SCIENTIFIC OPINION

Scientific Opinion on the Role of Tick Vectors in the Epidemiology of Crimean-Congo Hemorrhagic Fever and African Swine Fever in Eurasia[1]

EFSA Panel on Animal Health and Welfare[2, 3]

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The report provides an update on the role of the tick vectors in the epidemiology of African swine fever (ASF) and Crimean and Congo haemorrhagic fever (CCHF) in Eurasia, specifically to review of the geographical distribution of the relevant ticks with presentation of maps of their occurrence in Europe and Mediterranean basin; a description of the factors that define the relevant tick population dynamics and identify possible high risk areas in the EU; an update on the role of tick vectors associated with CCHF and ASF in Eurasia; and reviews available methods for the control of the relevant tick vectors. Data were collected through systematic literature review in a database from which maps of geographic distribution of ticks, CCHF virus and ASF virus were issued. The main vectors for CCHF are *Hyalomma* spp, Increase in the number of fragmented areas and the degradation of agricultural lands to bush lands are the two main factors in the creation of new foci of CCHF in endemic areas. Movement of livestock and wildlife species, which may carry infected ticks, contributes to the spread of the infection. The Middle East and Balkan countries are the most likely sources of introduction of CCHFV into other European countries. All the *Ornithodoros* species investigated so far can become infective with ASF virus and are perhaps biological vectors. These ticks are important in maintaining the local foci of the ASFV, but do not play an active role in the geographical spread of the virus. Wild boars have never been found infested by *Ornithodoros* spp. because wild boars normally do not rest inside protected burrows, but above the ground. There is no single ideal solution to the control of ticks relevant for CCHF or ASF. The integrated control approach is probably the most effective.

KEY WORDS

CCHF, ASF, vector, *Hyalomma*, *Ornithodoros*, tick-borne disease, Europe.

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SUMMARY

Introduction

The recent developments in the EU, especially the risk of introduction of African swine fever virus (ASF) from the East European countries and the Caucasus; and the threat that would represent the spread of the Crimean-Congo hemorrhagic fever virus (CCHF) in Europe; elicited a self-mandate of the EFSA's Panel of the Animal Health and Animal Welfare on the role of tick vectors associated with Crimean-Congo hemorrhagic fever and African swine fever in Eurasia. The occurrence of other tick-borne animal diseases and zoonoses in the EU was also considered as part of this mandate. The terms of reference of the self-mandate were:

- Provide an update on the role of the tick vectors in the epidemiology of ASF and CCHF in Eurasia, more in particular:
  - provide a review of the geographical distribution of the relevant ticks and produce maps of Eurasia displaying their occurrences;
  - review surveillance data to provide estimates of the relevant tick abundance and disease incidence in Eurasia;
  - describe the factors that define the relevant tick population dynamics and identify possible high risk areas in the EU for introduction considering the biological and ecological characteristics of the ticks and their ability to adapt to new areas;
  - provide an update on the role of the relevant vectors in the transmission and the maintenance of ASF and CCHF in Eurasia;
  - review available methods for the control of the relevant tick vectors.
- Provide a general overview of the geographic distribution of ticks which have proven involvement in the transmission of animal diseases and zoonoses in Eurasia.

The mandate resulted in two scientific reports: this one on "The role of ticks in the epidemiology of CCHF and ASF in Eurasia" (addressing the first point); and another on "The geographic distribution of tick-borne infections and their vectors in Europe and the other regions of the Mediterranean basin", covering the second point.

The methodology

Part of the presented information derived from findings of two previous scientific reports (CFP/EFSA/AHAW/2007/02 and CFP/EFSA/AHAW/2008/04) obtained in accordance with Article 36 of Regulation (EC) No 178/ 2002[4]. The section on ASF was elaborated in parallel with EFSA scientific opinion on African swine fever (EFSA Journal 2010; 8(3); 1556). In addition, a systematic literature review was performed in order to gather data on tick and tick-borne pathogens distribution. The review included searching in the databases integrated in ISI web of knowledge and Pubmed for the scientific papers published during the last 10 years. The *ad hoc* experts, also, contributed with relevant scientific papers regardless of the timeframe, which were also submitted to the same procedure of systematic literature review. Historical data on ticks' distribution (approximately from year 1970 to 2000) was obtained from the integrated consortium of ticks and tick-borne diseases

[4] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

(European project, ICTTD3). All these data were stored in a database that allowed the creation of distribution maps either for ticks or for tick-borne pathogens. The maps have the limitation of representing only what was found through the systematic literature review process or in the ITTCD3 database.

The results for CCHF

Relevant tick species were identified and their role in transmission of the viruses is discussed. The main vectors are *Hyalomma* spp. but at local level *Dermacentor* spp. and *Rhipicephalus* spp. have proven capacity of maintaining the CCHFV through their life cycle and transmit it to the vertebrates. However the role of *I. ricinus* (the most widely distributed tick in Europe) in the transmission of CCHFV has not been ascertained. Several studies indicate that argasid (soft) ticks are not competent CCHFV vectors.

Maps are provided describing the geographic distribution of the ticks involved in the epidemiology of CCHF, as well as the distribution of CCHFV. It is unknown so far the reason for the limited geographical spread of CCHF cases to countries in the South East of Europe and Middle East, although the tick vectors of CCHFV (*Hyalomma marginatum*, *Dermacentor marginatus*, *Rhipicephalus bursa*) are much wider spread in Europe.

Surveillance data are scattered and inconsistent in terms of diagnostic capability and host species including humans. Data on tick abundance is lacking, specifically for *Hy. marginatum*. Infection can be monitored in ticks using various methods of collection. Movement of livestock and wildlife species, which may carry infected ticks, contributes to the spread of the infection. Migratory birds may not be sufficient to establish new foci of infection. The absence of clinical signs and the lack of reliable diagnostic tests to detect the infection in mammals and birds make the confirmation of the infection in animals difficult. This report has not considered the movement of viraemic domestic or wild animals as a source of spread infection because it is out of the remit of the mandate.

Disease ecology, including its vectors, was described. Mammals such as rodents, hedgehogs, hares, are considered the principle reservoirs of CCHFV. Ungulates are important hosts for adult tick vector species. Vireamic periods, however, in ruminants appear to be short which may reduce the transmission of this virus to other hosts. In the tick populations the infection is maintained by transstadial and transovarial transmissions. Co-feeding transmission has not been proven in a natural setting. Increase in the number of fragmented areas and the degradation of agricultural lands to bush lands are the two main factors in the creation of new foci of CCHF in endemic areas. A complex chain of interactive factors exists that makes causes for changes in the infection level often difficult to assess. Climate change is only one of the factors associated with the spread of the infection. High risk areas were identified according to scientific evidence, specifically related to the vector ecology. The Middle East and Balkan countries are the most likely sources of introduction of CCHFV into other European countries. There are existing risk models for other tick-borne diseases, such as tick-borne relapsing fever (TBRF), and ASF. These two models serve the purpose of identifying gaps in knowledge and data that can be applied for CCHF risk modelling.

Measures of control of the relevant ticks were presented with scientific evidence of their efficiency. There is no single ideal solution to the control of ticks. The integrated control approach is probably the most effective.

The results for ASF

Relevant tick species were identified. Of all the invertebrates tested up to the present only the soft ticks of the genus *Ornithodoros* are susceptible to ASFV infection either naturally or experimentally. Other soft ticks remain untested under laboratory conditions. Hard ticks or other blood feeding invertebrates have not been shown to be a vector of ASFV. All the *Ornithodoros* species (*O. erraticus*, *O. moubata* /*porcinus*, *O. coreaceus*. *O. turicata*, *O. puertoricensis*, *O. parkeri* and *O. savignyi*) investigated so far can become infective and are perhaps biological vectors of ASF. Of these species, some are found only in Afrotropical and Neotropical regions. Only the *O. erraticus* complex is found in the European, trans-Caucasus countries (TCC) and Russian Federation territories.

Maps are provided describing the geographic distribution of the ticks involved in the epidemiology of ASF, as well as the distribution of ASFV.

To our knowledge there is no systematic monitoring of the occurrence of *Ornithodoros* ticks in EU.

The ecology of the disease was revised. The *O. erraticus* complex, because of its long life (up to 15 years) and strong resistance to starvation and persistence of infection for up to 5 years, is important in maintaining the local foci of the ASFV (and lead to endemicity in a region). However, they do not play an active role in the geographical spread of the virus. *Ornithodoros* ticks feed mainly on animal species living in burrows, such as rodents and reptiles. Pigs are mostly accidental hosts, from which the ticks can be infected. The epidemiological role played by soft ticks becomes important where pigs are managed under traditional systems, including old shelters/sties with crevices. Wild boars have never been found infested by *Ornithodoros* spp. because wild boars normally do not rest inside protected burrows, but above the ground. Due to the limited available data on associated factors with the distribution of soft ticks, prediction of their potential distribution is difficult to construct. EFSA scientific opinion on African swine fever (EFSA Journal 2010; 8(3); 1556) elaborates on the risk of introduction of ASFV in the EU.

Measures of control of the relevant ticks were discussed. Eradication of *O. erraticus* from the old pig sties is invariably unsuccessful. The use of acaricides on pigs and their habitat can reduce the level of infestation in the premises but does not avoid the pigs of becoming infected by ASF virus if they are bitten by a virus infective tick.

Recommendations

CCHF

The current database on ticks' distribution should be maintained. Scientific information can be included in the database only if the following items are clearly specified: the scientific names of the organisms studied (vectors or pathogens), origin of samples (vegetation, host), type of samples (blood, serum, organs, etc), geo-reference of the sample, diagnostic tools used to identify the ticks, date, and history of the samples if applicable (imported host or vectors, travel history).

There is a need for a standard case definition of CCHFV infection in vertebrates and arthropods. It is essential to notify the presence of vectors during outbreaks and the disease notification should include the possible involvement of vectors.

Mapping of endemic areas and assessment of future risk areas are necessary for appropriate surveillance and control of CCHF. Vector collection with reliable identification methods and tools (morphological keys and DNA-based tick species identification) should be available in all countries where CCHFV occur or can be expected to emerge in the near future.

The presence of CCHFV in a geographic area can be monitored by PCR on ticks collected on the animals and in the environment (questing ticks)

Frequent serosurveys of grazing animals, including livestock and wildlife, in CCHFV free areas neighbouring or bordering known high risk areas (i.e. the southern Balkans and Turkey) is another approach for determining recent introduction of the virus. A prerequisite for such approaches, however, would be the development of reliable and specific diagnostic assays to detect antibodies in a range of host species.

A risk model for the introduction and spread of CCHFV, including gaps in existing knowledge, should be considered regarding the available similar models such as ASF and TBRF. Pathways other than tick transmission for the introduction and spread of infection should be considered in this risk model.

To prevent virus infection to humans by ticks, general guidelines on personal protection should be available to the general public in affected countries.

Integrated tick control measures should be developed at international level.

Acaricides should be used in accordance with the best practice that minimise the development of resistance by the tick species.

ASF

The ASF recommendations are in agreement to those presented in EFSA scientific opinion on African swine fever (EFSA, 2010)

Recommendations for research

CCHF

The role of other tick species, specifically *I. ricinus*, in the transmission of CCHFV should be investigated under natural conditions.

The role of European wild life, especially wild ungulates, in the spread of CCHFV needs to be investigated.

The necessary biotic and abiotic conditions for the maintenance of CCHFV in nature require further investigation.

In order to understand the epidemiology, further knowledge on the pathogenesis and distribution of the virus in mammalian hosts should be obtained

ASF

Different *Ornithodoros* spp. may play various roles in the epidemiology of ASF. It would need further investigations

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BACKGROUND

Article 36 of the European Parliament and Council Regulation (EC) No 178/2002, foresees the possibility to financially support a networking of organisations operating in the fields within the EFSA's mission. Under this framework, a call for proposals was published on the EFSA website in August 2007 (CFP/EFSA/AHAW/2007/02) to review three specific animal diseases (African Horse Sickness, African Swine Fever and Classical Swine Fever) and to provide an evaluation of the distribution of arthropod vectors and their potential for transmitting exotic or emerging vector-borne animal diseases and zoonoses. In August 2008 another call for proposals was published (CFP/EFSA/AHAW/2008/04) to review Epizootic hemorrhagic disease and Crimean-Congo hemorrhagic fever.

The topics for the call were selected respecting previous recommendations from the Animal Health and Welfare Panel and/or were based on recent occurrences of vector-borne animal diseases and zoonoses in the EU. The proposed topics were considered to be in line with the Community Animal Health Policy (CAHP), since one of the key elements of the CAHP is the early detection of exotic and new/emerging disease threats. The scientific reviews will support the preparedness for issuing scientific opinions on exotic or emerging diseases and/or their vectors.

The focus of this mandate is on the role of the tick vectors in the epidemiology of Crimean-Congo hemorrhagic fever and African swine fever. Ticks are arthropods (Order Ixodida) that suck blood from animals and humans. Two major families can be distinguished: the hard ticks (Family Ixodidae), includes the genera *Amblyomma, Dermacentor, Hyalomma, Ixodes, Rhipicephalus* (previously called *Boophilus*); and the soft ticks (Family Argasidae), including the genera *Ornithodoros* and *Argas* amongst others.

Ticks occur around the world and can be mechanical and/or biological vectors of bacteria, protozoan parasites, and viruses. In many cases the presence of the disease is linked to the presence of vector-competent tick species in a specific area. The movement of livestock, humans and undetected ticks in planes, lorries and ships may be associated with the introduction of exotic vector-borne microbes.

Most species of hard tick (Ixodidae) feed on different hosts (one host for each evolutionary stage), and can remain attached to them for several days and be carried over large distances which partly explain their importance as vectors of disease agents.

Soft ticks (Argasidae), instead, feed for short periods on their hosts, varying from several minutes to days, depending on such factors as life stage, host type, and species of tick. The feeding behaviour of many soft ticks can be compared to that of fleas or bedbugs, as they often reside in the nest of the host, feeding rapidly when the host return.

The abundance, feeding patterns, longevity of the ticks, and environmental factors such as vegetation, temperature and rainfall, play a role in the transmission of disease agents. The presence and persistence of tick-borne diseases depend on biological and ecological relationships between vertebrates, ticks and disease agents. All of these factors should be taken into account when trying to determine the importance of a particular species as a potential vector.

In regions and countries where tick-borne diseases are present, abundance, seasonality and distribution of the ticks can be assessed by catching ticks on the usual hosts and by collecting methods of unfed ticks in the environment. Control and prevention of pathogens transmitted by ticks may be through the application of acaricide on the hosts or in the environment.

Crimean-Congo haemorrhagic fever (CCHF) is a zoonotic viral disease that is asymptomatic in infected animals, but a serious threat to humans. The virus is mainly transmitted by tick species of the genus *Hyalomma*. Some species of the genera *Dermacentor* and *Rhipicephalus*, however, have also been shown to be capable of transmitting CCHFV. Various mammals and possibly some avian species serve as amplifying hosts on which the feeding ticks can be infected with the virus. Animal-to-human (for example, through contact with infected animal blood or ticks), and human-to-human (by contact with infectious blood or body fluids) transmission also can occur.

African swine fever (ASF) is a highly contagious virus infection of domestic and wild pigs/*suidae* that can be transmitted by certain soft tick species of *Ornithodoros*. There are different epidemiological scenarios depending on the specific circumstances in each geographical area regarding virus strains, host susceptibility, biological vector presence and/or vector interaction with susceptible hosts. In the Iberian Peninsula, *O. erraticus* was the main vector, associated with domestic pigs. In Sardinia and some African countries, the virus is maintained by free range/backyard pigs that have recovered from infection and remain in a carrier state. In the Caucasus, contacts between diseased wild boars and free ranging pigs seem to play an important role in the spread of ASF and the role of ticks still needs to be clarified.

Because the paucity of data on vector competence for many tick species and the lack of information on the effect of environmental factors on CCHFV transmission, studies are needed to evaluate relevant tick species as well the various factors that might affect virus transmission. Also the role of ticks in the epidemiology of ASF in the Caucasus needs to be elucidated.

TERMS OF REFERENCE

- Provide a general overview of the geographic distribution of tick species which have proven involvement in the transmission of animal diseases and zoonoses in Eurasia.

- Provide an update on the role of the tick vectors in the epidemiology of African swine fever and Crimean-Congo hemorrhagic fever in Eurasia[5], more in particular:

  - provide a review of the geographic distribution of the relevant tick species and produce maps of Eurasia displaying their occurrences;

  - review surveillance data to provide estimates of the relevant tick abundance and disease incidence in Eurasia;

  - describe the factors that define the dynamics of the relevant tick species and identify possible high-risk areas in the EU for introduction considering the biological and ecological characteristics of the ticks and their ability to adapt to new areas;

  - provide an update on the role of the relevant vectors in the transmission and the maintenance of viruses of Crimean-Congo hemorrhagic fever and African swine fever in Eurasia;

  - review available methods for the control of the relevant tick vectors.

[5] Eurasia, in this report, is referred to the following areas: Europe, a buffer zone of 600 km along the Russian Federation border, the Caucasus region, North Africa, Turkey, and Middle East.

ASSESSMENT

1. Introduction

This report addresses the second bullet point of the terms of reference. It is structured in two parts: one on the role of ticks in the epidemiology of Crimean-Congo hemorrhagic fever (CCHF); and the other on the role of ticks on the epidemiology of African swine fever (ASF).

Crimean-Congo hemorrhagic fever is a viral zoonosis transmitted by ticks, widely distributed in Africa, Asia and Eastern Europe that may cause severe outbreaks in humans (WHO 2009). The infection can usually occur without obvious clinical signs in animals but can be life-threatening to humans. The virus is mainly transmitted by tick species of the genus *Hyalomma.* Some species of the genus *Dermacentor* and *Rhipicephalus*, however, have also been shown to be capable of transmitting Crimean-Congo hemorrhagic fever virus **(**CCHFV). Various mammals and probably some avian species serve as amplify hosts that can subsequently infect ticks feeding on these hosts. Animal-to-human, such as through contact with infected animal blood or ticks, and human-to-human, by contact with infectious blood or body fluids, transmission also can occur. Nevertheless meat from butchered animals do not pose a risk for the consumer as in this substrate the CCHFV is inactivated by a drop in pH, as occurs during the maduration process that the meat undergoes after slaughter (Swanepoel and Burt 2004). There are limited data and research findings on vector competence of several tick species, specifically the favourable environment factors for effective CCHFV transmission. A recent scientific report on CCHF (CFP/EFSA/AHAW/2008/04) elaborated on existing literature and research finding on this disease with special emphasis on the role of ticks.

There is limited information about the global impact of this disease on human population. There is, however, several evidence of its significant health effect when an outbreak is reported. For instance between 2002 and 2008 the Ministry of Health of Turkey reported 3128 Crimean-Congo hemorrhagic fever cases with 5% of case-fatality rate (ECDC, 2008). Earlier several outbreaks were reported from Africa with scattered data in terms of the health impact of this disease. In recent years new cases continue to appear in the known historical areas (Crimean peninsula, the Caucasic area, and in the Balkans)

Gonzalez (Gonzalez et al. 1998) experimentally demonstrated that in sheep viraemia appeared for a period of zero to nine days and also that pre-immunized sheep develop in experimental condition limited but effective viraemia.

African swine fever is caused by a complex DNA virus classified in the Asfarviridae family, genus *Asfivirus*, which can infect different species of soft ticks and wild and domestic pigs. In the vertebrate hosts, the aetiological agent replicates preferentially in monocyte and macrophage cells and causes a range of clinical signs from peracute to chronic or unapparent forms of disease. EFSA report 2010 has addressed the various issues related to this disease and its agent. One of the potential modes of transmission of this virus between pigs is through the soft ticks, specifically *Ornithodoros* spp. In addition these tick species can play a major role in the persistence of the virus in specific geographical locations (EFSA, 2010). The assessment below focuses on the role of *Ornithodoros* spp. as vectors for this disease. The ecological, geographical distribution, control measures, and other biological characters of these vectors are presented in the current report.

2. Tick species as vectors of CCHF

There are several field studies present in literature performed in various geographical areas where CCHF has been reported in outbreaks.

Findings from sero-epidemiological surveys and attempts to isolate the virus from wild and domestic vertebrates suggested a wide range of role of the different vertebrates in the maintenance and the spread of the virus and infection. The role these vertebrates could be as either virus reservoirs or non-reservoirs but they are significant contributors to the tick vector population density and/or epidemiologically important members of the biocenose. Virus isolation rates from various tick species revealed the degree of risk to human health presented by these ticks. Demonstration of trans-stadial and trans-ovarial transmission of the virus by ticks provided evidence of the biological properties permitting CCHFV survival from season to season. These biological properties are significantly different in ecological and zoogeographical zones and in one-host, two-host, and three-host ticks (Hoogstraal 1979). The table 6 of the scientific review on CCHF (CFP/EFSA/AHAW/2008/04) lists some references and features on field studies on CCHFV vector competence for hard tick species of CCHF virus ticks as vectors for CCHF

2.1. Reservoirs

The biology of vertebrates implicated in the ecology of CCHF is important in understanding the transmission cycles of the infection. Humans become infected when they come in contact with the natural CCHFV enzootic cycles. The qualitative and quantitative aspects of the biology of each vertebrate involved in maintaining the enzootic cycle of the virus are not fully understood since there is a wide diversity of ecological and zoogeographical habitats of CCHF foci, (Hoogstraal 1979; Linthicum and Bailey 1994). There is a variety in the level of the human infection with CCHFV with the potential to be locally severe that can contribute to human's exposures to vertebrates, increased densities of vertebrates, and tick species. This is evidence in the observations of the outbreaks in the Crimean and Turkey (Hoogstraal 1979;Linthicum and Bailey 1994; Ergonul 2006).

2.2. Vector competence of hard tick species for CCHFV

Hyalomma species are considered the main vectors of CCHFV, however, the virus has been isolated from several other species of ticks. Potential tick vectors off CCHFV in general are: *Hyalomma marginatum, Hy. rufipes, Hy. truncatum, Hy. scupense* (synonym *Hy. detritum*), *Hy. anatolicum, Hy. excavatum, Hy. dromedarii, Hy. impeltatum, Ixodes ricinus, Dermacentor marginatus, Rhipicephalus sanguineus, Rh. bursa and Rh. rossicus.* Table 2 of the scientific review on CCHF (CFP/EFSA/AHAW/2008/04) summarizes data on vectorial competence of different tick species. The host preferences are not the limiting factor for the spread of the infection since this spread is linked to the maintenance of the virus in the environment.

From a European perspective, some of these species are candidates for a risk of spread of the pathogen. In this sense the most important potential vector in Europe is *Hyalomma marginatum*, a species widespread in Mediterranean-type landscapes. Furthermore, both *Dermacentor marginatus* and *Rhipicephalus bursa* are species with a wide distribution in the Mediterranean basin, the latter being already implicated in the transmission of human cases in Greece (Papadopoulos and Koptopoulos, 1980). It is difficult to ascertain the real implications of *Ixodes ricinus* in the transmission of the pathogen under natural conditions. While the species has been examined under laboratory conditions (Hoogstraal 1979, Watts et al 1988), the wide range of this tick in Europe even at northern latitudes, and the lack of association of this species with areas of active transmission of the virus, may support the conclusion that the tick is not important in the maintenance and diffusion of natural foci of CCHFV.

Ticks were suspected of transmitting Crimean-Congo hemorrhagic fever virus (CCHFV) shortly after the disease was formally described in the mid-1940s and inoculation of tick suspensions into human volunteers confirmed that ticks contained a filterable agent that caused CCHF. The CCHFV was detected in *Hyalomma marginatum* shortly after the disease was described. Since the initial studies, CCHFV has been isolated from ticks in several occasions (Hoogstraal, 1979). To date, CCHFV has been isolated from at least 30 species of ticks belonging to both argasids (soft ticks) and ixodids (hard ticks). Only members of three ixodid genera, namely *Dermacentor, Hyalomma* and *Rhipicephalus* have been shown to be able to maintain CCHFV through its life cycle and transmit the virus to other animals (Hoogstraal, 1956; Swanepoel, 1998; Swanepoel et al., 1998). In contrast, several studies indicate that argasid (soft) ticks are not competent CCHFV vectors (Shepherd et al 1989; Logan et al 1989a; Durden et al 1993).

The mere isolation of virus from an arthropod, however, does not incriminate it as an actual vector. Although relatively few studies have examined the ability of ticks to actually transmit CCHFV, those that have considered vector competence have consistently shown that ixodid ticks, particularly members of the genus *Hyalomma* (Table 2 of the scientific review on CCHF CFP/EFSA/AHAW/2008/04), are highly susceptible to infection with CCHFV and that infected ticks can transmit it by bite. Published report suggests that *Hyalomma* spp. are the main vectors, since CCHFV can infect the tissues of the tick and be transmitted in both trans-stadial and trans-ovarial ways. The high susceptibility of *Hyalomma* to CCHFV is indirectly confirmed by these observations and reports. The known distribution of the virus broadly coincides with the global distribution of these tick species. Infection with the virus is acquired by *Hyalomma* spp. feeding on an infected host. It is important to state that viraemic periods in both wild and domestic animals are short (up to one week according to Swanepoel and Burt 2004), so the infection within generations of ticks seems to be better maintained by transstadial and transovarial transmissions, as mentioned before. Transovarial transmission of the virus can occur with different effectiveness according to its strain. In the absence of susceptible vertebrate hosts, the virus can be maintained in ticks for extended periods by transovarial and transstadial transmission. In these conditions ticks can be considered vectors of CCHFV and reservoirs as well. Although there are few available studies, there is still a lack of field and laboratory data on the role of ticks in maintaining CCHFV in the environment. The understanding of the cycle of CCHF virus transmission needs further field investigations, especially in Europe (ECDC). Viral isolates were also obtained from field-collected eggs and unfed immature stages of *Hy. marginatum*, demonstrating evidence of transovarial (from infected mother through the eggs), and transstadial (i.e. from larvae to nymph to adult) transmissions (Watts et al., 1988; Zeller et al., 1994). Unpublished studies also suggest that viral RNA has been detected by PCR in larvae hatched from infected females of *Hy. marginatum* (A. Gargili and K. Midilli, pers. comm. contacted through telephone call by the WG member Estrada-Peña on April 28th 2010)

- The main vector is *Hyalomma* spp, but at local level *Dermacentor* spp and *Rhipicephalus* spp have proven capacity of maintaining the CCHFV through their life cycle and transmit it to the animals
- In contrast, several studies indicate that argasid (soft) ticks are not competent CCHFV vectors
- It should be investigated if any other tick species especially *I. ricinus* may transmit CCHFV under natural conditions (there is no evidence of natural transmission of CCHFV from *I. ricinus* by now). Viremic periods in domestic animals appear to be short which may influence the transmission cycle of this virus to other hosts.

Virus-tick interaction (transmission cycles)

Infection or even transmission competence *per se* are not necessarily sufficient for persistent cycles if the pattern of attachment by different tick stages would not allow sufficient amplification (Randolph and Rogers 2007). Although trans-ovarial transmission does occur, its efficiency as the only mechanism to maintain the virus in the tick population has not been yet proved (Wilson et al. 1991). It is hypothesised that co-feeding transmission may be an efficient form to sustain the infection level in nature, as it occurs in other tick-borne viral diseases (such as tick-borne encephalitis). In these conditions, at least two tick stages must feed on any one host. Non-viremic transmission of infection between ticks is believed to be facilitated by factors present in tick saliva and has been demonstrated under laboratory conditions with infected CCHFV adult fed together with non infected immature *Hy. truncatum* ticks and *Hy. impeltatum* ticks on non viremic mammals (Burt and Swanepoel 2005). In a national park in Cape Province, South Africa, there was a complete separation between immature stages of *Hy. turanicum* and *Hy. truncatum* on hares (the most highly infested host species), ground-feeding birds or small rodents; and adult ticks on zebra and eland (*Taurotragus oryx*). This pattern of tick biology indicates the distinct possibility that, while ruminants may be vital in feeding adult ticks and therefore supporting tick populations, smaller vertebrates may be the principle maintenance hosts (Randolph and Rogers 2007). An additional complication for understanding CCHF is the variability shown by *Hy. truncatum* and *Hy. rufipes* between a two-host feeding pattern (i.e. both larvae and nymphs feeding from the same host and adults from a second) and three-host feeding pattern (each stage feeding on a different host), apparently depending on the species of larva1 hosts (Rando1ph and Rogers 2007).

2.3. Factors influencing vector competence for CCHFV

The following factors are related to the vector competence for CCHF:

- The potential pathogenicity of the vector;
- The viraemia in the host;
- The biology of the hard tick:
    - Co-feeding;
    - Predilection body sites to feed on the host;
    - Host preferences;
    - Environmental factors as the temperature (that would affect the extrinsic incubation period), and the humidity for the vectors;
    - Transovarian and transstadial transmission.

- Co-feeding transmission may be an efficient form to sustain the infection level in nature, but it has never been satisfactorily proven (Estrada-Peña [WG member], personal communication)
- Ruminants are important hosts for adult ticks; however small and medium sized mammals are considered the principle reservoirs of CCHFV.

2.4. Laboratory studies for virus-tick interaction and transmission

The experimental studies on CCHF are not numerous; this is probably due to the necessity to have high safety leve of laboratory (BSL4) to work with this virus. The majority of these studies were performed by South African and French researchers who worked in West Africa. The table 5 of the scientific review on CCHF (CFP/EFSA/AHAW/2008/04) lists some references and information on experimental studies on CCHF.

Table 3 of the scientific review on CCHF (CFP/EFSA/AHAW/2008/04) summarized data generated from experimental infection in ticks (Linthicum and Bailey, 1994) while comprehensive data on vectorial competence are shown in table 2 of the scientific review on CCHF (CFP/EFSA/AHAW/2008/04). The CCHF viral infection has been demonstrated for many species feeding upon viremic vertebrates, Engorged nymphs of *Hy. rufipes*, *Hy. truncatum,* and *Rh. evertsi* became infected after intracoelomic inoculation (Shepherd et al 1989). Transmission of CCHFV to a vertebrate host has been demonstrated for many species (Linthicum and Bailey 1994). Larvae of *Hy. truncatum* and *Hy. impeltatum* were infected while co-feeding with corresponding CCHFV-infected adults on non-viremic guinea pigs (non-viremic transmission) (Gordon et al., 1993).

Okorie (1980) demonstrated that six hours after intracoelomic inoculation of 3.5 mouse LD_{50} of CCHFV into *Hy. rufipes* nymphs and adults. The eclipse period in adults lasted for over 24 h after inoculation. In both nymphs and adults, the peak content of virus was obtained by day 4 post-inoculation. The peak virus content in nymphs was much higher than that in adults inoculated directly. This suggests that CCHFV replicates faster in the immature nymphal stage of *Hy.rufipes* than in the adult stage. During metamorphosis of nymphs to adults, a drop in virus titre, followed by an increase in the new stage was revealed. This drop in virus titre could be explained by the fact that tissues of ectodermal origin are destroyed by lysis and replaced during metamorphosis. The increase in the titre could therefore be due to re-establishment of infection in the new tissues. The CCHFV titer in *Hy. rufipes* increased after a blood meal. This may be due to the increased cell activity after feeding, when old epithelial cells slough into the lumen and new cells replace them. This cell activity might have favoured replication of CCHFV, as previously suggested. Another important observation was that unfed male and female adults had about the same virus content until after engorgement, when the level of virus in the females became higher than in the males. The detection of CCHFV in the unfed adults, 185 days post-inoculation, shows that the virus persists for a long time in *Hy. rufipes*.

In conclusion it has been shown that the transmission of CCHF virus was trans-stadially to nymphs and adults, and horizontally to guinea pigs serving as host of nymphs and adults. Sexual transmissionof CCHFV has also been observed from male to female *Hy. truncatum.* Experimental studies demonstrated transstadial transmission of CCHFV consistently in all ticks infected by feeding on a viremic vertebrate (Linthicum and Bailey 1994). Although these experimental studies on the role of transovarial transmission in infected ticks are not conclusive and they may depend upon the used strain of CCHFV, there is consistency in demonstrating that the transovarial transmission is different among tick species.

- The infection within the generations of ticks is maintained by transstadial and/or transovarial transmissions.
- Transovarial transmission depends on the involved strains of CCHFV and on the tick species (Zeller et al 1997).

2.5. Life cycle

Stages of development of ixodid ticks (hard ticks) include egg, a six-legged larva, one eight-legged nymphal instar, and eight-legged male and female (adults). Life cycles of ticks are classified according to the number of times the three feeding stages change hosts. The species are classified as one-host, two-host, or three-host ticks.

Mating normally occurs on the host. It is a stepwise process normally regulated by sex pheromones. Females and males feed in a short blood meal for a few days (3 to 4 days for females and fewer days for the males) and then the female attract the male by releasing sex pheromones. After the transfer of the spermatophore (sperm container), females continue feeding until repletion (duration is species dependent) and drop from the host. Males remain on the host for several weeks. It is during this phase that infected males would be able to transmit some pathogens (virus) intrastadially (i.e. from one host to another host).

Three-hosts tick species

This is the commonest type of life cycle of hard ticks. Larvae develop in the eggs until ready to hatch, a period which depends on temperature, and which may last some weeks (Estrada-Peña et al. 2004). These ticks feed to engorgement as larvae, drop off their host to the ground, and moult into nymphs. Nymphs attach to another host animal, feed to engorgement, and drop to the ground. After moulting into adults, each female and male attaches to a host, mates, completes engorgement, and drops to the ground, where eggs are oviposited. Ticks that have recently hatched from eggs or from moulting have soft bodies and are inactive for one to two weeks until the external body wall hardens. The life cycle of three host ticks is slow, from six months to several years.

