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Original article

Importation of *Hyalomma marginatum*, vector of Crimean-Congo haemorrhagic fever virus, into the United Kingdom by migratory birds

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ABSTRACT

Hyalomma marginatum ticks are an important vector of Crimean-Congo haemorrhagic fever virus which can result in a severe and potentially fatal disease in humans. Given the continued emergence of clinical cases in Eurasia and focalised upsurges of *H. marginatum* populations in Europe, it seemed prudent to assess the potential of this vector species to be introduced into the United Kingdom. Immature forms of *H. marginatum* are frequent ectoparasites of passerine birds many of which migrate from Africa to the UK each spring. Incoming birds were inspected for ticks during the spring migration in 2010 and 2011. A total of 68 ticks was collected from 971 birds (29 bird species), 21% (14) of the ticks were identified as *H. marginatum*. *Oenanthe oenanthe* (Northern wheatear) and *Sylvia communis* (Whitethroat) were found to be infested by this tick in both years and with multiple ticks. Single specimens were also removed from *Acrocephalus schoenobaenus* (Sedge warbler) and *Phoenicurus phoenicurus* (Common redstart) in 2010. This study provides the first contemporary evidence for substantial importation of this tick species into the UK.

Introduction

Migratory birds are often implicated in the transport of ticks and their associated pathogens. Ticks by themselves have relatively little mobility, however, when attached to migratory birds they may be transported across barriers such as rivers, deserts and mountains, oceans and continents. This method of dispersal and introduction is considered most important for one- and two-host ticks due to the longer length of time they are attached to their hosts. It is well documented that birds transport ticks harbouring *Borrelia* (Hasle et al., 2011; Olsén et al., 1993, 1995), tick-borne encephalitis virus (Waldenström et al., 2007), *Anaplasma* and *Rickettsia* (Hildebrandt et al., 2010) throughout many parts of Europe. There has been less attention given recently to the role of migratory birds in introducing ticks infected with Crimean-Congo haemorrhagic fever (CCHF) virus into Europe.

In the last decade CCHF virus has been recognised as a growing problem in Eurasia affecting several Eastern European countries with an upsurge of cases in Kosovo (Jameson et al., 2012) and the emergence of human clinical cases in Greece (Papa et al., 2008), Turkey (Karti et al., 2004), Georgia (Zakhashvili et al., 2010), and India (Mishra et al., 2011). The virus has been detected or isolated from over 30 species of ticks, however, this does not denote their active involvement in the transmission and/or maintenance of the virus in natural cycles (Ergonul and Whitehouse, 2007). Only 12 tick species from the genera *Dermacentor*, *Hyalomma*, and *Rhipicephalus* have been confirmed as vectors in the field with the remaining only proven in the laboratory setting. *Hy. marginatum* Koch, 1844, is considered to be of most importance for transmission of CCHF virus in Europe (Hoogstraal, 1979; EFSA, 2010).

Commonly known as the Mediterranean *Hyalomma*, *Hy. marginatum* is widely distributed in North Africa and Asia where it is reported from Algeria, Armenia, Azerbaijan, Egypt, Ethiopia, Georgia, Iran, Iraq, Israel, Morocco, Sudan, Syria, Tunisia, and Turkey. It is also present in Southern and Eastern Europe having been recorded in Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, France, Greece, Italy, Kosovo, Macedonia, Moldova, Montenegro, Portugal, Romania, Russia, Serbia, Spain, and Ukraine (Hoogstraal, 1979; Estrada-Peña et al., 2004; Walker et al., 2003; EFSA, 2010). There are several historical records of *Hy. marginatum* being imported into Europe on migratory birds including Sweden (Brinck et al., 1965), Czech Republic (Černý and Balat, 1957), Norway (Mehl et al., 1984), Slovakia (Černý and Balat, 1989), Finland (Nuorteva and Hoogstraal, 1963), and Germany (Walter et al., 1979). More recent records have also been reported in Slovenia (Tovornik, 1990) and Switzerland (Papadopoulos et al., 2001;

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Poupon et al., 2006). It is not currently considered to be a resident species in the United Kingdom (UK), although several immatures (8 records) were collected in the 1960s and 1970s from migratory birds (Martyn, 1988) and one adult male on a horse imported from Portugal (Jameson and Medlock, 2011).

Hy. marginatum is a two-host tick with both larvae and nymphs feeding without leaving the host. As a direct result, the same tick may be attached to the host for 12–26 days. It is this long length of attachment which enables them to be passively transported from the birds' wintering grounds in Africa along migration routes to countries such as the UK (Rechav et al., 1987).

For several tick species implicated in the maintenance and transmission of CCHF virus, birds, in particular ground-feeding passerines, are important hosts for the immature stages (Kaiser et al., 1974; Hoogstraal et al., 1961). In general, birds are considered to be refractile hosts and unimportant in the virus cycle other than to act as hosts to the tick vectors; however, findings by Zeller et al. (1994) and Vatansever (2010) suggest otherwise. Both authors report detection of virus from *Hyalomma* spp. nymphs feeding on birds [*Alectoris chukar* (Chukar partridge), *Numidia meleagris* (Blue-helmet guinea-fowl), *Lamprotornis caudatus* (Long-tailed glossy starling), and *Tockus erythrorhynchus* (Red-beaked hornbill)]. Such results indicate that either birds are capable of infecting feeding ticks or that transovarial transmission may have a role in virus amplification. Until further studies are conducted on the direct role that birds have in CCHF virus amplification, it could conservatively be assumed that it is only the attached infected ticks which may introduce the virus into the UK.

