Ticks and Tick-borne Diseases 10 (2019) 101272

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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

Original article

Assessing the role of migratory birds in the introduction of ticks and tick-borne pathogens from African countries: An Italian experience

Ilaria Pascucci[a,*], Marco Di Domenico[a], Giulia Capobianco Dondona[a], Annapia Di Gennaro[a], Andrea Polci[a], Andrea Capobianco Dondona[a], Elisa Mancuso[a], Cesare Cammà[a], Giovanni Savini[a], Jacopo G. Cecere[b], Fernando Spina[b], Federica Monaco[a]

[a] Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale" Campo Boario, 64100, Teramo, Italy
[b] Area Avifauna Migratrice, Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA), via Ca' Fornacetta 9, I-40064, Ozzano Emilia, BO, Italy

ARTICLE INFO

Keywords:
Tick-borne pathogens
Migratory birds
Flavivirus

ABSTRACT

The continuous flow of billions of birds between Africa and Europe creates an "ecological bridge" between physically remote areas. Migratory birds fly south from their breeding grounds during late summer/fall and fly back in spring. These movements regulate the spread of internal and external parasites, as well as pathogens of potential public health concern. The aim of the present study was to investigate the possible introduction of exotic tick species and tick-borne pathogens into Europe via migratory birds. At the bird observatory of Ventotene island (Italy), 443 feeding ticks were collected from 249 birds captured and ringed during their northbound migration in spring 2013. Each tick was identified by morphological and molecular methods and then tested for bacterial and viral pathogens: *Borrelia burgdorferi* s.l., *Rickettsia* spp., *Ehrlichia ruminantium* and *Coxiella burnetii*, Crimean Congo haemorrhagic fever virus (CCHFV) and *Flavivirus*. Morphological and molecular identification confirmed *Hyalomma rufipes* as the most abundant species among the collected arthropods (366/443; 82.6%) followed by *Hyalomma marginatum* (10/433; 2.3%). *Rickettsia aeschlimannii* was identified in 158 ticks, while one engorged *Amblyomma variegatum* nymph was infected with *Rickettsia africae*. The other bacteria were not detected in any specimen. Among viruses, RNA belonging to West Nile virus and other *Flavivirus* were detected whereas all ticks were negative for CCHFV RNA. These results confirm how migratory birds play a role in carrying *Rickettsia*-infected ticks, as well as viruses of zoonotic importance, from Africa into Europe. To what extent tick species are capable of establishing a permanent population once introduced in naïve areas, is far from defined and deserve further investigation.

1. Introduction

Twice a year, more than two billions of birds fly between breeding grounds in Europe and their non-breeding areas in Africa (Hahn et al., 2009). During the long trips, birds can act as dispersal vehicles of plants (Cecere et al., 2010), invertebrates (Frisch et al., 2007) and pathogens (Owen et al., 2006) between continents. Many migratory avian species can be host of immature ticks of medical and veterinary importance and may play a significant role in the geographic distribution of ticks and pathogens. Birds easily cross geographical and ecological barriers, and move faster than wingless hosts. Therefore, birds can potentially spread pathogens far beyond their original home ranges.

Migration is part of the wide phenomenon of bird movements and it can be defined as the regular seasonal flight between breeding and non-breeding grounds (Berthold, 2000). According to this definition, most migratory European birds can be classified as short-distance migrants when they move between European breeding areas and the southern non-breeding Western Palearctic region (Europe and Northern Africa) and long-distance or trans-Saharan migrants when they spend the non-breeding period in sub-Saharan Africa. Birds are vehicles of ticks and related pathogens and they could sustain both viraemic and non-viraemic tick-borne pathogen transmission. Non-viremic transmission occurs during co-feeding as demonstrated in other vertebrates for some tick-borne pathogens (Labuda and Randolph, 1999; Voordouw, 2015).

Moreover, birds are recognized as reservoirs of West Nile virus (WNV) and Usutu virus (USUV) and the presence of different WNV genetic lineages and clades (Monaco et al., 2015; Savini et al., 2008; Sotelo et al., 2009) in the Mediterranean basin is attributable to the movement of birds in areas with abundance of mosquitoes and ticks (Kolodziejek et al., 2014; Lwande et al., 2014). In the present study

* Corresponding author.
E-mail address: i.pascucci@izs.it (I. Pascucci).

https://doi.org/10.1016/j.ttbdis.2019.101272
Received 30 November 2018; Received in revised form 29 July 2019; Accepted 15 August 2019
Available online 16 August 2019
1877-959X/

Fig. 1. Location of Ventotene island in the Mediterranean Basin.

ticks have been collected in spring on the island of Ventotene from staging migrants. The study aims to investigate the role of migratory birds in the introduction of exotic tick species and tick-borne pathogens namely *Ehrlichia ruminantium*, *Rickettsia* spp., *Borrelia burgdorferi* s.l., *Coxiella burnetii*, Crimean Congo haemorrhagic fever virus (CCHFV), and the role of bird-associated ticks as bio-indicators of avian infections, namely mosquito-borne *Flavivirus,* focusing on WNV and USUV.

2. Materials and methods

2.1. Sample collection

Fieldwork was carried out in Ventotene island, an important stop-over site for migrating birds, located in the central Tyrrhenian Sea (coordinates: 40°48'0"N 13°26'0"E, area: 1.54 km^2; Fig. 1). During spring migration, a huge number of migratory birds, mainly songbirds, reach Ventotene after a non-stop flight from Northern African coast (Spina et al., 1993) and spend there a variable lapse of time, from few hours to few days, depending on weather and body conditions (Goymann et al., 2010) resting or feeding on insects or nectar (Cecere et al., 2010) before continuing their northbound journey (Tenan and Spina, 2010).

Birds were captured from the 2nd of April to the 22nd of May 2013 by nine 2.5 m high mist-net transects up to 150 m of length,. Once captured, a total of 3444 birds were identified at species level, sexed, aged, measured, marked with metal rings according to standard ringing procedures and inspected for the presence of tick parasites. All procedures were performed by authorized expert bird-ringers. A total of 443 feeding ticks were collected from 249 birds. Ticks were then stored in 70% ethanol for subsequent laboratory analyses.