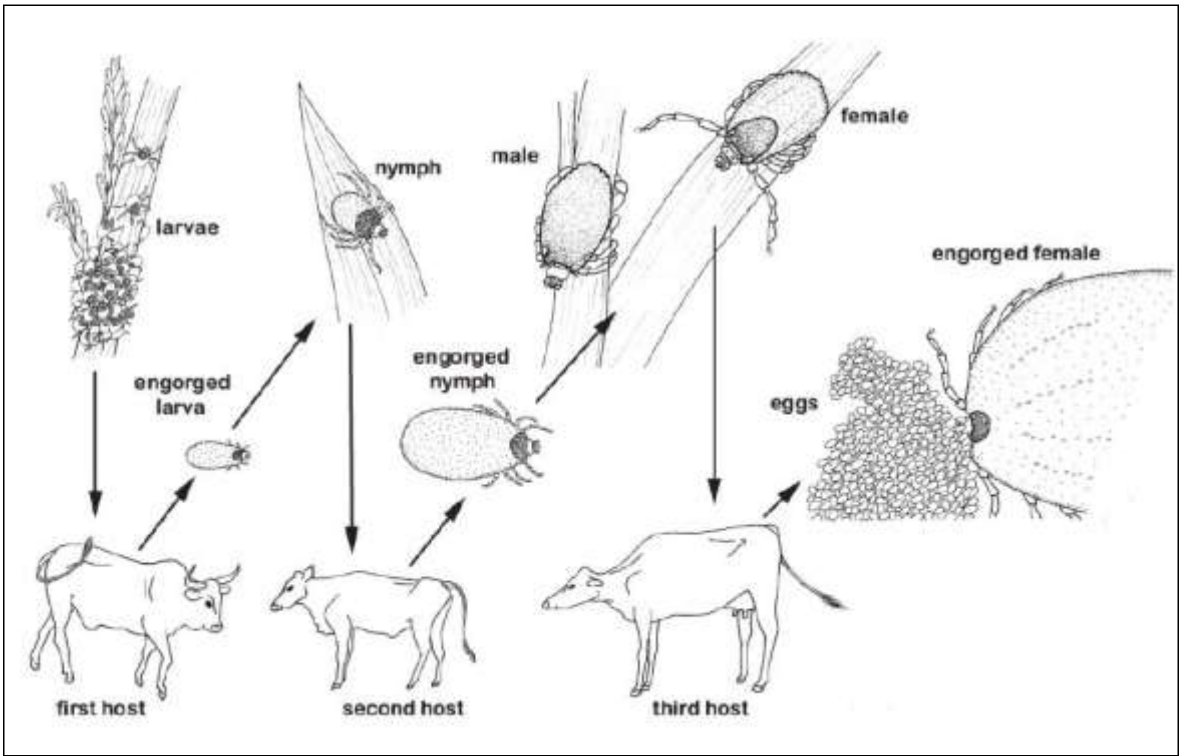


Figure 1: Life cycle of three-host tick species, from Walker et al 2003.

In Eurasia, the following three-host tick species have been associated with CCHFV: *I. ricinus, Ha. punctata, D. marginatus, Rh. pumilio, Rh. rossicus, Rh. sanguineus*, and *Rh. turanicus*. These three-host species are considered to be primarily involved in the ecology of CCHFV as enzootic vectors of the virus and not in transmission to humans. Although very few tick species have been incriminated in the transmission of CCHFV to humans, almost all the species associated with the virus are known to feed on humans under some circumstances. This is the reason for the uncertainty in considering *I. ricinus* as a primary vector of the virus, since it is widespread in Europe and is a common parasite of humans across its geographical range, where no clinical cases are observed.

Two-hosts tick species

As in all types of life cycles, eggs are laid on soil. Larvae hatch after several weeks of development and quest for a host. When they have feed completely they remain attached to the host and moulting occurs there. The nymphs then feed on the same host and after engorgement drop off the host and moult into adults on the soil. After hardening, the adults quest for a second host, attach, feed, mate and only the fed, mated females drop to oviposit in the natural environment. Males remain on the host for several weeks to mate with other females and eventually die. This type of life cycle is common for species of the genus *Hyalomma, Rhipicephalus evertsi* and *Rhipicephalus bursa*. Variations of this general pattern exist. In some species, e.g., *Hyalomma anatolicum excavatum,* a two-host life cycle may change into a three-host life cycle depending on the host on which the ticks feed on.

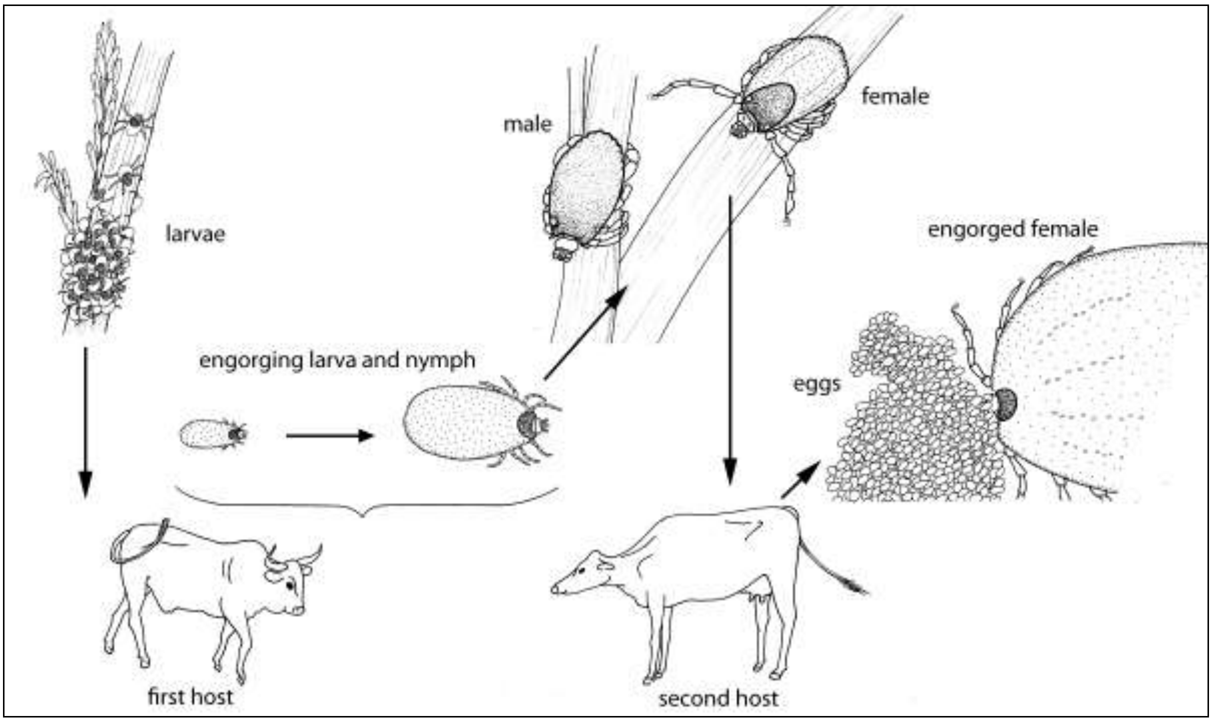


Figure 2: Life cycle of two-host tick species. Modified from Walker et al. 2003.

Two-host tick species can be regarded as two subgroups based on whether or not they utilize the same or different species of hosts to develop into the immature and adult stages. Species that attach as larvae and adults to the same species of host (monotropic) include *Rh. bursa*, *Rh. e. evertsi*, *Hy. detritum*, *Hy. anatolicum*, and sometimes *Hy. truncatum*. All stages of *Rh. e. evertsi* normally infest domestic or wild herbivores, but larvae may sometimes infest small mammals such as rodents and hares (telotropic) (Hoogstraal 1956). *Hy. detritum* commonly feed on cattle and horses. The adults feed in the summer and the nymphs undergo a winter diapause (Hoogstraal 1956). Tick species in which the immatures and adults feed on two dissimilar host species (ditropic) include *Hy. m. marginatum*, *Hy. turanicum*, and *Hy. rufipes*. Larvae and nymphs of *Hy. marginatum* feed primarily on small wild mammals and birds, while adults are commonly found on cattle, horse, sheep, goat, and camel (Hoogstraal 1956). *Hy. rufipes* adults are most commonly found on domestic cattle but also on horses, sheep, goats, and large wild animals like buffalo and giraffe. Immatures feed on a variety of birds and also on hares (Linthicum and Bailey 1994).

Hy. marginatum exists in Europe: Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, France, Greece, Italy, Macedonia, Moldova, Montenegro, Portugal, Romania, Russia, Serbia, Spain, Ukraine; Asia: Armenia, Azerbaijan, Georgia, Iran, Iraq, Israel, Syria, Turkey and Turkmenistan; Africa: Algeria, Egypt, Libya, Morocco and Tunisia. The records of this species from further south in Africa (e.g. Nigeria) and north in Europe (e.g. Finland) are most probably a consequence of its immature stages being transported there by migratory birds.

Hy. rufipes is distributed on Europe: Macedonia, Malta, Russia (Astrakhan region), Ukraine (Zmeiniy island); Asia: Iran, Israel, Kazakhstan, Oman, Russia (southern Dagestan), Saudi Arabia, Syria, Tajikistan, Turkmenistan, Uzbekistan, Yemen. The natural distribution of the species is Sub-Saharan Africa and adjacent regions of Africa and Arabia along the Red Sea. The records of *Hy. rufipes* from Europe and most regions of Asia and North Africa (except Egypt) are an apparent consequence of the dissemination of the immature stages by migratory birds from Africa.

Hy. turanicum exists in Asia: Afghanistan, Bahrain, India, Iran, Iraq, Jordan, Kazakhstan, Kuwait, Kyrgyzstan, Nepal, Oman, Pakistan, Qatar, Saudi Arabia, Syria, Tajikistan, Turkmenistan, Uzbekistan and Yemen; Africa: Egypt**.**

One-hosts tick species

From an evolutionary point of view, this is the most advanced type of life cycle that occurs in all species of the *Boophilus* sub-genus of the *Rhipicephalus* genus but also in the winter tick *Dermacentor albopictus,* the latter mainly being present in Canada. All the stages remain on the host after the larvae have attached. The adults will change position on the same host for mating. Thus all three feedings of any individual tick occur on the same individual host. The "on-host" part of the life cycle of one-host ticks is usually rapid (between 21 and 22 days). The advantage of one, and to a lesser extend also two-host ticks, is the relatively protected environment that the hosts offer to the vulnerable larval and nymphal stages. In this way, the immature stages are not exposed to hostile climatic conditions, reducing mortality significantly. In many tick species (mainly three-host ticks), dormancy regulates the life cycle to avoid unfavourable climatic conditions and thus reducing mortality as well.

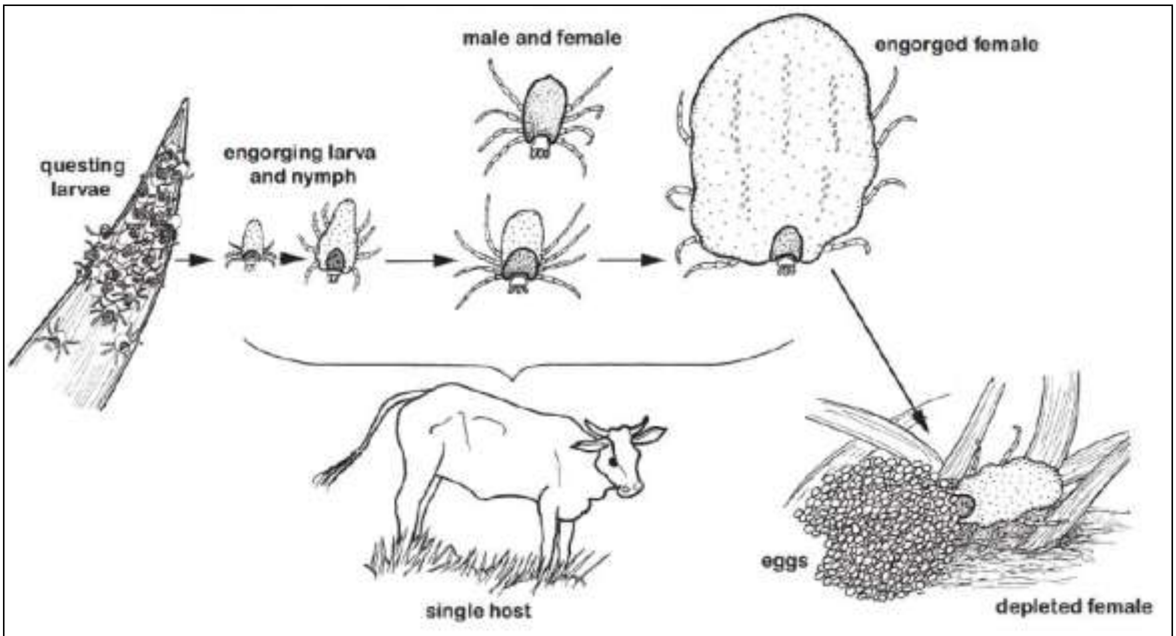


Figure 3: Life cycle of the one-host tick species, from Walker et al 2003.

All stages of the species in the subgenus *Boophilus* are completed on the same vertebrate host and they rarely feed on humans. The role of one-host tick species in the ecology of CCHFV is still unclear.

Life cycle influence on the epidemiology of the disease

The life cycle, together with the biology and the behavior of the vector tick can affect the epidemiology of the disease. Endophylic ticks (with the host range constituted by rodents, hares, hedgehogs) could be good for the maintenance but are not suitable for the dispersion and consequently for the geographic spread of CCHFV. Exophilic competent vectors that have as host animals able to move longer distance are more suitable for the geographical spread of the virus.

- Three-host ticks have the longest life cycle (months to years) than other one or two host ticks, and can perpetuate the presence of the virus for longer periods.

- One-host ticks are the most evolved, and the ones with the shortest life cycle. The off host period is limited to the eggs and questing larvae, favouring survival of the tick population. However, they are also more vulnerable to the treatment with acaricides.
- The host offers an appropriate environment to the immature stages.

2.6. Identification

Identification of ticks can be based on different criteria. Morphology, which can be some times the only criterian, but also in a more holistic approach it may be based on host species, predilection sites, geographical occurrence, seasonality, and others. Morphological identification is based on presence / absence of eyes; the presence of anal plates; ornamentation on the legs and scutum; shape, size and the patterns of the scutum, and other unique characteristics.

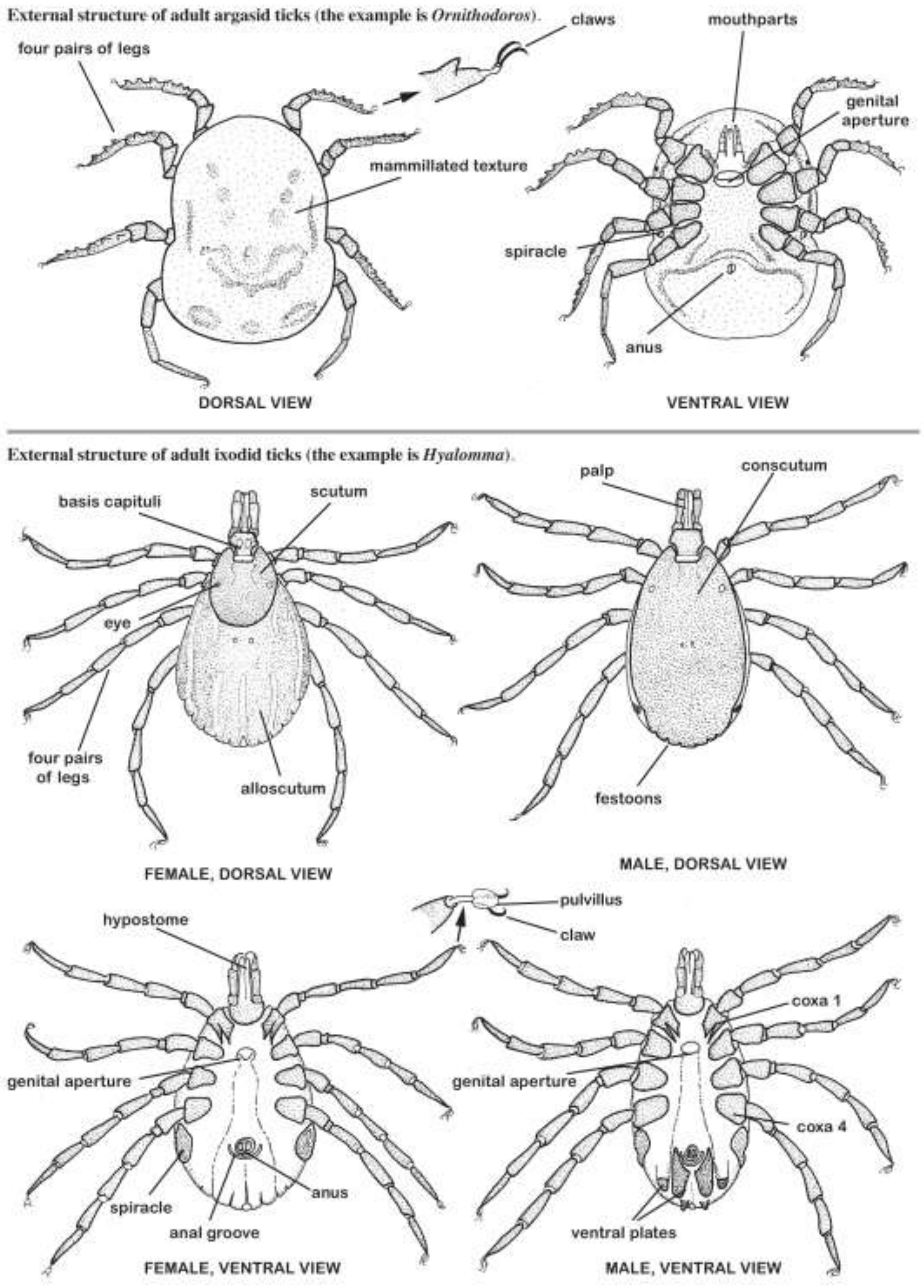


Figure 4: Main anatomical characteristics and terms used to describe the morphology of adult hard ticks, from Walker et al 2003.

Table 1: Generically distinguishing features of hard ticks

Feature	*Hyalomma*	*Rhipicephalus*	*Rh. (Boophilus)*	*Ixodes*	*Dermacentor*	*Haemaphysalis*	*Amblyomma*	*Rhipicentor**	*Aponomma**	*Margaropus**
Size (unfed adults, total length)	large (0.5-0.8cm)	medium (0.3-0.6cm)	small (0.1-0.3cm)	small (0.2-0.3)	medium-large (0.4-0.9cm)	small (0.1-0.3cm)	very large (0.5-1.0cm)	medium (0.3-0.5)	large (0.5-0.6cm)	small (0.1-0.3cm)
Mouthparts	long	Short to medium	very short	long	medium	short	very long	medium	long	very short
Basis captuli		hexagonal	hexagonal	with ventral auriculae	rectangular	rectangular		hexagonal		
Ornate	no	no, except for 4 spp.	no	no	yes	no	yes	no	some species	no
Eyes	yes	yes	yes	no	yes	no	yes	yes	no	yes
Festoons	yes	yes	no	no	yes	yes	yes	yes	yes	no
Adanal plates	yes	yes	yes	no	no	no	none, or very small	no	no	yes
Sub-anal plates	yes	no	no	no	no	no	no	no	no	no
Anal groove	posterior, chalice-shaped	posterior, chalice-shaped	posterior, vertical line	anterior, semicircular or u-shaped	posterior, chalice-shaped	posterior, chalice-shaped	posterior, semicircular	posterior, chalice-shaped	posterior, chalice-shaped	absent
Caudal process	no	present in some species	yes on some	no	no	no	no	no	no	yes, pilose posterior margin
Coxae I	bifid	bifid			large, bifid			large, bifid		
Coxae IV					very large			very large, with spurs		
Legs	banded			grouped anteriorly	banded		banded			banded, enlarged segments in males

(*) These genera are currently not present in European member states, but they may be imported in the future.

2.6.1. Genus Hyalomma

Tick species of the genus *Hyalomma* are found in Africa, southern Europe and Asia. Many species have been redescribed recently based on morphological characteristics (Apanaskevich and Horak, 2005, 2006, 2007, 2008a, 2008b, 2008c, 2009, Apanaskevich et al, 2008a, 2008b, and 2009). These characteristics may however vary with populations, host, and other environmental factors making it difficult to identify them. *Hyalomma* ticks are mainly two- and three-host species (Matthysse and Colbo, 1987).

The genus is characterised by ticks of large size, long mouthparts, pale to dark scutum and pale rings on their legs. All species have convex eyes, festoons and the males have anal plates.

Hyalomma marginatum

Hyalomma marginatum is a hard tick species complex occurring in southern Europe, southern Asia and most of Africa. It is characteristic of steppe, savannah and lightly wooded hill and valley biotopes with fairly low humidity. *Hy. marginatum* sensu lato (s.l.) species are the most important vectors of CCHFV. There are four species in this complex which differ in their geographical distribution: *Hy. turanicum, Hy. isaaci, Hy. rufipes,* and *Hy. marginatum*. The later one is the only European species in the complex (Merck and Co., 2008). *Hy. marginatum* is a two-host species moulting from larva to nymph on its first host (especially hares, hedgehogs and birds) and infesting the second host (mainly sheep, goats, swine, cattle and horses) as an adult. Both larvae and nymphs feed without leaving the host and spend several days on its host. The immature stays for a long time on the host (12–26 days) which enables them to be passively transported by migrating birds over hundreds or even thousands of kilometers (Manilla 1998c; Rechav et al. 1987). Photographs of *Hy. marginatum* adults are provided in appendix I.

Hyalomma marginatum (s.l.) species are the most important vectors of CCHFV. There are four species in this complex which differ in their geographical distribution: *Hy. turanicum, Hy. isaaci, Hy. rufipes,* and *Hy. marginatum*. The later one is the only European species in the complex (Merck and Co., 2008)

Hyalomma rufipes

Adults *Hy. rufipes* are large ticks with dark-brown bodies, long mouthparts, a heavily punctated scutum, small, round and bright eyes, and long, red and white banded legs. They differ from *Hy. truncatum*, which is present in the same geographic area, in that the whole scutum is punctated and in the males it is more circular than the rather elongate shape of the latter species. Furthermore, *Hy. rufipes* differ from *Hy. marginatum* for its more hairy spiracular plate. However it is virtually impossible to distinguish between the species by means of the naked eye (Manilla 1998c; Norval and Horak 2004). Apanaskevich and Horak (2008) published more recently a determination key to diferenciate these two species.

Hyalomma truncatum

Adults of *Hy. truncatum* are medium-sized ticks with long mouthparts and dark-brown bodies, beady eyes, and long, red and white banded legs. The posterior surface of the scutum in males is characterized by a depression containing numerous large punctuations; otherwise it is comparatively smooth (Norval and Horak 2004).

Hyalomma detritum

Adult *Hy. detritum* are medium-sized ticks with long mouthparts, a very dark-brown, smooth, shiny scutum, and long reddish or yellowish-brown legs which may have paler bands. This tick species is the most abundant on cattle in the sub-humid and the semi-arid zones of Sub- and North Saharan Africa, with a high prevalence. In the humid area, *Hy. detritum* population represents only 4% of tick sampling. All the stages are endophilic and are usually found in wall crevices and cracks, under the rocks and even under the dried manure.

Hyalomma anatolicum* and *Hyalomma excavatum

The study of the ecology, development and epizootic role of these two similar species is very difficult. The taxonomic status of these species is not even uniformly agreed on today. Their occurrence within the same geographical area, the presence of hybrid forms, morphological variations and impossibility of distinguishing the larvae and nymphs complicate this work. Ecological conditions apparently determine which form will develop in a given location (Liebish et al. 1989; Apanaskevich 2003),

A recent study which had compared the morphology of each stage of the two species of several specimens belonging to different collection, allows to definitely separate two specific identities *Hy. anatolicum* and *Hy. excavatum* (Apanaskevic and Horak 2005).

Hyalomma dromedarii

Adult *Hy. dromedarii* are large ticks with long mouthparts. The scutum of the male is characterized by posterior grooves and ridges. The colour of these ticks varies from yellow-brown to nearly black. The legs are paler than the scutum and may be ringed by paler bands.

Hyalomma impeltatum

Hyalomma impeltatum needs to be distinguished from *Hy. dromedarii*, with which it shares similar morphological features, hosts, and geographical areas. The scutum of female *Hy. impeltatum* has a distinctly sinuous posterior margin compared to slightly sinuous in *Hy. dromedarii*. The genital aperture is bordered on each side by a slight bulge that gives the genital area a trilobed appearance distinctive to this species (Estrada-Peña et al. 2004).

2.6.2. Genus Rhipicephalus

The genus *Rhipicephalus* is probably of African origin with several species found in Europe and Asia. One species, *Rh. sanguineus* is cosmopolitan most probably because its specific host preference, dogs. Most of the ticks are three-host species except for *Rh. bursa*, which is two-host. The species which are part of the subgenus *Boophilus* are all one-host tick species.

Rhipicephalus presents short mouthparts, hexagonal basis capituli, brown scutum although some species (mainly African) might have coloured ornamentation, eyes, festoons and azdanal and accessory adanal plates. The identification of many species and immature stages is cumbersome because of their similar morphology.

Rhipicephalus rossicus

Rhipicephalus rossicus is a medium sized, light to medium-brown tick belonging to the *Rhipicephalus sanguineus* group. The broadly elongate spiracles and relatively impunctate scuta in both male and female and a small circular deep set porose area in the female are distinctive features that allow to separate it from *Rh. sanguineus* (Walker et al 2000).

Rhipicephalus sanguineus

Rhipicephalus sanguineus is universally known as “the kennel tick” (Manilla 1998b; Walker et al. 2000; Estrada-Peña et al. 2004). It is a medium sized, pale yellowish-brown or reddish-brown tick.

The *Rh. sanguineus* group comprises several tick species. The biosystematic status of the majority of them has been confused (Walker et al. 2000; Estrada-Peña et al. 2004). Two species of the group which closely resemble *Rh. sanguineus* are *Rh. camicasi*, and *Rh. turanicus*. Females of *Rh. sanguineus* are differentiated from those of *Rh. camicasi* and *Rh. turanicus* by the genital aperture which is usually a broadly V shape in *Rh. sanguineus* compared to a narrow U in *Rh. camicasi* and *Rh. turanicus*. Both sexes of *Rh. sanguineus* have spiracle plates with tails, which are narrow, less than the width of the adjacent festoon. In *Rh. turanicus*, these tails are broad. Males of *Rh. sanguineus* do not have a depression of the cervical fields compared the small depression there in *Rh. camicasi* (Walker et al. 2000; Estrada-Peña et al. 2004).

2.6.3. Genus Ixodes

Ixodes has a large number of species and the greatest natural geographic distribution of any ixodid genus. It occurs throughout the tropical and temperate regions (Matthysse and Colbo, 1987). They are all three-host ticks.

The genus is characterised by long mouthparts, auriculae latero-ventrally on the basis capituli, absence of eyes, festoons and adanal plates, An anal groove is conspicuous and anterior to the anus. Their legs also appear to be grouped anteriorly.

Ixodes ricinus

This is a species found in the Palaearctic region in a wide range of environments, typically involving sites with an adequate degree of relative humidity. However, it can colonize even Mediterranean type areas in northern Africa and western Iberian Peninsula. This species has been intensively studied in Europe because of its role in the transmission of a wide range of pathogens to domestic animals and humans (Estrada-Peña et al. 2004). It was supposed to be involved in the transmission of CCHFV in the forests of Moldavia and Bulgaria (Hoogstraal 1979). However, its implication in the infection has never been demonstrated. Unique morphological characters in *I. ricinus* is the presence of a pre-anal groove (Prostriata) which is absent in the rest of the tick species considered herein.

2.6.4. Genus *Dermacentor*

Dermacentor ticks are also widely distributed throughout the world. Most of the species are three-host ticks although *D. albipictus,* a North American species*,* presents a one-host life cycle.

Dermacentor ticks have short but broad mouthparts and a rectangular basis capituli. Eyes and festoons are present. Males have no anal plates but large coxae, particularly the fourth pair.

Dermacentor marginatus* and *Dermacentor reticulatus

The characteristics of the genus *Dermacentor* are: short mouthparts with a basis capituli of straight lateral margins, both sexes usually have white enamel ornamentation and the males have very large fourth coxae. These species have white enamel on the scutum or conscutum (Manilla 1998b, Estrada-Peña *et al*. 2004). Both species can be found in Europe and in the Mediterranean region. *D. reticulatus* mainly feeds on dogs. Sometimes both species can be found on the same host; therefore, separation of both species from each other is important. In both sexes, the most important feature is the presence of a palpal spur in *D. reticulatus*, which is absent in *D. marginatus*. In the females, most prominent details are the shape of porose areas, the shape of the gap between internal and external spurs on coxa I and the size of the lips in the genital aperture. In the males, cornua are long in *D. reticulatus* (short in *D. marginatus*) and the lateral groove is in the form of punctations only in *D. reticulatus* (there is no groove visible) (Estrada-Peña et al. 2004).

2.7. Host preferences

2.7.1. Host seeking strategies

Ticks have different ways of finding hosts: most exophilic ticks such as *I. ricinus, Dermacentor spp.* or *Hy. marginatum* employ the ambush strategy, climbing onto weeds, grasses, bushes, or other leafy vegetation to wait for passing hosts. The height to which the ticks climb depends on several factors. The later stages, especially the adults, climb higher in the vegetation where they are more likely to encounter larger animals such as deer, carnivores, and humans. Since the water balance is the limiting factor for the activities of these ticks, the ticks remain on the undersides of leaves or other protective cover with their legs folded. Here they can remain for many hours until increasing desiccation causes a descent to the cooler, humid ground layer where they can replenish lost body water (Sonenshine, 2005). In many species, larvae, that are most sensitive to the desiccation (Manilla, 1998d), remain closest to the ground layer where they are most likely to encounter small mammals, ground-feeding birds, and other small vertebrate hosts (Sonenshine, 2005) or cluster together on the vegetation. Host-seeking ticks of many species respond to a wide range of stimuli such as: shadows, vibrations, odours, tactile signals, and other stimuli indicating the presence of a host, whereupon they extend their forelegs anterolaterally and cling to the hair or clothing of the passing host (Sonenshine, 2005). The dragging method for collecting ticks from vegetation takes advantage of the questing behaviour. They cling to the cloth and do not immediately distinguish it from a living host. Even sound can attract ticks. The sounds produced by barking dogs have been reported to attract the brown dog tick (*Rhipicephalus sanguineus*), while sounds in the range emitted by cattle are known to attract larvae of the cattle tick (*Rh. (B.) microplus*) (Sonenshine 2005).

The hunter strategy is the method used by several species that feed on large mammals such as the desert inhabiting, xerophilic camel tick (*Hy. dromedarii*) or the southern African *Amblyomma* ticks. These ticks remain buried in soil, sand, or duff where they are protected from extreme heat and desiccation. However, when excited by host odours, the ticks emerge and run rapidly across the ground to attack these hosts, often up to 2 or 3 m away. Hunter ticks can discriminate dark shapes against the bright background of the sky and they are easily excited by carbon dioxide. Carbon dioxide in high concentrations is believed to act as a general excitant but may not facilitate identification of the source. In addition, tick pheromones and possibly host odours can provide the directional information that leads the ticks to cattle or other ungulate hosts (Barré et al. 1997). Odours are detected by olfactory sensilla, especially the prominent multiporose sensillum in the Haller's organ on the first tarsus (Sonenshine 2005).

Most of endophilic ticks (like *Ixodes hexagonus* and *Hyalomma detritum*) usually wait for their host in nests, caves, or other environmental settings and when hosts come close to the ticks, they crawl to the host (Manilla 1998d).

2.7.2. Host preference

Ticks do not feed equally on all vertebrate animals. Many tick species show some degree of host specificity, accepting only a limited variety of animals as a candidate blood source. Such ticks are host specific, i.e., they are restricted to a particular class, order, or even genus of vertebrates as hosts (Sonenshine 1994). Host specificity extends across a broad range of extremes from those that are highly host specific at one end to those that exhibit little, if any, specificity at the other. The latter are termed low or non-specific or opportunistic tick species. Often, such tick species will feed on a particular class of vertebrates, e.g. birds, but no other vertebrate animals. Many species exhibit restricted host ranges in one or two stages of the life cycle, but a much broader range of hosts in another period of the life cycle. The generalists constitute the opposite end of the host specificity spectrum. Such non-specific ticks feed readily on virtually all terrestrial vertebrates, although amphibians are rarely used. Some examples of the preferred hosts for ticks of importance in the transmission of CCHFV are detailed below. Sometimes, it is not a strict specificity, but depends on the availability of a given host in the territory colonized by the tick. True, or physiological host specificity, is the result of:

1. the tick's ability to recognize and respond to specific host-originated compounds (kairomones), especially odours such as CO_2, NH_3, lactic acid, and other volatiles, reinforced by thermal and contact stimuli characteristic of the host body;

2. pharmacologically active compounds in tick saliva that enable the ticks to suppress (or evade) host homeostatic mechanisms, thereby enhancing blood flow into the feeding pool but minimizing inflammatory responses that reveal presence of the tick (Sonenshine, 1994).

Host preference is also affected by ecological determinants that have in influence on the resistance to the desiccation:

1. the habitat or habitats in which the ticks quest or hunt for hosts;

2. the questing height, i.e., the elevation above the ground at which ticks rest while waiting for passing hosts;

3. the time of the day when ticks actively seek hosts.

Therefore ticks that can tolerate substantial water loss may quest in relatively dry, meadow environments during daylight hours and climb relatively high on to grassy or weedy vegetation. Resistance to the water loss is not only dependent to the species but in many cases also to the developmental stages (Sonenshine 1994; Manilla 1998d; Sonenshine 2005). Additionally, the life cycle of most tick species is synchronised with prevailing environmental conditions, by any form of dormancy, in such a way that the most vulnerable stages (eggs and larvae) are present during the least hostile climatic conditions.

Hyalomma marginatum: the immatures have been collected on wild and domestic birds (Passeriformes, Falconiformes, Galliformes, Gruiformes, Charadriformes, Strigiformes, Coraciformes), European brown hare (*Lepus europeus*), hedgehog, and wild ungulates. Adult stages have been reported on domestic and wild ungulates, horses and humans.

Hyalomma rufipes: the main hosts for adults are domestic and wild ungulates. The hosts for immatures are the scrub hare (*Lepus saxatilis*) and birds.

