Preventive Veterinary Medicine 149 (2018) 47–52

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

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

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

Claudio De Liberato[a], Raffaele Frontoso[a,*], Adele Magliano[a], Alessandro Montemaggiori[b], Gian Luca Autorino[a], Marcello Sala[a], Andrew Bosworth[c], Maria Teresa Scicluna[a]

[a] Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Via Appia Nuova 1411, 00178 Rome, Italy
[b] Department of Biology and Biotechnology "C. Darwin", Sapienza University - Zoology, Viale dell'Università 32, 00185 Rome, Italy
[c] National Infection Service, Public Health England, Porton Down, Salisbury, Wiltshire, United Kingdom

ARTICLE INFO

Keywords:
Crimean-Congo haemorrhagic fever
Tick
Migratory birds
Serological survey
Sheep
Italy

ABSTRACT

Crimean-Congo haemorrhagic fever (CCHF), endemic in Africa, Asia, Eastern Europe and the Middle East, is caused by a tibovirus (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 tibovirus 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

* Corresponding author.
E-mail address: raffaele.frontoso@izslt.it (R. Frontoso).

https://doi.org/10.1016/j.prevetmed.2017.10.014
Received 30 May 2017; Received in revised form 25 October 2017; Accepted 25 October 2017

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 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).

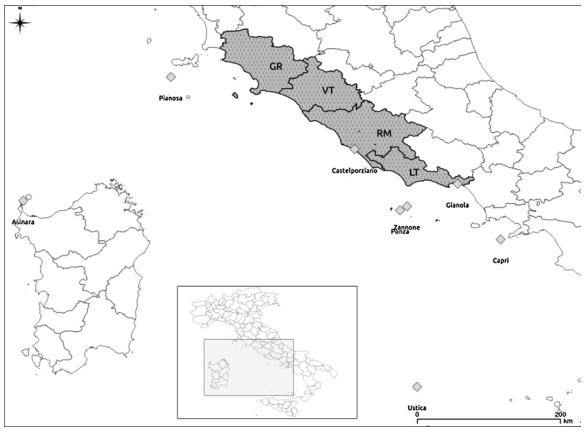

Fig. 1. Sampling area.
Legend
The dotted grey areas represent the provinces in which sheep were sampled (GR = Grosseto; LT = Latina; RM = Rome; VT = Viterbo). Grey diamonds indicate the ticks sampling sites (Castelporziano and Gianola are on the mainland, the remaining are islands). Localisation of the sampling area within Italy is displayed in the central box.

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

Table 1
Species and number of bird checked for ticks, number of specimens infested by *Hyalomma* sp., percentage of infestation and number of detected *Hyalomma* sp. for each species.