2.2. Tick identification

Preliminary morphological identification of ticks was carried out with a stereomicroscope using the dichotomous key described by Manilla (1998).

Ticks collected on wild birds are generally immature and their morphological identification at species level is challenging. Consequently, except for the few nymphs belonging to the *Ixodes* genus, which are morphologically identifiable, all the ticks belonging to other genera were identified at the species level by molecular methods. Ticks were homogenized using Tissue-lyser (Qiagen, Germany) and nucleic acids were extracted with Maxwell 16 LEV simply RNA blood kit (Promega, USA), according to manufacturer's protocol. This kit allows to purify both DNA and RNA. Quality control was performed by the Nanodrop spectrophotometer ND1000 (ThermoFisher Scientific, USA).

A small region of the *12S r*RNA was amplified and sequenced to identify the species (Toma et al., 2014).

Five microliters of extracted DNA were added to the reaction mix prepared with: buffer 1X, $MgCl_2$ 2 mM, dNTPs 200 µM, primers 0,3 µM each, nuclease free water (for a final volume of reaction mix of 45 µl) and the Taq Gold 0.625 U (Thermo Fisher Scientific, USA).

Cycling conditions consisted of an initial denaturation (10 min at 95 °C) followed by 45 cycles of denaturation (30 s at 95 °C), annealing (30 s at 55 °C), and extension (30 s at 72 °C). A final extension at 72 °C for 7 min completed the amplification.

PCR products were checked through electrophoresis on 1% agarose gel and then purified using Expin TM PCR SV Kit (GeneAll Biotechnology, Korea), according to manufacturer's instructions. Purified samples were quantified by Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). Sequencing of the amplicons was conducted by BigDye® Terminator v3.1 Cycle Sequencing Kit

(Applied Biosystems, USA), and Agencourt CleanSEQ Kit (Beckman Coulter, USA) for purification. Analyses were completed on 3130XL Genetic Analyzer (Applied Biosystems, USA).

Sequences were assembled with Contig Express (Vector NTI suite 9.1; Invitrogen, USA) and compared with those available in GenBank database using the Basic Local Alignment Search Tool (BLAST) software.

2.3. Pathogen detection

Specific primers and TaqMan probes were used to detect *B. burgdorferi* s.l. (Michelet et al., 2014), *Rickettsia* spp. (Kato et al., 2013), *R. aeschlimannii* (Jiang et al., 2012), *E. ruminantium* (Steyn et al., 2008) and *C. burnetii* (Panning et al., 2008) in every single tick. real-time PCR reactions were performed on 7900 H T Fast real-time PCR System (Applied Biosystems, USA) using the TaqMan Fast Universal PCR Master Mix 2X (Applied Biosystems, USA) following the manufacturer's instructions. To identify the species of *Rickettsia* spp. DNA positive samples, but negative for *R. aeschlimannii,* a fragment of *ompA* or *ompB* was sequenced according to the protocol of Santibáñez et al. (2013). Sequencing of the PCR products was performed as described above.

The presence of viral pathogens was investigated in pooled nucleic acids except for those extracted from ticks identified as *Hyalomma marginatum,* which were tested individually. Ten µl of nucleic acids extracted from individual ticks were pooled according to the hosting bird and the tick species up to a maximum of 5 individuals. If positive or doubt, individual RNA from the pools was tested to confirm and identify the infected tick. A total of 267 pools and 10 *H. marginatum* individuals were screened for viral RNA by specific real-time RT-PCR assays. In particular, CCHFV was investigated by using the commercial assay RealStar® CCHFV RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) according to the manufacturer's instructions.

Flavivirus were detected according to the protocol of Scaramozzino et al. (2001), whereas WNV and USUV were investigated with the assays of Del Amo et al. (2013) and Cavrini et al. (2011) respectively.

3. Results

From the 3444 screened birds, 249 (7.2%), belonging to 26 species (22 Passeriformes and 4 non-Passeriformes), were infested by ticks. All the parasitized birds were regular migratory species and the 23 species hosting infected ticks were trans-Saharan migrants.

No ticks were found on the few species resident in the island.

Among the 443 ticks collected, most of them were nymphs (228 individuals; 51.5%), the remaining ticks consisted of 214 larvae (48.3%), and 1 adult (0.2%).

Morphological and molecular identification confirmed *Hyalomma rufipes* as the most abundant species of the collection (366/443; 82.6%) followed by *H. marginatum* (10/433; 2.3%). Table 1 provides the details of tick species collected during the study.

Rickettsia spp. were detected in 159 tick samples by real-time PCR.

Specifically, *R. africae* was detected in one engorged *Amblyomma variegatum* nymph collected from an Icterine warbler (*Hippolais icterina*), while *R. aeschlimannii* was identified in 158 ticks resulting the most abundant pathogen detected in this study (Table 2). In particular, it was identified in *H. rufipes* (n = 140), *H. marginatum* (n = 5), *Hyalomma* spp. (n = 12) and *H. truncatum* (n = 1) collected from 23 different species of trans-Saharan birds (Table 2) whereas *E. ruminantium, C. burnetii* and *B. burgdorferi* s.l. were not detected in any of the tested ticks.

Flavivirus RNA was detected in 6 pooled samples and 4 single arthropods of *H. rufipes*, as well as in one engorged nymph of *H. marginatum*, all collected from long-distance migrators (Table 3). WNV RNA was identified in two nymphs, one *H. marginatum* and one *Amblyomma* sp., that also tested positive for *Flavivirus* RNA; these ticks were respectively collected from a Western yellow wagtail (*Motacilla flava*) and an Icterine warbler, both trans-Saharan migrants (Table 3). All ticks tested negative for USUV and CCHFV RNA.

