), Bhanja virus (BHAV) targeting the S Segment gene (Table 1), CCHFV targeting the S Segment gene (Atkinson et al., 2012), Piroplasms (*Babesia/Theileria*) targeting the 18S rRNA gene (de Marco et al., 2017), and *Rickettsia* targeting the citrate synthase gene (Stenos et al., 2005). PCR mastermix and cycling for detection of AHFV differed from conditions reported by Atkinson et al. (2012) by replacing 10 µl of reaction mix and 0.8 µl SS III RT/taq enzyme mix with 5 µl TaqMan Fast Virus and using the AHFV primers and probe reported in Table 1. PCR mastermix and cycling conditions for the detection of BHAV were identical to those reported by Atkinsonand coworkers (2012), but using the BHAV primers and probe reported in Table 1. Sequencing libraries were prepared using an Illumina Nextera XT Library Prep Kit (Illumina, Cambridge, UK) and sequenced on an Illumina MiSeq.

Table 1
Primers and probe sequences used for detection of Alkhurma virus and Bhanja virus.

Molecular test	Primers set	Primer sequence Sequence (5'–3')
Alkhurma virus	Forward	5’ GGTGCACCGAGACTGGTT 3’
	Reverse	5’ GGTCAGCGTGGTTCCA 3’
	Probe	5’ FAM GACCTCTCCTTGCCATGGCGACA BHQ1 -3’
Bhanja virus	Forward	5’ ACACAAAGAAGCCGCKAACG 3’
	Reverse	5’ CCGCCAACAACCTCATCATC 3’
	Probe	5’ FAM-CACTGATATCCTTAAGGAGTTCGGTGAGG-BHQ1 3’

3. Results & discussion

The tick was morphologically identified as a male *Hyalomma rufipes* based upon dense patterning of punctations on the scutum, short lateral grooves, dense spiracular setae, shape of the spiracular plate, and shape and arrangement of the adanal plates. Morphological identification was confirmed by molecular analysis (with 99% identity to the accession number KU130624). The tick tested positive for *Rickettsia aeschlimannii*, which was confirmed by direct sequencing of the nucleic acid extract, with 0.82% of total reads generated mapped to a *Rickettsia* reference sequence (CP003342.1) and a consensus sequence covering 82% of the genome was obtained. Phylogenetic analysis of the citrate synthase (*gltA*) gene confirmed the sequence as *R. aeschlimannii* (Fig. 1).

This spotted fever group rickettsia was first isolated from *H. marginatum* in Africa in 1997, but was also suggested likely to be present in ticks in the Mediterranean at the time (Beati et al., 1997). In addition to *Hyalomma* ticks being found infected with *R. aeschlimannii*, including a recently detected *H. marginatum* in Austria (Duscher et al., 2018), many other tick species from different genera have also been found infected elsewhere in the world (Chisu et al., 2017; Igolkina et al., 2018; Mutai et al., 2013; Omondi et al., 2017; Oteo et al., 2006; Pereira et al., 2018; Portillo et al., 2008; Tomassone et al., 2010; Tosoni et al., 2016), including ticks imported (or likely imported) into Europe via migratory birds (Hornok et al., 2013; Mancini et al., 2015; Rumer et al., 2011; Toma et al., 2014; Wallménius et al., 2014). Despite the presence of the pathogen in ticks in multiple European countries, only a small number of human cases of disease have been reported in Africa (Portillo et al., 2015; Pretorius et al., 2004) and only two cases have been reported in Europe; one in Greece (Germanakis et al., 2013) and one in Italy (Tosoni et al., 2016). Disease presentation is usually mild (Pretorius et al., 2004) and treatable with antibiotics, but may also be underreported due to similarities with other spotted fever group infections (Raoult et al., 2002). This pathogen is not known to cause significant disease in horses, and horses are not known to act as a reservoir of infection. The tick also tested positive for *Midichloria mitochondrii* and *Francisella persica*, two tick-borne bacteria which are not known to be pathogenic.

Neither the infested horse, nor other horses in the stable were found to have a history of travel and no further ticks were detected on any of the horses. This suggests that the tick may have entered the UK on a migratory bird during spring as a fully-fed nymph, which successfully

