



Monitoring for the possible introduction of Crimean-Congo haemorrhagic fever virus in Italy based on tick sampling on migratory birds and serological survey of sheep flocks



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ABSTRACT

Crimean-Congo haemorrhagic fever (CCHF), endemic in Africa, Asia, Eastern Europe and the Middle East, is caused by a tivirus (CCHFV) transmitted in particular by the *Hyalomma* genus of the Ixodidae family that can remain attached to the host for up to 26 days, which in case of migratory birds allows long distance carriage. Although CCHF in domestic ruminants is usually subclinical, they may become reservoirs and act as sentinels for the introduction and/or circulation of CCHFV.

In this study, possible CCHFV introduction and circulation in Italy were monitored by tick sampling on migratory birds and by a serosurvey conducted on sheep. While bird tick sampling was conducted in thirteen ringing sites of Central and Southern Italy, the serosurvey was performed on flocks grazing in coastal provinces of Central Italy that are stop over areas for birds flying from Africa, where *Hyalomma* ticks and CCHFV are endemic, to Central and Northern Europe.

A total of 282 ticks (80.8% were *Hyalomma* spp.) were collected from 139 (0.28%) migratory birds of the 50,325 birds checked with 0.22% infested by *Hyalomma* spp., involving 22 avian species with a mean number of 1.6 *Hyalomma* spp. per infested bird.

For the serosurvey, 540 sheep sera were randomly collected that resulted all negative when examined by an indirect IgG ELISA, employing a recombinant antigen coded by the CCHFV S gene.

While the present study confirmed the introduction of CCHFV potential vectors in Central Italy, transported by migratory birds arriving from endemic areas, the serosurvey results did not put in evidence the concomitant arrival of the virus in the study area during the survey period. In general, in areas potentially at risk of CCHFV introduction and circulation, structured serological monitoring of susceptible domestic animals represents a rational system for an early detection of virus circulation.

1. Introduction

Crimean-Congo haemorrhagic fever (CCHF) is one of the most widespread tivirus associated diseases, with human cases occurring in Africa, Asia, Eastern Europe and the Middle East (EFSA, 2010). It is considered “emerging” at a global scale (Messina et al., 2015) and evidence of this is that CCHF autochthonous human cases were recently reported for the first time in September 2016, in Western Europe (ECDC, 2016), several thousand kilometres westward respect to the nearest endemic areas. CCHF is caused by a virus (CCHFV) of the *Orthonairovirus* genus, family Bunyaviridae, which is transmitted by

several tick species of the Ixodidae family, especially those of the *Hyalomma* genus (Horak et al., 2001). *Hyalomma* spp. are two-host ticks, moulting from larva to nymphs while attached to their first host, a small mammal or a ground dwelling bird (Randolph and Rogers, 2007). The ticks can remain attached to the primary host for a maximum of 26 days, which in the case of migratory birds, allows them to be transported even over long distances (Hillyard, 1996) that is a well-documented occurrence in many European countries (Molin et al., 2011; Jameson et al., 2012; Mancini et al., 2013). The species *Hyalomma marginatum* is considered in Europe as the most relevant CCHFV vector (Hoogstraal, 1979) and infection in tick populations is

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maintained by both trans-stadial and trans-ovarial transmission. Humans are infected either through tick bites or due to direct contact with infected blood of a mammalian host (Gale et al., 2011).

CCHFV antibodies were detected in many wild and domestic animals, such as European hare (*Lepus europaeus*), house mouse (*Mus musculus*), cow, goat, sheep, donkey, horse, and pig (Nalca and Whitehouse, 2007). Although CCHF in livestock is generally subclinical, domestic ruminants may become reservoirs because of virus transmission from feeding adult ticks in rural environments which poses a high human health risk due to possible direct transmission (Ozkul, 2009; Gale et al., 2010). Despite this, they can act as sentinels for the arrival and/or circulation of CCHFV especially in non-endemic areas (EFSA, 2010). In fact, by the time CCHF human cases are diagnosed, this would represent the tip of an iceberg with underlying ongoing enzootic cycles that more or less involve different mammal host species, as stated by Randolph and Ergonul (2008).

CCHFV can spread over long distances and potentially be introduced in new areas, transported by vectors attached to migratory birds flying thousands of kilometres during their spring migration from current endemic areas such as in those south of the Sahara, Greece and Turkey (Karti et al., 2004; Papa et al., 2008). Several bird species breeding during summer in Europe fly back in autumn south of Sahara. It is estimated that 2.1 billion song and near-passerine birds arrive each spring in Europe (Hahn et al., 2009). On these bases, the risk of CCHFV introduction by this route is considered possible for many countries of Western Europe (Gale et al., 2010) in which naïve populations of the vector are already present (Mild et al., 2010). In view of this, several European countries are in constant alert of the possible arrival of CCHFV, albeit many authors in the last years have downsized the effective CCHFV risk introduction via migratory birds carrying infected ticks (EFSA, 2010; Gale et al., 2010, 2011; Estrada-Peña et al., 2011). In most parts of Europe, where *H. marginatum* is permanently resident, spring temperatures are not sufficiently high for nymphs arriving on migratory birds to moult into adults (Gray et al., 2009). Moreover, Gale et al. (2011) defined that the probability of arrival in Europe of an infected nymph on a migratory bird is 10^{-4} , even if the author does not relate this data to a time interval. Therefore, every spring the probability of an infected nymph arriving on a bird is very low i.e., 1:10000. Nevertheless, introduction of CCHFV infected ticks from Africa through this route was considered the possible explanation of virus recovery in Turkey in 2002 (Leblebicioglu et al., 2014) and in Spain in 2010 and 2016 (Estrada-Peña et al., 2012; ECDC, 2016). Moreover, due to the continuing climatic changes occurring even in the Mediterranean, this region could become permissive for bird transported infected nymphs of the genus *Hyalomma*, to moult on arrival into adults (Gray et al., 2009) which would then potentially infect their mammal hosts with CCHFV.