Table 1
Results of tick identification. All the ticks were differentiated at species level by means of *12S* partial sequence analysis, but *Ixodes frontalis* by morphology; the 54 *Hyalomma* sp. ticks failed in species identification for both morphological and molecular methods. The *12S* partial sequence analysis of the 4 inconclusive *Amblyomma* gave 100% identity with *Amblyomma* sp.

Species	Stages				
	Larvae	Nymphs	Adults	Total	(%)
Amblyomma sp.	–	4	–	4	0.9
Amblyomma variegatum	–	1	–	1	0.2
Hyalomma truncatum	1	2	–	3	0.6
Hyalomma marginatum	3	7	–	10	2.3
Hyalomma rufipes	172	194	–	366	82.6
Hyalomma sp.	38	16	–	54	12.2
Ixodes ricinus	–	3	–	3	0.7
Ixodes frontalis	–	1	1	2	0.4

4. Discussion

The spread of ticks and tick-borne pathogens outside their geographical habitat by migratory birds has been well documented for strictly ornithophilic (Hornok et al., 2016) as well as for opportunistic ticks feeding on birds at larval and nymphal stages (Hasle, 2013; Smith et al., 1996). This has already been demonstrated for *H. marginatum* and *H. rufipes*, that are frequently found on birds flying from Africa (Jaenson et al., 1994; Jameson et al., 2012) and, to a lesser extent, for some species of the genus *Amblyomma* (European Food Safety Authority, 2010a).

Tick species identified in the present survey confirmed the bird migratory behaviour as one of possible drivers for dispersal of these parasites along the migratory flyways (Toma et al., 2014; Wallménius et al., 2014). The predominance of *Hyalomma* ticks of the *H. marginatum* complex and particularly of the species *H. rufipes* in our samples confirms the results of previous studies carried out in Ventotene island (Di Lecce et al., 2018; Toma et al., 2014). The geographic distribution of *H. rufipes* is mainly in sub-Saharan Africa where it is one of the most common in the *Hyalomma* genus, feeding on cattle, antelopes, horses and other large herbivores (European Food Safety Authority, 2010b). Its dispersal along migratory routes has been used to explain its occasional presence in central Europe (Chitimia-Dobler et al., 2016; Nijhof et al., 2007) as well in the Mediterranean basin (Ruiz-Fons et al., 2006), where an established small population of *H. rufipes* has been recently described in the West Aegean region of Turkey (Bakirci et al., 2011). The second most abundant species was the Eurasian *H. marginatum*, which is one of the most common species of the tick fauna in the Mediterranean region, including Italy. This species has frequently been found on migratory birds whose southernmost movements are usually limited to the Mediterranean basin. Nevertheless, we collected *H. marginatum* also from long-distance migrants (Table 3) suggesting a possible encounter between ticks and birds also at stop-over sites in North Africa, where birds shortly stop to rest before resuming their flight across the Mediterranean.

The presence of the *H. marginatum* has been reported with increasing frequency in the last decades along the northernmost latitudes, in Germany (Kampen et al., 2007), in the United Kingdom (Jameson and Medlock, 2009) and in Russia (Movila et al., 2013). However, it is well known that relatively dry and warm regions are the preferred habitat of *H. marginatum* (Manilla, 1998) and it is unlikely that the limited number of individuals carried by migratory birds may establish a population in free areas outside the ideal temperature range for the species (European Food Safety Authority, 2010b; Gray et al., 2009).

Amblyomma ticks are common in sub-Saharan Africa feeding on a

Table 2
Details of *Rickettsia* spp. detection and species identification. Results of molecular test for bacterial pathogens on ticks, *Rickettsia africae* was identified by *ompB* partial sequence, while neither *ompA* nor *ompB* successfully identified at species level *Rickettsia* spp. *R. aeschlimannii* in all the 158 positive ticks, was identified by means of specific real-time PCR.

Bird species	Tick species	Larval stage	*Rickettsia* spp.
European turtle dove (*Streptopelia turtur*)	*Hyalomma rufipes*	larva (2)	*R. aeschlimannii*
	Hyalomma sp.	larva (1)	*R. aeschlimannii*
Common cuckoo (*Cuculus canorus*)	*Hyalomma rufipes*	nymph (1)	*R. aeschlimannii*
European nightjar (*Caprimulgus europaeus*)	*Hyalomma marginatum*	nymph (2)	*R. aeschlimannii*
Eurasian wryneck (*Jynx torquilla*)	*Hyalomma rufipes*	larva (2)	*R. aeschlimannii*
	Hyalomma sp.	larva (1)	*R. aeschlimannii*
Eurasian golden oriole (*Oriolus oriolus*)	*Hyalomma rufipes*	nymph (4)	*R. aeschlimannii*
Willow warbler (*Phylloscopus trochilus*)	*Hyalomma rufipes*	larva (11)	*R. aeschlimannii*
		larva (9)	*R. aeschlimannii*
	Hyalomma sp.	larva (5)	*R. aeschlimannii*
		larva (2)	*R. aeschlimannii*
Wood warbler *(Phylloscopus sibilatrix)*	*Hyalomma rufipes*	nymph (13), larva (7)	*R. aeschlimannii*
		larva (1)	*R. aeschlimannii*
	Hyalomma sp.	larva (1)	*R. aeschlimannii*
Great reed warbler (*Acrocephalus arundinaceus*)	*Hyalomma rufipes*	nymph (1), larva (1)	*R. aeschlimannii*
		larva (1)	*R. aeschlimannii*
Sedge warbler (*Acrocephalus schoenobaenus*)	*Hyalomma rufipes*	nymph (3)	*R. aeschlimannii*
Reed warbler (*Acrocephalus scirpaceus*)	*Hyalomma rufipes*	larva (1)	*R. aeschlimannii*
Icterine warbler (*Hyppolais icterina*)	*Amblyomma variegatum*	nymph (1)	*R. africae*
	Hyalomma rufipes	nymph (2), larva (4)	*R. aeschlimannii*
Garden warbler (*Sylvia borin*)	*Hyalomma rufipes*	larva (4)	*R. aeschlimannii*
Common whitethroat (*Sylvia communis*)	*Hyalomma rufipes*	nymph (16), larva (4)	*R. aeschlimannii*
Subalpine warbler (*Sylvia cantillans*)	*Hyalomma rufipes*	larva (1)	*R. aeschlimannii*
Common nightingale (*Luscinia megarhynchos*)	*Hyalomma rufipes*	nymph (2), larva (1)	*R. aeschlimannii*
	Hyalomma sp.	larva (1)	*R. aeschlimannii*
Spotted flycatcher (*Muscicapa striata*)	*Hyalomma rufipes*	larva (2)	*R. aeschlimannii*
European pied flycatcher (*Ficedula hypoleuca*)	*Hyalomma rufipes*	nymph (9), larva (5)	*R. aeschlimannii*
Collared flycatcher (*Ficedula albicollis*)	*Hyalomma rufipes*	nymph (2)	*R. aeschlimannii*
Common redstart (*Phoenicurus phoenicurus*)	*Hyalomma marginatum*	nymph (2)	*R. aeschlimannii*
	Hyalomma rufipes	nymph (7), larva (2)	*R. aeschlimannii*
	Hyalomma sp.	larva (2)	*R. aeschlimannii*
Whinchat (*Saxicola rubetra*)	*Hyalomma rufipes*	nymph (11), larva (8)	*R. aeschlimannii*
	Hyalomma rufipes	larva (1)	*R. aeschlimannii*
	Hyalomma truncatum	larva (1)	*R. aeschlimannii*
Northern wheatear (*Oenanthe oenanthe*)	*Hyalomma rufipes*	nymph (1)	*R. aeschlimannii*
Western yellow wagtail (*Motacilla flava*)	*Hyalomma rufipes*	larva (1)	*R. aeschlimannii*
	Hyalomma marginatum	nymph (1)	*R. aeschlimannii*
Tree pipit (*Anthus trivialis*)	*Hyalomma rufipes*	nymph (1)	*Rickettsia sp.*