Hyalomma truncatum: the main hosts of the adults are large domestic and wild ungulates of the orders Artiodactyla and Perissodactyla. Smaller animals including carnivores, rodents, hares, hedgehogs, birds and reptiles, as well as primates and humans are rarely infested. The hosts of the immature stages are hares and rodents.

Hyalomma detritum: domestic cattle and horse, sheep, goat and camel

Hyalomma anatolicum: immature stages parasitize large domestic and wild animals; adults feed mainly on livestock and wild ungulates as deer.

Hyalomma excavatum: the immatures feed on hedgehogs, rodents and hares; adults feed mainly on burrowing rodents.

Hyalomma impeltatum: the immatures have been collected on rodents, hare or ground birds; The adult stages have been reported on all large domestic animals particularly cattle and camel.

Rhipicephalus rossicus: hedgehog, hare and rodents are the hosts for the immature stages; adult stages can be found on domestic animals, hedgehog and occasionally in human.

Ixodes ricinus: very wide host range, mammals, birds and even lizards. It has been recorded on about 237 hosts (Gern 1994). It is the most frequent in Europe among the about 30 tick species that may feed on humans.

Dermacentor marginatus: Immature stages have been found on small mammals as rodents, medium sized carnivores, birds, and human; domestic ruminants and wild ungulates, wild boar for the adults.

Rhipicephalus sanguineus: dog and wild carnivores; occasionally human, cattle, goat and wild carnivores.

- There is a wide host range of the tick species that are able to transmit CCHFV.
- *Ixodes ricinus* is the most frequent in Europe among tick species that feed on humans. This species has the widest host range, either in mammals, birds or even lizards.

2.8. Field monitoring approaches

Actually there is no surveillance system in place for tick vectors in Europe. There is a lack of standardised longitudinal data in most of the countries. There is a need for standardised protocols for tick collection including appropriate tick identification and tick-borne pathogens detection. The OIE Terrestrial Animal Health Code (OIE, 2009) lays down the general principles that should be followed for surveillance of arthropod vectors of animal diseases. The most commonly used tick collection methods are based on manual collection from hosts and suitable habitats with dragging and / or flagging methods.

2.8.1. Collection of ticks from live or dead hosts

Tick specimens are frequently obtained from their hosts. It is seldom feasible to examine the entrie body of a livestock or pet for ticks, but in some studies the animal is cast to the ground or held in a crush then one half of the body is searched fully. It is often more efficient to examine a sample of fixed areas of the host. This is very useful for ticks which are known to have sites where they prefer to feed (= predilection sites). For example, on a herd of cattle in the western Mediterranean parts, one can expect to find *Rh. turanicus* adults on the ears; *Hy. marginatum* and *Hy. lusitanicum* adults on the dewlap, axillae, udder and groin; *Rh. (Boophilus) annulatus* generally on the shoulders, dewlap, and belly (Estrada-Peña et al., 2004). An effective way to detect adult ticks, especially when they are engorging, is to feel the hair coat of the host with the palm of the hand. Smaller domestic animals in a clinic can be examined in the same way. To find immature ticks or unfed adults the hair can be parted systematically using forceps as a comb (Latif and Walker 2004).

Small mammals, particularly small rodents, can be live-trapped in particular habitats by specially constructed “live rodent traps” baited with sunflower seeds and pieces of fruit (apple). The traps must be inspected at short, hourly intervals to minimize the risk that more than one animal is caught in the trap (since two animals in one trap may harm and try to kill each other). Any trapped animal is kept inside the trap cage which is placed indoors in a quiet environment, and placed over a water-filled tray. The trapped small mammal is offered seeds and water *ad libitum* and may be released after 2-3 days, when usually no more ticks will detach from it. Ethical clearance, usually from the University’s Committee on Ethics in Animal Research is necessary prior to this type of live animal trapping and maintenance of living vertebrates.

Ticks can also be collected from recently killed animals: the dead body or the skin of a hunter-killed vertebrate may be put into a plastic bag shortly after the animal has been shot, which will then be transported to the laboratory. The plastic bag used for transportation is inspected for any presence of ticks which are then recorded and preserved. In a special, separate room where, preferably the temperature is somewhat below ordinary room temperature the dead animal or the skin of it is hung a few dm above a plastic or metal tray half-filled with water. Ticks detaching from the dead animal or skin will drop into the water where the ticks can be collected with forceps. The water tray should be inspected at least every 6 hr and any tick therein recorded, put into labelled vials and stored in a plastic bag with high humidity in a refrigerator (+4 to +8 C).To remove ticks from host skin whilst retaining their good condition for identification is necessary to use good quality steel forceps. The forceps is used to grip the tick firmly over its scutum and mouthparts as closely to the host skin as possible, and then pull strongly and directly out from the skin. Usually the mouthparts will be removed with the rest of the tick and often with a plug of cement. Teeth may be damaged during removal of the mouthparts of the tick from the host. For the identification of some genera observation of the arrangement of the teeth is necessary. Generally, for more easy identification is useful to have males in addition to females. If the ticks are required live for further studies they should be placed in strong tubes containing a piece of damp paper.

The ticks should be kept cool in a refrigerator or over ice but, in the latter case, take care not to freeze them fatally. To preserve the specimens at the collection site place them directly into 70% alcohol. To label collection tubes in the field the best method is to use a lead pencil to write a small label on white card. This label is placed inside the tube with the ticks. Labels on the outside of the tubes should only be written on tape wrapped completely around the tube. Field collection data should include date, site, collector, host species and other information relevant to the study.

2.8.2. Collection of ticks from environment

Some species of ticks can be collected whilst they are unfed and questing on vegetation. If they are sufficiently dense in numbers adult can be picked from grass stems (Latif and Walker 2004; Estrada-Peña *et al*. 2004). More often, it is efficient to use a drag, which mimics a passing host. This consists of a cloth 1m square which is dragged slowly across the vegetation for 5 m to 10 m (or 10 seconds of walking using a stop-watch; or a longer distance if tick density is low). Larvae, nymphs and adults will grip onto the dragging cloth temporarily and can be collected with a forceps. A white cloth of cotton is effective for dragging and / or flagging. For dragging a cord is fitted with a bar at the front and a cord for pulling it. For flagging a very strong frame is attached to the white cloth is used to sweep the vegetation. The efficience of the methods is species depended. For *Ixodes ricinus* mainly immatures (larvae and nymphs) are caught; whereas for *Dermacentor* these methods are more successful for adult ticks. Traps using carbon dioxide as an attractant can be used to collect “hunter” ticks (Gray 1985).

Endophilic ticks can be collected directly from the nests or shelters of their hosts using forceps to probe in cracks and under pieces of dry dung, spiders webs etc. This is very effective for moulting nymphs and adults of *Hyalomma* ticks in cattle housing (Estrada-Peña et al. 2004; Latif and Walker 2004). CO_2 traps have been used to catch *Ornitthodoros moubata* in warthog burrows. During collection it is necessary to protect the whole body from tick bites by wearing long trousers tucked inside the boots or trousers tucked inside stockings.

In conclusion various methods for collection and monitoring ticks have been used. Reliable method will depend on the lifecycle of the species of interest, host species, and the precision (presence/absence vs. density) of measuring the tick’s presence.

2.8.3. Reporting a surveillance study

Data extraction from scientific literature in terms of presence and density of ticks is a daunting task, not because of the number of articles but mainly because of the un-standardised way in which some scientists present their data. Especially older articles sometimes lack precision especially related to geo-location, diagnostic method, collection methods etc. It would therefore be useful to determine a standardized way of data reporting.

These data are essential for modelers and epidemiologists to present vector or disease ranges, produce risks map, or analyse and understand the epidemiological situation of those vector-pathogens-host relationship.

Scientific papers should at least clearly specify the scientic names of the organisms studied (vectors or pathogens), origin of samples (vegetation, host), type of samples (blood, serum, organs, etc), geo-reference of the sample, diagnostic tools used to identify the samples, date, and history of the samples if applicable (imported host or vectors, travel history). Without a precise description of the data, they have limited use.

2.9. Factors influencing the abundance of CCHFV vectors

Because of the ubiquity of the putative vertebrate hosts of CCHFV, host availability is unlikely to be a limiting ecologica1 factor in the distribution, or even the prevalence, of the CCHF virus. During the co-feeding process, ticks can be infected with some arboviruses from the neighbouring feeding, infective ticks - via the host - without necessity of viraemia in the host. Such co-feeding transmission has been reported as the main transmission route for TBEV. This may add both qualitatively (more species than conventionally recognized acting as hosts) and quantitatively to the transmission potential (Randolph and Rogers 2007). Co-feeding transmission has been suggested to be important for the maintenance of CCHFV (Nuttall and Labuda, 2008).

The principal environmental factors responsible for the enzootic distribution of CCHFV within the steppe, savanna, semi-desert, and foothill ecotypes of the Palearctic region are those that enhance the prevalence of *Hyalomma* ticks. In an old report, which had no follow up, the hypothesis was that in the deciduous forests of Moldavia *Dermacentor* and *Rhipicephalus* species have displaced *Hyalomma* species as vectors, probably because of changes in the cattle herding practices (Hoogstraal 1979). It is unlikely that *I. ricinus* participates in the transmission of the virus, since this tick species is widespread and abundant in areas of Europe, where no cases have been reported.

In a given region, CCHFV transmission is observed mainly in limited foci where climatic factors and/or environmental changes are conducive to the survival of *Hyalomma* ticks and their hosts. CCHFV foci may be along river floodplains with rich grasslands (Astrakhan Oblast), shrub and woodland vegetation (Uzbekistan), desert or semi-desert, sparse forests scattered in desert (Kazakhstan), and forests and thickets of rough steppe lands (Rostov Oblast) (Linthicum and Bailey 1994; Watts et al. 1988).

- Host availability is unlikely to be a limiting ecological factor in the distribution, or even the prevalence of CCHFV. This is becauses of the abundance in enzootic areas of vertebrate hosts of competent vectors which are at the same time potential CCHFV hosts.
- Environmental factors including climate can affect the enzootic distribution of CCHFV by changing the abundance and / or the distribution of the vectors.

2.9.1. Abiotic factors

Abiotic factors are more likely to determine patterns of epidemiological risk, because they affect the rates of growth of many of the tick population processes, which are critica1 to the dynamics of tick-borne disease systems (Randolph and Rogers 2007).

2.9.1.1. Environmental conditions

Many of the Eurasian competent vectors of CCHFV belong to the Mediterranean tick fauna. The Mediterranean region is a zone of complex biogeography, because the diversity of habitats and the sudden landscape changes which are a consequence of the altitudinal variation.

The relief is also a contributing factor to this variability. Drastic changes in elevation may contribute to severe variations in climate. The variability of the climate forms the base for a species-rich thick fauna (Estrada-Peña et al. 2004).

Arid or semiarid steppes of central Asia are also appropriate environment for some of *Hyalomma* tick vectors of CCHF

In general, CCHF outbreaks have evolved against a background of favourable climatic factors and environmental changes beneficial for the survival of great numbers of *Hyalomma* species and hosts of both their immature and adult stages. In the former Soviet Union, environmental changes may have contributed to CCHF outbreaks. For instance, neglect of agricultural lands, introduction of susceptible military personnel or new settlers into an infected area, wide scale collectivisation of agriculture in the socialist countries, changing pasture patterns, converting floodplains to farmland, and flood control led to CCHF outbreacks during times of war.

During World War 2, after the occupation of Crimea (1941–1944), normal agricultural practices were disrupted and the common sport of hunting the European hares ceased. When Soviet troops reoccupied the hilly Crimean steppes in 1944, hares had become excessively abundant and neglected pastures had become overgrown with weeds, and the first outbreak after the Second World War was documented (Ergonul, 2006). However, this explanation cannot be applied to the recent and still ongoing epidemic in Turkey. The main vector, *Hy. marginatum* has a distribution range larger than the current area where disease occcurs. If we accept that the first cases of the disease appeared around 2002 in a region where agricultural changes happened, similar to those described previously, it is difficult to explain how the disease spread into large areas of the country, without such a kind of agricultural background. One should expect that the virus primarily colonized a territory with similar characteristics, as reported for previous outbreaks in the 20th century. However, data assembled by the Ministry of Health of Turkey, show that the disease is spreading within the range of preferred habitat of the vector, and is well outside the areas of neglected agricultural lands. More gradual environmental changes such as wide-scale changes in agricultural practices, changing pasture patterns, conversion of flood plains and marshy deltas to farmland and pastures, and flood control measures can create new habitats for vectors and hosts, and lead to increased pathogen transmission. The same changes could also amplify the populations of wild vertebrate hosts and thereby create new foci.

- In the case of *Hy. marginatum*, gradual environmental changes such as, changing pasture patterns, conversion of flood plains and marshy deltas to farmland and pastures, and flood control measures can create new habitats for vectors and hosts, and lead to an increase in the disease agent transmission. This may be due to the increased human activities and exposure to the infected vectors.

2.9.1.2. Climate influences

In temperate regions the foci of enzootic CCHF are typically in areas characterized by warm summers and relatively mild winters (Hoogstraal 1979). However, the only extensive study carried out on the relationships between climate and the disease, proved that these foci are not associated with a given climate niche (Estrada-Peña et al., 2009). In Turkey, the disease is reported in areas of high habitat fragmentation with small agricultural fields, and high abundance of some wild hosts, like hares and wild boars. The climate trends in the country had no influence on the spread of the disease. The only factor positively and unequivocally associated to the increase of human cases is the increase in habitat patchiness (fragmentation) and the degradation of agricultural lands to bush lands.

Studies on the ecology of *Hy. marginatum* have shown that there are at least four different subpopulations (isolated populations) of the species in the Mediterranean basin, each one regulated by different climate variables (Gray et al 2008). This variability was observed when comparing statistically the climate patterns with the distribution of the ticks, but it has not been proved by morphological or genetic methods yet. Thus, these factors are important for the tick populations' phenology. In such a sense, populations in the core of the Mediterranean basin (northern Africa and southern Europe) are regulated by the rainfall and the evapo-transpiration in summer. The decrease of rainfall and the increase of evapo-transpiration would impact on the available habitat for the tick, allowing its spread towards northern latitudes. However, large populations in the Eastern Europe, the Balkans, areas of Turkey and the Caucasus, are regulated by the minimum temperatures in late autumn. It has been proposed that low temperatures in late autumn would preclude the moulting of nymphs, therefore overwintering as engorged instars with a high mortality, since moulted adults are more resistant to the cold (Gray et al 2008). However, warmer autumns would allow the moulting of engorged nymphs, decreasing the mortality of the population and allowing for a slow and gradual spread of the tick onto neighbour territories.

In Eurasia and northern Africa CCHF foci are found in arid areas, i.e., deserts and semi-deserts. In Africa on the whole, CCHF enzootic areas can range from wet central African forests to the very sparsely vegetated, arid Sahelian areas of West Africa (Linthicum and Bailey 1994). These areas, together with the Caucasus and Middle East, are of concern for the spread of the CCHFV into Europe because of the migrations of birds to Europe.

In Mauritania, Saluzzo *et al*. (1985) reported that the geographic distribution of CCHFV closely fits the distribution of *Hy. marginatum*, the most frequent vector in southern Mauritania; however, it is replaced in the very arid semi-desertic regions of northern Mauritania by *Hy. dromedarii,* which is not usually infected with the virus in the area. *Hy. dromedarii* is very well adapted to the desert environment (Hoogstraal, 1956), and able to withstand low humidity and extremes in temperatures.

- It seems that there is no direct association between CCHF foci and climate factors within the distribution range of *Hyalomma*. This conclusion however derives from only one extensive study carried out on the relationships between climate and the disease.
- Main regulatory factors other than adequate temperatures to molt and to quest are the rainfall and evapotranspiration in western Mediterranean, and the late autumn temperatures in eastern Mediterranean. The tick prefers the lowest range of rainfall in western European Mediterranean and the highest range in northern Africa. In eastern Mediterranean, low autumn temperatures would prevent the molting of engorged nymphs, with a high mortality of engorged instars in winter
- Increase in habitat patchiness and the degradation of agricultural lands to bush lands are the two main factors in the creation of new foci of CCHF in endemic areas.

2.9.1.3. Implications of climate change and modelling

There are indications that climate change in some areas is increasing the range and abundance of certain potential vectors of human infections in general. Thus, in Europe, changes in climate during the last decades appear to cause an expanding range, to higher latitudes and altitudes, as well as affect the population density of *I. ricinus* (Tälleklint and Jaenson 1998; Lindgren et al., 2000; Materna et al 2005; Lindgren and Jaenson 2006). The reasons are presumably that the changing climate, which shortens the winters and increases the length of the vegetation period, is beneficial to this tick species both directly, and indirectly, by increasing tick development rate, and by increasing the abundance and geographical range of important hosts of the adult ticks, i.e., deer and hares. However, it is difficult to attribute climate change as the main cause of the increasing incidence of CCHF. Climate change models are required that take into account the dynamic biological processes involved in vector abundance and pathogen transmission affecting the complex ecology and epidemiology of CCHF. Recently, studies on possible effects on climate change on *Hy. marginatum* distribution and on the influence of climate change on the occurrence of tick-borne disease have been reviewed by Gray et al. (2008).

Although migratory birds are carriers of immature *Hyalomma* ticks and could potentially introduce them into currently *Hyalomma*-free areas in the spring, their climate requirements and current climate data do not suggest that they can become established. Mid-March and early April are the main periods of mass arrival of birds in Spain on their way to northern Europe (Gray et al, 2008). Immature *Hy. marginatum* are found on local - no migratory- birds in central Spain around late May and early June, which is too late for northern African and southern European populations of *Hy. marginatum* to mix because of the current climate barriers imposed by their respective climate requirements at the moment of bird migration. If climate change includes the predicted temperature increases, *Hy. marginatum* ticks may become established in northern latitudes but it is debatable whether initial introduction will occur as a result of bird migration alone because only very small numbers of ticks and immature stages, would be involved. It is more likely that the establishment of *Hy. marginatum* will mainly result from the introduction of adult females feeding on wild and domestic ruminants via the Middle East and the Balkans, where there is much uncontrolled movement of livestock (Gray et al. 2008). The role of European wildlife, especially wild ungulates, has not been investigated in the spread of CCHFV. More research is needed on this topic. If these hypotheses are valid, the tick could probably spread into northern latitudes, mainly around a wide area near to Balkans. This seems to be the area predicted to have a kind of climate change which would turn large areas in the North from unsuitable to suitable.

Climatic models have been built to predict possible dispersion and establishment of ticks in CCHF free areas but the real effectiveness of such models is still to be defined since many other factors such as vegetation patterns or host abundance operating at different levels restrict the effective dispersal and establishment of potential invaders (Gray et al, 2008).

The basic concept underlying species occurrence modelling is the definition of the ecological niche: each species is found within specific ranges for environmental variables that support individual survival and reproduction. Species occurrence can be predicted by inclusion of appropriate climate variables in what are commonly referred to as climate suitability models (CSMs). The relationships are generalized from a sample of correlations of species presence with specific values of environmental variables. However, these CSM are unsuitable if an adequate understanding of the factors influencing the transmission of an infection is necessary. The several variables involved in such processes (such as densities of hosts, and host-seeking infected ticks), and a perception of the small scale of foci are only adequately addressed with models designed to describe seasonal dynamics (Gray et al. 2008).

While some biological models have been produced for tick species such as *Rh. (B.) microplus*, *A. americanum*, and *I. scapularis*, none is currently available for tick species occurring in Europe. Such models are a priority to adequately understand the impact of climate change on tick populations, provided that adequate data are used for important components such as host densities and microhabitat suitability (Gray et al. 2008). However, despite the absence of such data in climate suitability models, these models have proved useful in the elucidation of foci for the recent outbreak of CCHF in Turkey. They identified habitat fragmentation as a critical factor in the spatial and temporal distribution of human cases. In highly fragmented habitats, the risk of contact between humans and infected ticks appears highest. The spatial distribution map for *Hyalomma* ticks in Turkey is currently used to focus surveillance studies on areas with higher than expected number of CCHF cases. These areas are currently intensively surveyed for CCHFV in *Hyalomma* ticks on wild and domestic livestock to improve risk maps for this important disease which is expected to continue to cause outbreaks in Turkey in the years to come (EstradaPeña et al., 2007).

Other climatic model based on temperature and rainfall predicted that large areas of Europe would potentially be affected by an increase in the climate suitability for *Rhipicephalus* spp. and *Hy. marginatum* after an increase in temperature and decrease in rainfall. Decreasing temperatures in Europe are predicted to result, however, in habitat loss for *Rh*. (*B.) annulatus*, *Rh. bursa*, and *Hy. marginatum*. Gray et al (2008) stress that this is only an evaluation of the climate suitability for these tick species, since changes in vegetation, host availability, and animal movements were not included in the analyses (Gray et al. 2008).

Transmission of virus to humans occurs when there is an overlap of activities between reservoir, vector, and humans, and differs according to the pathogens and the location. Climate change may impact all of these factors and how they interact. Although changes in climate will directly affect tick survival, activity, and development, there is no sufficient evidence that rising temperatures will result in a greater abundance of ticks by simply increasing rates of development. Changes in development rates, however, will make tick cohorts available with changing patterns of seasonal activity. Indirect effects of climate change will impact the number of infected ticks by affecting the vegetation. Climate change may also influence disease risk by affecting the long-term use of land for farming, tourism, etc. Weather patterns influence short-term human behaviour, such as picnicking and berry and mushroom picking.

Climate effects are more easily noticeable close to the geographical distribution limits of both vector and disease (cf. *I. ricinus* in northern Europe (Tälleklint and Jaenson 1998; Lindgren et al., 2000; Materna et al 2005; Lindgren and Jaenson 2006)). The magnitude of the effects of climate change in an endemic area is determined not only by ecological conditions but may be influenced by socioeconomic factors, human migration and settlement, ecosystems and biodiversity, migrating patterns of birds, land-use and land cover changes, human cultural and behavioural patterns, and immunity of the population.

A complex chain of processes exists that makes the precise factors responsible for changes in disease incidence often difficult to assess. Current research is undergoing in attempts to match datasets collected for different purposes in order to reduce confounding variables. It is evident that data from long-term studies on disease incidence, tick biology, tick distribution and tick abundance, host abundance and distribution, and relevant vegetation biology, specifically in relation to climate change, are required. Such data will permit the development of models to predict future tick-borne disease occurrences, which take account of dynamic biological processes instead of simply the likelihood of occurrence of climate suitability for particular tick species (Gray et al. 2008).

- A complex chain of processes exists that makes the precise factors responsible for changes in disease incidence often difficult to assess.
- There are many migratory birds bringing high numbers of ticks, into new areas. However, this may not always be sufficient for the tick to become established in new areas.

- The establishment of *Hy. marginatum* will mainly result from the movement of livestock wich will carry adult tick females feeding on wild and domestic ruminants
- Middle East and the Balkans may be the origin of introduction of CCHFV into other European countries.
- No risk model is yet available for any European tick species. Data from long-term studies on disease incidence, tick biology, tick distribution and tick abundance, host abundance and distribution, and relevant vegetation biology, specifically in relation to climate change, are required.
- Despite the absence of data in climate suitability models, these models have proved useful in the elucidation of foci for the recent outbreak of CCHF in Turkey. They identified habitat fragmentation as a critical factor in the spatial and temporal distribution of human cases. In highly fragmented habitats, the risk of contact between humans and infected ticks appears highest

2.9.2. Biotic factors

To maintain the CCHFV in the environment, infected ticks have to maintain sufficient vira1 load during development and moulting. The virus must replicate in cells of the salivary glands for onward transmission to the next host. Each of these steps depends on the ability of a pathogen to overcome the many intrinsic biological (molecular, cellular, physiological, and physical) barriers during its passage from host to host via the vector. Even then, although biologically possible, this cycle may not proceed with sufficient force to support persistent transmission cycles. That depends on the quantitative balance of the rates of all the processes involved in each complete transmission cycle (Randolph and Rogers 2007).

In comparison to other tick borne virus such as Tick Borne Encephalitis virus, little is known quantitatively about the necessary biotic conditions for the maintenance of CCHFV in nature and the potential or proven role of the various biologically competent tick species are still not clear (Randolph and Rogers 2007).

There are several aspects of the biology of CCHFV vectors that could affect their abundance. Most of what is known has been determined from studies in the laboratory with very little field research completed. These factors are:

1. Oviposition: Ixodid ticks oviposit once in a life time. Eggs are laid without regard to location at on time approximately 1-3 weeks after detaching from the host. For the main vectors this period has been assessed by laboratory trials. The period of oviposition in *Hy. impeltatum* can range from 16 to 50 days (Logan et al. 1989b). Usually, numerous eggs are laid, although the number can vary tremendously. *Hy. marginatum* female can lay from 4,300 to 15,500 eggs, while Knight et al. (1978) found that *H. rufipes* females can lay from 3,184 to 13,180 eggs. *Hy. impeltatum* female can lay from 713 to 15,904 eggs with a mean hatch rate of 84% (Logan et al. 1989b). *Hy. truncatum* females lay from 4,434 to 8,210 eggs with a mean hatch rate of 48% (Linthicum et al. 1991).

2. Fecundity: The overall fecundity of a tick population in a given year can be expressed as a product of the total number of eggs oviposited by individual females of a generation that successfully hatch and the number of generations completed during that year. In nature, the shortest time required to complete a tick generation may be 4 or 5 months; however, climatic conditions and host availability may increase generation time and decrease the overall fecundity of a given population. Hoogstraal (1979) reported that *Hy. marginatum*, *Hy. truncatum*, and other *Hyalomma* species in Eurasia complete only one generation per year because of the severe winter climate.

3. Density and biting activity: Outbreaks of CCHF coincide with an elevated population density and increased feeding of *Hyalomma* ticks. During the outbreaks in Bulgaria, for example, adult *Hy. marginatum*, the principal vector, appeared, reached maximum population densities, and declined in close association with the occurrence of human cases, peak numbers of cases, and decreasing number of cases, respectively (Hoogstraal 1979). In the Central Asian republics of the former USSR, the seasonal distribution of CCHF cases were closely associated with the seasonal dynamics of *Hy. anatolicum*, the suspected vector (Linthicum and Bailey 1994).

4. Longevity: Adult ticks can survive for very long periods of time, months, or years, without blood feeding, if climatic conditions and availability of hosts are not favourable. *Hy. marginatum* can survive for more than 800 days without a blood-meal under optimal laboratory conditions. Adult *Hy. truncatum*, *Hy. impeltatum*, *Hy. rufipes*, *A. variegatum*, *Rh. appendiculatus*, *Rh. e. evertsi*, *Rh. pulchellus,* and *Rh. sanguineus* have been held in the laboratory without blood-feeding for up to 1 year (Linthicum and Bailey 1994).

5. Life cycle and host preference: Different types of life cycles have great influence in the maintenance of CCHFV. They have been described for each species considered. CCHF disease epidemiology appears to be controlled by ticks with a long life cycle (Linthicum and Bailey 1994). It is important to understand biological parameters such as number of hosts parasitized by an individual tick during its lifetime, the potential variety of hosts parasitized, and the degree of host specificity. Because of the diversity of ecological and zoogeographic habitats of CCHF foci; the host preferences of potential tick vectors; the qualitative and quantitative aspects of the biology of each vertebrate involved in maintaining the enzootic cycle of the virus must be determined (Hoogstraal 1979; Linthicum and Bailey 1994).

6. Host immunity and host resistance: The subject of acquired host resistance is known for the hard tick species. The effects of resistance range from simple rejection by the host lowered engorged weight, prolongation of feeding time, interference with feeding to death of the tick on the host. Many hard tick species can induce a host reaction to repeated tick feedings, which results in resistance; however, observations in *Hyalomma* species conflict. Domestic rabbits did not develop resistance to *Hy. excavatum* and *Hy. dromedarii*, but they did develop resistance to *D. marginatus* and *Rh. sanguineus*, while guinea pigs developed a limited resistance to *Hy. truncatum* larvae (Linthicum and Bailey 1994). Genetic resistance has been described in West African indigenous cattle; studies conducted in N'Dama and Gobra zebu cattle in The Gambia showed that N'Dama possess a higher degree of resistance than Gobras against adult ticks species belonging to two genera characterized by long hypostome, such as *A. variegatum*, *Hy. truncatum* and *Hy. rufipes* (Mattioli et al. 2000).

- Quantitative data on the necessary biotic conditions for the maintenance of CCHFV in nature do not exist.
- The potential or proven role of the various biologically competent tick species is still unclear.
- Outbreaks of CCHF coincide with an elevated population density and increased feeding activity of *Hyalomma* ticks

2.10. Estimates of relevant tick abundance and prevalence of ticks infected with CCHFV.

Due to ubiquity of the putative vertebrate hosts of CCHFV, and their common infestation by ticks of many genera including *Hyalomma*, host availability is unlikey to be a limiting ecological factor in the distribution or even the prevalence, of this virus. Tick abundance is depending on several environmental factors such as climatic factors, change of land use, human behaviour, and availability of hosts, among others.

Following the CCHF outbreaks several field surveys have been carried out. Vatansever and collaborators described (Vatansever et al. 2008) passive surveillance for tick bites in humans undertaken in the city of Istanbul (Turkey) in the summer and autumn of 2006. In the monitoring from 1,054 reported tick bites, most were females of *I. ricinus* (27%) and nymphs of *Hy. aegyptium* (50%). A few adults of *Hyalomma m. marginatum, Rh. sanguineus* and *D. marginatus* were also recorded. Climate features at 1-km resolution (monthly minimum temperatures in late summer and autumn and rainfall) and vegetation features at high resolution (density and heterogeneity of forest-type vegetation as well as distance of reporting site to these vegetation features) have been used to explain high reporting clusters for both *Ixodes* and *Hyalomma*. In fact while *Ixodes* is highly reported in dense highly heterogeneous vegetation patches, *Hyalomma* is commonly found in areas far from forest-type features and in the small, relatively dry vegetation patches within the urban fabric (Vatansever et al. 2008).

Again, an investigation on Ixodid ticks has been performed to investigate the distribution of ticks in Turkey in 2007. *Ixodes* spp. were mostly seen in high rainfall and the intensive forest area of northern Turkey. Ticks of the genera *Haemaphysalis, Hyalomma, Boophilus, Dermacentor, and Rhipicephalus* are widespread throughout Anatolia. During the survey some species have been found to be sporadic: *A. varigeatum* in Hatay province (border to Syria), *B. kohlsi* in Southeastern Turkey (border of Syria), *Ornithodoros* in Central and East Anatolia, and *Otobius megnini* found in East Anatolia (Malatya Province) (Aydin and Bakirci 2007).

Following the first outbreak, investigation of vector and virus circulation (performing serology in cattle) in Anatolia in 2007 a survey on tick collected from cattle has been performed recording 80% of *Hy. m. marginatum* (Ertuk 2007).

Pascucci and collaborators (Pascucci and Cammà 2007) in a survey on tick bites on humans performed in a local hospital in central Italy recorded 166 tick bites registering high prevalence of *Ixodes ricinus* (85%), but also with 4% of tick bites referred to *Hy.m.marginatum*.

Prevalence of infected ticks

In an endemic area of Turkey, Vatansever et al., (2010) reported a prevalence of infected *Hy. marginatum* questing ticks of 16.43% (46 out of 276 ticks) by using semi-nested PCR technique. Within the 6 localities surveyed in the said endemic area, prevalence of infected ticks ranged from 6% up to 30%. Viral load was determined by qRT-PCR in 6 positive samples with comparatively low, medium and high visual band intensity on the gels. Viral load ranged from 4.88×10^{1} to 8.64×10^{4}. The significance of these different viral loads in terms of hability to transmit the infection is still unclear.

- Tick abundance data associated with CCHF is lacking specifically for *Hy.m.marginatum*.
- Surveillance systems for CCHF and subsequently its vectors are also lacking. Exceptions for collection of data are during outbreaks but without data of tick abundance.
- It is essential to notify the presence of vectors during outbreaks and the disease notification should include the involvement vectors.

2.11. Geographic distribution

The following maps have been made up using three sources of data: A systematic literature review (see appendix G) based on scientific papers retrieved from the databases integrated in ISI web of knowledge and Pubmed in the last 10 years, as well as on a pool of scientific papers considered relevant by the WG experts, coming from their private collections, regardless of the time frame; and published historical data (approximately from years 1970 - 2000) of the integrated consortium on ticks and tick-borne diseases (ICTTD3 European project). Such historical data was not available for tick-borne pathogens. The signs in red colour indicate cases extracted from the systematic literature review (last 10 years). The green colour indicates historical cases (older than 2000). Dots correspond to coordinates (latitude/longitude). Stars correspond to cases where coordinates were not indicated and even could not be found because the name of the location given in the corresponding paper was equivocal (several toponyms existed with the same name). Stars are placed either in the middle of the smallest administrative region described in the scientific paper (for the countries that do not have official NUTS); or in the middle of the NUTS containing the specified location. Countries or areas that are not showing cases of presence of this tick are not necessarily free of it. Rather the species may not be presented in available literature for this report.