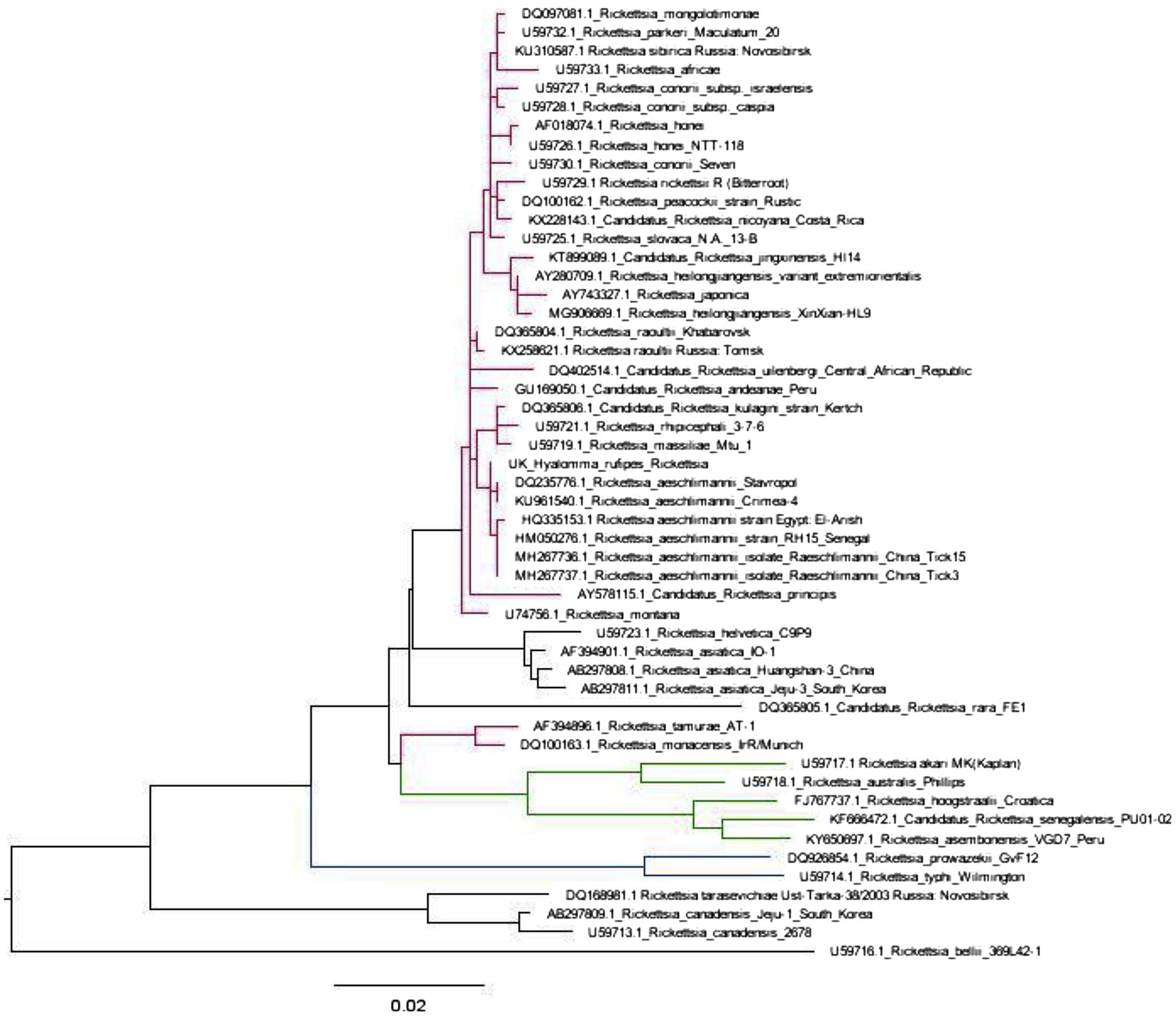

Fig. 1. Maximum-likelihood phylogenetic tree comparing representative *Rickettsia gltA* sequences. *Rickettsia* groups are highlighted as follows: Red – Spotted Fever Group, Green –Transitional Group and Blue – Typhus Group. Evolutionary history was inferred by using the Maximum-likelihood method and Tamura-Nei model in MEGA 6.06 (Kumar et al., 2018) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

moulted and attached to a horse. Swallows are known to nest in the stables of the infested horse, after travelling to the UK from Africa for the summer, and as swallows are known hosts of *H. rufipes* (Matsumoto et al., 2004), this may have been the source of a nymph that later moulted to a male and fed on the horse. Although this theory cannot be confirmed, this may be the first detection of a *Hyalomma* nymph moulting in the UK climate. Despite follow-up, no further *Hyalomma* ticks were detected, suggesting that this may have been an isolated case; much like those reported in other European countries where similar records of adult *H. rufipes* have been reported (Chitimia-Dobler et al., 2016; Hornok and Horváth, 2012; Nijhof et al., 2007; Ruiz-Fons et al., 2006).