wide range of hosts, including reptiles. In particular, *A. variegatum*, the tropical bont tick – TBT, is widespread in tropical areas up to the southern part of the continent. It has a three-host life cycle, with larvae, nymphs and adults feeding on separate hosts and immatures frequently feeding on birds (European Food Safety Authority, 2010b). Thus, the sporadic finding of immature on long-distance migratory birds in Italy and Greece is not surprising (Albanese et al., 1971; Papadopoulos et al., 1996; Toma et al., 2014; Wallménius et al., 2014). Conversely, the recent report of an adult male of *Amblyomma variegatum* in Corsica (Cicculli et al., 2019) suggests possible risk of establishment of a wild *Amblyomma* population in southern Europe. Hence, *Amblyomma* can be considered one of the major threats for the area as foreseen by the analysis of environmental and climatic parameters in different predictive models (Pascucci et al., 2007; Sutherst and Maywald, 1985).

Besides, the tick collected in the French island was infected by *Rickettsia africae,* the causative agent of the African tick bite fever, the most widespread tick-borne rickettsiosis in sub-Saharan Africa. Similarly, we identified *R. africae* in a nymph of *A. variegatum* even though the most abundant bacterium was *R. aeschlimannii*. Since its detection in Morocco in 1997 from *H. marginatum* (Beati et al., 1997), it

Table 3
Details of the tick specimen positive to the *Flavivirus* and WNV specific RT-PCRs.

Bird species	Tick species	Larval stage	Target	N. ticks/pool
European nightjar (*Caprimulgus europaeus*)	*H. marginatum*	nymph	*Flavivirus*	1
Western yellow wagtail (*Motacilla flava*)	*H. marginatum*	nymph	*Flavivirus*/WNV	1
Wood warbler (*Phylloscopus sibilatrix*)	*H. rufipes*	nymph	*Flavivirus*	4
Wood warbler (*Phylloscopus sibilatrix*)	*H. rufipes*	nymph	*Flavivirus*	3
Wood warbler (*Phylloscopus sibilatrix*)	*H. rufipes*	nymph	*Flavivirus*	4
Icterine warbler (*Hippolais icterina*)	*Amblyomma* sp.	nymph	*Flavivirus*/WNV	1
Icterine warbler (*Hippolais icterina*)	*H. rufipes*	nymph	*Flavivirus*	1
European pied flycatcher (*Ficedula hypoleuca*)	*H. rufipes*	nymph	*Flavivirus*	3
European pied flycatcher (*Ficedula hypoleuca*)	*H. rufipes*	nymph	*Flavivirus*	4
European pied flycatcher (*Ficedula hypoleuca*)	*H. rufipes*	larva	*Flavivirus*	1
European pied flycatcher (*Ficedula hypoleuca*)	*H. rufipes*	larva	*Flavivirus*	3
European pied flycatcher (*Ficedula hypoleuca*)	*H. rufipes*	nymph	*Flavivirus*	1
Whinchat (*Saxicola rubetra*)	*H. rufipes*	nymph	*Flavivirus*	1

has been responsible for outbreaks of human spotted fever in Italy and is considered an important emerging pathogen (Tosoni et al., 2016). Overall, the prevalence for *Rickettsia* spp. (36%) in our samples is comparable with the one reported in previous studies by Wallménius et al. (2014) and Toma et al. (2014).

Coxiella burnetii was not detected in any collected tick even though the pathogen is worldwide distributed and recognizes a wide range of tick vectors. Puzzling results were reported by previous studies: *C. burnetii* DNA was not detected in ticks collected on resident and short and long-distance migratory birds in Camargue (Socolovschi et al., 2012), whereas it was detected in 2.7% of ticks collected on resident birds in Slovakia (Berthová et al., 2016), in 20% of ticks from migrating and local birds in Cyprus (Ioannou et al., 2009) and in 30.6% of ticks on migratory birds in Italy (Toma et al., 2014).