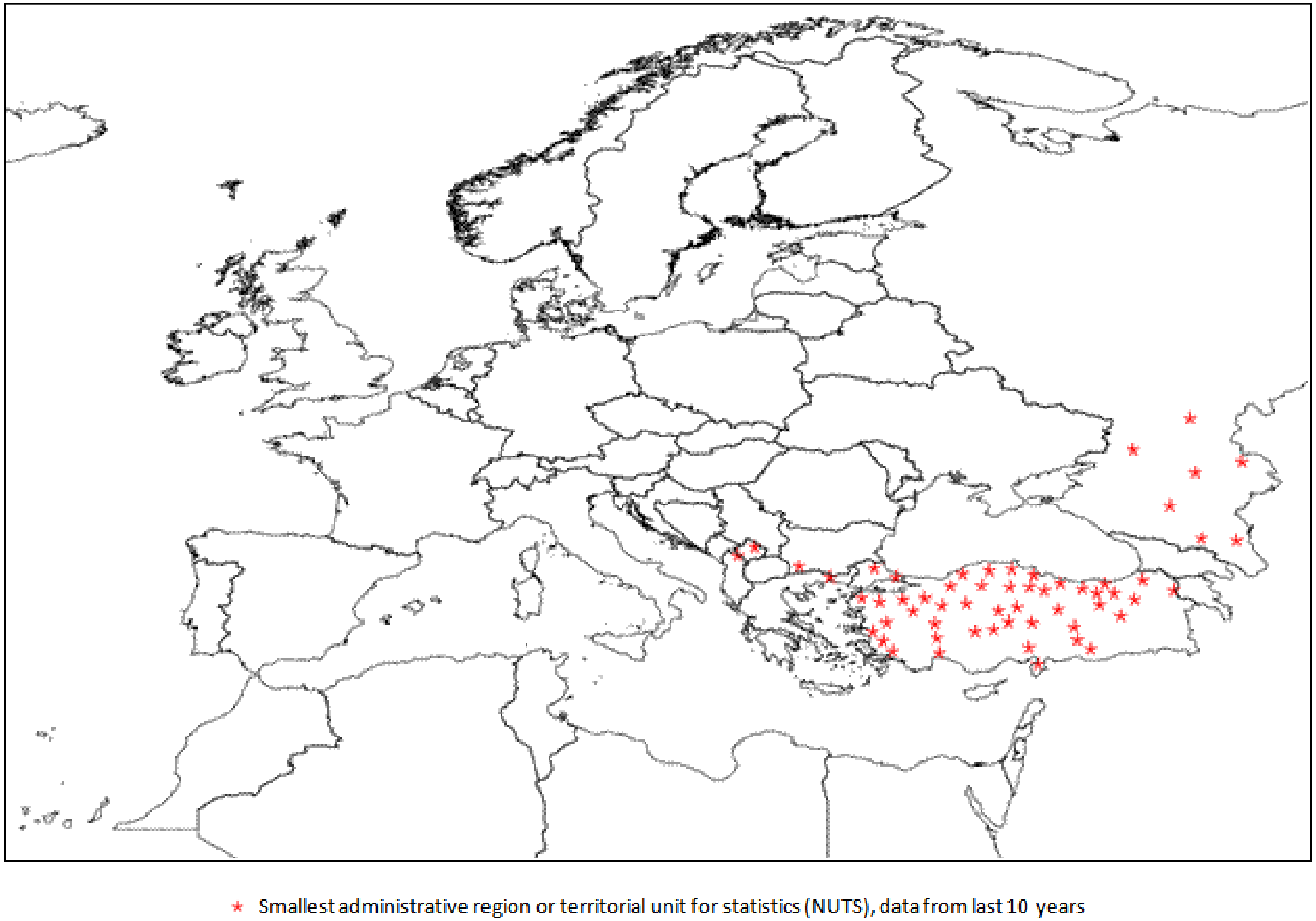
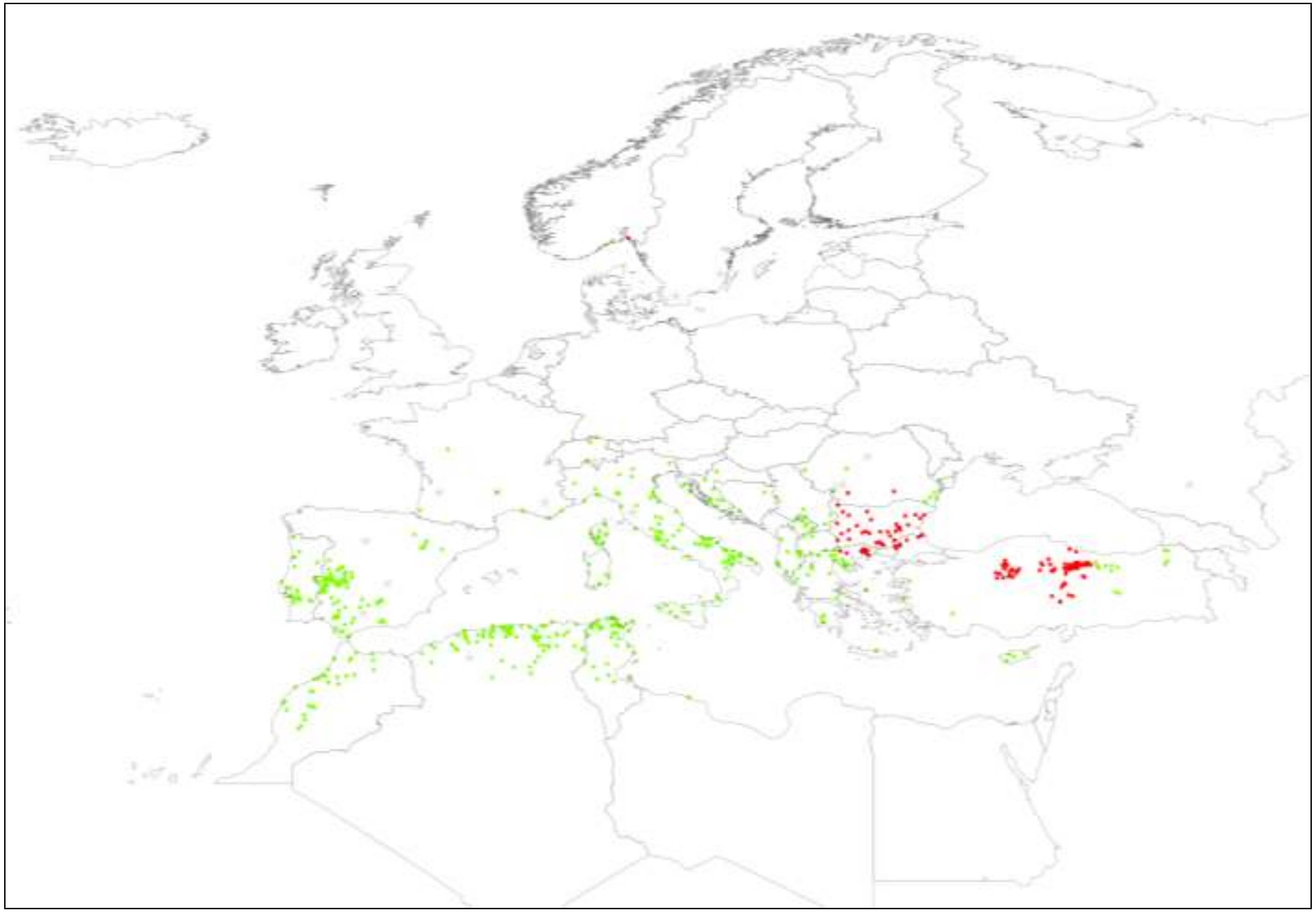


Figure 5: Map of the reported occurrence of CCHFV

Critical assessment of the map of the reported occurrence of CCHFV:

Clinical cases of CCHF in humans, as well as infected ticks under natural (field) conditions have been recorded only in an area covering southern Russia, Turkey, Albania, Kosovo, Greece, Romania and Bulgaria. The disease is widespread in Turkey, following the areas where the main tick vector, *Hyalomma marginatum*, exists. In other countries of the Balkans, the disease has been scarcely reported. No published reports exist for the distribution and incidence of the disease in the Balkans and Russian areas other than reports collated from Promed Mail organization (www.promedmail.org). It is likely that the virus circulates in the Balkans (as suggested by the recent first report of CCHF in humans in Greece [Papa et al., 2008]). This is likely the most exposed area for spread of the virus to other parts of Europe. Both the virus and the signs of clinical infection in humans have been partly surveyed in Europe with negative results. This is a cause of concern, since we ignore the reasons why the disease (or the virus) is absent in places where the tick vectors (se the comments on *Hyalomma marginatum* and other hard tick) are widespread.



* Smallest administrative region or territorial unit for statistics (NUTS), data from last 10 years
● Coordinate (latitude/longitude), data from last 10 years
● Coordinate (latitude/longitude), historical data (before 2000)

Figure 6: Map of the reported occurrence of *Hyalomma marginatum*

Critical assessment of the map of reported occurrence of *Hyalomma marginatum*:

The tick is well known from the Mediterranean region, where it can populate areas in northern Africa and southern Europe. The tick is restricted to the Oromediterranean, since too cold or too dry climate may restrict the colonization of potentially available areas. Accurate records exist for the tick in wide areas from Turkey to Portugal. The presence of the tick has been recorded from southern Russia and neighbour Republics (but not documented with any geographical information). The tick is also known from other sites in northern Africa, but those records lack adequate referencing. Scattered records of the tick exist in northern Europe, but these records came from migratory birds, and they do not represent established populations. Migratory birds may bring immature ticks into free areas in spring, but the current climate in the Scandinavian countries jeopardizes their establishment. Concerns exist about the probable spread of the tick into northern latitudes, therefore spreading into areas where the tick is currently absent. Permanent populations of the tick are currently established around 46ºN, and they seem to be restricted in movements because the Alps and Balkans mountain ranges. In any case, considerable interest exists about the possible spread of the tick, and more field studies are necessary to understand the basic phenological processes behind the observed distribution.

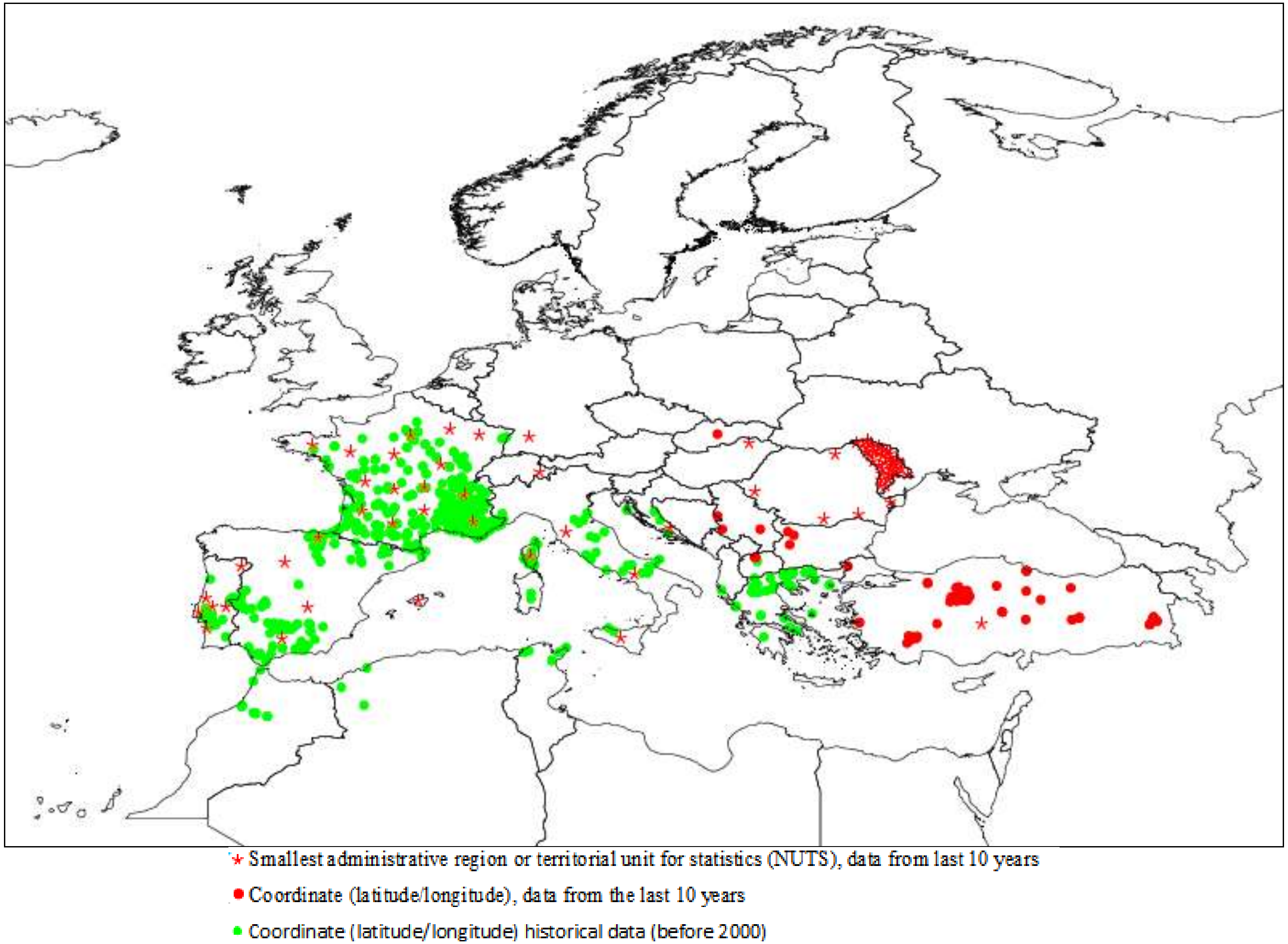


Figure 7: Map of the reported occurrence of *Dermacentor marginatus*.

Critical assessment of the map of the reported occurrence of *Dermacentor marginatus*:

There are no conclusive studies about the involvement of *Dermacentor marginatus* in the transmission of CCHF virus among animals and humans. However, the area of distribution of the tick overlaps in many parts with that of *Hyalomma marginatum* (the proven vector) and *Rhipicephalus bursa* (a suspected vector of some CCHF viral strains). *Dermacentor marginatus* is well known from the Mediterranean region, and it seems the tick can spread further to its previously known distribution area. The tick is present in areas northern of the Balkans, being recorded from as northern as Hungary and southern Germany. The tick *Dermacentor reticulatus*, which has a northern distribution in Europe, has been wrongly reported as *D. marginatus* in parts of Germany. Therefore, the actual northern limits of this tick are not well known. The tick seems to be widespread in France, in areas of a climate similar to that found in southern Germany. It has not been recorded from Belgium or The Netherlands. Established populations are well known in southern Russia, but there not geographically accurate reports about the issue.

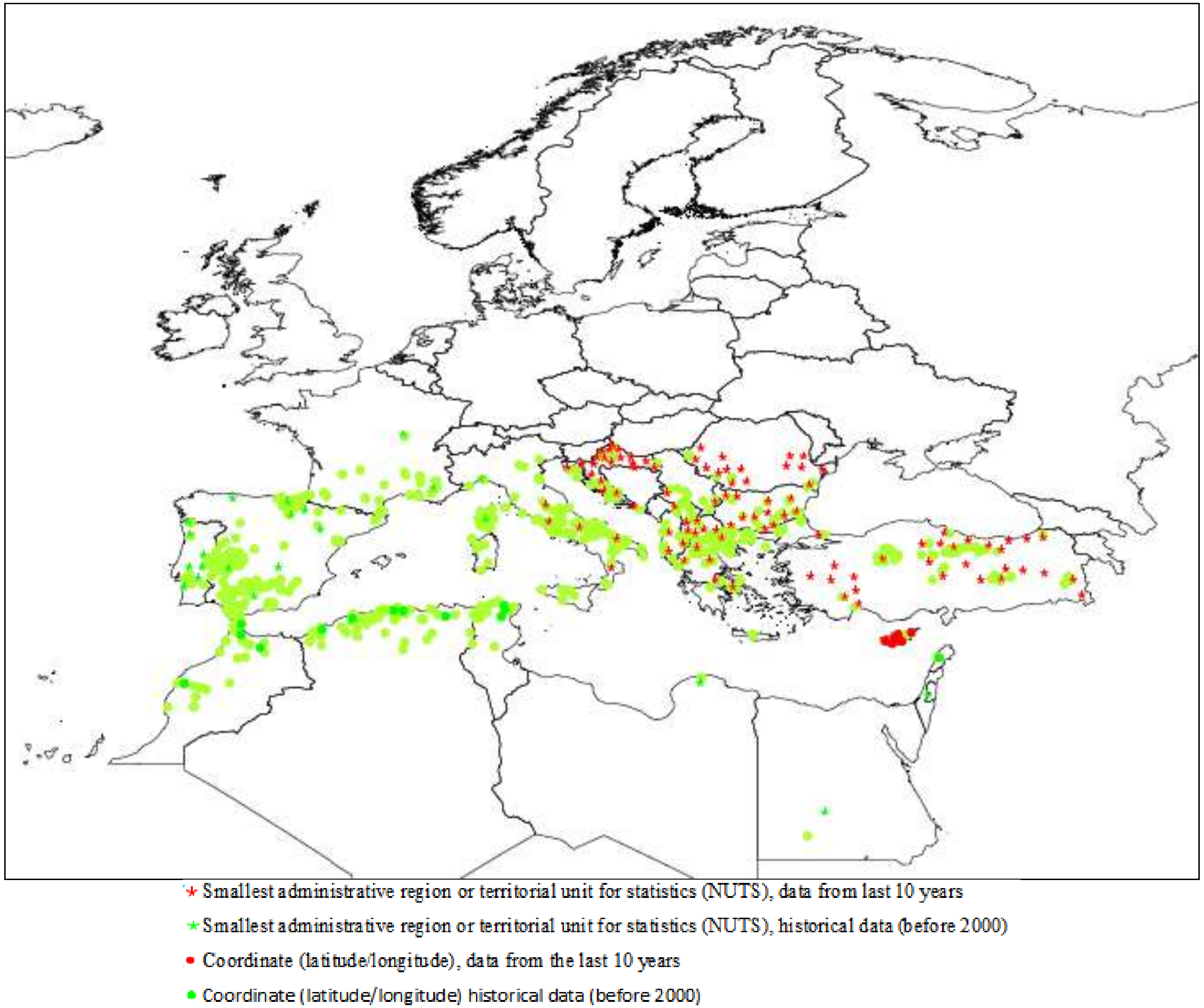


Figure 8: Map of the reported occurrence of *Rhipicephalus bursa*

Critical assessment of the reported occurrence of *Rhipicephalus bursa:*

This tick is the putative vector of the strain AP-92 of CCHF virus in the Balkans area. The viral strain was isolated from such a tick species, and one human case has been reported from Turkey. However, it has been not empirical association of the tick with any human case of the disease. The distribution of *Rhipicephalus bursa* overlaps with that of *Hyalomma marginatum* and *Dermacentor marginatus* in parts of its Mediterranean range. Such a distribution extends to approximately 45ºN latitude. However, the former prefer drier areas, and the latter colonizes colder sites. It is interesting to note the different distribution area of both *D. marginatus* and *Rh. bursa* in France, where a similar collection effort on both species has been carried out in the last 30-40 years of tick field collections. *Rh. bursa* is more abundant in the Oromediterranean than *D. marginatus*, while the latter can colonize colder and more humid sites. The tick seems to be absent from many parts of southern Russia, probably replaced by other ticks species, but this extreme has not been reported.

2.12. The risk of introduction and the spread of CCHF in Europe

2.12.1. Plausible paths for the spread of CCHF into Europe via ticks

This section refers only to the ticks' pathways. We are aware that the animal and human movement can contribute to the spread of the disease, however theses issues are considered out of the remit of this report's mandate.

The following sources can be considered for the introduction and spread of CCHF into Europe. These are based on our current knowledge of the ecology of the disease and its vectors. These paths are through:

- nymphs of the tick carried via migratory birds from Africa;
- nymphs of the tick on birds moving in the East to the West fly zone –specifically from current endemic areas to non endemic areas;
- ticks on ungulates that are moving from current endemic areas to non endemic areas.

The following description details the movement of birds and ungulates from the various geographical sources with the potential scenarios for the introduction of CCHFV:

1. **Birds coming from Africa.**

Annually millions of birds fly from Africa to Europe, using three main routes: the western route along the Atlantic coast of Africa; the central route along the Nile river; and the eastern route through the Red Sea (Hoogstraal et al 1963).

Although, some birds fly directly from sub-Saharan Africa to Europe, they are not potential sources for the introduction of CCHFV. These particular birds carry *Hyalomma rufipes* which are tropical ticks, and they will not survive even in the Mediterranean climate conditions. Thus, the potential for *Hy. rufipes* that are infected by CCHFV in Africa, to be introduced to Europe, survive, and spread the virus is minimal.

The introduction of the CCHF through the western route is related to small size birds that fly in short distance segments due to their size. They usually land in western Africa (specifically in Mauritania and Morocoo) nearby the sea, with the potential to have the infestation with immature ticks prior to completion of their journey to Europe. Due to the frequent bird stops, and the limited tick feeding period (i.e. not longer than 20 days), the tick burden at their final destination is reduced. The same scenario can happen with the birds flying across the Nile river.

2. **Birds movement in the East to the West flight zones – specifically from current endemic areas to non endemic areas.**

These flights are extremely rare, and are not related to specific recognized pattern. Although, limited information is known about any possible bird flight patterns from the Eastern or Western Europe the potential number of ticks to be introduced via such a casual paths is extremely low (Snow et al, 1997). This is true even if these birds fly in the season when nymphs are active.

3. Movements of wild or domestic animals, especially ungulates.

The uncontrolled movements of wild or domestic ungulates, in the southern area of Balkans (See CCHFV map), may spread infected tick populations into naive areas. These Balkans areas are the most "serious" in spreading the virus to the rest of European countries that are currently infested with the vector species.

2.12.2. Surveillance approaches for monitoring and controlling of CCHF.

Approaches for surveillance should consider the endemicity of the infection, the level of risk of introduction of the infection, and distribution of mammals in the geographic area of interest. The absence of clinical manifestations in domestic and wild animals, the short viraemia and lack of reliable serological diagnostic assay to detect the infection in these animals complicates the surveillance design.

Appendix H (Modified from table 4 of CFP/EFSA/AHAW/2008/04) provides data on serological surveillance on CCHFV in domestic animals. A number of these reports have obtained positive results in several animal species (i.e. serologically positive sheep ranging from 3 to 77 %). There is, however, inconsistency in the approach of the surveillance systems, diagnostic tests including case definition, and in the selection of the tested population.

In spite of the several serological surveys on domestic animals (Appendix H), no reliable standardized diagnostic methods are available for serological monitoring.

Surveillance of viraemia by PCR in animals is not a reliable method to prevent CCHFV spread in animals. The presence of CCHFV in a geographic area can be monitored by PCR on ticks collected on the animals and in the environment (questing ticks). Since large scale surveillance is not feasible, the use of dead and hunted animals is considered the best approach.

Birds are important in transporting immature *Hyalomma* ticks during spring migration from Africa to Southern Europe. They act as carriers of ticks but not harbouring the virus. Usually only the immature stages of the vector species feed on birds. Adults feed on ungulate hosts and are rarely found in birds. For the establishment of new *Hyalomma* population in Europe mammals infected with adults are necessary. Spring migratory birds may not represent a high risk of introduction of *Hyalomma* since these ticks are nearly all immature (Hoogstraal et al, 1963, Manilla 1982, Gray et al 2008, Jaenson, WG member, personal communication).

Acording to Gray et al (2008) "Although migratory birds are carriers of immature *Hyalomma* ticks and could potentially introduce them into currently *Hyalomma*-free areas in the spring, their climate requirements and current climate data do not suggest that they can become established. Mid-March and early April are the main periods of mass arrival of birds in Spain on their way to northern Europe. Data obtained from the Climate Research Unit (UK) show that average temperature in that period is 16-17 C in Morocco and Mauritania, 9-11 C in southern Spain, and 5-6 C in southern Germany. According to data on molting of engorged nymphs under laboratory conditions, about 300 C cumulative degrees above the developmental zero (14-16 C) are necessary to complete the molt. Nymphs that engorge at the time of migratory bird arrival in early spring would need much longer to molt in southern Germany, with a consequent increase in mortalty, than in north-western Africa, where only a few weeks are required."

Domestic and wild ungulates may represent a greater risk than migratory birds for the introduction of infected ticks, but the latter possibility should not be ignored. Some institutions have initiated colaboration with bird ringers to collect ticks to be tested for viral infection. This initiative in Sweden has been incorporated as a component of their surveillance system (Jaenson, personnal communication).

2.12.3. Risk of introduction and spread

The following facts are known and can support in determining the risk of introduction and spread of Crimean-Congo Hemorrhagic Fever (CCHF):

- Several tick species are able to transmit the CCHFV. The virus is mainly transmitted by tick species of the genus *Hyalomma*. Some species of the genera *Dermacentor* and *Rhipicephalus*, however, have also been shown to be capable of transmitting CCHFV.

- There is, however, a wide uncertainty about the important role of these ticks in the transmission since the number of human cases reported from Europe are scattered with limited background information about the type of tick exposure.

- Some of these tick species are currently present in some regions of Europe (See the maps of tick distribution). When analyzing the maps, it is important to realize that countries or areas that are not showing cases of presence of a particular tick or tick-borne pathongen are not necessarily free of it. Rather the species may not be presented in available literature for this report.

- There is the potential for direct transmission of the virus through contaminated meat that originate from infected but subclinical animals. According to ECDC factsheet and per this report –Chapter 1, CCHF-, meat itself is not the source of infection because the virus is inactivated by post-slaughter acidification of the tissue; and CCHFV does not survive cooking. There is no published information on the survival of the CCHFV in bone marrow. The ECDC stresses the role of infected blood and other tissues of livestock in the transmission of the disease. Farmers, veterinarians and abattoir workers in endemic areas are at higher risk. Healthcare workers are the second most affected group when nursing CCHF patients. Thus, the disease cycle can be maintained through this route without the involvement of ticks. This route of transmission is unlikely to occur in a non-endemic zone.

- Most human cases have been reported from Turkey, where most affected people are farmers and agriculture workers.

- Although there is an existing risk model for similar diseases, such as tick-borne relapsing fever (TBRF), this model lacks both certainty and sufficient biological variability (ECDC Report on TBRF). This model, however, served the purpose of identifying gaps in the knowledge and data for TBRF.

- The EFSA's most recent report for African swine fever (ASF) includes a unique approach to risk assessment for its introduction and spread into Europe. This approach for CCHF is necessary but there are limited scientific experience and observations for the application of this approach. Taking into consideration the zoonotic aspect of CCHF, the lack of clinical signs in animals, and the lack of standardized diagnostic tools, the risk assessment approach of ASF should be adapted to CCHF.

In conclusion, the above facts indicate that a risk assessment model can be developed based on available qualitative data and opinions to determine the risk of spreading CCHF in Europe. This model will be considered for human risk but there is animal involvement in the biology of this disease. The Working Group discussed this potential model but it was not considered as part of the mandate of the current task. Further information and expertise are required to construct such a model.

2.13. Hard tick prevention and control in relation to CCHF

2.13.1. Education to Increase Awareness

General education is perhaps the most important means of prevention strategy towards tick bites and tick-borne infections. Important knowledge concerns tick biology, for instance, that a single tick may transmit several species of pathogens during a single feeding occasion, and that prompt removal of attached ticks will reduce the risk that transfer of such amounts of pathogens takes place that will cause overt disease. The risk of tick bites is certainly reduced by knowledge about and avoidance of heavily tick-infested areas, and that ticks usually occur on the ground and on low (<1.5 m) vegetation. Furthermore, the risk is reduced by wearing protective clothing, including boots with trousers tucked inside the boots. Personal prophylactic methods, based on chemical tick repellents, are available but none is 100% effective. Thus, all ticks are not repelled by the application of "barriers" on the skin or clothes, of tick repellent chemicals such as NN-diethyl-3-methyl benzamide (DEET), Icaridin (KBR 3023) and p-menthane-3,8-diol (PMD). Clothes impregnated with permethrin usually repel ticks effectively but such usage of this chemical is not permitted in all countries. After visiting a tick-infested area all parts of the body should be checked for ticks and any tick present should be removed as soon as possible, preferably with a fine-pointed forceps. Any delay in removing a tick will potentially increase the dose of pathogen(s) present in the tick's saliva being transferred to the host. To remove ticks potentially infected with the CCHF virus gloves on the hands should be used; the CCHF virus is exceedingly infectious; infection via minor ulcers or cracks on the skin may take place.

In general, for prevention of tick bites and tick-borne infections the use of Integrated Pest Management (IPM) may be more effective than reliance on a single magic bullet (Piesman et al. 2006). Moreover, the evolution of resistance to the majority of the chemical groups of acaricides and insecticides; the fact that few new pesticides are available; environmental side-effects; presence of residues of acaricides and insecticides in food for human consumption; opposition from environmentalists; and increased costs have brightened the future for "alternative strategies". They include methods based on anti-tick vaccines (Willadsen 2008), genetically tick-resistant breeds of livestock, pasture rotation, and semiochemicals (pheromones, allomones and kairomones) alone or in combination with limited use of acaricides, e.g which combines extracts of the tarsal gland of deer with an acaricide to attract and kill *Ixodes* ticks (Sonenshine 2008). The use of biocontrol agents (BCAs), including pathogens, nematodes, parasitoids and predators is another strategy which may have a bright future, especially for tick control in restricted habitats such as gardens and parks. One advantage is the specificity of several BCAs to target pest species but a disadvantage is the slow activity of BCAs in comparison to acaricides. Among the different strategies based on BCAs for tick control the use of entomopathogenic fungi may be the most promising (Samish et al. 2008). Effective and safe vaccines for human use against important tick-borne infections such as CCHF are obviously needed.

To enable appropriate surveillance and control of CCHF and other medically important tick-borne infections mapping of endemic areas and assessment of future risk areas, e.g., based on geographical information systems are necessary. Resources for vector surveillance, including DNA-based tick species identification should be strengthened in all countries where CCHF or other important tick-borne infections occur or are expected to emerge in the near future.

- After visiting a tick-infested area all parts of the body should be checked for ticks and any tick present should be removed as soon as possible, preferably with a fine-pointed forceps.
- To remove ticks potentially infected with the CCHF virus gloves on the hands should be used; the CCHF virus is exceedingly infectious; infection via minor ulcers or cracks on the skin may take place.

2.13.2. The use of acaricides for control of CCHF

For many decades tick control has relied strongly on chemical acaricides. Chemical acaricides, if correctly applied, are efficient and cost effective but misuse or inappropriate usage have caused serious problems. The main groups of acaricides used for tick control on livestock were the organochlorines, organophosphates and carbamates (George et al. 2008) but are no longer permitted or not advisable to use due to persistence and accumulation in food webs, toxicity to mammals and/or cross-resistance with other chemical groups of acaricides. Nearly all acaricides now available for control of ticks on domesticated mammals are limited to the pyrethroids, benzoyl-phenyl ureas, macrocyclic lactones, spinosad, fipronil, and natural acaricides/insecticides from the neem tree *Azadirachta indica*. Their use is often restricted by being applied onto ear-tags or necklaces, but large quantities of acaricides are still used as sprays or dips for control of ticks on livestock, mainly in the subtropics and tropics.

Acaricides based on permethrin or deltamethrin can be used to kill potentially CCHFV-infected ticks which come into contact with human clothing. However, control of CCHF by treating livestock with acaricides is often impractical; particularly under the extensive farming conditions which prevail in many of the relatively dry areas where *Hyalomma* ticks species are most prevalent.

Acaricides for use on cattle are usually applied in dipping tanks or baths (Stachurski and Lancelot 2006) but sometimes dipping baths are employed for control of e.g., *Hyalomma* ticks on the legs and feet of sheep. Spray races are often used due to their advantage of limiting the amount of acaricide used; the cattle pass through a heavy low pressure spray and become soaked with acaricidal fluid, but body parts such as ears and groin may not be effectively treated by this method (Latif and Walker 2004). Small numbers of livestock can be treated with hand-held sprayers. The usual type is a pressurised container as a back-pack with a spray lance. It delivers a heavy wetting spray which soaks the whole animal. This type of sprayer is comparatively cheap, but often delivers excessive amounts of acaricide, which is lost. In contrast, selective treatment of preferred attachment sites of ticks may be delivered with small domestic sprayers. However, with such partial treatments there is the risk of promoting acaricidal resistance to evolve (Latif and Walker 2004, George et al. 2008). Following the outbreak of CCHF in an ostrich abattoir in South Africa in 1996, it was decided that ostriches should be treated with pyrethroids and kept in a tick-free environment for two weeks prior to slaughter to reduce the risk of exposing abattoir workers to ticks infected with CCHFV (Swanepoel et al. 1998).

Pour-on formulations of acaricides contain high quality oil which spreads through the greasy hair coat of livestock. Pour-on formulations are relatively expensive, but there is little wastage of acaricide and they may be cheaper in the long term. Oil formulations can also be applied as selective spot-on treatments. Similarly, if an acaricide is formulated with oil, then the ears and other sites can be treated. The pour-on formulations can also be used with applicators to treat wild ungulates in game reserves. The animals are attracted to a baited feeding station and will come into contact with a porous surface soaked with the pour-on oil (Latif and Walker 2004). A similar method is now used for control of tick vectors of Lyme borreliosis in the US (Piesman et al. 2006).

When a tick population is exposed to an acaricide it is likely that certain tick individuals will be selected for, especially if the application is irregular for a long time. Moreover, some mutant individuals may arise which are able to survive the normal dose of acaricide. If the acaricide is continuously used in the same way, the surviving individual ticks will give rise to an acaricide resistant population which will soon become the predominant tick genotype in the area. The presence of this resistant tick population will greatly reduce the effectiveness of the acaricide. This can be overcome, in the short term, by using a different type of acaricide. However, the risk will now be that a tick population will appear which is resistant to two or more types of chemicals/active ingredients. Tick resistances to acaricides have been detected in Africa, South America and Australia, but not in Europe. Studies on tick resistance to chemical/active ingredients of acaricides should be performed at

the European level. One-host ticks are more susceptible to develop acaricide resistance due to their short life cycle.
To avoid or to slow down the onset of this problem there are certain rules to follow:

- when acaricides are used they should be stored as recommended and be used as soon as possible to be of full strength as specified by the manufacturer;
- all ticks on treated animals should be killed;
- Acaricides should preferably be used according to the rotating principle to avoid or delay resistances, according to official veterinary advice.

The most common classes/groups of acaricides are:

- the synthetic pyrethroids encompass many useful compounds (e.g. deltamethrin, fenvalerate and flumethrin). However, there may be restrictions on their sale in some countries in order to reduce the rate at which new acaricides become poorly effective due to resistance.
- the phenylpyrazole group, e.g. fipronil which is formulated for control of ticks on dogs;
- botanical materials (e.g. extract of the neem tree containing azadirachtin).

As mentioned previously the above-mentioned groups of acaricides are no longer permitted or not advisable to use due to persistence and accumulation in food webs, great toxicity including carcinogenicity of some compounds to mammals, and/or cross-resistance with other chemical groups of acaricides.