Bird species	N. of birds controlled for ticks' presence	N. of birds infested by *Hyalomma* sp. and% of infestation	N. of *Hyalomma* sp.	Bird species	N. of birds controlled for ticks' presence	N. of birds infested by *Hyalomma* sp. and% of infestation	N. of *Hyalomma* sp.
Circus aeroginosus	2			*Turdus phylomelos*	74		
Circus pygargus	4			*Cisticola juncidis*	1		
Accipiter nisus	1			*Locustella naevia*	2		
Falco tinnunculus	28			*Acrocephalus schoenobaenus*	351	2 (0.57)	7
Falco subbuteo	4			*Acrocephalus scirpaceus*	63		
Alectoris rufa	5			*Acrocephalus arundinaceus*	158	1 (0.63)	1
Coturnix coturnix	57			*Hippolais icterina*	4884	3 (0.61)	3
Phasianus colchicus	3			*Hippolais plyglotta*	6		
Burhinus oedicnemus	1			*Sylvia sarda*	1		
Larus michahellis	24			*Sylvia undata*	11		
Streptopelia decaocto	1			*Sylvia conspicillata*	3		
Streptopelia turtur	275			*Sylvia cantillans*	1493	1 (0.67)	1
Cuculus canorus	21	1 (4.75)	1	*Sylvia melanocephala*	329		
Tyto alba	4			*Sylvia communis*	5998	38 (0.63)	55
Otus scops	61	1 (1.65)	2	*Sylvia borin*	9489	1 (0.15)	1
Asio otus	3			*Sylvia atricapilla*	687		
Caprimulgus europeus	179			*Phylloscopus inornatus*	1		
Apus apus	1			*Phylloscopus bonelli*	5		
Merops apiaster	372			*Phylloscopus sibilatrix*	4289	17 (0.4)	26
Coracias garrulus	3			*Phylloscopus collybita*	341		
Upupa epops	107	1 (0.93)	1	*Phylloscopus trochilus*	3229	1 (0.4)	1
Jynx torquilla	145			*Regulus regulus*	6		
Calandrella brachydactila	13			*Regulus ignicapillus*	2		
Alauda arvensis	6			*Muscicapa striata*	2510	1 (0.4)	1
Riparia riparia	250			*Ficedula albicollis*	372		
Ptyonoprogne rupestris	2			*Ficedula semitorquata*	3		
Hirundo rustica	1632			*Ficedula hipoleuca*	3906	6 (0.15)	8
Hirundo daurica	10			*Aegithalos caudatus*	3		
Delichon urbica	281			*Cyanistes caeruleus*	5		
Anthus campestris	21			*Parus major*	5		
Anthus trivialis	318	4 (1.26)	8	*Certhia brachydactyla*	1		
Anthus pratensis	13			*Oriolus oriolus*	222	4 (1.83)	6
Motacilla flava	159	2 (1.26)	9	*Lanuis collurio*	30		
Motacilla alba	4			*Lanius senator*	186	2 (1.77)	2
Troglodytes troglodytes	4			*Sturnus vulgaris*	5		
Prunella modularis	5			*Passer italiae*	141		
Erithacus rubecula	372			*Passer hispanolensis*	18		
Luscinia megarinchos	603	2 (0.33)	6	*Passer montanus*	1		
Luscinia svecica	2			*Fringilla coelebs*	13		
Phoenicurus ochrurus	53			*Serinus serinus*	45		
Phoenicurus phoenicurus	1770	26 (1.47)	45	*Carduelis chloris*	79		
Saxicola rubetra	3828	22 (0.57)	41	*Carduelis carduelis*	77		
Saxicola torquata	9			*Carduelis spinus*	2		
Oenanthe oenanthe	520	2 (0.38)	2	*Carduelis cannabina*	22		
Oenanthe hispanica	21	1 (4.7)	1	*Coccothraustes coccothraustes*	2		
Monticola saxatilis	15			*Emberiza cirlus*	3		
Monticola solitaris	2			*Emberiza hortulana*	9		
Turdus merula	23			*Miliaria calandra*	6		

free-ranging sheep of 1153 farms having at least 100 individuals. The sampled sheep were clinically healthy lactating animals with at least two years of likely exposure in stop over areas of birds flying in spring from Africa to Central and Northern Europe.

The study population was considered as homogeneous for risk of infection all over the study area as they were all free-ranging flocks with similar managing conditions. As the study aim was to reveal the presence of at least one CCHFV ELISA seropositive animal in the study population, the sample size was empirically determined for the detection of infection assuming an expected minimum CCHFV seroprevalence of 1%, with a 95% probability and 100% sensitivity and specificity of the ELISA employed. These parameters were defined in view of the absence of reports about CCHFV circulation in Italy. Given these assumptions, if at least 300 animals from a population of 487.000 sheep were sampled and all were seronegative, the predicted probability that the population is infected at a prevalence of 1% is 0.05.

A simple random sampling method was adopted with the selection of the sera from the list referring to those stored during the 2013 National Brucellosis Eradication Program.