Finally, the lack of detection of *B. burgdorferi* s.l. and *E. ruminantium* DNA could be explained by the small number of tick vectors collected during the study(*Ixodes* and *Amblyomma*).

The high number of positive results for *Flavivirus* RNA confirms that ornithophilic ticks like *H. rufipes* may serve as bio-indicator of avian infection by mosquito-borne *Flavivirus* such as WNV and USUV. Even though in some specimens it was not possible to identify the viruses to species level, the results address the need for continuous monitoring of their potential introduction.

Of particular interest is the detection of WNV RNA in one *H. marginatum* collected from a Western yellow wagtail (*Motacilla flava*) during the study period. The bird belongs to the Passeriformes order, which includes species highly susceptible to WNV infection, capable of developing viremia and transmit the virus to vectors (Komar et al., 2003).

The broad spectrum of *H. marginatum* hosts together with the high number of vertebrates susceptible to WNV infection may enhance the transmission of the virus to local susceptible hosts allowing its introduction into novel areas. Ticks of the genus *Amblyomma* also showed the potential to carry viral pathogens since WNV RNA was detected during the laboratory investigations.

The lack of CCHFV RNA detection in any of our specimen can be associated with a low level of viral circulation in the non-breeding sites where the virus is endemic or to the relatively small number of sampled ticks. Nevertheless, the finding does not exclude the potential of novel introductions by migratory birds. In fact, CCHFV has been recently detected in Italy in a *H. rufipes* nymph collected from a Whinchat (*Saxicola rubetra*) (Mancuso et al., 2019) as well as being sporadically reported in ticks collected from migratory birds in Greece (Lindeborg et al., 2012), Morocco (Palomar et al., 2013) and Turkey (Al-Abri et al., 2017). Migratory birds could indeed be the responsible of the recent CCHFV introduction in Spain (Estrada-Peña et al., 2012). Therefore, availability of more information on the role played by birds in the dispersal of the virus is unanimously considered crucial by researchers (Spengler et al., 2018). Both *H. rufipes* and *H. marginatum* immatures spend extraordinary long time on the birds (12–26 days) (European Food Safety Authority, 2010a) and this could allow passive transport of live virus over long distance.

The presence of resident or established tick populations and the circulation of tick-borne pathogens in Ventotene island have never been investigated, so far. There is a low risk of the establishment of a viable population of exotic ticks due to the lack of suitable hosts (large herbivores) in the island and winter climatic conditions. However, since the Mediterranean region is highly vulnerable to climatic changes, it's essential to carry out further research and to develop possible surveillance strategies.

5. Conclusions

Considering an influx of more than two billions of birds linking Africa and Europe (Hahn et al., 2009), millions of ticks are probably transported every year, and the introduction of exotic species could be more frequent than expected. Our findings confirm the assumption that migratory birds may play a role in spreading ticks and tick-borne pathogens and, consequently, contribute to expand their geographic distribution. The high number of real-time PCR positive samples of *R. aeschlimannii* deserves particular attention and should raise awareness for public health

Even though the tick dispersal does not imply the establishment of the different species in new territories, global warming can move the northern limit of their original distribution range, together with possible new risks of pathogen introduction.

Funding

This work has been supported by the research grant RF-2009-1547447: Origin and evolution of recent vector-borne virus incursions in the Mediterranean basin from the Ministry of Health Research, Italy.

Ethical statement

1) This material has not been published in whole or in part elsewhere;
2) The manuscript is not currently being considered for publication in another journal;
3) All authors have been personally and actively involved in substantive work leading to the manuscript, and will hold themselves jointly and individually responsible for its content.

Declaration of Competing Interest

None.

Acknowledgement

We gratefully acknowledge the support of Dr. Sara Riello and all volunteers operating the ringing station of Ventotene. We warmly thank the Riserva Naturale Statale "Isole di Ventotene e S. Stefano" for logistic and financial support. Results from the Progetto Piccole Isole ISPRA

References

Al-Abri, S.S., Al Abaidani, I., Fazlalipour, M., Mostafavi, E., Leblebicioglu, H., Pshenichnaya, N., Memish, Z.A., Hewson, R., Petersen, E., Mala, P., Nhu Nguyen, T.M., Rahman Malik, M., Formenty, P., Jeffries, R., 2017. Current status of Crimean-Congo haemorrhagic fever in the World Health Organization Eastern Mediterranean Region: issues, challenges, and future directions. Int. J. Infect. Dis. 58, 82–89. https://doi.org/10.1016/j.ijid.2017.02.018.

Albanese, M., Bruno Smiraglia, C., Lavagnino, A., 1971. Notizie sulle zecche di Sicilia con segnalazione di *Hyalomma detritum* e *Amblyomma variegatum*. Riv. Parassitol. 32, 273–276.

Bakirci, S., Saralyi, H., Aydin, L., Latif, A., Eren, H., Karagenç, T., 2011. *Hyalomma rufipes* (Koch, 1844) infesting cattle in the West Aegean region of Turkey. Turkish J. Vet. Anim. Sci. 35, 359–363. https://doi.org/10.3906/vet-0910-160.

Beati, L., Meskini, M., Thiers, B., Raoult, D., 1997. *Rickettsia aeschlimannii* sp. nov., a new spotted fever group rickettsia associated with *Hyalomma marginatum* ticks. Int. J. Syst. Bacteriol. 47, 548–554. https://doi.org/10.1099/00207713-47-2-548.

Berthová, L., Slobodník, V., Slobodník, R., Olekšák, M., Sekeyová, Z., Svitálková, Z., Kazimírová, M., Špitalská, E., 2016. The natural infection of birds and ticks feeding on birds with *Rickettsia* spp. and *Coxiella burnetii* in Slovakia. Exp. Appl. Acarol. 68, 299–314. https://doi.org/10.1007/s10493-015-9975-3.

