

Virus investigation in ticks from migratory birds in Italy

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SUMMARY

The role of migratory birds in circulation tick-borne viruses needs to be better defined. In order to assess the potential role of migratory birds in exotic virus spread, we conducted a study to identify ticks collected from migratory birds in the Central Region of Italy, and performed molecular investigation for Crimea-Congo Hemorrhagic Fever (CCHFV), West Nile fever (WNV) and Usutu (USUV) in the vectors.

A total of 137 competent ticks were collected with predominance of *Hyalomma* species. Although, negative results were obtained for all viruses considered, the high proportion of *Hyalomma* ticks highlights the potential risk for the dissemination of tick-borne viruses through infested migratory birds.

KEY WORDS: Migratory birds, Ticks, *Hyalomma*, *Ixodes ricinus*, CCHFV, WNV, USUV.

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Migratory birds are infested by infectious disease vectors, such as ticks and mosquitoes, and may play an important role in the dispersal of vectors and microbial agents, contributing to their introduction in previously unaffected or naïve geographical areas (Georgopoulou *et al.*, 2008).

We conducted a study to describe ticks collected from migratory birds in the Lazio Region, Central Italy, in order to assess their potential role in exotic virus dispersal. Moreover, we performed molecular analyses in the vectors to detect the occurrence of unusual tick-borne viral agents in our country, such as CCHFV, or West Nile fever virus (WNV) and Usutu virus (USUV) for which it will be interesting to investigate ticks for the other agents that could be carried by ticks.

Ring activities were carried out on Ventotene and Ponza from April 2010 to May 2011, when

huge numbers of migrating birds stage on the small islands during their spring migration, and in Castel Di Guido and in Paliano in October 2010, during the post-breeding period which coincides with the beginning of autumn migration. Ticks were collected and identified on the base of the morphology, then stored at -80°C in order to search CCHFV, WNV, and USUV viruses using molecular tools. Real-Time PCR analyses were conducted as previously describe methods for all viruses (Drosten *et al.*, 2002; Jost *et al.*, 2011; Lanciotti *et al.*, 2000).

Overall, 137 ticks were collected on 41 birds belonging to 17 species, 4 of them being partial migrants and 13 long-distance migrants (Table 1). All ticks collected in the Lazio Region were at early stages: 132 nymphs belonging to genus *Hyalomma*; 3 nymphs and 2 larvae belonging to *Ixodes ricinus* (Table 1). The molecular analysis showed negativity for all viruses investigated.

Our study shows that the prevailing ticks on partial and long-distance migratory birds are *Hyalomma* nymphs, which represent the most competent species for CCHFV. Recently, CCHFV has broadened its geographical range within the Mediterranean basin and the Balkans, as demon-

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TABLE 1 - Birds species and ticks.

Bird species n = 41 (n)	N° <i>Hyalomma</i> spp. (%)	N° <i>Ixodes ricinus</i> (%)
Partial migrants		
<i>Erithacus rubecula</i> (2)	0 (0.0)	4 (80.0)
<i>Turdus merula</i> (4)	4 (3.0)	0 (0.0)
<i>Buteo buteo</i> (1)	7 (5.3)	0 (0.0)
<i>Sylvia atricapilla</i> (2)	2 (1.5)	0 (0.0)
Long distance migrants		
<i>Anthus trivialis</i> (3)	6 (4.5)	0 (0.0)
<i>Motacilla flava</i> (1)	2 (1.5)	0 (0.0)
<i>Phoenicurus phoenicurus</i> (2)	2 (1.5)	0 (0.0)
<i>Saxicola rubetra</i> (5)	20 (15.2)	0 (0.0)
<i>Sylvia communis</i> (7)	18 (13.6)	0 (0.0)
<i>Ficedula hypoleuca</i> (2)	2 (1.5)	0 (0.0)
<i>Muscicapa striata</i> (1)	1 (0.8)	0 (0.0)
<i>Oenanthe hispanica</i> (1)	1 (0.8)	0 (0.0)
<i>Phylloscopus trochilus</i> (1)	1 (0.8)	0 (0.0)
<i>Hippolais polyglotta</i> (1)	1 (0.8)	0 (0.0)
<i>Luscinia megarhynchos</i> (6)	25 (18.9)	0 (0.0)
<i>Lanius senator</i> (1)	1 (0.8)	0 (0.0)
<i>Pernis apivorus</i> (1)	39 (29.5)	1 (20.0)

strated by outbreaks occurred in Bulgaria, Albania, Kosovo, and Turkey (Ergönül 2012). The determinants of CCHFV spread to previously unaffected areas are still partially undefined. Since the prevalence of CCHFV is low even in endemic areas, the lack of detection of CCHFV sequences in *Hyalomma* ticks collected from migratory birds in our study might be due to the relatively small sample size. Thus, our negative findings are not surprising.

Before drawing conclusions, the possible limits of our study should be mentioned. Firstly, some tick-borne viruses, such as TBE, were not considered. On the other hand, we studied mosquito-borne viruses such as WNFV and USUV, for which it is not demonstrated that ticks act as a reservoir; however, some tick species have the potential to act as passive vectors for those viruses.

Third the study had limited power due to the small sample size.

In conclusion, we did not detect CCHFV nor WNFV and USUV in *Hyalomma* ticks collected on migratory birds in Central Italy. However, the high proportion of *Hyalomma* ticks found on migratory birds highlights the potential for the spread of CCHFV and other tick-borne viruses through infested migratory birds.

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