The importance of *H. rufipes* as a vector of CCHFV is paramount, and the feeding behaviour of this species on birds that migrate over large distances (Hoogstraal et al., 1961) means that the UK could potentially be reached by CCHFV infected *H. rufipes* (England et al., 2016). Whilst the specimen tested negative, migratory bird-associated importation of *H. rufipes* nymphs infected with CCHFV has been reported in Greece (Lindeborg et al., 2012). To the best of our knowledge, none of the importation or detection events in the literature have resulted in newly established populations of *H. rufipes* or CCHFV cases outside the normal endemic geographical range. Each event does, however, highlight the potential for vectors of deadly pathogens to move across large distances and survive, at least for a short period, and successfully feed on hosts in new areas.

One of the major limitations for establishment of *H. rufipes* in northern Europe is current climatic conditions that can impact upon the moulting process of fully-engorged nymphs which may drop off migratory birds into a new geographical region (Capek et al., 2014; De Liberato et al., 2018; Hornok and Horváth, 2012). Similar species are suggested to have established in new areas in southern Europe, likely following importation from migratory birds; as evidenced by the presence of *H. marginatum* in southern France (Vial et al., 2016). The detection of *H. rufipes* on a horse in Dorset highlights the need for continued vigilance and follow-up of any unusual ticks that might be found infesting horses (or other large hosts) as such animals could act as sentinel indicators for the successful moulting of imported CCHFV-infected nymphs in the UK. The southerly location of Dorset in England makes it a key location for migratory birds arriving in the UK along with the ticks they may transport. If this tick detection was a result of importation on a migratory bird and moulting in the UK environment, the unusually warm weather experienced during the summer of 2018 may have been a factor; as it likely was in Germany in 2015 (Chitimia-Dobler et al., 2016) and during a more recent detection of *H. marginatum* in Austria during 2018 (Duscher et al., 2018). The mean maximum temperature anomaly compared with 1981–2010 climate data was 2.5–3.5 °C higher each month during May-July 2018, with mean temperature 1.5–2.5 °C warmer. The UK Met Office reported this as the second warmest July since 1910 (Met Office, 2018). Enhanced surveillance of ticks on horses or other large animals should commence during the period August-October, when nymphs that may have been brought into the UK on spring migrating birds could have had time to moult, and adults would be expected to be most active (Hornok and Horváth, 2012). Ticks should be removed and sent to PHE's TSS for identification and further investigation, if necessary.

4. Conclusion

This demonstrates for the first time likely evidence of successful moulting of a non-native vector of CCHFV, *H. rufipes*, in the UK during the summer of 2018. Further importations of nymphs of this species into the UK are possible due to the high number of migratory birds entering the country annually. Establishment of this tick in Northern Europe is suggested to be currently limited by its cooler climate, which may affect the metamorphosis of nymphs to adults. The unusually warm weather experienced in the UK during the summer of 2018 may have supported this and such weather patterns could be experienced under future climate change scenarios. If other *H. rufipes* nymphs were imported and moulted into adults which successfully fed and mated on horses in the yard affected, oviposition and successful hatching of larvae during spring 2019 could be possible. A follow-up survey in Dorset should be conducted during spring 2019 to detect any continued *H. rufipes* activity. Enhanced surveillance should also include attempts to detect *Hyalomma* in other areas of southern England where migratory birds are known to transport ticks (Jameson et al., 2012), as moulting of *Hyalomma* nymphs in the UK during 2018 would not have been limited to Dorset. The simultaneous detection of *H. marginatum* in Austria (Duscher et al., 2018) may suggest that further records of *Hyalomma* could also be reported in other non-endemic areas of Europe during 2019. Horses are important large mammal hosts for feeding adults of *H. rufipes*, and enhanced surveillance of these animals in the months following the arrival of spring migrant birds is recommended, particularly during and following warmer summers when nymphal moulting could potentially be supported in the UK. Any unusual ticks found in the UK can be sent to PHE's TSS (https://www.gov.uk/guidance/tick-surveillance-scheme) for identification and follow-up investigation, to help monitor the importation or detection of non-native tick vectors in the UK.

Acknowledgements

The authors would like to thank Martin Peaty BVSc of The Barn Equine Surgery, Three Legged Cross, Wimborne, Dorset who detected and submitted the unusual specimen for identification, and also the owners of the horse for sharing information on the travel history of the horse. JMM is partly funded by the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Environmental Change and Health at the London School of Hygiene & Tropical Medicine in partnership with Public Health England (PHE), and in collaboration with the University of Exeter, University College London, and the Met Office; and partly funded by the NIHR HPRU on Emerging Infections and Zoonoses at the University of Liverpool in partnership with PHE and Liverpool School of Tropical Medicine. The authors would also wish to thank the PHE Genomic Services Development Unit for performing sequencing. The authors would also like to acknowledge the EU Framework Horizon 2020 Innovation Grant (EVAg, No. 653316), and the Department for Environment Food and Rural Affairs (DEFRA), Scottish Government and Welsh Government through grants SV3045 and SE4113 for funding. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, the Department of Health or PHE.

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