Cavrini, F., Della Pepa, M.E., Gaibani, P., Pierro, A.M., Rossini, G., Landini, M.P., Sambri, V., 2011. A rapid and specific real-time RT-PCR assay to identify Usutu virus in human plasma, serum, and cerebrospinal fluid. J. Clin. Virol. 50, 221–223. https://doi.org/10.1016/j.jcv.2010.11.008.

Cecere, J.G., Matricardi, C., Frank, B., Imperio, S., Spina, F., Gargallo, G., Barboutis, C., Boitani, L., 2010. Nectar exploitation by songbirds at mediterranean stopover sites. Ardeola 57, 143–157.

Chitimia-Dobler, L., Nava, S., Bestehorn, M., Dobler, G., Wölfel, S., 2016. First detection of *Hyalomma rufipes* in Germany. Ticks Tick. Dis. 7, 1135–1138. https://doi.org/10.1016/j.ttbdis.2016.08.008.

Cicculli, V., de Lamballerie, X., Charrel, R., Falchi, A., 2019. First molecular detection of *Rickettsia africae* in a tropical bont tick, *Amblyomma variegatum*, collected in Corsica, France. Exp. Appl. Acarol. 77, 207–214. https://doi.org/10.1007/s10493-018-00336-2.

Del Amo, J., Sotelo, E., Fernández-Pinero, J., Gallardo, C., Llorente, F., Agüero, M., Jiménez-Clavero, M.A., 2013. A novel quantitative multiplex real-time RT-PCR for