2.2.2. Serological assay

The serological survey was carried out adapting an indirect human

ELISA to sheep (Dowall et al., 2012). The assay employs a recombinant nucleoprotein (rNP) as antigen and an anti-sheep IgG monoclonal antibody (Mab), conjugated with horseradish peroxidase (HRP) (Sigma-Aldrich®) as detector. The rNP expressed in Baculovirus is made up of 482 amino acids and is coded by the S gene of CCHFV (Dowall et al., 2012).

As the assay employed was modified from the one described in literature, a standardisation phase was conducted to define cut-off values to adopt for sheep sera, taking into account a diagnostic specificity (DSp) of 95%, with confidence level of 95% and a standard error of 0.05 (Jacobson, 1998). For this, 73 sera, presumably negative because collected from sheep where *Hyalomma* spp. are absent, were pooled and the optical densities (OD) of thirty replicates of this material were obtained to calculate the standard deviation of the method (SDm). The cut-off level of the assay was set as the sum of the average of the negative control (pool of sheep sera collected from *Hyalomma* spp. free areas), included in each run as a double replicate and two SDm. Two replicates of a 1/100 dilution of each sample was examined and the sample was defined as positive if its mean OD was equal or greater than the cut-off value.

The internal controls included in each run were a blank control, where only the rNP and the assay solution were distributed; a positive control represented by a CCHFV Mab; and a negative control prepared as described previously. In detail, immunoplates (Maxisorp, Nunc®) were coated with 100 µl/well of two µg/mL of rNP diluted in carbonate-bicarbonate buffer and incubated overnight at 2–8 °C. Unbound antigen was removed by washing three times with 300 µl/well of phosphate buffered saline (PBS) containing 0.05% Tween-20 (MP Biomedical®). Each well was then saturated with 200 µl of blocking buffer, consisting of PBS containing 10% skimmed milk powder and 0.05% Tween-20 (BioRad®). Plates were incubated for two hours at room temperature and then washed as already described.

Serum samples were diluted 1/100 in blocking buffer and examined as double replicates. After a two-hour incubation at room temperature, another washing cycle was performed and 100 µl of a HRP-Mab, used at the dilution recommended by the manufacturer, was added to each well. Following a one-hour incubation at room temperature, unbound antibody was removed by a washing step. A volume of 100 µl/well of 2.2′-azino-di[3-ethylbenzthiazoline sulfonate] (ABTS) substrate (Sigma-Aldrich®) was then added and the plates were incubated at room temperature for a further 25 min. The colorimetric reaction was stopped by the adding the ABTS stop solution, containing 1% sodium dodecyl sulphate (Applichem®). The sample OD was read at 450 nm, using a microplate spectrophotometer (Multiskan EX, Thermo Fisher Scientific, Waltham, MA).

3. Results

3.1. Tick sampling on migratory birds

During the study period, 50,325 birds belonging to 96 species (74 Passerines and 22 non-Passerines) were checked for the presence of ticks at the ringing stations. Overall, 282 ticks were collected from 139 migratory birds; among these, 228 (80.8%) belonged to the genus *Hyalomma*. Other genera/species detected were: *Haemaphysalis* spp., *Haemaphysalis punctata*, *Ixodes* spp., *Ixodes ricinus* and *Ixodes frontalis*. Considering the total number of birds checked over the two years, 0.28% of these were found infested with ticks and 0.22% with *Hyalomma* genus.

As expected, the majority of the ticks of this genus were nymphs (98%) with the exception of five adult specimens, all identified as *H. marginatum marginatum*.

Ticks of the genus *Hyalomma* were found on 22 migratory bird species (Table 1). The percentage of *Hyalomma* spp. infestation in birds varied from 0.15% (*Sylvia borin* and *Ficedula hypoleuca*) to 4.75% (*Cuculus canorus*). Regarding tick burden, maximum number of *Hyalomma* spp. found on a single host (a *Motacilla flava* and a *Sylvia communis*) was 8 and 103/139 birds (74.1%) harboured just one tick, with a 1.6 mean number of *Hyalomma* spp. per infested bird.