- the organophosphates (e.g. coumaphos and chlorfenvinphos). These acaricides were in use for many years, are very toxic to vertebrates and have caused problems with acaricide resistance.
- the carbamates (e.g. propoxur) have been used as acaricides.
- the formamidine group is represented by amitraz. This is an unusual acaricide because it is soluble in water and degrades rapidly, so it was made up fresh for each use.

2.13.3. Vaccines

An anti-tick vaccine has been available on the market for about a decade, as a recombinant vaccine against *Rh. (B.) microplus* based on a concealed gut antigen, Bm86. It has proved to be quite effective for control of *Rh. (B.) microplus* under natural field conditions in Australia, This vaccine will reduce tick reproductive capacity by up to 90% although its direct effect on tick mortality is lower (Willadsen 2006, 2008). Knowledge about tick physiology and biochemistry remains fragmentary. Many more potential antigens have been proposed than have been tested. Tick antigen targets studied to date are from a restricted range of functional classes. They include structural proteins, particularly from salivary glands, hydrolytic enzymes and their inhibitors, particularly those involved in haemostatic processes and a range of membrane-associated proteins of unknown function (Willadsen 2006, 2008). Because vaccines are expensive and involve considerable risk, a high level of efficacy is required to offset these negatives. Hence, there has been little industry enthusiasm for further commercialization of anti-tick vaccines. However, exciting new developments, such as the ability to disrupt the male engorgement factor or the administration of combined anti-tick and anti-pathogen vaccines, might change this picture (Sonenshine et al. 2006, Willadsen 2008). Our lack of understanding of what makes a particular tick species susceptible to vaccination is revealed by limited experience with the existing vaccine.

Although it uses an antigen from *Rh. (B.) microplus*, it is even more efficacious against *Rh. (B.) annulatus* than against the homologous species (Fragoso et al. 1998). Significant protection against *Rh. (B.) decoloratus* (de la Fuente et al. 1999) and strong protection against *Hy. dromedarii* (de Vos et al., 2001) have been reported, while it is ineffective against *Rh. appendiculatus*. Such effects appear not to correlate with the degree of sequence conservation of the antigen across tick species (Willadsen 2006).

2.13.4. Pheromone-assisted control

Novel strategies for pheromone-assisted tick control have been recently developed in which pheromone–acaricide-impregnated devices are used to lure and kill host-seeking ticks. Such strategies have proven effective in protecting livestock in southern Africa threatened by the “bont ticks”, *A. hebraeum* and *A. variegatum,* and by the risk of *Ehrlichia ruminantium* infection causing heartwater, which often has a relatively high mortality rate. In one study, cattle treated with pheromone–acaricide-impregnated tail tags showed up to 95% control of bont ticks in one three-month trial and up to 99% tick control during a second three-month trial period (Norval et al. 1996). Also of interest is more recent research for control of Lyme borreliosis vectors leading to the development of a tick-control device for use against *I. scapularis*. In this case, paste-like droplets impregnated with both acaricide and the components of the tick-assembly pheromone (guanine, xanthine and hematin) is used to create a device that can be applied to vegetation to attract and kill *I. scapularis* before they can infest humans or animals (Sonenshine et al. 2006, Sonenshine 2008).

2.13.5. Genetic resistance

In general, tick tolerance or tick resistance exhibited by certain livestock breeds might vary with the species of infesting tick; heterospecific resistance appears to be low or even absent among different genera of ticks, while a certain degree of cross-resistance is expressed to tick species belonging to the same genus (de Castro and Newson 1993). Genetic resistance has been described in West African N’Dama cattle. This race has a higher degree of natural resistance against ticks with a long hypostome, such as some *Amblyomma* and *Hyalomma* species, than to tick genera with a short hypostome. In *Bos indicus* breeds, the evidence for a genetic resistance trait to multihost ticks, such as *Rh. appendiculatus*, is not as strong as for the one-host ticks, i.e., subgenus *Boophilus* (Mattioli et al. 2000).

2.13.6. Biological control

The idea of using biological agents to control ticks is appealing, though the practical difficulties of doing so have to be resolved. Biological control agents are in principle highly desirable but their narrow host-specificity, often relatively low efficacy, costs of manufacture, certain application problems and sometimes low stability present serious challenges.

Particular focus has been on fungi of the genera *Beauveria* and *Metarhizium*. For example, in Kenya aqueous formulations of the spores of *Beauveria bassiana* and *Metarhizium anrisopliae* induced high mortality and reduced fecundity and egg hatchability in *Rh. appendiculatus* feeding on cattle. These formulations also caused high mortality in all life stages of *A. variegatum* and *Rh. appendiculatus* in the vegetation. The comparative ease by which the spores of these fungi can be produced and artificially disseminated makes them promising potential agents for the control of ticks (Norval and Horak 2004).

Domestic chickens are opportunistic predators of ticks and can be used in rural areas. In particular, the indigenous breeds of Galliformes, if allowed to scavenge amongst cattle, can consume considerable numbers of ticks (Latif and Walker 2004).

Moreover the coexistence on the same pasture of wild birds as red and yellow-billed Ioxpeckers (*Buphagus erythrorhynchus* and *Buphagus africanus*) also should be considered as control method. These birds, in fact, are virtually obligatory predators of ixodid ticks and they take large numbers of these parasites from both domestic cattle and from several wildlife species. The red-billed birds favour *Rh. (B.) decoloratus* and *Rh. appendiculatus* as food items, whereas yellow-billed oxpeckers prefer the latter tick and *Amblyomma* spp. (Bezuidenhout and Stutterheim 1980). This preference is probably due to the bigger beak of yellow-billed oxpeckers. As an aid to tick control on both wildlife and domestic cattle, red-billed oxpeckers have been reintroduced to several regions in which they originally occurred in South Africa, but from which they had in recent times disappeared (Van Someren 1951).

Arthropods can also be used for biological control of ticks: chalcid wasps of the genus *Ixodiphagus* are obligatory parasitoids of ixodid ticks and most species will oviposit and develop only in the nymphal stage of the tick. Several wasp larvae can successfully develop in a single engorged nymph, which is killed during this process. Two of the seven described species of these wasps occur in Africa, namely *I. hookeri* and *I. theilerae* (Hu et al. 1998; Mwangi et al.1997; Norval and Horak 2004).

2.13.7. Vegetation management

Ticks do not survive for long on pasture that is either heavily grazed and thus short and dry, or in areas where pasture land is rotated with crops. When intensively farmed land is fenced it is possible for cattle pastures to be cleared of ticks by a combination of management and acaricide use, and can then be maintained tick free. This method may be appropriate for control of *Rhipicephalus* (*Boophilus)* species and *Rh. appendiculatus*. Burning of pasture grasses may sometimes, but not always, kill many ticks. An extreme case of pasture management is zero-grazing of dairy cattle, but there is a risk of unexpected reintroduction of ticks on cut fodder, on wild mammals or birds, or on newly introduced animals. However, in the case of *Hyalomma* infestations of dairy cattle, the ticks are adapted to living within the cattle sheds, so these methods do not apply. Even if there is some use of spray treatments of the walls of dairy houses for the control of some *Hyalomma* ticks adapted to live indoors, it is more effective in the long term to render the walls smooth by plastering them with mortar (Latif and Walker 2004). This has proved to be useful in Australia and Africa.

There is no single, ideal solution to the control of ticks. Integrated control scenarios representing increased scientific and practical complexity can be developed and recommended. The integrated control approach is probably the most effective way to control ticks (Willadsen 2006; Jongejan and Uilenberg 1994; Young et al. 1988).

2.13.8. Conclusions and recommendations

- Effective control measures are needed against vectors of CCHFV. .
- To enable increased surveillance and appropriate control of CCHFV and its vectors mapping of endemic areas and assessment of future risk areas, e.g., based on geographical information systems are necessary.
- Vector surveillance and reliable identification methods (morphological keys and DNA-based tick species identification) should be strengthened where CCHFV occur or is expected to emerge in the near future.
- Integrated tick control measures should be developed at international level.

3. Tick species as vectors of ASFV in Eurasia

EFSA has recently produced a comprehensive report which has dealt with the role of ticks as vectors in the spread and the maintenance of ASF (EFSA, 2010).

3.1. Ornithodoros as vector

Only species of the genus *Ornithodoros*, belonging to the family of soft ticks (Argasidae) are able to transmit ASFV. All *Ornithodoros* species tested, up to the present (Table 2), have been proved to be competent vectors for ASFV. Some of these belong to the taxonomical group of *Ornithodoros erraticus*, a group of species distributed mainly in the Mediterranean basin and Middle East, including Transcaucasia and parts of Russia (Morel, 1969). Sanchez Botija (1963) demonstrated that *O. erraticus* found in pig sites can be a vector of ASFV in Europe, while Plowright et al. (1969a, 1969b) demonstrated that *O. moubata/porcinus* ticks associated with warthogs were also involved in virus transmission in Africa.

Some authors consider ASFV and *Ornithodoros* tick as co-evolving organisms. Actually, noticeable telomeric similarities in the genomes of ASFV and *Borrelia*, the latter sharing the same *Ornithodoros* tick host in Africa and considered an original pathogen of soft ticks, suggest that ASFV is also a primary organism of *Ornithodoros* ticks and co-adapt to its tick hosts (Hinnebusch and Barbour, 1991). This hypothesis could explain discrepancies concerning infection success rates noticeable between several past surveys. For example, De Tray (1963) reported a consistent establishment of the virus isolate “Uganda” in specimens of the *O. moubata* group (34/35 were infected) whereas another isolate “Tengani” only caused persistent infection in a small proportion of ticks (2/46 were infected). More recently, Kleiboeker et al. (1999) compared oral and intra-hemocoelic experimental infections of *Ornithodoros* ticks collected from warthog burrows in Kruger National Park and the Northern Transvaal region of South Africa, as well as ticks from Masai Mara Reserve in Kenya, by three different viruses from South Africa, Malawi and Zimbabwe, all originally isolated from ticks collected from the field. The oral infection conducted with the isolate from Malawi was self-limiting (decline of virus titers and number of ticks containing virus) while the others persisted. Regarding the cythopathology caused by the Malawi strain in infected ticks, the author suggested the non adaptation of the isolate to express specific genes which allow the production of large quantities of progeny virus without damaging the host cell. The reason why this virus was originally isolated from ticks could be the large opportunity for those ticks to feed on infected pigs with high viremic titers during an ASF outbreak and leakage of midgut contents into the hemocoel without tick mortality, instead of real adaptation of this virus isolate to the tick host (Kleiboeker et al., 1999). In 1988, Dixon and Wilkinson analysed genomes of ASFV isolates collected over a 2 year period from ticks inhabiting warthog burrows in four regions of Zambia and observed additional sequences in the region close to the left-end terminus of the genome, which are not observed in domestic pig isolates. The author concluded that virus replication in ticks and warthogs may require additional host-specific genes not necessary for multiplication in domestic pigs and that the introduction of virus from tick/warthog sources into domestic pig populations would remove the selection pressure for maintaining these genes (Dixon and Wilkinson, 1988). More recently, Burrage et al., (2004) and Alfonso et al., (2004) demonstrated that ASFV multigene family 360 genes in the left variable region of the genome encode a host range determinant required for efficient replication and generalization of infection in pigs and ticks. However, no more information is yet available on specific determinants to tick/warthog hosts, as it was previously suggested. In addition, it is unknown if ASFV is able to come back from the domestic to the sylvatic cycle, although some authors suggest recombination processes during co-infections in ticks may exist (Dixon and Wilkinson, 1988; Plowright, 1977).

3.2. Studies on *Ornithodoros* spp. as vectors

3.2.1. Field data

Ornithodoros erraticus and *O. moubata* are accepted as natural reservoirs for ASFV with a well established role in the maintenance of the infection in nature (Plowright et al, 1970a). Replication of ASFV in these tick species has been demonstrated, which allows these species to remain infectious for months and up to 5 years (Table 2), posing a threat of transmitting the virus each time they feed on pigs (Plowright et al, 1970b; Boinas, 1994).

The adults and nymphal stages 4 and 5 of *O. erraticus* remain infected for longer than the larvae up to nymph 3 (Basto et al., 2006; Boinas, 1994). It was reported that the prevalence of infected *O. erraticus* and the average titres of ASFV per tick decreased over time when pigs were not present (Boinas, 1994). The maintenance of virus infection in the tick can constitute risk if they are able to feed on pigs for periods of 40 weeks after an ASFV infective meal (Basto, et al., 2006). More recently, Vial et al (2007) demonstrated ASFV DNA in *O. sonrai* collected from the field in West Africa. *O. sonrai* is a different species that is morphologically undistinguishable from *O. erraticus*. They may copulate with *O. erraticus* but the progeny is not fertile (Estrada-Peña, WG member, personal communication). Therefore molecular analyses are required to differentiate them.

Amongst those tick species that were tested, various vectorial competences were observed for different ASFV isolates, which has been inferred from variations in the titres of virus obtained, clearance rates, and the contradictory reports on the mortality of colonies infected with different ASFV isolates (Boinas,1994; Hess *et al*., 1987, Endris *et al*., 1992, Mellor and Wilkinson, 1985). The vectorial competence of ticks for the ASFV strain currently circulating in the Caucasus is unknown.

3.2.2. Laboratory data

Experiments on the transmission of ASFV by soft ticks are limited. Experimental evidence shows that, in addition to *O. erraticus* and *O. moubata,* another five *Ornithodoros* species, which have been investigated so far, can be infected (Table 2). Four of them have been reported in North America and the Caribbean Basin: *O. coriaceus, O. turicata, O. parkeri* and *O. puertoricensis* (Hess et al., 1987) and one in Africa, *O. savignyi* (Mellor and Wilkinson, 1985). Transstadial transmission of the virus was confirmed in those species after four moults in *O. coriaceus*, and after one moult in *O. savignyi* and *O. parkeri* (Table 2). Transovarial transmission of the virus was demonstrated only for *O. puertoricensis* (Hess et al., 1987).

Table 2: Laboratory and field studies on *Ornithodoros* species to determine the vector capacity for ASFV

Species	Region	Max. DPI[1] virus presence	Virus transmission[2]				References
			Trans-Stadial[3]	Trans-ovarial	Sexual	To pigs	
O. coriaceus	North America	502 days	+ (4)	-	ND	+ 502 DPI	Hess et al., 1987; Endris et al., 1994.
O. turicata	North America	23 days	ND[4]	ND	ND	+ 23 DPI	
O. puertoricensis	North America	239 days	ND	+	ND	+ 239 DPI	
O. parkeri	North America	70 days	+ (1)	ND	ND	-	
O. savignyi	Africa	106 days	+ (1)	-	ND	+ 106 DPI	Mellor and Wilkinson, 1985
O. moubata	Africa	3 years	+ (5)	+	+	+ 3 years	[5]
O. erraticus/ Marocanus	Europe	5 years	+ (5)	-	+	+ 588 DPI	Hess *et al*., 1989; Endris *et al*., 1992; Boinas,1994

[1]DPI: Days post-infection; [2] only the maximum lengths of time for virus presence and transmission to pigs or horizontal or vertical transmission among tick populations are considered; .[3] Numbers of moults; [4]ND: no data; [5] (Kleiboeker, *et al*., 1998; 1999, Plowright, 1977; Plowright *et al*., 1970a, 1970b, 1974, 1994; Rennie *et al*., 2001.)

The experimentally infected specimens of *O. parkeri,* fed on susceptible pigs for 46 and 70 days, did not transmit ASFV, although virus was recovered from ticks in both sets of experiments. In one case, 6 experimentally infected ticks were reported to have an average titre of 10^3 HAD_{50}/tick (Haemadsortion 50% doses) (Hess *et al.,* 1987). The other four species of ticks infected pigs when fed between 23 and up to 502 days post-infection (Table 2). The virus was detected in the haemolymph and the salivary glands of some specimens of a colony of *O. turicata* at 20 days post-infection (DPI) and transmission was achieved when they fed on pigs at 23 DPI (Hess *et al*., 1987). No follow up, however, from this study exists, to indicate the potential transmission of the virus through these species.

The soft tick species present in the Trans Caucasus Countries and the Russian Federation have not been involved in experiments to demonstrate the transmission of ASFV. Because they all are included in the *O. erraticus* group, their role as potential vectors cannot be ruled out.

3.3. Vector competence for ASFV of soft tick species

3.3.1. Virus-tick interaction

Several factors can influence the vector competence of soft tick species for ASFV. Only those related to the ASFV- *Ornithodoros* spp. interactions, such as the kinetics of ASFV in soft ticks, the adaptation of ASFV to the vectors or the genetic diversification of ASFV are discussed below:

3.3.2. Kinetics of ASFV in soft tick species

Kleiboeker et al., (1998, 1999) experimentally infected different species of the *O. moubata* group with several virus isolates from Southern Africa and monitored.their kinetics in these arthropods. Initial ASFV replication occurred in phagocytic digestive cells of the midgut epithelium with subsequent infection after 15 days. Dissemination of virus from midgut to other tick tissues required 2 to 3 weeks and secondary sites of virus replication included hemocytes, connective tissue, coxal and salivary glands, and reproductive tissue. Viral titers in salivary gland and reproductive tissue were consistently the highest detected with 10^4 to 10^6 HAD_{50}/mg (Kleiboeker et al., 1998). In general, only low virus titres were found in field infected ticks: the highest titre was of $10^{4.3}$ HAD_{50}/tick (Basto et al., 2006; Boinas, 1995). In experimental studies with *O. erraticus*, ASFV infection rates generally decrease in orally infected ticks while in ticks inoculated with virus into the haemocoel it increases suggesting the existence of a gut barrier to infection (Boinas, 1995).

Persistent infection, characterized by active virus replication in ticks, has been observed for several months to several years (Greig, 1972; Plowright, 1970a, 1970b). In the pioneer work of Sanchez Botija (1963) in Spain, ASFV was isolated from *O. erraticus* 4 months after an outbreak of the disease and later the author reported that the virus persisted in ticks for up to 8 years after infection (Sanchez Botija, 1982). Specimens infected with the ASFV isolates were collected from pig farms that were abandoned more than 2 years and 9 months after depopulation following an outbreak in Portugal (Boinas, 1995). Some ticks were collected in a farm 2 years after an ASF outbreak and kept unfed in the laboratory for 3 years before they were assayed and infectious virus recovered (Boinas, 1995).

The adult ticks and the late nymphal stages are the most likely to be found infected in the field collections performed as time passes (Boinas, 1995). The proportion of virus isolations in these stages have an initial increase in time up to weeks 32 post outbreak while in the early nymphal stages it decreases in this period (Basto et al., 2006). Later the infection rates decreased in all stages (weeks 63 post outbreak). These field findings coincide with experimental studies that report a decrease in virus titres and infection rates at weeks 41 and 61 after experimental oral infection (Basto et al., 2006). The rate of decrease in virus infection rates in orally-infected ticks seems to be dependent on the initial infection titre and so the probability of persistent infection in ticks depends on the titre of the viraemia of infected pig as only the highest titres used to infect ticks in some experiments led to persistent infection in ticks (Boinas, 1995).

3.3.3. Genetic diversification of ASFV

By sequencing the C-terminal end of the p72 gene, Bastos et al., (2003) and Lubisi et al., (2005) observed higher genetic variations in genotypes directly isolated from *Ornithodoros* ticks and warthogs or genotypes circulating in East and Southern Africa, where the sylvatic cycle plays a crucial part in the epidemiology of the ASF. Some other genotypes were only found in domestic pigs and presented low genetic divergence (Lubisi et al., 2005). In Madagascar, using concatenated sequences of the p22 and p32 genes, Michaud (Michaud et al., 2007) detected relatively high genetic divergence between Malagasy virus isolates collected on domestic pigs from 1998 and 2003, compared to that observed on West African and European isolates since the 1970s. In Madagascar, it has been suspected that the introduced virus was adapted to local bush pigs and *Ornithodoros* ticks, leading to its accelerated diversification. Such diversification phenomenon reported in several African countries has been previously analysed by Dixon and Wilkinson (1988) on Zambian virus isolates from *Ornithodoros* ticks. A considerable genetic diversity was observed between virus isolates from ticks collected from same regions and even same warthog burrows; this diversity resulted from peculiar point mutations and was all along the length of the genome, instead of insertions/deletions in the region close to the left-hand terminus of the genome usually observed for host filter (Dixon and Wilkinson, 1988). Regarding these results, ticks would be able to enhance the diversification of ASFV and the emergence of new virulent isolates to domestic pigs. However, no information is yet available on the location and the expression of genetic diversification. In addition, this process does not seem compatible with the persistence of ASFV in Iberian *Ornithodoros* ticks and observed genetic homogeneity of ASFV in Europe.

3.4. Biology of *Ornithodoros* spp.

3.4.1. Identification

Identification of species of *Ornithodoros* is difficult since most species are not morphologically different. Wallis and Miller (1983) conducted electrophoretical enzyme analysis to differentiate between some of the species. The adults of most species of *Ornithodoros* in Eurasia have a similar morphology, rendering the identification on that stage difficult. Larvae are the most adequate stage to provide with an adequate determination. According to the morphological features outlined by Filippova (1966) ticks in the *Ornithodoros erraticus* complex can be separated by the setal dorsal complement in the larvae, reduced to only two pairs of dorsal setae. Currently, there is no accurate way to separate the different species in the complex. Furthermore, there are no adequate DNA data of the species found in the wide distribution range of the complex, and there are strong evidences pointing to a greater diversity than currently reported. Appendix J provides a photograph of an adult male of *O. erraticus*.

3.4.2. Life cycle

The life cycle of *O. erraticus* includes the stages of eggs, larvae, nymphs (3 to 5 stages) and adults (see figure 9. The larvae and each of the nymphal stage need a blood meal before moulting to the next stage of life cycle. The adults need to have blood meals to be able to reproduce. The females lay about 120 eggs in each oviposition (Encinas-Grandes *et al*., 1993) and up to 6 gonotropic cycles can occur.

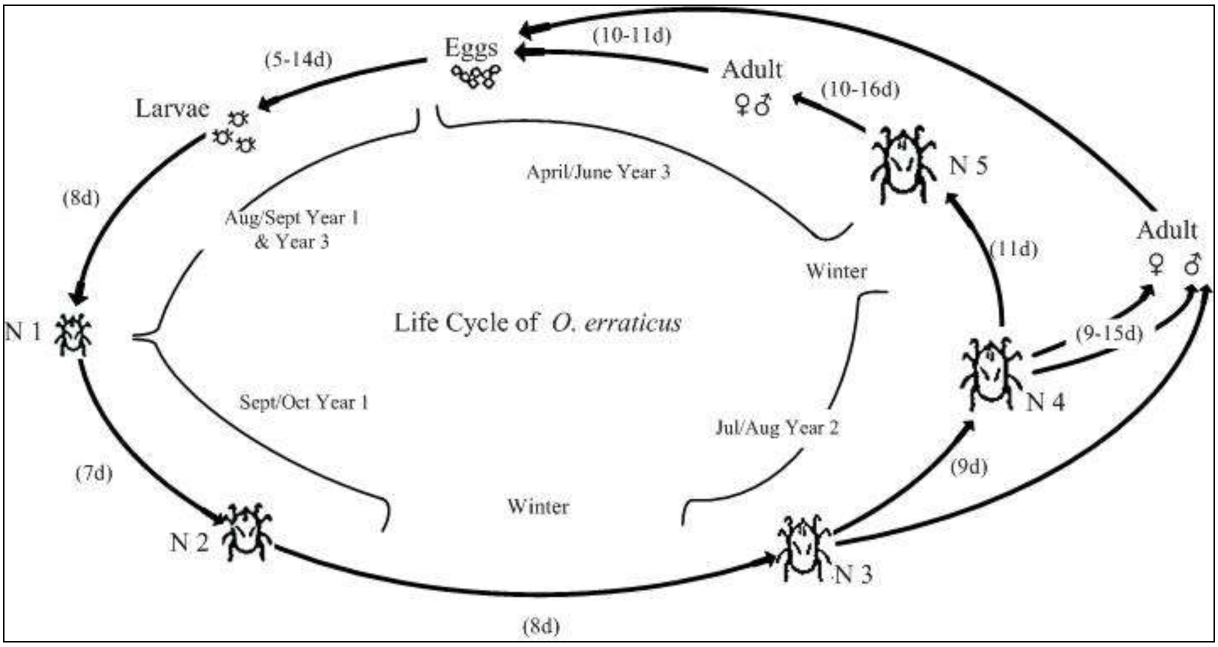


Figure 9: Life cycle of *O. erraticus* in the field (Spain) and in the laboratory (adapted from Boinas, 1994)

Although under laboratory conditions the complete cycle can occur in only 154 days (el Shoura, 1987), in the field the tick has a seasonal activity associated with prevailing temperature and humidity. In winter, the tick is inactive, and activity only starts when minimum external temperatures are within the range 10-13 ºC, consequently completion of the cycle takes from 2 to 4 years in the Iberian Peninsula (Caiado et al., 1990; Encinas-Grandes et al., 1993; Fernandez Garcia, 1970; Oleaga-Perez et al., 1990).

All soft ticks (not only the ones involved in ASFV transmission) are able to resist long starvation periods of up to 5 years for large nymphs and adults (Fernandez Garcia, 1970; Oleaga-Perez et al., 1990, Boinas, 1994) and their life span, if regularly feeding, can last up to 15 years (Encinas et al., 1999).

3.4.3. Host preference

The natural hosts of the *Ornithodoros* species complex are rodents. Pigs must be considered accidental hosts, because they simply share the same habitat. In other words, ticks find a good habitat in premises where pigs are kept. Other accidental/alternative hosts include humans, sheep and chickens (Tendeiro, 1962). In Portugal engorged ticks were found in birds nests on infested premises, indicating that birds also may act as alternative hosts for these vectors/arthropods (Boinas, 1995). *O. erraticus* was not reported from sporadic collections from European wild boar habitat in regions where ticks persisted adjacent to domestic pigs (Louza et al., 1989). This is because the ticks need a burrow or a protected shelter, while wild boars rest on the surface of the ground.

3.5. Geographic distribution of *O. erraticus* spp. in Europe, the Mediterranean Basin, the TCC, the RF

In Europe, the Mediterranean Basin, the TCC countries or the RF the only *Ornithodoros* species that had been reported historically were those belonging to the *O. erraticus* complex (*O. alactagalis, O. asperus, O. normandi, O. pavlovskyi, O. tartakovskyi,O. tholozani, O.erraticus, O.lahorensis and O.sonrai)* (Table 3). Presence of ticks of the *O. erraticus* group has been reported in the Caucasus; however, knowledge of their distribution and host preferences is limited (Table 3). The ecological niche of these ticks in the region has not been adequately determined.

Table 3: Historical records on *Ornithodoros* spp. in Europe, the Mediterranean Basin, and the Caucasus.

Ornithodoros species	Country reported	Reference
O. alactagalis	Armenia, Azerbaijan, Georgia, Iran, Northern Caucasus, Transcaucasia, Turkey	Filippova, 1966; Gugushvili, 1972; Gugushvili, 1973; Gugushvili, 1973; Ismailova *et al.* 1981; Kadatskaya, and Talybov, 1979; Shustrov, 1956.
O. asperus	Algeria, Italy, Morocco, Portugal, Spain, Tunisia	Filippova, 1966.
O. erraticus	Algeria, Egypt, Italy, Morocco, Portugal, Spain, Tunisia	Baltazard, 1937; Baltazard, *et al.* 1950; Blanc and Brunean, 1956; Blanc and Bruneau 1954; Boinas *et al.,* 2004; Boinas, 1995; Caminopetros and Triantaphyllopoulos, 1936; Cordero Del Campillo, M. 1974; Davis and H. Hoogstraal 1954; Hoogstraal *et al*. 1954; Khalil *et al.* 1984; Martinez Pereda. and Espinosa 1997; Morel, 1968; Estrada-Peña, 2005; Oleagaperez *et al.* 1990; Perez-Sanchez i 1994 ; Shoukry *et al.* 1993.
O. lahorensis	Armenia, FYROM, Kazakhstan, Kosovo, Syria, Turkey	Akopyan, *et al*.,1981; Aydin and Bakirci, 2007; Campana, Y., 1946; Golem, 1951; Gutzevich, 1948; Hoffmann *et al*., 1971; Inci *et al*., 2002; Kalkan, 1982; Kusov, 1962; 1966; Kusov, *et al*., 1966; Liebisch, 1975; Milutinovic, *et al*., 1997; Pavlov, 1944; Payzin and Akkay, 1952; Payzin, 1949; Sayin, *et al*., 1997; Sayn, *et al*., 2009;
O. pavlovskyi	Kazakhstan, Kirghizia, Tajikistan, Turkmenistan, Uzbekistan	Filippova, 1966
O. sonrai	Egypt, Morocco, Mauritania	Morel, 1968; Vial, pers. comm. (the presence of *O. sonrai* in Morocco is controversial)

Ornithodoros species	Country reported	Reference
O. tartakovskyi	Daghestan, Iran, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan	Filippova, 1966; Pospelova-Shtrom, 1941; 1949; 1963.
O. tholozani	Cyprus, Daghestan, Egypt, Irak, Iran, Israel, Jordan, Kazakhstan, Kyrgyzstan, Lebanon, Libya, Syria, Turkey, Ukraine, USSR	Assous and Wilamowski, 2009; Avivi, *et al*., 1973; Davis. and Hoogstraal, 1956; L'vov *et al*. 1975; Morel, 1968; Pospelova-Shtrom, 1941; 1949
O. verrucosus	Armenia, Georgia, Russian Federation,	Maruashvili, G. M. (1965); Pavlovskii, E. N. (1936); Gugushvili, 1972 and 1973; Shustrov, 1956.

The *O. erraticus* group is important in maintaining the local foci of ASFV; however, they do not play an active role in the geographic spread of the virus. This is because the involved ticks feed for short times and drop off the host, and commonly at night times when animals are resting. Therefore, the likelihood that these ticks move with an infected host to new premises or territories is minimal or should be considered negligible. Once a focus is established, soft ticks may have a role as local reservoirs of ASFV, as already observed in the Iberian Peninsula where repeated outbreaks occurred in premises infested with ticks (Perez-Sanchez et al.,1994; Arias and Sanchez-Vizcaino, 2002). The Spanish ASF eradication programme showed that in areas of outdoor pig production, where infected ticks occurred, the time to achieve eradication was significantly longer that in areas without ticks (Arias and Sanchez-Vizcaino, 2002). To our knowledge, no report is available to indicate the role of local soft tick populations in the Caucasus in regards to ASF.

Maps of the geographical distribution of ASFV and *Ornithodoros* spp.

The following maps have been made up using three sources of data: A systematic literature review (see appendix G) based on scientific papers retrieved from the databases integrated in ISI web of knowledge and Pubmed in the last 10 years, as well as on a pool of scientific papers considered relevant by the WG experts, coming from their private collections regardless of the time frame; and published historical data (approximately from years 1970 - 2000) of the integrated consortium on ticks and tick-borne diseases (ICTTD3 European project). Such historical data was not available for tick-borne pathogens. The signs in red colour indicate cases extracted from the systematic literature review (last 10 years). The green colour indicates historical cases (older than 2000). Dots correspond to coordinates (latitude/longitude). Stars correspond to cases where coordinates were not indicated and even could not be found because the name of the location given in the corresponding paper was equivocal (several toponyms existed with the same name). Stars are placed either in the middle of the smallest administrative region described in the scientific paper (for the countries that do not have official NUTS); or in the middle of the NUTS containing the specified location. Countries or areas that are not showing cases of presence of this tick are not necessarily free of it. Rather the species may not be presented in available literature for this report.

Figure 10: Map of reported occurrence ASFV

Critical assessment of the map of reported occurrence of ASFV:

The few existing records of the ASFV have been obtained from active surveillance of the infection in domestic pigs, wild boars and soft ticks associated to foci of outbreaks. The exception to this is the island of Sardinia, considered endemic for ASFV. In this island the tick *Ornithodoros* is not present, and no records of wildboar infested with ticks do exist. Therefore, ticks do not play a role in the maintenance of ASFV in that island. The endemicity is mainly related to the traditional extensive production system. The distribution recorded in the map is expected to be biased because the culinary habits in wide regions of northern Africa, and parts of eastern Mediterranean, driving to the absence of the domestic pig as the main host of the virus. The most recent outbreaks are in the Caucasus. The Island of Sardinia is considered endemic for ASFV. The cases represented in Portugal and Spain were eradicated in 1999 and 1995 respectively.