the simultaneous detection and differentiation of West Nile virus lineages 1 and 2, and of Usutu virus. J. Virol. Methods 189, 321–327. https://doi.org/10.1016/j.jviromet.2013.02.019.
Di Lecce, I., Bazzocchi, C., Cecere, J.G., Epis, S., Sassera, D., Villani, B.M., Bazzi, G., Negri, A., Saino, N., Spina, F., Bandi, C., Rubolini, D., 2018. Patterns of *Midichloria* infection in avian-borne African ticks and their trans-Saharan migratory hosts. Parasite Vector 11, 1–11. https://doi.org/10.1186/s13071-018-2669-z.
Estrada-Peña, A., Palomar, A.M., Santibáñez, P., Sánchez, N., Habela, M.A., Portillo, A., Romero, L., O.J, 2012. Crimean-Congo hemorrhagic fever virus in ticks, southwestern Europe, 2010. Emerg. Infect. Dis. 18, 179–180. https://doi.org/10.3201/eid1801.111040.
European Food Safety Authority, 2010a. Scientific opinion on geographic distribution of tick-borne infections and their vectors in Europe and the other regions of the Mediterranean Basin. EFSA J. 8, 1723. https://doi.org/10.2903/j.efsa.2010.1723.
European Food Safety Authority, 2010b. Scientific opinion on the role of tick vectors in the epidemiology of Crimean-Congo hemorrhagic fever and African swine fever in Eurasia. EFSA J. 8, 1–156. https://doi.org/10.2903/j.efsa.2010.1703.
Frisch, D., Green, A.J., Figuerola, J., 2007. High dispersal capacity of a broad spectrum of aquatic invertebrates via waterbirds. Aquat. Sci. 69, 568–574. https://doi.org/10.1007/s00027-007-0915-0.
Goymann, W., Spina, F., Ferri, A., Fusani, L., 2010. Body fat influences departure from stopover sites in migratory birds: evidence from whole island telemetry. Biol. Lett. 6, 478–481. https://doi.org/10.1098/rsbl.2009.1028.
Gray, J.S., Dautel, H., Estrada-Peña, A., Kahl, O., Lindgren, E., 2009. Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip. Perspect. Infect. Dis. 1–12. https://doi.org/10.1155/2009/593232.
Hahn, S., Bauer, S., Liechti, F., 2009. The natural link between Europe and Africa - 2.1 billion birds on migration. Oikos 118, 624–626. https://doi.org/10.1111/j.1600-0706.2008.17309.x.
Hasle, G., 2013. Transport of ixodid ticks and tick-borne pathogens by migratory birds. Front. Cell. Infect. Microbiol. 3, 1–6. https://doi.org/10.3389/fcimb.2013.00048.
Hornok, S., Flaisz, B., Takács, N., Kontschán, J., Csörgo, T., Csipak, A., Jaksa, B.R., Kováts, D., 2016. Bird ticks in Hungary reflect western, southern, eastern flyway connections and two genetic lineages of *Ixodes frontalis* and *Haemaphysalis concinna*. Parasite. Vector. 9, 1–9. https://doi.org/10.1186/s13071-016-1365-0.
Ioannou, I., Chochlakis, D., Kasinis, N., Anayiotos, P., Lyssandrou, A., Papadopoulos, B., Tselentis, Y., Psaroulaki, A., 2009. Carriage of *Rickettsia* spp., *Coxiella burnetii* and *Anaplasma* spp. by endemic and migratory wild birds and their ectoparasites in Cyprus. Clin. Microbiol. Infect. 15, 158–160. https://doi.org/10.1111/j.1469-0691.2008.02207.x.
Jaenson, T.G., Talleklint, L., Lundqvist, L., Olsen, B., Chirico, J., Mejlon, H., 1994. Geographical distribution, host associations, and vector roles of ticks (Acari Ixodidae, argasidae) in Sweden. J. Med. Entomol. 31, 240–256.
Jameson, L.J., Medlock, J.M., 2009. Results of HPA tick surveillance in Great Britain. Vet. Rec. 165https://doi.org/10.1136/vr.165.5.154-a. 154–154.
Jameson, L.J., Morgan, P.J., Medlock, J.M., Watola, G., Vaux, A.G.C., 2012. Importation of *Hyalomma marginatum*, vector of Crimean-Congo haemorrhagic fever virus, into the United Kingdom by migratory birds. Ticks Tick. Dis. 3, 95–99. https://doi.org/10.1016/j.ttbdis.2011.12.002.
Jiang, J., You, B.J., Liu, E., Apte, A., Yarina, T.R., Myers, T.E., Lee, J.S., Francesconi, S.C., O'Guinn, M.L., Tsertsvadze, N., Vephkhvadze, N., Babuadze, G., Sidamonidze, K., Kokhreidze, M., Donduashvili, M., Onashvili, T., Ismayilov, A., Agayev, N., Aliyev, M., Muttalibov, N., Richards, A.L., 2012. Development of three quantitative real-time PCR assays for the detection of *Rickettsia raoultii*, *Rickettsia slovaca*, and *Rickettsia aeschlimannii* and their validation with ticks from the country of Georgia and the Republic of Azerbaijan. Ticks Tick. Dis. 3, 326–330. https://doi.org/10.1016/j.ttbdis.2012.10.004.
Kampen, H., Poltz, W., Hartelt, K., Wölfel, R., Faulde, M., 2007. Detection of a questing *Hyalomma marginatum marginatum* adult female (Acari, Ixodidae) in southern Germany. Exp. Appl. Acarol. 43, 227–231. https://doi.org/10.1007/s10493-007-9113-y.
Kato, C.Y., Chung, I.H., Robinson, L.K., Austin, A.L., Dasch, G.A., Massunga, R.F., 2013. Assessment of real-time PCR assay for detection of *Rickettsia* spp. And *Rickettsia rickettsii* in banked clinical samples. J. Clin. Microbiol. 51, 314–417. https://doi.org/10.1128/JCM.01723-12.
Kolodziejek, J., Marinov, M., Kiss, B.J., Alexe, V., Nowotny, N., 2014. The complete sequence of a West Nile virus lineage 2 strain detected in a *Hyalomma marginatum marginatum* tick collected from a song thrush (*Turdus philomelos*) in Eastern Romania in 2013 revealed closest genetic relationship to strain Volgograd 2007. PLoS One 9. https://doi.org/10.1371/journal.pone.0109905.
Komar, N., Langevin, S., Hinten, S., Nemeth, N.M., Edwards, E., Hettler, D.L., Davis, B.S., Bowen, R.A., Bunning, M.L., 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg. Infect. Dis. 9, 311. https://doi.org/10.3201/eid0903.020628.
Labuda, M., Randolph, S.E., 1999. Survival strategy of tick-borne encephalitis virus: cellular basis and environmental determinants. Zentralblatt für Bakteriol. 289, 513–524. https://doi.org/10.1016/S0934-8840(99)80005-X.
Lindeborg, M., Barboutis, C., Ehrenborg, C., Fransson, T., Jaenson, T.G.T., Lindgren, P.-E., Lundkvist, Å., Nyström, F., Salaneck, E., Waldenström, J., Olsen, B., 2012. Migratory birds, ticks, and Crimean-Congo hemorrhagic fever virus. Emerg. Infect. Dis. 18, 2095–2097. https://doi.org/10.3201/eid1812.120718.
Lwande, O.W., Venter, M., Lutomiah, J., Michuki, G., Rumberia, C., Gakuya, F., Obanda, V., Tigoi, C., Odhiambo, C., Nindo, F., Symekher, S., Sang, R., 2014. Whole genome phylogenetic investigation of a West Nile virus strain isolated from a tick sampled from livestock in north eastern Kenya. Parasite. Vector 7, 1–10. https://doi.org/10.1186/s13071-014-0542-2.
Mancuso, E., Toma, L., Polci, A., d'Alessio, S.G., Di Luca, M., Orsini, M., Di Domenico, M., Marcacci, M., Mancini, M., Spina, F., Goffredo, M., Monaco, F., 2019. Crimean-Congo hemorrhagic fever virus genome in tick from migratory bird, Italy. Emerg. Infect. Dis. 25, 1418–1420. https://doi.org/10.3201/eid2507.181345.