3.2. Serological survey

The number of sheep sera analysed were 540 and were collected within the following provinces: Grosseto, Rome and Latina and Montalto di Castro, the target coastal municipality in the province of Viterbo (Fig. 1). All sera resulted ELISA negative.

4. Discussion

To the best of our knowledge, this is the first serosurvey conducted in Italy for CCHFV. Sheep are known to seroconvert to CCHFV (Hassanein et al., 2004; Nalca and Whitehouse, 2007) and are consequently considered suitable sentinels for monitoring and detecting virus introduction/circulation *foci* in new and non-endemic areas (Mostafavi et al., 2012; Schuster et al., 2016). In Central Italy, *H. marginatum* is endemic and the target population of sheep selected for this study were from flocks grazing in warm and dry coastal areas, where, theoretically, ticks arriving on migratory birds could survive, moult to adults and seek a blood meal. The method employed appears to be a valid screening test for its simplicity and safety and because it does not require particular biosafety measures. As the NP variability is lower than 4%, the assay is expected to be sensitive and to detect the majority of CCHFV strains circulating globally; nevertheless, further validation should be performed to better assess its diagnostic characteristics. Given that the present results indicate that in the study area CCHFV is below the detection limit of the survey, this suggests that the virus has not recently arrived in the region or, even if introduced in ticks, transmission to autochthonous vertebrate hosts did not occur (Gray et al., 2009).

The aim of our survey was to perform the first Italian retrospective screening of a possible CCHFV circulation in the entire coastal sheep population of Latium and Tuscany. The random sampling design adopted was practical because it used sera routinely collected under another disease surveillance schemes. It should be a suitable approach if the assumption of random spread of the virus among extensively grazed sheep is valid. In such a situation, there wouldn't be significant spatial or even flock-level clustering of virus circulation. Given this assumption, and having tested 540 randomly selected sheep, we are 95% confident that the sample would be able to detect the presence of infection prevalence above 0.6%.

For these purposes, tick vector spatial distribution is also crucial to focus the sampling activities in the areas at major risk of virus spread.

Tick survey on birds was carried out during the spring migration, when 2.1 billion songbirds and near-passerine birds enter Europe, from Central and Southern Africa (Hahn et al., 2009) after crossing or wintering in areas where CCHFV is endemic. Similar to what was reported by previous surveys (Molin et al., 2011; Mancini et al., 2013), the majority of ticks detected were immature stages of the genus *Hyalomma*; the only exception to these findings are the results of Jameson et al. (2012) obtained in the United Kingdom, where only 21% of recovered ticks were *Hyalomma* spp. Both in Capri, Italy (Molin et al., 2011) and in the UK (Jameson et al., 2012), the percentage of birds found infested with at least one tick was much higher (5.45% and 2.7% respectively) compared to the 0.28% reported in the present study. However, both studies were carried out in single ringing sites, while in our study, 13 different sites (5 sites in 2013 and 8 sites in 2014), distributed over a large area of Italy, were controlled and the total number of birds checked (50,325 over two sampling seasons in the present study) was much higher when compared to the previous studies: 7453 in Capri (Molin et al., 2011) and less than 1000 in UK (Jameson et al., 2012). This huge sampling volume allows a more realistic estimation of the number of birds infested with ticks arriving in Italy during the spring migration even if according to the ornithologists' personal

observations, an unusual low number of ticks on migrating birds characterized the two sampling seasons.

In the present study, twenty-two bird species were found harbouring *Hyalomma* spp. ticks, similar to what reported by Mancini et al. (2013), who found 17 species infested with *Hyalomma* spp. which differed from the findings in UK (Jameson et al., 2012) where only 4 infested species were found. Regarding tick burden, infested birds generally harboured a mean ranging from 1.2 (Jameson et al., 2012) to 3.3 (Mancini et al., 2013). All of the species found infested by *Hyalomma* spp. were transaharian migrants, which pass over areas where CCHFV is endemic during their spring migration. Of all the bird species identified in this study, 45.8% regularly fly over CCHFV endemic areas of Africa (Spina and Volponi, 2008a,b; BirdLife's Global Species Programme, 2015). Data recovery of the single birds ringed in Italy confirm that many species found harbouring *Hyalomma* ticks pass through countries endemic for CCHFV; among these are the *Sylvia borin* specimens recovered in Mali and Egypt, *Hippolapis icterina* in Egypt and Democratic Republic of Congo and *Phylloscopus trochilus* in Democratic Republic of Congo and Tanzania (Spina and Volponi, 2008b).