Adult ticks carried on imported livestock (Jameson and Medlock, 2009) could represent another introductory route of CCHFV into European states; as a matter of fact, thousands of meat horses are annually imported from Eastern Europe to Italy.

In this scenario, an innovative approach was adopted to monitor CCHFV introduction and circulation in Italy, targeting two epidemiological phases of the virus:

- 1) introduction: monitored by tick sampling on migratory birds to evaluate the arrival of potential CCHFV vectors in Italy from endemic areas of Africa and Eastern Europe. This would provide data on the relative prevalence of *Hyalomma* spp. on migratory birds arriving from endemic areas, considered a prerequisite for assessing the probability of infected ticks entering a country (Gale et al., 2010), as strongly advised by Jameson et al. (2012) for areas with resident *Hyalomma* populations;
- 2) circulation: monitored by a serological survey conducted on sheep flocks aimed at detecting CCHFV circulation in coastal areas of Latium and Tuscany, regions of Central Italy, where migratory birds

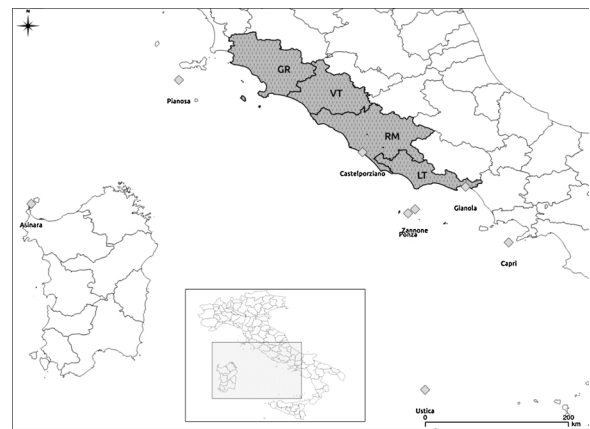


Fig. 1. Sampling area.

Legend

The dotted grey areas represent the provinces in which sheep were sampled (GR = Grosseto; LT = Latina; RM = Rome; VT = Viterbo). Grey diamonds indicate the ticks sampling sites (Castelporziano and Gianola are on the mainland, the remaining are islands). Localisation of the sampling area within Italy is displayed in the central box.

stop over and where the tick vector is present. Indeed, the serological CCHFV monitoring of susceptible animal populations is considered as a valid indicator for the spread of the virus in a territory (Schuster et al., 2016).

2. Materials and methods

2.1. Tick sampling on migratory birds

Tick sampling was conducted between March 20th to May 20th of 2013 and 2014 during the spring migration of birds arriving from Africa, which were captured by professional ornithologists for regular ringing activities. Regardless of the captured species, the birds were checked for the presence of ticks. The capturing sites are represented in Fig. 1 and were the following: Castelporziano (natural area 20 km south of Rome), Pianosa island (Central Tuscany), Ponza and Zannone islands (Southern Latium) and Asinara island (Sardinia) in 2013 and again Castelporziano, Ponza and Zannone, Pianosa, Asinara and Ustica island (Sicily), Gianola (coastal site in southern Latium) and Capri island (Naples) in 2014. Data regarding all bird species winter ranges are available (Spina and Volponi, 2008a,b; BirdLife's Global Species Programme, 2015).

During tick removal, the ornithologists wore individual protective clothing to minimize the risk of exposure to infectious agents that birds and their parasites could be harbouring.

On collection, ticks were preserved in 70% ethanol and transferred to the laboratory for their identification, performed according to Manilla (1998) and Iori et al. (2005). Relative to the *Hyalomma* genus, regarded as the most relevant vector for CCHF transmission, only a generic identification was possible as it is difficult to classify accurately the nymphs at the species level.

2.2. Serological survey

2.2.1. Study area, study population and sampling method

The serosurvey in sheep was set up in the coastal provinces of Latium and Tuscany regions (Central Italy). This was possible because the sheep blood samples collected from these two Italian Regions, within a national program for the control and eradication of brucellosis, are examined at the regional state laboratory where the authors operate. The data on the sheep population of the study area were extrapolated from those reported in June 2013 on the National Data Base (BDN) for livestock registration, defining a study population of 487,000

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