Manilla, G., 1998. Fauna d'Italia—Ixodida. . Edizioni Calderini de Il Sole 24 ORE Edagricole, Bologna.
Michelet, L., Delannoy, S., Devillers, E., Umhang, G., Aspan, A., Juremalm, M., Chirico, J., van der Wal, F.J., Sprong, H., Boye Pihl, T.P., Klitgaard, K., BÃ dker, R., Fach, P., Moutailler, S., 2014. High-throughput screening of tick-borne pathogens in Europe. Front. Cell. Infect. Microbiol. 4, 1–13. https://doi.org/10.3389/fcimb.2014.00103.
Monaco, F., Goffredo, M., Briguglio, P., Pinoni, C., Polci, A., Iannetti, S., Marruchella, G., Di Francesco, G., Di Gennaro, A.P., Pais, M., Teodori, L., Bruno, R., Catalani, M., Ruiu, A., Lelli, R., Savini, G., 2015. The 2011 West Nile disease outbreak in Sardinia region. Italy. Vet. Ital. 51, 5–16. https://doi.org/10.12834/VetIt.260.2386.2.
Movila, A., Alekseev, A.N., Dubinina, H.V., Toderas, I., 2013. Detection of tick-borne pathogens in ticks from migratory birds in the Baltic region of Russia. Med. Vet. Entomol. 27, 113–117. https://doi.org/10.1111/j.1365-2915.2012.01037.x.
Nijhof, A.M., Bodaan, C., Postigo, M., Nieuwenhuijs, H., Opsteegh, M., Franssen, L., Jebbink, F., Jongejan, F., 2007. Ticks and associated pathogens collected from domestic animals in the Netherlands. Vector-Borne Zoonotic Dis. 7, 585–596. https://doi.org/10.1089/vbz.2007.0130.
Owen, J., Moore, F., Panella, N., Edwards, E., Bru, R., Hughes, M., Komar, N., 2006. Migrating birds as dispersal vehicles for West Nile virus. Ecohealth 3, 79–85. https://doi.org/10.1007/s10393-006-0025-9.
Palomar, A.M., Portillo, A., Santibáñez, P., Mazuelas, D., Arizaga, J., Crespo, A., Gutiérrez, Ó., Cuadrado, J.F., Oteo, J.A., 2013. Crimean-Congo hemorrhagic fever virus in ticks from migratory birds. Morocco. Emerg. Infect. Dis. 19, 260–263. https://doi.org/10.3201/eid1902.121193.
Panning, M., Kilwinski, J., Greiner-Fischer, S., Peters, M., Kramme, S., Frangoulidis, D., Meyer, H., Henning, K., Drosten, C., 2008. High throughput detection of *Coxiella burnetii* by real-time PCR with internal control system and automated DNA preparation. BMC Microbiol. 8, 1–8. https://doi.org/10.1186/1471-2180-8-77.
Papadopoulos, B., Morel, P., Aeschlimann, A., 1996. Ticks of domestic animals in the Macedonia region of Greece. Vet. Parasitol. 63, 25–40.
Pascucci, I., Conte, A., Scacchia, M., 2007. Use of geographic information systems to identify areas at risk of introducing *Amblyomma variegatum* and *A. Hebraeum* to Italy. Vet. Ital. 43, 655–661.
Ruiz-Fons, F., Fernández-de-Mera, I.G., Acevedo, P., Höfle, U., Vicente, J., de la Fuente, J., Gortazár, C., 2006. Ixodid ticks parasitizing Iberian red deer (*Cervus elaphus hispanicus*) and European wild boar (*Sus scrofa*) from Spain: geographical and temporal distribution. Vet. Parasitol. 140, 133–142. https://doi.org/10.1016/j.vetpar.2006.03.033.
Santibáñez, S., Portillo, A., Santibáñez, P., Palomar, A.M., Oteo, J.A., 2013. Usefulness of rickettsial PCR assays for the molecular diagnosis of human rickettsioses. Enferm. Infecc. Microbiol. Clin. 31, 283–288. https://doi.org/10.1016/j.eimc.2012.08.001.
Savini, G., Monaco, F., Calistri, P., Lelli, R., 2008. Rapid communications phylogenetic analysis of West Nile virus isolated in Italy in 2008. Eurosurveillance 13, 260967–260968.
Scaramozzino, N., Crance, J., Jouan, A., de Briel, D., Stoll, F., Garin, D., 2001. Comparison of *Flavivirus* universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of Flaviviruses targeted to a conserved region of the NS5 gene sequences. J. Clin. Microbiol. 39, 1922–1927. https://doi.org/10.1128/JCM.39.5.1922.
Smith, R.P., Rand, P.W., Lacombe, E.H., Morris, S.R., Holmes, D.W., Caporale, D.A., 1996. Role of bird migration in the long-distance dispersal of *Ixodes dammini*, the vector of Lyme disease. J. Infect. Dis. 174, 221–224. https://doi.org/10.1093/infdis/174.1.221.
Socolovschi, C., Reynaud, P., Kernif, T., Raoult, D., Parola, P., 2012. Rickettsiae of spotted fever group, *Borrelia valaisiana*, and *Coxiella burnetii* in ticks on passerine birds and mammals from the Camargue in the south of France. Ticks Tick. Dis. https://doi.org/10.1016/j.ttbdis.2012.10.019.
Sotelo, E., Fernandez-Pinero, J., Llorente, F., Agüero, M., Hoefle, U., Blanco, J.M., Jiménez-Clavero, M.A., 2009. Characterization of West Nile virus isolates from Spain: new insights into the distinct West Nile virus eco-epidemiology in the Western Mediterranean. Virology 395, 289–297. https://doi.org/10.1016/j.virol.2009.09.013.
Spengler, J.R., Bente, D.A., Bray, M., Burt, F., Hewson, R., Korukluoglu, G., Mirazimi, A., Weber, F., Papa, A., 2018. In: Second International Conference on Crimean-Congo Hemorrhagic Fever. Antiviral Res. 150. pp. 137–147.
Spina, F., Massi, A., Montemaggiori, A., Baccetti, N., 1993. Spring migration across central Mediterranean: general results from the "Progetto Piccole Isole". Die Vogelwarte 37, 1–94.
Steyn, H.C., Pretorius, A., McCrindle, C.M.E., Steinmann, C.M.L., Van Kleef, M., 2008. A quantitative real-time PCR assay for *Ehrlichia ruminantium* using pCS20. Vet. Microbiol. 131, 258–265. https://doi.org/10.1016/j.vetmic.2008.04.002.
Sutherst, R.W., Maywald, G.F., 1985. A computerised system for matching climates in ecology. Agric. Ecosyst. Environ. 13, 281–299. https://doi.org/10.1016/0167-8809(85)90016-7.
Tenan, S., Spina, F., 2010. Timing and condition-related effects on recapture probability, mass change and stopover length of spring migrating songbirds on A small Mediterranean island. Ardeola 57, 121–132.
Toma, L., Mancini, F., Di Luca, M., Cecere, J.G., Bianchi, R., Khoury, C., Quarchioni, E., Manzia, F., Rezza, G., Ciervo, A., 2014. Detection of microbial agents in ticks collected from migratory birds in central Italy. Vector-Borne Zoonotic Dis. 14, 199–205. https://doi.org/10.1089/vbz.2013.1458.
Tosoni, A., Mirijello, A., Ciervo, A., Mancini, F., Rezza, G., Damiano, F., Cauda, R.,

Gasbarrini, A., Addolorato, G., 2016. Human *Rickettsia aeschlimannii* infection: first case with acute hepatitis and review of the literature. Eur. Rev. Med. Pharmacol. Sci. 20, 2630–2633.

Voordouw, M.J., 2015. Co-feeding transmission in Lyme disease pathogens. Parasitology 142, 290–302. https://doi.org/10.1017/S0031182014001486.

Wallménius, K., Barboutis, C., Fransson, T., Jaenson, T.G., Lindgren, P.E., Nyström, F., Olsen, B., Salaneck, E., Nilsson, K., 2014. Spotted fever *Rickettsia* species in *Hyalomma* and *Ixodes* ticks infesting migratory birds in the European Mediterranean area. Parasit. Vectors 7, 318. https://doi.org/10.1186/1756-3305-7-318.