Different percentages of infestation were found on the various species (from 0.15% to 4.75%) suggesting that some birds are more prone to being infested by *Hyalomma* spp. ticks, probably because of different ecological habits during spring migration (e.g. some species may spend more time on the ground or in bushes, where *Hyalomma* spp. larvae are able to infest the host). Another possible explanation could be that transaharian birds adopt different migration strategies where, even if crossing areas where *Hyalomma* spp. are present, not all species necessarily stop or stop for enough time to become infested (Lemke et al., 2013; Pilastro et al., 1998).

5. Conclusions

Due to its public health impact, CCHFV epidemiology should be investigated in detail and its potential spread constantly monitored. The exclusive detection of the viral nucleic acid in ticks arriving on migratory birds would not prove their ability to introduce the virus in new areas (Gray et al., 2009). The additional adoption of a serological survey in sheep was aimed at resolving this issue; seropositive animals would confirm that the virus was introduced and was able to circulate. The proposed serosurveillance system conducted on samples that are collected for other purposes could represent a valid and relatively cheap method that can be used as an indicator of viral circulation over an extensive area.

Acknowledgments

Research granted by the Italian Ministry of Health, Project Code RF-2009-1539631 “New and emerging vector borne infectious diseases in Italy: epidemiology, surveillance, diagnosis and impact on the safety of blood and organ donations”

Authors would like to thank all the ornithologists and the bird ringers who contributed to ticks collection and the coordinators of each ringing station: Giuseppe Landucci (Castelporziano), Nicola Maggi (Pianosa), Danilo Pisu (Asinara), Natalino Cuti (Ustica), Massimo Sacchi (Zannone), Dario Piacentini (Capri), Fabio Giannetti (Gianola) and Massimiliano Cardinale (Ponza). Authors are also grateful to Dr. Roberto Nardini for the map of Fig. 1 and Dr. Stuart Dowall for revising the manuscript. Views expressed in this manuscript are those of the authors and do not necessarily reflect those of the employing institutions.

References

BirdLife's Global Species Programme 2015 online resource (http://www.birdlife.org/datazone/species) (last accessed 20 May 2017).

Dowall, S.D., Richards, K.S., Graham, V.A., Chamberlain, J., Hewson, R., 2012. Development of an indirect ELISA method for the parallel measurement of IgG and IgM antibodies against Crimean-Congo haemorrhagic fever (CCHF) virus using recombinant nucleoprotein as antigen. J. Virol. Methods 179, 335–341.

European Centre for Disease Prevention and Control (ECDC), 2016. Crimean–Congo Haemorrhagic Fever in Spain – 8 September 2016. Stockholm.

European Food Safety Authority (EFSA), 2010. 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, 1–280.

Estrada-Peña, A., Martínez Avilés, M., Muñoz Reoyo, M.J., 2011. A population model to describe the distribution and seasonal dynamics of the tick *Hyalomma marginatum* in the Mediterranean basin. Transbound. Emerg. Dis. 58, 213–223.

Estrada-Peña, A., Palomar, A.M., Santibáñez, P., Sánchez, N., Habela, M.A., Portillo, A., Romero, L., Oteo, J.A., 2012. Crimean-Congo haemorrhagic fever virus in ticks, southwestern Europe 2010. Emerg. Infect. Dis. 18, 179–180.