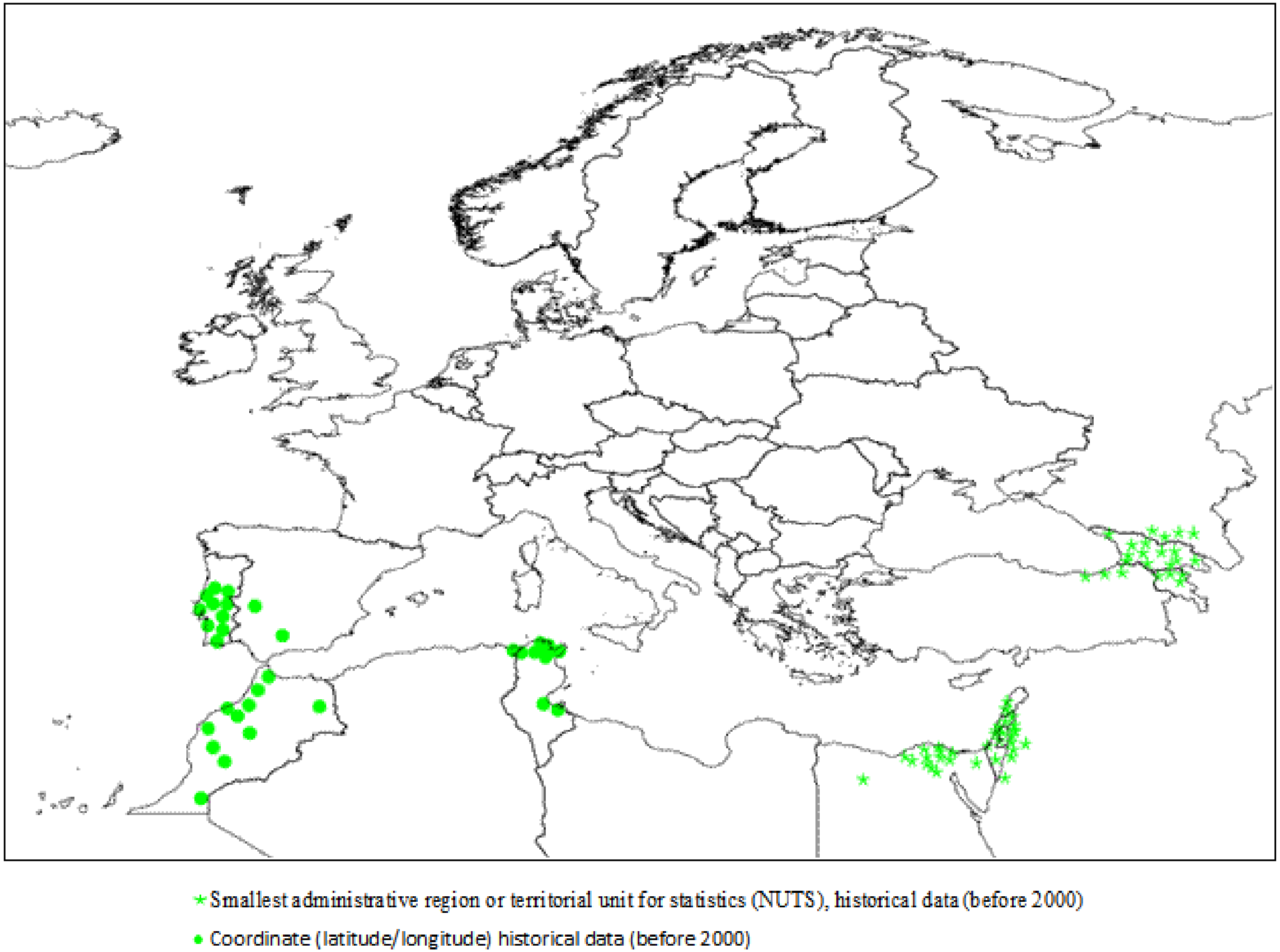


Figure 11: Map of the reported occurrence of *Ornithodoros* spp.

Critical assessment of the map of the reported occurrence of *Ornithodoros* spp:

The map covers the known distribution of the species in the genus *Ornithodoros* allegedly involved in the transmission of the African swine fever. The reported distribution of these ticks is poor, as they seem to be restricted to parts of the western Mediterranean and portions of Georgia, Armenia and Turkey. Species in the genus are known to be present also in parts of Morocco, Tunisia, and Egypt. Currently, there is a fragmentary knowledge about the distribution of the species, being hypothesized that *O. erraticus* would be present in the Iberian Peninsula, and parts of Morocco, while *O. sonrai* would be the species found in Egypt and southern Morocco. The mechanisms operating on the species separation in Morocco are unknown. It is interesting to note that there are not recent records (years 2000-2010) of the ticks in Europe, and the map has been carried out with historical records (years 1950-2000). This is because the tick has received some attention with the epidemics of the ASF in Europe. All the species in the group are xerophilous ticks, therefore living in open and dry habitats, commonly associated to rodent burrows.

3.6. Population dynamics of *Ornithodoros* spp.

3.6.1. Factors influencing the abundance of *Ornithodoros* spp.

3.6.1.1. Ability of *Ornithodoros* spp. to adapt to new areas

Because of the peculiar composition of their cuticle, African *Ornithodoros* ticks like other species of the Argasidae family can adapt to drier external conditions (<20% RH) and higher critical temperatures (63 C for *O. moubata*) than hard ticks, which explains their widely geographical distribution in African sub-Saharan countries (xerophilic type) (Morel, 1969). However, the absence of hard shell on soft-bodied ticks constrains or restricts their development and life cycle. All *Ornithodoros species* colonize underground or protected habitats (endophilic type that reduces the influence of the macro-climate on their geographic distribution (Morel, 1969). These parasites are classified as being endophilous and nidicolous since they live in the habitat of the host (Sonenshine, 1993). *Ornithodoros savignyi*, however, is an exception to this and lives at ground level and is widely distributed in sub-Saharan desert areas thanks to a particularly high critical temperature tolerance (up to 75 C).

Consequently, *Ornithodoros* spp. are mainly found in burrows of small mammals and other vertebrates, dugouts and trenches habited by wild animals, caves especially those in which excreta, litters or nests are present (Morel, 1969; Rodhain, 1976). Some species could adapt to domestic conditions and have been found in chicken pens, small ruminant sheds, pigsties and human dwellings with mud walls and floors (Walton, 1962; Rodhain, 1976).

All these different underground habitats provide optimal temperature and humidity necessary for the survival and development of *Ornithodoros* ticks. As a consequence, it is reasonable to consider that habitat preference may constitute a more important factor than host selection for *Ornithodoros* ticks, leading to their apparent host ubiquity. Studying distribution patterns of *O. porcinus domesticus* in human dwellings in East Africa, Walton (1962) noticed that ticks were either found in humid and cold highlands, humid and hot coasts and also in dry and hot lowlands. In the first area, ticks were found only in traditional buildings where the cooking fire was warming and drying the soil. In the second and third areas, ticks were colonizing cracks where external climate was buffered to provide cool temperature and higher humidity. Consequently, *Ornithodoros* ticks depend to a certain limit on the external climate but seem to be able to adapt to, apparently, unfavourable conditions by selecting underground habitats that may provide microclimatic optima. Hoogstraal et al., (1954) observed the same patterns for *O. sonrai* in Egypt, which never colonizes dry pure sand burrows while it is found in the same areas when sand-clay or silt-sand soils maintain a relative humidity from close rivers or annual rainfall.

It was never reported that soft ticks can move by itself outside buildings or its burrows. Soft tick species can only move when attached to the host. Transfer can be explained either by the transfer of utensils contaminated with the parasite or by the passive transfer of soft ticks feeding on animals being moved. This could only be responsible for transfer over short distances, since the time of feeding is generally short, 10 to 30 minutes (Fernandez Garcia, 1970), and possibly even shorter when the animal is in movement unless trapped in a skin fold, etc.

3.7. Other potential tick vectors

There have been several studies evaluating other blood-sucking invertebrates as mechanical vectors of ASFV. These included several species of lice, mites, flies and ixodid (hard) ticks. Transmission of ASFV by a mechanical vector was achieved with the pig louse, *Haematopinus suis* (Sanchez Botija and Badiola, 1966). Nonetheless, several other researchers were unable to demonstrate transmission of ASFV by this louse species (Heuschele and Coggins, 1965; Kovalenko et al., 1967; Montgomery, 1921). Further, ASFV could not be isolated from specimens of two warthog lice species, *Haematopinus phachochoeri* and *Haematomyzus hopkinsky*, that were collected from ASFV infected warthogs (Plowright, 1977; Thomson *et al*., 1980). Another experimentally-proven potential mechanical vector of ASFV that transmitted the virus to pigs after 24 hours was the stable fly, *Stomoxys calcitrans,* kept at 25-26 C (Mellor *et al*., 1987, Webb, 1990).

The following ixodid ticks have been tested for the transmission of the ASFV to pigs with negative results: *Rhipicephalus* spp. (Sanchez Botija, 1963), *Rh. simus*, *Rh. bursa, Amblyomma variegatum* (Kovalenko et al., 1967, Plowright, 1977), *Hyalomma* spp. (Plowright et al., 1994), *A. americanum* and *A. cajennense* (Groocock et al., 1980). Although ASFV was detected in these last two tick species for 4-7 days after feeding on viraemic blood, they did not transmit virus to pigs. Adults of a calliphorid fly species, *Auchmeromyia luteola,* collected near infected warthog burrows were tested for ASFV with negative results (Plowright, 1977, Thomson et al., 1980). Adults of flea species, as well as the Diptera, sandflies *Phlebotomus* spp., biting midges *(Culicoides* spp.) and black flies (*Simulium* spp.) collected from warthog burrows also were not infected with ASFV (Thomson et al., 1980)

According to Plowright et al. (1994), mosquitoes (*Anopheles* spp.) and unspecified flies (no other information was provided in the original paper) failed to transmit virus by feeding on pigs. The blood sucking kissing bug species, *Triatoma gerstaeckeri,* also failed to transmit the virus, but transstadial passage was demonstrated and ASFV was recovered 41 days later (Hess et al., 1987).

3.8. Soft tick prevention and control in relation to ASF

Eradication of *O. erraticus* from the old pig sties is invariably unsuccessful. This is due to the tick’s long life and long survival without feeding, the existence of accidental hosts other than pigs and the possibility of penetrating into the cracks and surfaces of the buildings, where they are not accessible to acaricides and to the use of fire torch on the surfaces or ground. These factors have been the major reason to abandon *O. erraticus* infested pig farms after an ASF outbreak; and th have led to avoiding the use of this type of building to shelter pigs in the Iberian peninsula (Boinas, 1994). The use of endectocides in pigs can reduce the level of infestation in the premises but does not avoid that the pigs become infected by ASFV if they are bitten by an infective tick.

The measures applied in practice include not housing pigs in infested buildings. When such buildings are located in the area of a pig herd, the premises can be isolated with fences to prevent the access of pigs or even destroyed and new premises rebuilt in another location (Arias and Sanchez-Vizcaino, 2002). No effective vaccine against the ticks exists but there are promising studies on salivary glands extracts and "concealed" gut antigen extracts (Astigarraga et al., 1997, Astigarraga et al., 1995, Manzano-Roman et al., 2006; 2007).

3.9. Conclusions and recommendations

- Of all the invertebrates tested up to the present only the soft ticks of the genus *Ornithodoros* are susceptible to ASFV infection either naturally or experimentally. Other soft ticks remain untested under laboratory conditions. Hard ticks or other blood feeding invertebrates have not been shown to be a vector of ASFV.

- All the *Ornithodoros* species (*O. erraticus, O. moubata /porcinus, O. coreaceus. O. turicata, O. puertoricensis, O. parkeri* and *O. savignyi*) investigated so far can become infective and perhaps will be biological vectors of ASF. Of these species, some of them are found only in Afrotropical and Neotropical regions.

- Different *Ornithodoros* spp. may play various roles in the epidemiology of ASF
- Only the *O. erraticus* complex is found in the European and TCC and RF territories. Morphological characteristics alone are not sufficient to differentiate some of these *Ornithodoros* species.
- The *O. erraticus* complex, because of its long life (up to 15 years) and long resistance without feeding and persistence of infection for up to 5 years, is important in maintaining the local foci of the ASFV (and lead to endemicity in a region). However, they do not play an active role on the geographical spread of the virus.
- *Ornithodoros* ticks feed mainly on animal species living in burrows, such as rodents and reptiles. Pigs are mostly accidental hosts, from which the ticks can be infected. The epidemiological role played by soft ticks becomes important where pigs are managed under traditional systems, including old shelters/sties with crevices.
- Wild boars have never been found infested by *Ornithodoros* spp. because they normally do not rest inside of protected burrows, but over the ground.
- Due to the limited available data on associated factors with the distribution of soft ticks, prediction of their potential distribution is difficult to construct.
- Eradication of *O. erraticus* from the old pig sties is invariably unsuccessful.
- The use of acaricides on pigs and their habitat can reduce the level of infestation in the premises but does not avoid the pigs of becoming infected by ASF virus if they are bitten by a virus infective tick.

3.10. Predictions of vector and vector-borne disease occurrence

Predictions of vector and vector-borne disease occurrence are based on determining the appropriate combination of habitat conditions for vectors and reservoirs (habitat including climate and other ecological factors), and the susceptible host. Two major concepts can influence the predictions:

1) the natural nidality of transmissible diseases, independent of humans, infectious diseases exist in nature under a combination of local conditions and in the presence of vertebrates and arthropods that act as vectors and/or reservoirs of the pathogenic agent; and

2) the fundamental ecological niche of a species, defined as a set of environmental conditions within which a species can survive and persist. This niche consists of a multi-dimensional hypervolume that determines suitable habitat for the species but is distinct from the distribution of the species plotted in the geographical space.

Under the first concept, and provided strict assumptions on disease transmission dynamics, it is possible to approximate disease distribution using some indices, such as those based on vector presence and/or abundance. According to the second concept, such potential vector presence and/or abundance should be predicted instead of being described, in order to extend predictions into non sampled areas or future periods. This method uses habitat suitability based on favourable or tolerable environmental conditions for a species survival, development and dynamics.

In terms of the soft ticks, to date, efforts to model climate suitability for soft ticks have been restricted to *Otobius megnini* (Estrada-Peña et al., in press) while studies are on-going to map the distribution and define potential climate niches of ticks of the *O. porcinus - O. moubata* group (Vial and Estrada-Peña, pers. communication). Considering the peculiar biology and ecology of argasid ticks, it seems difficult to adapt predictions of suitable habitats for hard ticks to soft ticks. The major feature that often discriminates these groups of organisms in relation to their environment is the nidicolous life-style of argasid ticks. These ticks are limited to specialized host-related niches and have evolved well-adapted life history traits that insure their persistence in their microhabitat. For example, the short blood feeding period for nymphs and adults may result in low dispersal capacity, or at least “step by step” dispersion, and thus in a more limited distribution of soft tick species and their associated pathogens.

Regarding to hard ticks, several modelling strategies have been developed aimed to understand the basic behavior of some tick-borne pathogens. In the same way, some models addressed basic questions about the climate suitability for hard ticks, as a proxy for predictive mapping. The first category of maps aimed to predict the geographical range of tick species is based on records of a given tick species. Such a kind of models use a statistical approach, trying to infer the expected range of the species (or population) after the collections of the tick in a more or less wide territory. A second category is aimed to develop equations able to characterize each stage of the ticks’ life cycle, then using actual climate data to project the stability of the population looping until a stable stage is reached. Both models have pros and cons. It is difficult to obtain every parameter for the complete life cycle of a tick species, mainly regarding the mostly unknown factors operating the mortality of feeding stages on hosts. Further on this, the density and abundance of a given host population must to be carefully addressed before to project those models as predictive outputs. On the other hand, statistical models may infer a good estimation of the known range of the tick, but they don't account with the adaptation of the population to different climate characteristics, as the basic behavior of a liking population.

Further on the issue of hosts, it is arduous to develop even a simple model aimed to project and predict estimations of infection with a given tick-borne pathogen. This is because an extreme variability observed in the field studies, linked to the focal nature of tick-borne pathogens foci. Therefore, studies developed in a given territory may, or may not, be of applicability to another part of the tick or pathogen range, because subtle variations in tick or hosts densities, as well as host refractoriness to infection.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

CCHF

- The main vectors are *Hyalomma* spp, but at local level *Dermacentor* spp and *Rhipicephalus* spp have proven capacity of maintaining the CCHFV through their life cycle and transmit it to the vertebrates. However the role of *I. ricinus* (the most widely distributed tick in Europe) in the transmission of CCHFV has not been ascertained.

- Several studies indicate that argasid (soft) ticks are not competent CCHFV vectors

- Mammals such as rodents, hedgehogs, hares, are considered the principle reservoirs of CCHFV. Ungulates are important hosts for adult tick vector species. Viraemic periods, however, in ruminants appear to be short which may reduce the transmission of this virus to other hosts.

- In the tick populations the infection is maintained by transstadial and transovarial transmissions. Co-feeding transmission has not been proven in a natural setting.

- Increase in the number of fragmented areas and the degradation of agricultural lands to bush lands are the two main factors in the creation of new foci of CCHF in endemic areas.

- A complex chain of interactive factors exists that makes causes for changes in the infection level often difficult to assess. Climate change is only one of the factors associated with the spread of the infection.

- The absence of clinical signs and the lack of reliable diagnostic tests to detect the infection in mammals and birds make the confirmation of the infection in animals difficult.

- Surveillance data are scattered and inconsistent in terms of diagnostic capability and host species including humans. Infection can be monitored in ticks using various method of collection.

- Movement of livestock and wildlife species which may carry infected ticks ought to contribute to the spread of the infection. Migratory birds may not be sufficient to establish new foci of infection. We do not consider the movement of viremic domestic or wild animals because it is out of the remit of the mandate

- The Middle East and Balkan countries are the most likely sources of introduction of CCHFV into other European countries.

- There are existing risk models for other tick-borne diseases, such as tick-borne relapsing fever (TBRF), and ASF. These two models serve the purpose of identifying gaps in knowledge and data that can be applied for CCHF risk modelling.

- There is no single ideal solution to the control of ticks. The integrated control approach is probably the most effective.

ASF

- Of all the invertebrates tested up to the present only the soft ticks of the genus *Ornithodoros* are susceptible to ASFV infection either naturally or experimentally. Other soft ticks remain untested under laboratory conditions. Hard ticks or other blood feeding invertebrates have not been shown to be a vector of ASFV.

- All the *Ornithodoros* species (*O. erraticus, O. moubata /porcinus, O. coreaceus. O. turicata, O. puertoricensis, O. parkeri* and *O. savignyi*) investigated so far can become infective and are perhaps biological vectors of ASF. Of these species, some are found only in Afrotropical and Neotropical regions.

- Only the *O. erraticus* complex is found in the European, trans-Caucasus coountries and and Rusian Federation territories. Morphological characteristics alone are not sufficient to differentiate some of these *Ornithodoros* species.

- The *O. erraticus* complex, because of its long life (up to 15 years) and strong resistance to starvation and persistence of infection for up to 5 years, is important in maintaining the local foci of the ASFV (and lead to endemicity in a region). However, they do not play an active role in the geographical spread of the virus.

- *Ornithodoros* ticks feed mainly on animal species living in burrows, such as rodents and reptiles. Pigs are mostly accidental hosts, from which the ticks can be infected. The epidemiological role played by soft ticks becomes important where pigs are managed under traditional systems, including old shelters/sties with crevices.

- Wild boars have never been found infested by *Ornithodoros* spp. because wild boars normally do not rest inside protected burrows, but above the ground.

- Due to the limited available data on associated factors with the distribution of soft ticks, prediction of their potential distribution is difficult to construct.

- Eradication of *O. erraticus* from the old pig sties is invariably unsuccessful.

- The use of acaricides on pigs and their habitat can reduce the level of infestation in the premises but does not avoid the pigs of becoming infected by ASF virus if they are bitten by a virus infective tick.

RECOMMENDATIONS

CCHF

- The current database on ticks' distribution should be maintained. Scientific information can be included in the database only if the following are clearly specified: the scientific names of the organisms studied (vectors or pathogens), origin of samples (vegetation, host), type of samples (blood, serum, organs, etc), geo-reference of the sample, diagnostic tools used to identify the ticks, date, and history of the samples if applicable (imported host or vectors, travel history).

- There is a need for a standard case definition of CCHFV infection in vertebrates and arthropods. It is essential to notify the presence of vectors during outbreaks and the disease notification should include the possible involvement of vectors.

- Mapping of endemic areas and assessment of future risk areas are necessary for appropriate surveillance and control of CCHF. Vector surveillance and reliable identification methods and tools (morphological keys and DNA-based tick species identification) should be available in all countries where CCHFV occur or can be expected to emerge in the near future.

- The presence of CCHFV in a geographic area can be monitored by PCR on ticks collected on the animals and in the environment (questing ticks)

- Frequent serosurveys of grazing animals, including livestock and wildlife, in CCHFV free areas neighbouring or bordering known high risk areas (i.e. the southern Balkans and Turkey) is another approach for determining recent introduction of the virus. However, a prerequisite for such approaches would be the development of reliable and specific diagnostic assays to detect antibodies in a range of host species.

- A risk model for the introduction and spread of CCHFV, including gaps in existing knowledge, should be considered regarding the available similar models such as ASF and TBRF. Pathways other than tick transmission for the introduction and spread of infection should be considered in this risk model.

- To prevent virus infection to humans by ticks, general guidelines on personal protection should be available to the general public in affected countries.

- Integrated tick control measures should be developed at international level.

- Acaricides should be used in accordance with the best practice that minimise the development of resistance by the tick species.

ASF

- The ASF recommendations are in agreement to those presented in EFSA scientific opinion on African swine fever (EFSA, 2010)

Recommendations for research

CCHF

- The role of other tick species, specifically *I. ricinus*, in the transmission of CCHFV should be investigated under natural conditions.

- The role of European wildlife, especially wild ungulates, in the spread of CCHFV needs to be investigated.

- The necessary biotic and abiotic conditions for the maintenance of CCHFV in nature requires further investigation.

- In order to understand the epidemiology, further knowledge on the pathogenesis and distribution of the virus in mammalian hosts should be obtained

ASF

- Different *Ornithodoros* spp. may play various roles in the epidemiology of ASF. It would need further investigations.

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APPENDICES

APPENDIX A: CCHFV GEOGRAPHIC DISTRIBUTION DATA.

See table appendix L for the related complete reference.

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Albania	Kukës		723	
Bulgaria	Blagoevgrad	Gotse Delchev	1109	
Bulgaria	Blagoevgrad	Gotse Delchev	1412	
Greece	Anatoliki Makedonia kai Thraki		1413	
Greece	Anatoliki Makedonia kai Thraki	Rodopi	721	
Kosovo	Pristina		1402	
Russia	Astrakhan'		1068	
Russia	Astrakhan'		1404	
Russia	Astrakhan'		635	
Russia	Dagestan		1403	
Russia	Dagestan		1411	
Russia	Ingush		1411	
Russia	Kalmyk		1401	
Russia	Kalmyk		1404	
Russia	Kalmyk		1408	
Russia	Rostov		1068	
Russia	Rostov		634	
Russia	Rostov	Dubovskiy rayon	1407	
Russia	Rostov	Martynovskiy rayon	1414	
Russia	Rostov	Orlovskiy rayon	1405	
Russia	Rostov	Orlovskiy rayon	1414	
Russia	Rostov	Peschanokopskiy rayon	1407	
Russia	Rostov	Proletarskiy rayon	1407	
Russia	Rostov	Remontnenskiy rayon	1407	
Russia	Rostov	Sal'skSal'skiy rayon	1405	
Russia	Rostov	Tselinnyy rayon	1406	
Russia	Rostov	Tsimlyanskiy rayon	1406	
Russia	Rostov	Zimovnikovskiy rayon	1406	
Russia	Rostov	Zimovnikovskiy rayon	1414	
Russia	Stavropol'		1404	
Russia	Stavropol'		1404	
Russia	Stavropol'		635	
Russia	Stavropol'	Apanasenkovskiy rayon	1410	
Russia	Stavropol'	Apanasenkovskiy rayon	1414	
Russia	Stavropol'	Arzgirskiy rayon	1414	
Russia	Stavropol'	Blagodarnenskiy rayon	1410	
Russia	Stavropol'	Budennovsk	1408	
Russia	Stavropol'	Budennovskiy rayon	1414	
Russia	Stavropol'	Ipatovskiy rayon	1414	
Russia	Stavropol'	Izobil'nenskiy rayon	1410	
Russia	Stavropol'	Krasnogorodskiy rayon	1409	
Russia	Stavropol'	Krasnogorodskiy rayon	1410	
Russia	Stavropol'	Novoaleksandrovskiy rayon	1414	

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Russia	Stavropol'	Novoselitskiy rayon	1410	
Russia	Stavropol'	Petropavlovskiy rayon	1414	
Russia	Stavropol'	Rostov	1400	
Russia	Stavropol'	Stepnovskiy rayon	1414	
Russia	Volgograd		1068	
Russia	Volgograd		1404	
Russia	Volgograd	Oktyabr'skiy	1408	
Turkey	Adana		1399	3
Turkey	Adiyaman		1399	
Turkey	Afyon		1399	4
Turkey	Aksaray		1399	
Turkey	Amasya		1342	44
Turkey	Ankara		1399	20
Turkey	Artvin		1399	31
Turkey	Artvin		708	3
Turkey	Aydin		1399	14
Turkey	Balikesir		1399	2
Turkey	Bayburt		1399	3
Turkey	Bilecik		1399	3
Turkey	Bingöl		1399	
Turkey	Bolu		1399	14
Turkey	Bursa		1399	
Turkey	Çanakkale		1399	
Turkey	Çankiri		1399	38
Turkey	Çorum		1399	117
Turkey	Erzincan		1399	21
Turkey	Erzurum		1399	73
Turkey	Erzurum		708	2
Turkey	Eskisehir		1399	6
Turkey	Giresun		1102	8
Turkey	Giresun		1399	25
Turkey	Gümüshane		1399	70
Turkey	Gümüshane		991	
Turkey	Hatay		1399	
Turkey	Icel		1399	2
Turkey	Isparta		1399	2
Turkey	Istanbul		1399	
Turkey	Istanbul		649	4
Turkey	Izmir		1399	4
Turkey	Karabuk		1399	18
Turkey	Kars		1399	
Turkey	Kastamonu		1399	42
Turkey	Kayseri		1399	5
Turkey	KIRIKKALE		1399	4
Turkey	Kirklareli		1399	2
Turkey	Kirsehir		1399	3
Turkey	Konya		1399	4
Turkey	Kütahya		1399	8
Turkey	Malatya		1399	4
Turkey	Manisa		1399	2
Turkey	Mugla		1399	3
Turkey	Mus		1399	2

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Turkey	Nevsehir		1399	2
Turkey	Ordu		1102	15
Turkey	Osmaniye		1399	
Turkey	Samsun		1399	11
Turkey	Sanliurfa		1399	3
Turkey	Sinop		1399	3
Turkey	Sivas		1399	114
Turkey	Tokat		1102	219
Turkey	Tokat		708	2
Turkey	Trabzon		708	2
Turkey	TUNCELI		1399	2
Turkey	VAN		1399	
Turkey	Yozgat		1399	95
Turkey	Zinguldak		1399	

(*) if more than one.

APPENDIX B: *HYALOMMA MARGINATUM* GEOGRAPHIC DISTRIBUTION DATA.

See appendix L for the related complete reference.

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Albania	Elbasan	Elbasanit	0	
Albania	Elbasan	Librazhdit	0	
Albania	Korçë	Korçës	0	
Albania	Korçë	Pogradecit	0	2
Albania	Lezhë	Lezhës	0	
Albania	Vlorë	Sarandës	0	
Albania	Vlorë	Vlorës	0	3
Algeria	Aïn Defla	Ain Benian	0	
Algeria	Aïn Defla	Ain Lechiakh	0	
Algeria	Aïn Defla	Arib	0	
Algeria	Aïn Témouchent	Ain Kihel	0	
Algeria	Alger	Ain Benian	0	
Algeria	Alger	Ouled Chebel	0	
Algeria	Annaba	Annaba	0	
Algeria	Batna	Ain Touta	0	
Algeria	Batna	Batna	0	
Algeria	Batna	Chemora	0	
Algeria	Batna	Ksar Bellezma	0	
Algeria	Batna	Ras El Aioun	0	
Algeria	Batna	Seriana	0	
Algeria	Beja	Bejaia	0	
Algeria	Biskra	Besbes	0	
Algeria	Biskra	El Ouitaya	0	
Algeria	Biskra	Ouled Djellal	0	
Algeria	Biskra	Tolga	0	
Algeria	Blida	Bouaarfa	0	
Algeria	Blida	Bouinan	0	
Algeria	Blida	Soumaa	0	
Algeria	Bouira	Ain El Hadjar	0	
Algeria	Bouira	Ain Turk	0	
Algeria	Bouira	Djebahia	0	
Algeria	Bouira	El Asnam	0	
Algeria	Bouira	Lakhdaria	0	2
Algeria	Bouira	Sour El Ghouzlane	0	3
Algeria	Boumerdès	Boudouaou	0	
Algeria	Boumerdès	Corso	0	
Algeria	Boumerdès	Isser	0	
Algeria	Chlef	Bouzeghaia	0	
Algeria	Chlef	Chlef	0	2
Algeria	Chlef	Sendjas	0	
Algeria	Chlef	Sidi Akkacha	0	
Algeria	Chlef	Taougrit	0	
Algeria	Chlef	Tenes	0	
Algeria	Constantine	Constantine	0	
Algeria	Constantine	El Khroub	0	
Algeria	Djelfa	M'Liliha	0	
Algeria	El Tarf	El Aioun	0	
Algeria	Guelma	Ain Rekada	0	
Algeria	Guelma	Hammam Debagh	0	2

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Algeria	Jijel	Chekfa	0	
Algeria	Jijel	Jijel	0	
Algeria	Jijel	Jijel	110	
Algeria	Jijel	Jijel	110	
Algeria	Laghouat	Laghouat	0	
Algeria	M'Sila	Ain Errich	0	
Algeria	Mascara	Ain Ferah	0	
Algeria	Médéa	Berrouaghia	0	
Algeria	Médéa	Boughezoul	0	
Algeria	Médéa	Medea	0	
Algeria	Mila	Chelghoum Laid	0	
Algeria	Oran	Oran	0	
Algeria	Oran	Oued Tlelat	0	
Algeria	Oum el Bouaghi	Meskiana	0	
Algeria	Relizane	Relizane	0	
Algeria	Relizane	Sidi M'Hamed Benaouda	0	
Algeria	Sétif		110	
Algeria	Sétif		110	
Algeria	Sétif	Ain Arnat	0	5
Algeria	Sétif	Ain Lahdjar	0	
Algeria	Sétif	El Eulma	0	
Algeria	Sétif	Guellal	0	
Algeria	Sétif	Mezloug	0	
Algeria	Sétif	Ouled Saber	0	
Algeria	Sétif	Setif	0	
Algeria	Sidi Bel Abbès	Dhaya	0	
Algeria	Skikda	Ouled Attia	0	
Algeria	Tébessa	Bir El Ater	0	
Algeria	Tébessa	Boulhaf Dyr	0	
Algeria	Tébessa	Tebessa	0	4
Algeria	Tiaret		129	
Algeria	Tipaza	Hadjout	0	
Algeria	Tissemsilt	Lardjem	0	
Algeria	Tissemsilt	Theniet El Had	0	
Algeria	Tizi Ouzou	Boghni	0	
Algeria	Tizi Ouzou	Draa-Ben-Khedda	0	
Algeria	Tizi Ouzou	Tadmait	0	
Algeria	Tizi Ouzou	Tizi Ouzou	0	2
Algeria	Tizi Ouzou	Tizi-Ghenif	0	
Algeria	Tizi Ouzou	Zekri	0	
Bosnia and Herzegovina	Federacija Bosna i Hercegovina	Tuzla	0	
Bulgaria	Blagoevgrad	Bansko	0	2
Bulgaria	Burgas	Karnobat	0	
Bulgaria	Burgas	Malko Tarnovo	0	
Bulgaria	Burgas	Tsarevo	0	
Bulgaria	Haskovo	Svilengrad	0	
Bulgaria	Kardzhali	Ardino	0	
Bulgaria	Kardzhali	Kardzhali	0	2
Bulgaria	Kardzhali	Momchilgrad	0	2
Bulgaria	Kyustendil	Rila	0	
Bulgaria	Lovech	Troyan	0	

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Bulgaria	Montana	Montana	0	
Bulgaria	Pazardzhik	Pazardzhik	0	
Bulgaria	Pernik	Breznik	0	
Bulgaria	Pleven	Pleven	0	
Bulgaria	Plovdiv	Plovdiv	0	
Bulgaria	Plovdiv	Saedinenie	0	
Bulgaria	Shumen	Preslav	0	
Bulgaria	Sliven	Sliven	0	
Bulgaria	Sofia	Samokov	0	
Bulgaria	Sofia	Zlatitsa	0	
Bulgaria	Stara Zagora	Chirpan	0	
Bulgaria	Stara Zagora	Haskovo	69	
Bulgaria	Stara Zagora	Radnevo	0	
Bulgaria	Varna	Dolni Chiflik	0	
Bulgaria	Varna	Provadiya	0	
Bulgaria	Varna	Varna	0	
Bulgaria	Vidin	Dimovo	0	3
Bulgaria	Vratsa	Vratsa	0	
Bulgaria	Yambol	Tundzha	0	3
Bulgaria	Yambol	Yambol	0	2
Croatia	Grad Zagreb		0	2
Croatia	ibensko-Kninska		0	
Croatia	Istarska		0	
Croatia	Karlovacka		0	
Croatia	Primorsko-Goranska		0	3
Croatia	Splitsko-Dalmatinska		0	3
Croatia	Splitsko-Dalmatinska		781	
Croatia	Zadarska		0	6
Cyprus	Famagusta		0	
Cyprus	Nicosia		0	2
Cyprus	Paphos		0	2
France	Aquitaine	Pyrénées-Atlantiques	0	
France	Aquitaine	Pyrénées-Atlantiques	1280	
France	Centre	Indre-Et-Loire	0	2
France	Corse		1280	
France	Corse	Corse-Du-Sud	0	14
France	Corse	Haute-Corse	0	7
France	Languedoc-Roussillon	Lozère	0	
France	Languedoc-Roussillon	Pyrénées-Orientales	1280	
France	Provence-Alpes-Côte-d'Azur	Alpes-Maritimes	0	
France	Provence-Alpes-Côte-d'Azur	Bouches-Du-Rhône	0	7
France	Provence-Alpes-Côte-d'Azur	Var	0	
France	Provence-Alpes-Côte-d'Azur	Var	1280	
Germany	Baden-Württemberg	Freiburg	1302	
Greece	Anatoliki Makedonia kai Thraki		735	
Greece	Anatoliki Makedonia kai Thraki		735	
Greece	Anatoliki Makedonia kai Thraki	Drama	0	4
Greece	Anatoliki Makedonia kai Thraki	Evros	0	5
Greece	Anatoliki Makedonia kai	Kavala	0	4