Three tick species which could possibly introduce CCHF virus into the UK are *Haemaphysalis punctata* (found mainly in Europe but also North Africa), *Hy. marginatum* (Europe, North and East Africa), and *Hy. rufipes* (Central and South Africa, sparse in North Africa). Historical tick records for the UK show importations of both *Hy. marginatum* and *Ha. punctata* have occurred via migratory birds, but not *Hy. rufipes* (Martyn, 1988). For the purpose of this study, it was decided to focus on the importation of *Hy. marginatum* ticks due to the importance of this tick species in CCHF virus transmission cycles in Eurasia, the lack of conclusive evidence for *Ha. punctata* as a vector in the absence of *Hyalomma* spp. and the unlikely event of survival of *Hy. rufipes* in the temperate conditions of the UK. Larvae and nymphs are the most likely stages of *Hy. marginatum* to be found on birds with adults seldom if ever found to be feeding on such hosts (Hoogstraal, 1979).

Given the evidence for increased circulation of CCHF virus within Europe, it is prudent to conduct an assessment on the future potential for introduction of the vector and/or virus. The purpose of this study was to investigate if migratory birds continue to introduce *Hy. marginatum* ticks into the UK and if so on which bird species, their infestation rates, and their likely origin.

Materials and methods

Identification of bird species considered most likely to be parasitized by *Hy. marginatum* was conducted through a literature review using the terms "*Hyalomma*" AND "tick" AND "bird" in PubMed. In addition the ICTTD TickBase (www.icttd.nl/index.php?id=10) was searched for all records of *Hy. marginatum* from birds in Africa and the Mediterranean. The complete list of all bird species was edited to only include those which were known spring migrants to the UK.

Portland Bird Observatory in Dorset, England (50.55°N 2.44°W) was identified as the most suitable site for the study due to its consistent annual records of target bird species and its location on the south coast of England (one of the first landfall sites for migrating birds). Ticks were removed from birds caught for normal ringing purposes throughout spring migration (March to May 2010 and 2011). Passive and targeted surveillance was undertaken. The former included inspection of birds caught in the observatory mist nets and the latter utilised spring traps baited with meal worms to capture insectivorous ground-feeding birds. All birds were identified to species level, and their sex, age, weight, and wing length recorded by Portland ornithologists after which they were passed to HPA entomologists for inspection for ticks. Efforts were made to reduce the stress to birds, by focusing on inspecting the head, ear canals, and neck which correspond to previous findings reported by Hoogstraal et al. (1961), who identified these areas as preferred by *Hyalomma* ticks. Any ticks found were removed using tweezers and placed in plastic vials containing 70% ethanol. Ticks were stored at room temperature until morphological identification was performed. All ticks were identified to species level according to Hillyard (1996), Walker et al. (2003), Estrada-Peña et al. (2004), and Apanaskevich and Horak (2008). Total RNA was extracted from all *Hyalomma* ticks using QIAamp Viral RNA Mini Kit (QIAGEN) according to the manufacturer's instructions and tested for presence of CCHF virus using real-time RT-PCR according to Atkinson et al. (in press).

Results

A total of 971 birds comprising 29 species was examined for ticks during the spring migrations of 2010 and 2011. The majority surveyed were *Phylloscopus trochilus* (Willow warbler) ($n = 531$), followed by *Sylvia atricapilla* (Blackcap) ($n = 97$), *Phylloscopus collybita* (Common chiffchaff) ($n = 86$), *Sylvia communis* (Whitethroat) ($n = 53$), *Oenanthe oenanthe* (Northern wheatear) ($n = 51$), *Phoenicurus phoenicurus* (Common redstart) ($n = 30$), and *Carduelis carduelis* (European goldfinch) ($n = 22$) with smaller numbers of a further 22 species (Table 1).

Sixty-eight ticks were removed from 53 infested birds of 9 species. Three species of ticks were identified: *Ixodes frontalis* ($n = 49$: 8 larvae, 40 nymphs (N), 1 female), *Hy. marginatum* ($n = 14$: 14 N), and *I. ricinus* ($n = 5$: 5 N) (Table 2). *I. frontalis* and *I. ricinus* ticks were collected from all months surveyed (March–May), whereas *Hy. marginatum* ticks were collected only in late April and May.

Whilst the majority of ticks collected was *I. frontalis*, the collection of 14 *Hy. marginatum* nymphs is worth special attention. *Hy. marginatum* constituted 21% of all ticks collected and was the tick species most commonly found to infest birds in multiple numbers with 5 and 3 nymphs collected from 2 *Oe. oenanthe* (Fig. 1) and 2 nymphs collected from 2 *Sy. communis*. Similar to previous findings by Hoogstraal et al. (1961), *Hy. marginatum* ticks were found only around the eyes, beak, and in the ears, whereas *Ixodes* spp. were also removed from the back of the neck and the body of the bird.

All *Hy. marginatum* specimens were subjected to real-time RT-PCR for detection of CCHF virus with no positives found.

Discussion

This study was undertaken during the spring migration when arriving birds are most likely to originate from areas known to support *Hyalomma* populations (i.e. Africa). Each spring, approximately 13 million birds migrate to Britain from Africa (Stone et al., 1997), however, *Hy. marginatum* ticks were only found on 4 species of birds in this study: *Oe. oenanthe* ($n = 8$), *Sy. communis* ($n = 4$), *Ac. schoenobaenus* ($n = 1$), and *Ph. phoenicurus* ($n = 1$). This was not unexpected as all species were included in the target bird species list, and our findings confirm historical records (Martyn, 1988). The British breeding populations of each infested bird species are reported to be 540,000, 931,000, 297,000, and 101,000 individuals, respectively (RSPB, 2011). With a mean relative intensity of 0.09,