Gale, P., Estrada-Peña, A., Martinez, M., Ulrich, R.G., Wilson, A., Capelli, G., Phipps, P., de la Torre, A., Muñoz, M.J., Dottori, M., Mioulet, V., Fooks, A.R., 2010. The feasibility of developing a risk assessment for the impact of climate change on the emergence of Crimean-Congo haemorrhagic fever in livestock in Europe: a review. J. Appl. Microbiol. 108, 1859–1870.

Gale, P., Stephenson, B., Brouwer, A., Martinez, M., de la Torre, A., Bosch, J., Foley-Fisher, M., Bonilauri, P., Lindström, A., Ulrich, R.G., de Vos, C.J., Scremin, M., Liu, Z., Kelly, L., Muñoz, M.J., 2011. Impact of climate change on risk of incursion of Crimean-Congo haemorrhagic fever virus in livestock in Europe through migratory birds. J. Appl. Microbiol. 112, 246–257.

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. http://dx.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. http://dx.doi.org/10.1111/j.1600-0706.2008.17309.x.

Hassanein, N.A., El-Azazy, O.M., Yousef, H.M., 2004. Detection of Crimean-Congo haemorrhagic fever virus antibodies in humans and imported livestock in Saudi Arabia. Trans. R. Soc. Trop. Med. Hyg. 91, 536–537.

Hillyard, P., 1996. Ticks of North-West Europe. In: Barnes, R.S.K., Crothers, J.H. (Eds.), Synopses of The British Fauna. The Natural History Museum, London, pp. 124–125.

Hoogstraal, H., 1979. The epidemiology of tick-borne Crimean-Congo haemorrhagic fever in Asia, Europe and Africa. J. Med. Entomol. 15, 307–417.

Horak, I.G., Swanepoel, R., Gummow, B., 2001. The distribution of Hyalomma spp. and human cases of Crimean-Congo haemorrhagic fever in South Africa (Environment. LCa, ed.). In: Proc. 10th Conference of The Association of Institutions for Tropical Veterinary Medicine. Copenhagen, Denmark. pp. 2001.

Iori, A., Di Giulio, A., De Felici, S., 2005. Zecche d'Italia. Rolando, Napoli.

Jacobson, R.H., 1998. Validation of serological assagys for diagnosis of infectious diseases. Rev. Sci. Tech. Int. Epiz. 17, 469–486.

Jameson, L.J., Medlock, J.M., 2009. Results of HPA tick surveillance in great britain. Vet. Rec. 165, 154.

Jameson, L.J., Morgan, P.J., Medlock, J.M., Watola, G., Vaux, A.G.C., 2012. Importation o *Hyalomma marginatu*m, vector of Crimean-Congo haemorrhagic fever virus, into the United Kingdom by migratory birds. Ticks Tick-Borne Dis. 3, 95–99.

Karti, S.S., Odabasi, Z., Korten, V., Yilmaz, M., Sonmez, M., Caylan, R., Akdogan, E., Eren, N., Koksal, I., Ovali, E., Erikson, B.R., Vincent, M.J., Nichol, S.T., Comer, J.A., Rollin, P.E., Ksiazek, T.G., 2004. Crimean-Congo haemorrhagic fever in Turkey. Emerg. Infect. Dis. 10, 1379–1384.

Leblebicioglu, H., Eroglu, C., Erciyas-Yavuz, K., Hokelek, M., Acici, M., Yilmaz, H., 2014. Role of migratory birds in spreading Crimean-Congo haemorrhagic fever. Turkey. Emerg. Infect. Dis. 20, 1331–1334.

Lemke, H.W., Tarka, M., Klaassen, R.H.G., A˚kesson, M., Bensch, S., Asselquist, D., Hansson, B., 2013. Annual cycle and migration strategies of a trans-Saharan migratory songbird: a geolocator study in the great reed warbler. PLoS One 8 (10), e79209. http://dx.doi.org/10.1371/journal.pone.0079209.

Mancini, F., Toma, L., Ciervo, A., Di Luca, M., Faggioni, G., Lista, F., Rezza, G., 2013. Virus investigation in ticks from migratory birds in Italy. New Microbiol. 36, 433–434.