Country	Admin 1	Admin 2	Reference ID	Number of entries*
	Thraki			
Greece	Dytiki Makedonia		735	
Greece	Dytiki Makedonia		735	
Greece	Dytiki Makedonia	Grevena	0	
Greece	Dytiki Makedonia	Kozani	0	
Greece	Ipeiros	Ioannina	0	3
Greece	Ipeiros	Preveza	0	
Greece	Kentriki Makedonia		735	
Greece	Kentriki Makedonia	Khalkidiki	0	5
Greece	Kentriki Makedonia	Khalkidiki	1303	
Greece	Kentriki Makedonia	Khalkidiki	1303	
Greece	Kentriki Makedonia	Khalkidiki	1303	
Greece	Kentriki Makedonia	Kilkis	0	4
Greece	Kentriki Makedonia	Pella	0	
Greece	Kentriki Makedonia	Serrai	0	2
Greece	Kentriki Makedonia	Thessaloniki	0	3
Greece	Kriti	Heraklion	0	
Greece	Peloponnisos	Arcadia	0	
Greece	Peloponnisos	Messinia	0	3
Greece	Stereá Elláda	Boeotia	0	
Greece	Stereá Elláda	Euboea	0	
Greece	Stereá Elláda	Fthiotis	0	
Greece	Thessalia	Larisa	0	
Italy	Abruzzo	L'Aquila	0	2
Italy	Abruzzo	Teramo	0	
Italy	Apulia	Bari	0	11
Italy	Apulia	Barletta-Andria-Trani	0	2
Italy	Apulia	Brindisi	0	2
Italy	Apulia	Foggia	0	19
Italy	Apulia	Lecce	0	2
Italy	Apulia	Taranto	0	2
Italy	Basilicata	Matera	0	7
Italy	Calabria	Cosenza	0	
Italy	Calabria	Reggio Di Calabria	0	3
Italy	Campania	Napoli	0	
Italy	Emilia-Romagna	Forli' - Cesena	0	
Italy	Emilia-Romagna	Parma	0	3
Italy	Emilia-Romagna	Ravenna	0	4
Italy	Emilia-Romagna	Rimini	0	
Italy	Friuli-Venezia Giulia	Udine	0	
Italy	Lazio	Latina	0	3
Italy	Lazio	Rieti	0	8
Italy	Lazio	Roma	0	14
Italy	Lazio	Viterbo	0	5
Italy	Liguria	Genova	0	2
Italy	Liguria	Savona	0	
Italy	Lombardia	Brescia	0	
Italy	Marche	Macerata	0	
Italy	Molise	Campobasso	0	6
Italy	Piemonte	Alessandria	0	
Italy	Piemonte	Torino	0	
Italy	Piemonte	Verbano-Cusio-Ossola	0	3

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Italy	Sardegna	Cagliari	0	
Italy	Sardegna	Carbonia-Iglesias	0	
Italy	Sardegna	Ogliastra	0	2
Italy	Sardegna	Oristano	0	2
Italy	Sicily	Agrigento	0	2
Italy	Sicily	Enna	0	
Italy	Sicily	Messina	0	
Italy	Sicily	Palermo	0	2
Italy	Sicily	Palermo	242	
Italy	Sicily	Palermo	366	
Italy	Sicily	Palermo	366	
Italy	Sicily	Palermo	366	
Italy	Sicily	Palermo	366	
Italy	Sicily	Palermo	998	
Italy	Sicily	Ragusa	366	
Italy	Sicily	Trapani	0	2
Italy	Toscana		1331	
Italy	Toscana	Florence	0	2
Italy	Toscana	Grosseto	0	
Italy	Toscana	Pisa	0	2
Italy	Trentino-Alto Adige	Trento	0	
Italy	Umbria	Perugia	0	2
Italy	Umbria	Terni	0	
Italy	Veneto	Venezia	0	
Italy	Veneto	Verona	0	
Kosovo	akovica	Decani	0	3
Kosovo	Gnjilane	Gnjilane	0	
Kosovo	Gnjilane	Vitina	0	
Kosovo	Kosovska Mitrovica	Kosovska Mitrovica	0	
Kosovo	Kosovska Mitrovica	Kosovska Mitrovica	346	
Kosovo	Kosovska Mitrovica	Kosovska Mitrovica	346	
Kosovo	Pristina	Lipljan	0	
Kosovo	Pristina	Priötina	0	
Libya	Al Marqab		0	
Macedonia	Eastern	Pehcevo	0	
Macedonia	Pelagonia	Bitola	0	2
Macedonia	Polog	Tetovo	0	2
Macedonia	Skopje	Centar	0	5
Macedonia	Southeastern	Lake Dojran	0	2
Macedonia	Southwestern	Ohrid	0	2
Macedonia	Vardar	Kavadartsi	0	
Morocco	Chaouia - Ouardigha	Ben Slimane	0	3
Morocco	Chaouia - Ouardigha	Settat	0	3
Morocco	Doukkala - Abda	El Jadida	0	
Morocco	Doukkala - Abda	Safi	0	3
Morocco	Fès - Boulemane	Zouagha-Moulay Yacoub	0	
Morocco	Gharb - Chrarda - Béni Hssen	Kénitra	0	3
Morocco	Grand Casablanca	Mohammedia	0	
Morocco	Marrakech - Tensift - Al Haouz	Al Haouz	0	3
Morocco	Marrakech - Tensift - Al	El Kela, des Sraghna	0	6

Country	Admin 1	Admin 2	Reference ID	Number of entries*
	Haouz			
Morocco	Marrakech - Tensift - Al Haouz	Marrakech	0	5
Morocco	Marrakech - Tensift - Al Haouz	Marrakech	0	
Morocco	Meknès - Tafilalet	Meknès	0	2
Morocco	Rabat - Salé - Zemmour - Zaer	Khémisset	0	7
Morocco	Rabat - Salé - Zemmour - Zaer	Skhirate-Témara	0	4
Morocco	Souss - Massa - Draâ	Taroudannt	0	
Morocco	Tanger - Tétouan	Chefchaouen	0	3
Morocco	Taza - Al Hoceima - Taounate	Al Hoceïma	0	
Morocco	Taza - Al Hoceima - Taounate	Taza	0	2
Portugal	Aveiro	Oliveira de Azeméis	0	
Portugal	Coimbra	Coimbra	0	
Portugal	Évora	Montemor-o-Novo	0	2
Portugal	Évora	Portel	0	4
Portugal	Guarda	Guarda	0	
Portugal	Leiria	Peniche	0	
Portugal	Lisboa	Alenquer	0	
Portugal	Portalegre	Marvão	0	
Portugal	Porto	Marco de Canaveses	0	
Portugal	Santarém	Benavente	0	
Portugal	Santarém	Coruche	0	
Portugal	Setúbal	Alcácer do Sal	0	15
Portugal	Setúbal	Grândola	0	
Portugal	Setúbal	Palmela	0	
Portugal	Setúbal	Set˙bal	0	
Portugal	Viana do Castelo	Paredes de Coura	0	
Romania	Alba		0	
Romania	Bucharest		0	
Romania	Constanta		0	8
Romania	Dolj		0	3
Romania	Gorj		769	
Romania	Mehedinti		769	
Romania	Mures		769	
Romania	Timis		0	3
Romania	Tulcea		0	
Romania	Tulcea		460	
Romania	Tulcea		769	
Russia	Stavropol'		1144	
Russia	Stavropol'		1144	
Serbia	Borski	Kladovo	0	2
Serbia	Macvanski	Ljubovija	0	2
Serbia	Pcinjski	Surdulica	0	
Serbia	Pirotski	Pirot	0	
Serbia	Ra ki	Novi Pazar	0	2
Serbia	Ra ki	Vrnjacka Banja	0	
Serbia	Toplicki	Kuröumlija	0	
Spain	Andalucía	Cádiz	0	6

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Spain	Andalucía	Cádiz	241	
Spain	Andalucía	Cádiz	241	
Spain	Andalucía	Cádiz	821	
Spain	Andalucía	Córdoba	0	3
Spain	Andalucía	Granada	0	3
Spain	Andalucía	Jaén	0	5
Spain	Andalucía	Sevilla	0	8
Spain	Aragón	Zaragoza	0	8
Spain	Castilla y León	Salamanca	0	
Spain	Castilla y León	Toledo	821	
Spain	Castilla y León	Toledo	821	
Spain	Castilla-La Mancha	Albacete	821	
Spain	Castilla-La Mancha	Ciudad Real	246	
Spain	Castilla-La Mancha	Cuenca	821	
Spain	Castilla-La Mancha	Toledo	0	
Spain	Castilla-La Mancha	Toledo	989	
Spain	Comunidad Foral de Navarra	Navarra	0	
Spain	Extremadura	Badajoz	0	14
Spain	Extremadura	Cáceres	0	50
Sweden	Gotland		1339	
Sweden	Kalmar		1339	
Sweden	Skåne		1339	
Switzerland	Ticino		1284	
Switzerland	Ticino		1284	
Tunisia	Ariana	Ettadhamen	0	
Tunisia	Ariana	Kalaat El Andalous	0	
Tunisia	Ariana	Sidi Thabet	0	3
Tunisia	Béja	Amdoun	0	
Tunisia	Béja	Béja Nord	0	
Tunisia	Béja	Béja Sud	0	
Tunisia	Béja	Nefza	0	3
Tunisia	Ben Arous (Tunis Sud)	Ezzahra	0	
Tunisia	Bizerte	Bizerte Nord	0	2
Tunisia	Bizerte	Bizerte Sud	0	
Tunisia	Bizerte	Ghar El Melh	0	
Tunisia	Bizerte	Ghazala	0	
Tunisia	Gabès	Hamma	0	
Tunisia	Gabès	Matmata	0	
Tunisia	Gafsa	Ksar	0	
Tunisia	Jendouba	Aïn Draham	0	2
Tunisia	Jendouba	Fernana	0	
Tunisia	Jendouba	Tabarka	0	
Tunisia	Kairouan	Kairouan Sud	0	
Tunisia	Kairouan	Sbikha	0	
Tunisia	Kebili	Kebili Nord	0	
Tunisia	Le Kef	Sakiet Sidi Youssef	0	2
Tunisia	Mahdia	Chebba	0	
Tunisia	Manubah	El Battan	0	
Tunisia	Manubah	Oued Ellil	0	
Tunisia	Manubah	Tebourba	0	
Tunisia	Médenine	Houmt Souk	0	
Tunisia	Monastir	Jammel	0	

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Tunisia	Nabeul	Haouaria	0	
Tunisia	Nabeul	Korba	0	2
Tunisia	Nabeul	Soliman	0	
Tunisia	Nabeul	Takelsa	0	
Tunisia	Sidi Bou Zid	Meknassi	0	
Tunisia	Tunis	Cité El Khadra	0	
Tunisia	Tunis	La Goulette	0	
Tunisia	Tunis	Sidi El Béchir	0	3
Tunisia	Zaghouan	Fahs	0	2
Tunisia	Zaghouan	Zriba	0	2
Turkey	Aksaray		1353	
Turkey	Ankara		0	25
Turkey	Ankara		1353	
Turkey	Ankara		1353	
Turkey	Ankara		844	
Turkey	Artvin		0	2
Turkey	Balikesir		0	
Turkey	Burdur		0	
Turkey	Çorum		0	6
Turkey	Elazig		0	3
Turkey	Erzurum		0	2
Turkey	Giresun		0	3
Turkey	Gümüshane		0	
Turkey	Izmir		0	
Turkey	Kayseri		0	8
Turkey	Kirklareli		0	
Turkey	Ordu		0	
Turkey	Samsun		0	2
Turkey	Sivas		0	12
Turkey	Tokat		0	45
Turkey	Yozgat		0	6

(*) if more than one.

APPENDIX C: *RHIPICEPHALUS BURSA* GEOGRAPHIC DISTRIBUTION DATA.

See appendix L for the related complete reference.

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Albania	Durrës	Durrësit	0	
Albania	Elbasan	Librazhdit	0	2
Albania	Korçë	Korçës	0	3
Albania	Lezhë	Lezhës	0	
Albania	Tiranë	Tiranës	0	2
Albania	Vlorë	Sarandës	0	
Albania	Vlorë	Vlorës	0	4
Algeria	Aïn Defla	Ain Benian	0	
Algeria	Aïn Defla	Ain Lechiakh	0	
Algeria	Aïn Defla	Arib	0	
Algeria	Aïn Defla	Ben Allal	0	
Algeria	Aïn Defla	Rouina	0	
Algeria	Aïn Témouchent	Ain Kihel	0	
Algeria	Aïn Témouchent	Terga	0	
Algeria	Alger	Ain Benian	0	
Algeria	Alger	Ain Benian	0	
Algeria	Alger	Bordj El Kiffan	0	
Algeria	Alger	Bouzareah	0	
Algeria	Alger	Ouled Chebel	0	
Algeria	Batna	Ain Touta	0	
Algeria	Biskra	El Ouitaya	0	
Algeria	Blida	Boufarik	0	
Algeria	Bouira	Ain Turk	0	
Algeria	Bouira	Lakhdaria	0	
Algeria	Bouira	Sour El Ghouzlane	0	2
Algeria	Boumerdès	Boudouaou	0	
Algeria	Boumerdès	Corso	0	
Algeria	Boumerdès	Dellys	0	
Algeria	Boumerdès	Isser	0	
Algeria	Boumerdès	Sidi Daoud	0	
Algeria	Chlef	Abou El Hassen	0	
Algeria	Chlef	Boukadir	0	
Algeria	Chlef	Bouzeghaia	0	
Algeria	Chlef	Chlef	0	2
Algeria	Chlef	Ouled Fares	0	
Algeria	Chlef	Sidi Akkacha	0	
Algeria	Chlef	Tenes	0	
Algeria	Constantine	Constantine	0	
Algeria	Djelfa	Benhar	0	
Algeria	El Tarf	El Aioun	0	
Algeria	Guelma	Hammam Debagh	0	
Algeria	Jijel	Jijel	0	
Algeria	Jijel	Taher	0	
Algeria	M'Sila	Ain Errich	0	
Algeria	M'Sila	Sidi M'Hamed	0	
Algeria	Mascara	Ain Ferah	0	
Algeria	Médéa	Medea	0	
Algeria	Mila	Mila	0	

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Algeria	Oran	Misserghin	0	2
Algeria	Oran	Oran	0	
Algeria	Relizane	Relizane	0	
Algeria	Relizane	Sidi M'Hamed Benaouda	0	
Algeria	Sétif	Ain Arnat	0	3
Algeria	Sétif	Setif	0	
Algeria	Sidi Bel Abbès	Dhaya	0	
Algeria	Sidi Bel Abbès	Sidi Bel Abbes	0	
Algeria	Tébessa	Bir El Ater	0	
Algeria	Tébessa	Tebessa	0	
Algeria	Tiaret		129	
Algeria	Tiaret	Meghila	0	
Algeria	Tiaret	Sougueur	0	2
Algeria	Tipaza	Douaouda	0	
Algeria	Tissemsilt	Theniet El Had	0	
Algeria	Tizi Ouzou		110	
Algeria	Tizi Ouzou	Ain-El-Hammam	0	
Algeria	Tizi Ouzou	Boghni	0	
Algeria	Tizi Ouzou	Draa-Ben-Khedda	0	
Algeria	Tizi Ouzou	Larbaa-Nath-Irathen	0	
Algeria	Tizi Ouzou	Tadmait	0	
Algeria	Tizi Ouzou	Tizi Ouzou	0	2
Algeria	Tizi Ouzou	Tizi-Rached	0	
Algeria	Tizi Ouzou	Zekri	0	
Algeria	Tlemcen	Tlemcen	0	2
Bulgaria	Blagoevgrad	Petrich	0	
Bulgaria	Blagoevgrad	Sandanski	0	2
Bulgaria	Burgas	Burgas	0	2
Bulgaria	Burgas	Kameno	0	
Bulgaria	Burgas	Malko Tarnovo	0	2
Bulgaria	Kardzhali	Ardino	0	2
Bulgaria	Montana	Montana	0	
Bulgaria	Pazardzhik	Septemvri	0	
Bulgaria	Plovdiv	Asenovgrad	0	
Bulgaria	Shumen	Preslav	0	
Bulgaria	Shumen	Shumen	0	2
Bulgaria	Stara Zagora	Haskovo	69	2
Bulgaria	Stara Zagora	Pavel Banya	0	
Bulgaria	Veliko Tarnovo	Veliko Tarnovo	0	
Bulgaria	Vratsa	Vratsa	0	
Bulgaria	Yambol	Tundzha	0	2
Bulgaria	Yambol	Yambol	0	
Croatia	Bjelovarska-Bilogorska		0	5
Croatia	Dubrovacko-Neretvanska		0	
Croatia	Grad Zagreb		0	2
Croatia	Istarska		0	
Croatia	Primorsko-Goranska		0	2
Croatia	Splitsko-Dalmatinska		0	5
Croatia	Splitsko-Dalmatinska		277	
Croatia	Splitsko-Dalmatinska		781	
Croatia	Vara dinska		0	

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Croatia	Zadarska		0	6
Cyprus	Famagusta		0	2
Egypt	Al Wadi al Jadid		0	
France	Aquitaine	Dordogne	0	2
France	Aquitaine	Landes	0	2
France	Aquitaine	Pyrénées-Atlantiques	0	5
France	Aquitaine	Pyrénées-Atlantiques	1280	
France	Bourgogne	Saône-et-Loire	0	
France	Corse		1280	
France	Corse	Corse-Du-Sud	0	12
France	Corse	Haute-Corse	0	9
France	Languedoc-Roussillon	Aude	0	3
France	Languedoc-Roussillon	Pyrénées-Orientales	0	6
France	Midi-Pyrénées	Aveyron	0	
France	Midi-Pyrénées	Lot	0	2
France	Provence-Alpes-Côte-d'Azur	Alpes-De-Haute-Provence	0	3
France	Provence-Alpes-Côte-d'Azur	Bouches-Du-Rhône	0	4
France	Provence-Alpes-Côte-d'Azur	Bouches-Du-Rhône	1280	
France	Provence-Alpes-Côte-d'Azur	Var	0	3
France	Provence-Alpes-Côte-d'Azur	Vaucluse	0	
France	Rhône-Alpes	Ardèche	0	2
France	Rhône-Alpes	Drôme	0	4
Greece	Anatoliki Makedonia kai Thraki		735	2
Greece	Anatoliki Makedonia kai Thraki	Drama	0	10
Greece	Anatoliki Makedonia kai Thraki	Evros	0	9
Greece	Anatoliki Makedonia kai Thraki	Kavala	0	10
Greece	Attiki	Attica	0	8
Greece	Dytiki Ellada	Achaea	0	2
Greece	Dytiki Makedonia		735	
Greece	Dytiki Makedonia		735	
Greece	Dytiki Makedonia	Florina	0	
Greece	Dytiki Makedonia	Grevena	0	10
Greece	Dytiki Makedonia	Kastoria	0	2
Greece	Dytiki Makedonia	Kozani	0	8
Greece	Ipeiros	Ioannina	0	
Greece	Ipeiros	Preveza	0	3
Greece	Kentriki Makedonia		735	
Greece	Kentriki Makedonia	Khalkidiki	0	13
Greece	Kentriki Makedonia	Khalkidiki	1303	
Greece	Kentriki Makedonia	Khalkidiki	1303	
Greece	Kentriki Makedonia	Khalkidiki	1303	
Greece	Kentriki Makedonia	Kilkis	0	8
Greece	Kentriki Makedonia	Pella	0	4
Greece	Kentriki Makedonia	Pieria	0	5
Greece	Kentriki Makedonia	Serrai	0	14
Greece	Kentriki Makedonia	Thessaloniki	0	12
Greece	Kriti	Rethymnon	0	7
Greece	Stereá Elláda	Boeotia	0	7
Greece	Stereá Elláda	Euboea	0	7
Greece	Stereá Elláda	Fokis	778	
Greece	Thessalia	Karditsa	0	7

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Greece	Thessalia	Larisa	0	
Israel	Jerusalem		1071	
Italy	Abruzzo	Chieti	0	2
Italy	Abruzzo	L'Aquila	0	8
Italy	Abruzzo	Teramo	0	2
Italy	Apulia	Bari	0	17
Italy	Apulia	Barletta-Andria-Trani	0	3
Italy	Apulia	Brindisi	0	
Italy	Apulia	Foggia	0	26
Italy	Apulia	Lecce	0	2
Italy	Apulia	Taranto	0	7
Italy	Basilicata	Matera	0	12
Italy	Calabria	Cosenza	0	
Italy	Calabria	Reggio Di Calabria	0	3
Italy	Campania		802	
Italy	Campania	Avellino	0	4
Italy	Campania	Caserta	0	3
Italy	Emilia-Romagna	Ravenna	0	4
Italy	Lazio	Latina	0	14
Italy	Lazio	Rieti	0	
Italy	Lazio	Roma	0	7
Italy	Liguria	Genova	0	
Italy	Liguria	Savona	0	
Italy	Marche	Ascoli Piceno	0	
Italy	Molise	Campobasso	0	6
Italy	Molise	Isernia	0	
Italy	Sardegna	Cagliari	0	
Italy	Sardegna	Carbonia-Iglesias	0	
Italy	Sardegna	Oristano	0	2
Italy	Sardegna	Sassari	0	4
Italy	Sicily	Agrigento	0	2
Italy	Sicily	Enna	0	
Italy	Sicily	Palermo	0	10
Italy	Sicily	Palermo	242	
Italy	Sicily	Palermo	366	
Italy	Sicily	Palermo	366	
Italy	Sicily	Palermo	366	
Italy	Sicily	Palermo	366	
Italy	Sicily	Palermo	998	
Italy	Sicily	Trapani	0	
Italy	Toscana	Grosseto	0	
Italy	Toscana	Livorno	0	3
Italy	Toscana	Pisa	0	
Italy	Umbria	Perugia	0	2
Italy	Veneto	Treviso	0	
Italy	Veneto	Verona	0	2
Kosovo	akovica	Decani	0	3
Kosovo	Kosovska Mitrovica	Kosovska Mitrovica	0	
Kosovo	Pristina	Obilic	0	
Kosovo	Uroöevac	Uroöevac	0	
Lebanon	Mount Lebanon	Matn	0	

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Libya	Al Jabal al Akhdar		0	
Macedonia	Pelagonia	Bitola	0	3
Macedonia	Polog	Tetovo	0	2
Macedonia	Skopje	Centar	0	4
Macedonia	Skopje	Zelenikovo	0	2
Macedonia	Southeastern	Lake Dojran	0	
Macedonia	Southwestern	Ohrid	0	2
Macedonia	Vardar	Kavadartsi	0	
Montenegro	Ro aje		0	
Morocco	Doukkala - Abda	Safi	0	2
Morocco	Fès - Boulemane	Zouagha-Moulay Yacoub	0	2
Morocco	Gharb - Chrarda - Béni Hssen	Kénitra	0	6
Morocco	Marrakech - Tensift - Al Haouz	Al Haouz	0	3
Morocco	Marrakech - Tensift - Al Haouz	El Kela, des Sraghna	0	5
Morocco	Marrakech - Tensift - Al Haouz	Essaouira	0	
Morocco	Marrakech - Tensift - Al Haouz	Marrakech	0	2
Morocco	Meknès - Tafilalet	Meknès	0	
Morocco	Tadla - Azilal	Béni Mellal	0	
Morocco	Tanger - Tétouan	Chefchaouen	0	2
Morocco	Tanger - Tétouan	Fahs Anjra	0	
Morocco	Tanger - Tétouan	Tanger-Assilah	0	2
Morocco	Tanger - Tétouan	Tétouan	0	
Morocco	Taza - Al Hoceima - Taounate	Taounate	0	2
Morocco	Taza - Al Hoceima - Taounate	Taza	0	2
Portugal	Braga		1143	
Portugal	Castelo Branco	Castelo Branco	0	3
Portugal	Évora	Montemor-o-Novo	0	
Portugal	Évora	Portel	0	
Portugal	Portalegre		1143	
Portugal	Portalegre	Marvão	0	3
Portugal	Porto	Marco de Canaveses	0	
Portugal	Santarém	Coruche	0	
Portugal	Setúbal	Alcácer do Sal	0	7
Portugal	Setúbal	Palmela	0	
Portugal	Viana do Castelo	Arcos de Valdevez	0	
Romania	Constanta		0	5
Romania	Dâmbovita		460	
Romania	Dolj		0	4
Romania	Timis		0	4
Romania	Tulcea		769	
Serbia	Borski	Kladovo	0	
Serbia	Juûno-Backi	Bac	0	
Serbia	Moravicki	Cacak	0	
Serbia	Ni avski	Doljevac	0	
Serbia	Pcinjski	Surdulica	0	2
Serbia	Pirotski	Pirot	0	2
Serbia	Ra ki	Kraljevo	0	
Serbia	Ra ki	Novi Pazar	0	3
Serbia	Ra ki	Vrnjacka Banja	0	
Serbia	Rasinski	Brus	0	
Serbia	Zlatiborski	Uûice	0	

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Spain	Andalucía	Cádiz	0	21
Spain	Andalucía	Cádiz	821	
Spain	Andalucía	Córdoba	0	14
Spain	Andalucía	Granada	0	9
Spain	Andalucía	Huelva	0	
Spain	Andalucía	Jaén	0	12
Spain	Andalucía	Jaén	611	
Spain	Andalucía	Jaén	611	
Spain	Andalucía	Jaén	611	
Spain	Andalucía	Jaén	611	
Spain	Andalucía	Sevilla	0	12
Spain	Aragón	Huesca	0	2
Spain	Aragón	Teruel	0	
Spain	Aragón	Zaragoza	0	
Spain	Castilla y León	Salamanca	0	3
Spain	Castilla y León	Toledo	821	
Spain	Castilla y León	Toledo	821	
Spain	Castilla y León	¡vila	0	
Spain	Castilla y León	Zamora	0	
Spain	Castilla-La Mancha	Albacete	0	2
Spain	Castilla-La Mancha	Albacete	821	
Spain	Castilla-La Mancha	Ciudad Real	0	3
Spain	Castilla-La Mancha	Guadalajara	0	
Spain	Castilla-La Mancha	Toledo	0	
Spain	Cataluña	Girona	0	3
Spain	Cataluña	Tarragona	0	5
Spain	Ceuta y Melilla	Ceuta	0	
Spain	Comunidad Foral de Navarra	Navarra	0	3
Spain	Extremadura	Badajoz	0	17
Spain	Extremadura	Cáceres	0	110
Spain	La Rioja	La Rioja	0	
Spain	País Vasco		77	
Spain	País Vasco	Álava	0	10
Spain	País Vasco	Guipúzcoa	0	8
Spain	País Vasco	Vizcaya	0	5
Spain	Principado de Asturias		821	
Tunisia	Ariana	Kalaat El Andalous	0	
Tunisia	Béja	Béja Sud	0	
Tunisia	Béja	Nefza	0	
Tunisia	Bizerte	Bizerte Sud	0	
Tunisia	Bizerte	Ghazala	0	
Tunisia	Bizerte	Sejnane	0	
Tunisia	Jendouba	Aïn Draham	0	
Tunisia	Jendouba	Ghardimaou	0	
Tunisia	Jendouba	Jendouba Nord	0	
Tunisia	Jendouba	Tabarka	0	
Tunisia	Le Kef	Nebeur	0	
Tunisia	Le Kef	Sakiet Sidi Youssef	0	
Tunisia	Nabeul	Soliman	0	
Tunisia	Nabeul	Takelsa	0	
Tunisia	Sidi Bou Zid	Meknassi	0	

Country	Admin 1	Admin 2	Reference ID	Number of entries*
Tunisia	Tunis	Carthage	0	
Tunisia	Tunis	Sidi El Béchir	0	
Tunisia	Zaghouan	Fahs	0	
Turkey	Afyon		186	
Turkey	Aksaray		1353	
Turkey	Ankara		0	20
Turkey	Ankara		1353	
Turkey	Ankara		1353	
Turkey	Artvin		0	
Turkey	Burdur		0	
Turkey	Burdur		1008	
Turkey	Çorum		0	
Turkey	Elazig		0	16
Turkey	Giresun		0	2
Turkey	Gümüshane		0	
Turkey	Istanbul		145	
Turkey	Kayseri		0	2
Turkey	Mus		22	
Turkey	Ordu		0	
Turkey	Samsun		0	
Turkey	Sivas		0	4
Turkey	Tokat		0	18
Turkey	Van		0	8
Turkey	Yozgat		0	5

(*) If more than one.

APPENDIX D: *DERMACENTOR MARGINATUS* GEOGRAPHIC DISTRIBUTION DATA.

See appendix L for the related complete reference.

Country	Admin 1	Admin 2	Reference	Number of entries*
Albania	Vlorë	Vlorës	0	
Algeria	Mostaganem	Mostaganem	0	
Algeria	Naâma	El Biod	0	
Algeria	Tlemcen	Tlemcen	0	
Bulgaria	Grad Sofiya	Stolichna	0	
Bulgaria	Montana	Montana	0	
Bulgaria	Stara Zagora	Haskovo	69	
Bulgaria	Vratsa	Vratsa	0	
Croatia	Primorsko-Goranska		0	2
Croatia	Splitsko-Dalmatinska		0	
Croatia	Splitsko-Dalmatinska		781	
Croatia	Zadarska		0	2
France	Alsace	Bas-Rhin	0	3
France	Aquitaine	Dordogne	0	4
France	Aquitaine	Dordogne	1280	
France	Aquitaine	Gironde	0	11
France	Aquitaine	Gironde	1280	
France	Aquitaine	Landes	0	17
France	Aquitaine	Landes	1280	
France	Aquitaine	Lot	1280	
France	Aquitaine	Lot-Et-Garonne	0	
France	Aquitaine	Lot-Et-Garonne	1280	
France	Aquitaine	Pyrénées-Atlantiques	0	7
France	Aquitaine	Pyrénées-Atlantiques	1280	
France	Auvergne	Allier	0	2
France	Auvergne	Allier	1280	
France	Auvergne	Cantal	0	3
France	Auvergne	Puy-De-Dôme	0	3
France	Auvergne	Puy-De-Dôme	1280	
France	Bourgogne	Côte-d'Or	0	10
France	Bourgogne	Côte-d'Or	1280	
France	Bourgogne	Nièvre	0	2
France	Bourgogne	Nièvre	1280	
France	Bourgogne	Saône-et-Loire	0	3
France	Bourgogne	Yonne	0	9
France	Bourgogne	Yonne	1280	
France	Bretagne	Ille-Et-Vilaine	0	2
France	Bretagne	Ille-Et-Vilaine	1280	
France	Bretagne	Morbihan	0	
France	Centre	Cher	0	2
France	Centre	Cher	1280	
France	Centre	Eure-Et-Loir	0	
France	Centre	Indre	0	2
France	Centre	Indre-Et-Loire	0	2
France	Centre	Indre-Et-Loire	1280	
France	Centre	Loiret	0	2
France	Champagne-Ardenne	Aube	0	
France	Champagne-Ardenne	Aube	1280	
France	Corse		1280	

Country	Admin 1	Admin 2	Reference	Number of entries*
France	Corse	Corse-Du-Sud	0	10
France	Corse	Haute-Corse	0	6
France	Franche-Comté	Jura	0	4
France	Île-de-France	Essonne	1280	
France	Île-de-France	Hauts-De-Seine	1280	
France	Île-de-France	Seine-Et-Marne	0	5
France	Île-de-France	Seine-Saint-Denis	1280	
France	Île-de-France	Val-D'Oise	0	
France	Île-de-France	Val-D'Oise	1280	
France	Île-de-France	Val-De-Marne	0	3
France	Île-de-France	Val-De-Marne	1280	
France	Île-de-France	Ville de Paris	0	
France	Île-de-France	Ville de Paris	1280	
France	Île-de-France	Yvelines	1280	
France	Languedoc-Roussillon	Aude	0	3
France	Languedoc-Roussillon	Gard	0	9
France	Languedoc-Roussillon	Hérault	0	3
France	Languedoc-Roussillon	Hérault	1280	
France	Languedoc-Roussillon	Pyrénées-Orientales	0	20
France	Languedoc-Roussillon	Pyrénées-Orientales	1280	
France	Limousin	Creuse	0	
France	Limousin	Haute-Vienne	0	4
France	Limousin	Haute-Vienne	1280	
France	Lorraine	Moselle	1280	
France	Midi-Pyrénées	Ariège	0	2
France	Midi-Pyrénées	Aveyron	0	12
France	Midi-Pyrénées	Aveyron	1280	
France	Midi-Pyrénées	Gers	0	3
France	Midi-Pyrénées	Gers	1280	
France	Midi-Pyrénées	Haute-Garonne	0	5
France	Midi-Pyrénées	Haute-Garonne	1280	
France	Midi-Pyrénées	Hautes-Pyrénées	0	3
France	Midi-Pyrénées	Lot	0	5
France	Midi-Pyrénées	Tarn	0	2
France	Midi-Pyrénées	Tarn	1280	
France	Midi-Pyrénées	Tarn-Et-Garonne	1280	
France	Pays de la Loire	Loire-Atlantique	0	
France	Pays de la Loire	Loire-Atlantique	1280	
France	Pays de la Loire	Maine-Et-Loire	0	2
France	Pays de la Loire	Maine-Et-Loire	1280	
France	Pays de la Loire	Mayenne	0	
France	Pays de la Loire	Vendée	0	7
France	Picardie	Oise	0	
France	Poitou-Charentes	Charente	0	2
France	Poitou-Charentes	Charente	1280	
France	Poitou-Charentes	Charente-Maritime	0	4
France	Poitou-Charentes	Charente-Maritime	1280	
France	Poitou-Charentes	Deux-Sèvres	0	4
France	Poitou-Charentes	Vienne	0	2
France	Poitou-Charentes	Vienne	1280	
France	Provence-Alpes-Côte-d'Azur	Alpes-De-Haute-Provence	0	26
France	Provence-Alpes-Côte-d'Azur	Alpes-Maritimes	0	15