Manilla, G., 1998. Acari Ixodida. Fauna d'Italia Vol. XXXVI. Calderini, Bologna.

Messina, J.P., Pigott, D.M., Golding, N., Duda, K.A., Brownstein, J.S., Weiss, D.J., et al., 2015. The global distribution of Crimean-Congo hemorrhagic feve. Trans. R. Soc. Trop. Med. Hyg. 109, 503–513.

Mild, M., Simon, M., Albert, J., Mirazimi, A., 2010. Towards an understanding of the migration of Crimean-Congo haemorrhagic fever virus. J. Gen. Virol. 91, 199–207.

Molin, Y., Lindeborg, M., Nyström, F., Madder, M., Hjelm, E., Olsen, B., Jaenson, T., Ehrenborg, C., 2011. Migratory birds, ticks and *Bartonella*. Inf. Ecol. Epidemiol. 1. http://dx.doi.org/10.3402/iee.v1i0.5997.

Mostafavi, E., Chinikar, S., Esmaeili, S., Amiri, F.B., Tabrizi, A.M.A., KhakiFirouz, S., 2012. Seroepidemiological survey of Crimean-Congo hemorrhagic fever among sheep in Mazandaran Province, Northern Iran. Vector Borne Zoonotic Dis. 12, 739–742.

Nalca, A., Whitehouse, C.A., 2007. Crimean-Congo haemorrhagic fever virus infection among animals. In: Ergonul, O., Whitehouse, C.A. (Eds.), Crimean-Congo Haemorrhagic Fever – A Global Perspective. SpringerDordrecht, The Netherlands, pp. 155–165.

Ozkul, A., 2009. Life threatening Crimean Congo haemorrhagic fever in Turkey: characteristics, epidemiology and future perspectives. In: Presented at Epizone 3rd Annual Meeting. Antalya, Turkey 13/14 May. pp. 2009.

Papa, A., Maltezou, H.C., Tsiodras, S., Dalla, V.G., Papadimitriou, T., Pierroutsakos, I.N., Kartalis, G.N., Antoniadis, A., 2008. A case of crimean-Congo haemorrhagic fever in Greece, june 2008. Euro Surveill. 13.

Pilastro, A., Macchio, S., Massi, A., Montemaggiori, A., Spina, F., 1998. Spring migratory

routes of eight trans-Saharan passerines through the central and western Mediterranean; results from a network of insular and coastal ringing sites. Ibis 140, 591–598. http://dx.doi.org/10.1111/j.1474-919X.1998.tb04704.x.
Randolph, S., Ergonul, O., 2008. Crimean-Congo haemorrhagic fever: exceptional epidemic of viral haemorrhagic fever in Turkey. Future Virol. 3, 303–306.
Randolph, S., Rogers, D., 2007. Ecology of tick-borne diseases and the role of climate. In: Ergonul, O., Whitehouse, C.A. (Eds.), Crimean-Congo Haemorrhagic Fever – A Global Perspective. SpringerDordrecht, The Netherlands, pp. 167–186.
Schuster, I., Mertens, M., Mrenoshki, S., Staubach, C., Mertens, C., Brüning, F., Wernike, K., Hechinger, S., Berxholi, K., Mitrov, D., Groschup, M.H., 2016. Sheep and goats as indicator animals for the circulation of CCHFV in the environment. Exp. Appl. Acarol. 68, 337–346.
Spina, F., Volponi, S., 2008a. Atlante della Migrazione degli Uccelli in Italia. 1. non-Passeriformi. Ministero dell'Ambiente e della Tutela del Territorio e del Mare. Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA) Tipografia CSR-Roma. 800 pp.
Spina, F., Volponi, S., 2008b. Atlante della Migrazione degli Uccelli in Italia. 2. Passeriformi. Ministero dell'Ambiente e della Tutela del Territorio e del Mare. Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA) Tipografia SCR-Roma. 632 pp.