Country	Admin 1	Admin 2	Reference	Number of entries*
France	Provence-Alpes-Côte-d'Azur	Alpes-Maritimes	1280	
France	Provence-Alpes-Côte-d'Azur	Bouches-Du-Rhône	0	20
France	Provence-Alpes-Côte-d'Azur	Bouches-Du-Rhône	1280	
France	Provence-Alpes-Côte-d'Azur	Hautes-Alpes	0	14
France	Provence-Alpes-Côte-d'Azur	Var	0	21
France	Provence-Alpes-Côte-d'Azur	Var	1280	
France	Provence-Alpes-Côte-d'Azur	Vaucluse	0	8
France	Rhône-Alpes	Ain	0	24
France	Rhône-Alpes	Ain	1280	
France	Rhône-Alpes	Ardèche	0	18
France	Rhône-Alpes	Ardèche	1280	
France	Rhône-Alpes	Drôme	0	23
France	Rhône-Alpes	Drôme	1280	
France	Rhône-Alpes	Haute-Savoie	0	3
France	Rhône-Alpes	Isère	0	34
France	Rhône-Alpes	Isère	1280	
France	Rhône-Alpes	Loire	0	4
France	Rhône-Alpes	Rhône	0	2
France	Rhône-Alpes	Rhône	1280	
France	Rhône-Alpes	Savoie	0	3
Germany	Baden-Württemberg		948	
Greece	Anatoliki Makedonia kai Thraki	Drama	0	2
Greece	Anatoliki Makedonia kai Thraki	Evros	0	3
Greece	Anatoliki Makedonia kai Thraki	Kavala	0	3
Greece	Attiki	Attica	0	2
Greece	Dytiki Makedonia	Florina	0	
Greece	Dytiki Makedonia	Grevena	0	9
Greece	Dytiki Makedonia	Kastoria	0	
Greece	Dytiki Makedonia	Kozani	0	3
Greece	Ipeiros	Preveza	0	3
Greece	Kentriki Makedonia	Khalkidiki	0	3
Greece	Kentriki Makedonia	Kilkis	0	
Greece	Kentriki Makedonia	Pella	0	
Greece	Kentriki Makedonia	Pieria	0	4
Greece	Kentriki Makedonia	Serrai	0	3
Greece	Kentriki Makedonia	Thessaloniki	0	
Greece	Peloponnisos	Arcadia	0	
Greece	Stereá Elláda	Euboea	0	
Greece	Stereá Elláda	Evritania	0	2
Greece	Stereá Elláda	Fthiotis	0	
Hungary	Borsod-Abaúj-Zemplén		443	
Hungary	Budapest		322	
Italy	Apulia	Foggia	0	8
Italy	Campania		802	
Italy	Campania	Avellino	0	
Italy	Campania	Caserta	0	
Italy	Emilia-Romagna	Forli' - Cesena	0	2
Italy	Emilia-Romagna	Ravenna	0	2
Italy	Lazio	Latina	0	
Italy	Lazio	Rieti	0	

Country	Admin 1	Admin 2	Reference	Number of entries*
Italy	Lazio	Roma	0	2
Italy	Marche	Macerata	0	
Italy	Molise	Campobasso	0	
Italy	Molise	Isernia	0	
Italy	Piemonte	Cuneo	0	
Italy	Sardegna	Nuoro	0	2
Italy	Sicily	Palermo	0	2
Italy	Sicily	Palermo	242	
Italy	Sicily	Palermo	366	
Italy	Sicily	Palermo	366	
Italy	Sicily	Palermo	366	
Italy	Sicily	Palermo	998	
Italy	Toscana		1331	
Italy	Toscana	Florence	0	
Italy	Umbria	Perugia	0	2
Macedonia	Skopje	Centar	0	5
Macedonia	Skopje	Zelenikovo	0	
Moldova	Anenii Noi		667	
Moldova	Balti		667	
Moldova	Basarabeasca		667	
Moldova	Bender		667	
Moldova	Briceni		667	
Moldova	Cahul		667	
Moldova	Calarasi		667	
Moldova	Cantemir		667	
Moldova	Causeni		667	
Moldova	Chisinau		667	
Moldova	Cimislia		667	
Moldova	Criuleni		667	
Moldova	Donduseni		667	
Moldova	Drochia		667	
Moldova	Dubasari		667	
Moldova	Edinet		667	
Moldova	Falesti		667	
Moldova	Floresti		667	
Moldova	Gagauzia		667	
Moldova	Glodeni		667	
Moldova	Hîncesti		667	
Moldova	Ialoveni		667	
Moldova	Leova		667	
Moldova	Nisporeni		667	
Moldova	Ocnita		667	
Moldova	Orhei		667	
Moldova	Rezina		667	
Moldova	Rîscani		667	
Moldova	Sîngerei		667	
Moldova	Soldanesti		667	
Moldova	Soroca		667	
Moldova	Stefan Voda		667	
Moldova	Straseni		667	
Moldova	Taraclia		667	
Moldova	Telenesti		667	

Country	Admin 1	Admin 2	Reference	Number of entries*
Moldova	Transnistria		667	
Moldova	Ungheni		667	
Morocco	Meknès - Tafilalet	Ifrane	0	2
Morocco	Rabat - Salé - Zemmour - Zaer	Khémisset	0	4
Morocco	Rabat - Salé - Zemmour - Zaer	Skhirate-Témara	0	2
Morocco	Tanger - Tétouan	Larache	0	
Morocco	Tanger - Tétouan	Tanger-Assilah	0	
Portugal	Aveiro	Oliveira de Azeméis	0	
Portugal	Beja	Mértola	0	
Portugal	Bragança		1143	
Portugal	Castelo Branco	Castelo Branco	0	4
Portugal	Évora	Évora	0	
Portugal	Leiria		1143	
Portugal	Lisboa		1143	
Portugal	Portalegre		1143	
Portugal	Santarém		1143	
Portugal	Santarém	Benavente	0	6
Portugal	Santarém	Coruche	0	
Portugal	Santarém	Santarém	0	
Portugal	Setúbal		1143	
Portugal	Setúbal	Alcácer do Sal	0	10
Portugal	Setúbal	Grândola	0	
Portugal	Setúbal	Grândola	1143	
Portugal	Setúbal	Palmela	0	6
Romania	Suceava		460	
Romania	Suceava		462	
Romania	Teleorman		460	
Romania	Teleorman		462	
Romania	Timis		177	
Romania	Tulcea		460	
Serbia	Macvanski	Ljubovija	0	
Serbia	Ni avski	Aleksinac	0	
Serbia	Zlatiborski	Priboj	0	
Slovakia	Kosicky		1349	
Slovakia	Zilinsky		1349	
Spain	Andalucía	Cádiz	0	7
Spain	Andalucía	Córdoba	0	12
Spain	Andalucía	Granada	0	11
Spain	Andalucía	Huelva	0	
Spain	Andalucía	Jaén	0	9
Spain	Andalucía	Jaén	611	
Spain	Andalucía	Jaén	611	
Spain	Andalucía	Málaga	0	3
Spain	Andalucía	Sevilla	0	
Spain	Aragón	Huesca	0	4
Spain	Aragón	Zaragoza	0	
Spain	Castilla y León	Soria	0	2
Spain	Castilla y León	Toledo	821	
Spain	Castilla-La Mancha	Albacete	0	
Spain	Castilla-La Mancha	Albacete	821	
Spain	Castilla-La Mancha	Cuenca	821	

Country	Admin 1	Admin 2	Reference	Number of entries*
Spain	Castilla-La Mancha	Guadalajara	821	
Spain	Castilla-La Mancha	Toledo	989	
Spain	Castilla-La Mancha	Toledo	989	
Spain	Cataluña	Girona	0	2
Spain	Cataluña	Lleida	0	4
Spain	Cataluña	Tarragona	0	
Spain	Comunidad de Madrid	Madrid	0	
Spain	Comunidad Foral de Navarra	Navarra	0	2
Spain	Extremadura	Badajoz	0	8
Spain	Extremadura	Cáceres	0	19
Spain	Islas Baleares	Baleares	1345	
Spain	La Rioja	La Rioja	0	3
Spain	País Vasco		77	
Spain	País Vasco	Álava	0	4
Spain	País Vasco	Guipúzcoa	0	5
Spain	País Vasco	Vizcaya	0	3
Switzerland	Graubünden		435	
Switzerland	Graubünden		435	
Switzerland	Graubünden		435	
Tunisia	Béja	Nefza	0	
Tunisia	Jendouba	Aïn Draham	0	2
Tunisia	Nabeul	Haouaria	0	
Tunisia	Nabeul	Takelsa	0	
Tunisia	Zaghouan	Zriba	0	2
Turkey	Aksaray		1353	
Turkey	Ankara		0	20
Turkey	Ankara		1353	
Turkey	Ankara		1353	
Turkey	Bolu		0	3
Turkey	Burdur		0	9
Turkey	Burdur		1008	
Turkey	Çorum		0	
Turkey	Elazig		0	6
Turkey	Giresun		0	
Turkey	Izmir		0	3
Turkey	Kayseri		0	
Turkey	Konya		0	
Turkey	Nevsehir		0	3
Turkey	Samsun		0	3
Turkey	Sivas		0	
Turkey	Tokat		0	
Turkey	Van		0	8

(*) If more than one.

APPENDIX E: ASFV GEOGRAPHIC DISTRIBUTION DATA.

See appendix L for the related complete reference.

Country	Admin 1	Admin 2	Reference ID
Armenia	Lori		4000
Armenia	Tavush		4000
Georgia	Ajaria	Batumi	4000
Georgia	Guria	Chokhatauri	4000
Georgia	Imereti	Bagdati	4000
Georgia	Kakheti	Akhmeta	4000
Georgia	Kvemo Kartli	Bolnisi	4000
Georgia	Mtskheta-Mtianeti	Akhalgori	4000
Georgia	Racha-Lechkhumi-Kvemo Svaneti	Ambrolauri	4000
Georgia	Samegrelo-Zemo Svaneti	Abasha	4000
Georgia	Samtskhe-Javakheti	Adigeni	4000
Georgia	Shida Kartli	Gori	4000
Georgia	Tbilisi	Tbilisi	4000
Italy	Sardegna	Cagliari	4000
Portugal	Beja	Aljustrel	122
Portugal	Beja		122
Portugal	Évora	Alandroal	122
Portugal	Évora		122
Portugal	Faro	Albufeira	122
Portugal	Portalegre	Alter do Chão	122
Portugal	Portalegre		122
Russia	Astrakhan'	Akhtubinsk	4000
Russia	Kalmyk	Chernozermel'skiy rayon	4000
Russia	Krasnodar	Abinskiy rayon	4000
Russia	Rostov	Aksayskiy rayon	4000
Russia	Stavropol'	Aleksandrovskiy rayon	4000
Russia	Volgograd	Alekseevskiy rayon	4000
Spain	Andalucía		0
Spain	Extremadura		0

APPENDIX F: *ORNITHODOROS* GEOGRAPHIC DISTRIBUTION DATA.

See table L for complete references.

Country	Admin 1	Admin 2	Reference ID
Algeria	El Tarf	Ain El Assel	0
Armenia	Lake Sevan		0
Armenia	Lori		0
Armenia	Shirak		0
Armenia	Tavush		0
Egypt	Ad Daqahliyah		0
Egypt	Al Buhayrah		0
Egypt	Al Gharbiyah		0
Egypt	Al Iskandariyah		0
Egypt	Al Isma`iliyah		0
Egypt	Al Minufiyah		0
Egypt	Al Qahirah		0
Egypt	Al Qalyubiyah		0
Egypt	Ash Sharqiyah		0
Egypt	Bur Sa`id		0
Egypt	Dumyat		0
Egypt	Kafr ash Shaykh		0
Egypt	Matruh		0
Egypt	Shamal Sina'		0
Georgia	Abkhazia	Gagra	1398
Georgia	Ajaria	Batumi	1398
Georgia	Guria	Chokhatauri	1398
Georgia	Imereti	Bagdati	1398
Georgia	Kakheti	Akhmeta	1398
Georgia	Kvemo Kartli	Bolnisi	1398
Georgia	Mtskheta-Mtianeti	Akhalgori	1398
Georgia	Racha-Lechkhumi-Kvemo Svaneti	Ambrolauri	1398
Georgia	Samegrelo-Zemo Svaneti	Abasha	1398
Georgia	Samtskhe-Javakheti	Adigeni	1398
Georgia	Shida Kartli	Gori	1398
Georgia	Tbilisi	Tbilisi	1398
Israel	Golan		0
Israel	HaDarom		0
Israel	Haifa		0
Israel	HaMerkaz		0
Israel	HaZafon		0
Israel	Jerusalem		0
Israel	Tel Aviv		0
Jordan	Ajlun	Ajloun	0
Jordan	Amman	Amman	0
Jordan	Aqaba	Aqaba	0
Jordan	Balqa	Al-Balqa	0
Jordan	Irbid	Aghwar Shamaliyyeh	0
Jordan	Jarash	Jarash	0
Jordan	Karak	Ayy	0
Jordan	Madaba	Dhiban	0
Jordan	Tafilah	Bsaira	0
Lebanon	An Nabatiyah	Hasbaya	0
Lebanon	Beirut	Beirut	0

Country	Admin 1	Admin 2	Reference ID
Lebanon	South Lebanon	Jezzine	0
Morocco	Chaouia - Ouardigha	Ben Slimane	0
Morocco	Doukkala - Abda	El Jadida	0
Morocco	Gharb - Chrarda - Béni Hssen	Kénitra	0
Morocco	Grand Casablanca	Casablanca	0
Morocco	Guelmim - Es-Semara	Assa-Zag	0
Morocco	Marrakech - Tensift - Al Haouz	Al Haouz	0
Morocco	Oriental	Berkane Taourirt	0
Morocco	Rabat - Salé - Zemmour - Zaer	Khémisset	0
Morocco	Souss - Massa - Draâ	Agadir-Ida ou Tanane	0
Morocco	Tadla - Azilal	Azilal	0
Morocco	Tanger - Tétouan	Chefchaouen	0
Palestina	Gaza	Deir Al-Balah	0
Palestina	West Bank	Bethlehem	0
Portugal	Beja	Aljustrel	0
Portugal	Castelo Branco	Belmonte	0
Portugal	Coimbra	Arganil	0
Portugal	Faro	Albufeira	0
Portugal	Leiria	Alcobaça	0
Portugal	Lisboa	Alenquer	0
Portugal	Portalegre	Alter do Chão	0
Portugal	Santarém	Mação	0
Portugal	Setúbal	Alcácer do Sal	0
Portugal	Évora	Alandroal	0
Russia	Chechnya	Achkhoy-Martanovskiy rayon	0
Russia	Ingush	Malgobekskiy rayon	0
Russia	Kabardin-Balkar	Baksanskiy rayon	0
Russia	North Ossetia	Alagirskiy rayon	0
Spain	Andalucía	Almería	0
Spain	Extremadura	Badajoz	0
Tunisia	Ariana	Ariana Médina	0
Tunisia	Ben Arous (Tunis Sud)	Ben Arous	0
Tunisia	Bizerte	Bizerte Nord	0
Tunisia	Béja	Amdoun	0
Tunisia	Gabès	Gabès Médina	0
Tunisia	Jendouba	Aïn Draham	0
Tunisia	Manubah	Borj El Amri	0
Tunisia	Médenine	Ben Guerdane	0
Tunisia	Nabeul	Beni Khalled	0
Tunisia	Tunis	Bab Bhar	0
Tunisia	Zaghouan	Bir Mchergua	0
Turkey	Artvin		0
Turkey	Rize		0
Turkey	Trabzon		0
Egypt	Al Jizah		1101

APPENDIX G: SYSTEMATIC LITERATURE REVIEW

To address this mandate, data originated basically from three different sources:

- A systematic literature review, based on scientific papers retrieved from the databases integrated in ISI web of knowledge and Pubmed in the last 10 years; and on a pool of papers considered relevant by the WG experts, coming from their private collections, regardless of the time frame;

- Published validated data from the integrated consortium on ticks and tick-borne diseases (ICTTD-3 European project, http://www.icttd.nl/index.php?id=2), collected by one of the experts of the WG

All data was collected in a database. The maps were issued from this database.

1) The systematic literature review:

Part of the data originated from a systematic literature review, in which we tried to approach the general principles of the systematic review method.

The review questions:

- Question 1: Which tick-borne infections occur in the region of concern?

- Question 2: Which tick species have proven involvement in the transmission of pathogens in the region of concern?

- Question 3: What are the geographical distributions of these tick species in the region of concern?

The experts of the WG agreed that the following list of ticks and tick-borne pathogens were the more relevant ones for the geographic area considered:

- **Ticks**:
    - *Argas persicus, A. reflexus;*
    - *Boophilus annulatus;*
    - *Dermacentor marginatus, D. reticulatus (synonym D. pictus);*
    - *Haemaphysalis punctata, Ha. inermis, Ha. concinna;*
    - *Hyalomma marginatum, Hy. scupense (synonym Hy. detritum), Hy. anatolicum, Hy. excavatum;*
    - *Ixodes ricinus, I. persulcatus, I. hexagonus, I. canisuga. I. uriae;*
    - *Ornithodorus erraticus; and*
    - *Rhipicephalus sanguineus and Rh. turanicus (Rh. sanguineus group), Rh. bursa.*
- **Pathogens**:
    - African swine fever (ASF virus)

- Anaplasmoses (*Anaplasma spp. Including A. phagocytophilum* synonym *Ehrlichia phagocytophila)*
- Babesioses (*Babesia spp.*)
- Crimean-Congo haemorrhagic fever (CCHF virus)
- Hepatozoonosis (*Hepatozoon canis*)
- Lyme borrelioses (*Borrelia burgdorferi* s.l. (several species are included in the complex, e.g., *burgdorferi, garinii, spielmanii, lusitaniae*)
- Rickettsioses (*Rickettsia spp. Erhlichia canis*)
- Recurrent fever or relapsing fever (*Borrelia hispanica*)
- Theilerioses (*Theileria parva, T. hirci, T. ovis, T. lestoquardi*).
- Tick-borne encephalitis group (TBEV and Louping ill virus)
- Bartonella (*Bartonella* spp., *B. henselae, B. quintana, B. vinsonii*)
- Q fever (*Coxiella*)
- African horse sickness (AHSV, tick-borne orbivirus)

The databases:

We searched in the databases integrated in ISI Web of Knowledge and Pubmed:

- ISI Web of Knowledge: includes CABI (CAB Abstracts and Global Health), Current Contents Connect, Food Science and Technology Abstracts, Journal Citation Reports, Web of Science. Derwent Innovations Index, Biological Abstracts, BIOSIS Previews, Inspec, MEDLINE, and Zoological Record. It contains proceedings and conferences (communications and posters).
- Pubmed: includes MEDLINE, PubMed citation of life science journals, and online books.

The strings:

We used two different strings: one for the ticks and another for the tick-borne pathogens. These strings were applied to the title and the abstract. The search was limited to the last 10 years, (since 2000), and it was updated to March 11th 2010, No language restrictions were set. The search in ISI Web of knowledge was made per topic.

Geographical scope: European countries (including non EU countries and a band zone of about 600 km in the European Russian Federation); Caucasian countries; Middle East and North African countries including the Western Sahara. These countries are individually named in the strings.

1) **Strings used for the ticks**:

1.1) **In ISI Web of knowledge** we used:

(*Argas* OR *Ornithodoros* OR *Dermacentor* OR *Haemaphysalis* OR *Hyalomma* OR *Ixodes* OR *Rhipicephalus* OR *Boophilus*)

AND

(Distribution OR presence OR occurrence OR reported)

AND

(Aland OR Albania OR Andorra OR Austria OR Belgium OR Bosnia and Herzegovina OR Bulgaria OR Croatia OR Cyprus OR Czech Republic OR Denmark OR Germany OR Spain OR Estonia OR Finland OR Faroe islands OR France OR Greece OR Hungary OR Ireland OR Italy OR Kosovo OR Latvia OR Liechtenstein OR Lithuania OR Luxembourg OR Macedonia OR Malta OR Montenegro OR The Netherlands OR Norway OR Poland OR Portugal OR Slovenia OR Romania OR San Marino OR Serbia OR Slovakia OR Switzerland OR Sweden OR United Kingdom OR Turkey OR Israel OR Palestine OR Jordan OR Lebanon OR Syria OR Morocco OR Algeria OR Tunisia OR Libya OR Egypt OR Western Sahara OR Armenia OR Belarus OR Georgia OR Moldova OR Ukraine OR Russia OR USSR)

1.2) **In Pubmed** the same strings were applied, but with this modification for searching in title and abstract:

((("(Argas"[Title/Abstract] OR "Ornithodoros"[Title/Abstract] OR "Dermacentor"[Title/Abstract] OR "Haemaphysalis"[Title/Abstract] OR "Hyalomma"[Title/Abstract] OR "Ixodes"[Title/Abstract] OR "Rhipicephalus"[Title/Abstract] OR "Boophilus)"[Title/Abstract])

AND

("distribution"[Title/Abstract] OR "presence"[Title/Abstract] OR "occurrence"[Title/Abstract] OR "reported"[Title/Abstract]))

AND

("2000"[Publication Date] : "3000"[Publication Date]))

AND

("(Aland"[Title/Abstract] OR "Albania"[Title/Abstract] OR "Andorra"[Title/Abstract] OR "Austria"[Title/Abstract] OR "Belgium"[Title/Abstract] OR "Bosnia and Herzegovina"[Title/Abstract] OR "Bulgaria"[Title/Abstract] OR "Croatia"[Title/Abstract] OR "Cyprus"[Title/Abstract] OR "Czech Republic"[Title/Abstract] OR "Denmark"[Title/Abstract] OR "Germany"[Title/Abstract] OR "Spain"[Title/Abstract] OR "Estonia"[Title/Abstract] OR "Finland"[Title/Abstract] OR "Faroe islands"[Title/Abstract] OR "France"[Title/Abstract] OR "Greece"[Title/Abstract] OR "Hungary"[Title/Abstract] OR "Ireland"[Title/Abstract] OR "Italy"[Title/Abstract] OR "Kosovo"[Title/Abstract] OR "Latvia"[Title/Abstract] OR "Liechtenstein"[Title/Abstract] OR "Lithuania"[Title/Abstract] OR "Luxembourg"[Title/Abstract] OR "Macedonia"[Title/Abstract] OR "Malta"[Title/Abstract] OR "Montenegro"[Title/Abstract] OR "The Netherlands"[Title/Abstract] OR "Norway"[Title/Abstract] OR "Poland"[Title/Abstract] OR "Portugal"[Title/Abstract] OR "Slovenia"[Title/Abstract] OR "Romania"[Title/Abstract] OR "San Marino"[Title/Abstract] OR "Serbia"[Title/Abstract] OR "Slovakia"[Title/Abstract] OR "Switzerland"[Title/Abstract] OR "Sweden"[Title/Abstract] OR "United Kingdom"[Title/Abstract] OR "Turkey"[Title/Abstract] OR "Israel"[Title/Abstract] OR "Palestine"[Title/Abstract] OR "Jordan"[Title/Abstract] OR "Lebanon"[Title/Abstract] OR "Syria"[Title/Abstract] OR "Morocco"[Title/Abstract] OR "Algeria"[Title/Abstract] OR "Tunisia"[Title/Abstract] OR "Libya"[Title/Abstract] OR "Egypt"[Title/Abstract] OR "Western Sahara"[Title/Abstract] OR "Armenia"[Title/Abstract] OR "Belarus"[Title/Abstract] OR "Georgia"[Title/Abstract] OR "Moldova"[Title/Abstract] OR "Ukraine"[Title/Abstract] OR "Russia"[Title/Abstract] OR "USSR)"[Title/Abstract])

2) **Strings used for the tick-borne pathogens**:

2.1) In ISI Web of Knowledge we used:

(African Swine Fever virus OR ASF virus OR ASFV OR Anaplasma OR A phagocytophilum OR Ehrlichia phagocytophila OR Babesia OR Crimean Congo Haemorrhagic Fever virus OR CCHF virus OR CCHFV OR Hepatozoon OR Lyme disease agent OR Borrelia OR B burgdorferi OR B garinii OR B spielmanii OR B lusitaniae OR Rickettsia OR R conorii OR Ehrlichia canis OR Borrelia hispanica OR B hispanica OR Theileria OR T parva OR T hirci OR T ovis OR T lestoquardi OR Tick borne encephalitis virus OR Louping ill virus OR TBE virus OR TBEV OR tick borne flavivirus OR TBEF OR TBEFV OR TBE group OR Francisella OR F tularensis OR Bartonella OR Q fever OR Coxiella OR African horse sickness virus OR AHSV OR tick borne orbivirus)

AND

(Distribution OR presence OR occurrence OR reported)

AND

(Aland OR Albania OR Andorra OR Austria OR Belgium OR Bosnia and Herzegovina OR Bulgaria OR Croatia OR Cyprus OR Czech Republic OR Denmark OR Germany OR Spain OR Estonia OR Finland OR Faroe islands OR France OR Greece OR Hungary OR Ireland OR Italy OR Kosovo OR Latvia OR Liechtenstein OR Lithuania OR Luxembourg OR Macedonia OR Malta OR Montenegro OR The Netherlands OR Norway OR Poland OR Portugal OR Slovenia OR Romania OR San Marino OR Serbia OR Slovakia OR Switzerland OR Sweden OR United Kingdom OR Turkey OR Israel OR Palestine OR Jordan OR Lebanon OR Syria OR Morocco OR Algeria OR Tunisia OR Libya OR Egypt OR Western Sahara OR Armenia OR Belarus OR Georgia OR Moldova OR Ukraine OR Russia OR USSR)

2.2) in Pubmed, the same strings were used but with the modification showed in point 1.2)

We used the reference management system EndNote. Duplicate references were deleted automatically and then checked manually. The search in ISI web of knowledge database and in Pubmed database produced a list of 2197 references.

First screening of the title and abstract of 2197 references:

The title and abstract were screened following the criteria described below:

Criterion	Included	Excluded
Concerns a tick species or a tick-borne pathogen occurrence in the area considered	Yes	No
Concerns a tick species with proven involvement in transmitting animal diseases or zoonoses	Yes	No
Contains geographic information on the distribution of the tick species or the tick-borne pathogen	Yes	No

After the fist screening 1222 references were considered relevant, 309 doubtful and 666 Non relevant. The doubtful references (title and abstract) were further revised by two WG experts who retrieved 10 relevant references. By checking the full text, other 43 initially doubtful references were considered as relevant. This produced a total of 1275 relevant references and 822 non relevant references.

We retrieved the full article of the relevant references, but we did not found all them. We miss:

6 articles in English language:

- 119 articles in languages other than English.
- These 125 articles are listed in appendix K.

Some relevant articles written in Hungarian, Italian, French, Spanish, German, Bulgarian and Dutch were considered.

Second screening and data extraction:

The second screening was performed in parallel with the data extraction. It was performed by two experienced veterinary parasitologists of the Veterinary School of the University of Zaragoza (Spain), one of them was a WG expert. The other WG experts were consulted when doubths arose. Apart from the three first criteria considered in the first screening, they checked:

Criterion	Included	Excluded
Language publication	English abstract and text available in English, French, German, Italian, Spanish, Hungarian, Bulgarian and Dutch	Abstract not available or text not in English, French, German, Italian, Spanish, Hungarian, Bulgarian or Dutch
Original work (not a review document)	Yes	No
Contains geographic information on the distribution of the tick species or the tick-borne pathogen	Yes	No
Does not specify a concrete geographic location. Rather refers it to the entire country.	No	Yes
Concerns a prompt importation of a tick species that does not reach sufficient epidemiological threshold for its establishment in the specified location.	No	Yes
Case-reports of human infections that, in order to protect the privacy of personal data, provide the address of a reference hospital instead of the residence of the infected patient.	No	Yes
Tick identification is unequivocal and appropriate.	Yes	No
The diagnostic method for the tick-borne pathogen is appropriate (serology, isolation, biological methods as PCR)	Yes	No

This second screening resulted in a total of 637 scientific papers considered appropriate to be included in the review. They are listed in appendix L, sorted by the identification number of the reference.

Data extraction:

Data was extracted to an excel spreadsheet containing the following fields:

- Tick genus and species, recorded as in the original paper
- Location of the tick: based on the nomenclature of statistical territorial units (NUTS) for the EU countries. For non EU countries that did not have the NUTS established, we recorded the

name of the location provided in the original report, at equivalent level of precision. The coordinates (latitude/longitude) were recorded if given in the article.

- If molecular techniques were used to identify the tick (yes/no)
- The source of the tick specimen: free living (questing), livestock, pet, human, wildlife (taxonomic order of the host)
- The corresponding bibliographic reference
- The tick-borne pathogen genus and species (as it appeared in the original work)
- Location of the pathogen: NUTS for European countries, For the countries that do not have the NUTS, the name of the administrative region at the level of precision provided in the scientific paper. Geographical coordinates if provided,
- Diagnostic/identification method of the pathogen: isolation, molecular, serology
- Source of the sample (pathogen): livestock, pet, human, wildlife (taxonomic order of the host), tick.
- The corresponding bibliographic reference

2) Other resources

- Gathering scientific papers from the personal collections of the WG members
- Integrated Consortium of Ticks and Tick-borne Diseases (ICTTD3): http://www.icttd.nl/index.php?id=2;
- Official reports of diseases, not indexed journals (at the knowledge of the WG members)
- PromedMail.org (for CCHF cases)
- OIE web page: data of the distribution of the ASF outbreaks in the Caucasus (accessed the 5 of May of 2010):
    - Russian Federation notified to the OIE from September 25 2009 to 21 April 2010: http://www.oie.int/wahis/public.php?page=event_summary&reportid=8462
    - Georgia reported to OIE during 2007 outbreak (no more reports since 2007): http://www.oie.int/wahis/public.php?page=event_summary&reportid=5720
    - Armenia reported to OIE, during 2007: http://www.oie.int/wahis/public.php?page=event_summary&reportid=6051
    - and during 2010: http://www.oie.int/wahis/public.php?page=event_summary&reportid=9032
    - Azerbaijan notified 2 outbreaks during 2008 but nothing later: http://www.oie.int/wahis/public.php?page=event_summary&reportid=6730
- Data from proceedings: EDEN international conference: Emerging vector-borne diseases in a changing European environment, Montpellier, France. 10-12 May 2010. http://international-conference2010.eden-fp6project.net/index.php/eden_colloque. Searched on June 4th 2010.

APPENDIX H: DATA ON SEROLOGICAL SURVEILLANCE ON CCHF IN DOMESTIC ANIMALS.

LOCATION	*SPECIES*	*SEROLOGY*	*YEAR*	*REFERENCE*
Albania	*Goats*	*Serological surveillance*	*2003-05*	*Papa A, Velo E, Papadimitriou E, Cahani G, Kota M, Bino S. 2009. Ecology of the Crimean-Congo Hemorrhagic Fever Endemic Area in Albania. Vector Borne Zoonotic Dis. Apr 29. [Epub ahead of print] (Papa et al. 2009)*
Central African Republic	*Zebu cattle*	*Serological surveillance*	*1992-93*	*Guilherme JM, Gonella-Legall C, Legall F, Nakoume E, Vincent J. Seroprevalence of five arboviruses in Zebu cattle in the Central African Republic. 1996. Trans R Soc Trop Med Hyg. Jan-Feb;90(1):31-3. (Guilherme et al. 1996)*
China	*Sheep, camels*	*Serological surveillance*	*2004-05*	*Sun S, Dai X, Muhetaer , et al. Epidemiology and Phylogenetic Analysis of the Crimean-Congo Hemorrhagic Fever Viruses in Xinjiang, China. 2009. J Clin Microbiol 47, 2536-2543.(Sun et al. 2009)*
China	*Sheep*	*Serological surveillance*	*2001*	*Qing T., Saijo M., Lei H., Niikura M., Maeda A., Ikegami T., Xinjung W., Kurane I., Morikawa S. 2003. Detection of immunoglobulin G to Crimean-Congo hemorrhagic fever virus in sheep sera by recombinant nucleoprotein-based enzyme linked immunosorbent and immunofluorescence assays. J Virol Methods 108 111-116 (Qing et al. 2003)*
Egypt	*Domestic buffaloes, cattle, sheep and goat, donkeys, horses, mules, pigs and dogs.*	*Serological surveillance*	*1977*	*Darwish M A, Imam I Z E, Omar F M and Hoogstraal H. 1977. A seroepidemiological survey for Crimean-Congo hemorrhagic fever virus in humans and domestic animals in Egypt. J. Egypt. Public Health Assoc. 52: 156-63. (Darwish et al. 1977)*
Greece	*Sheep*	*Serological surveillance*	*1971-76*	*Avsic-Zupanc. 2007. Epidemiology of CCHF in the Balkans. In: "Crimean Congo Haemorrhagic Fever-A global prespective" (Ergonul O, and Whitehouse CA, eds.) Springer 75-88.(Avsic-Zupanc 2007)*
Iran	*Dairy cattle*	*Serological surveillance*	*2006-2008*	*Lotfollahzadeh S, Nikbakht Boroujeni GR, Mokhber Dezfouli MR, Bokaei S, 2009. A Serosurvey of Crimean-Congo Haemorrhagic Fever Virus in Dairy Cattle in Iran. Zoonoses Public Health. 2009 Nov 13*
Iran	*livestock*	*Serological surveillance*	*2000-2007*	*Chinikar S, Goya MM, Shirzadi MR, GhiasiMS, Mirahmadi R, Haeri A, Moradi M, Afzali N, Rahpeyma M, Zeinali M, and Meshkat M, 2008. Surveillance and laboratory detection system of Crimean-Congo haemorrhagic fever in Iran. Transboundary and Emerging Diseases, 55, 200-204*
Iran	*Sheep and goat*	*Serological surveillance*	*2003-2005*	*Bokaie S, Mostafavi E, Haghdoost AA, Keyvanfar H., Gooya MM, Meshkat M, Davari A, and Chinikar S, 2008. Crimean Congo Hemorrhagic Fever in Northeast of Iran. Journal of Animal and Veterinary Advances, 7, 343-350.*
Iran	*Sheep*	*Serological surveillance*	*2008*	*Telmadarraiy Z., Moradi AR, Vatandoost H, Mostafavi E, Oshaghi MA, Zahirnia AH, Haeri A, and Chinikar S, 2008. Crimean-Congo Hemorrhagic Fever: A Seroepidemiological and Molecular Survey in Bahar, Hamadan Province of Iran. Asian Journal of Animal and Veterinary Advances, 3, 321-327*
Iran	*Cattle sheep and goats*	*Serological surveillance*	*1975*	*Saidi S, Casals J, Faghih MA. 1975. Crimean hemorrhagic fever-Congo (CHE'-C) virus antibodies in man, and in domestic and small mammals, in Iran. Am J Trop Med Hyg 24:353-357 (Saidi et al. 1975)*
Iraq	*Cattle sheep and goats*	*Serological surveillance*	*1975*	*Tantawi HH, Shony MO, Al-Tikriti SK. 1981. Antibodies to Crimean-Congo haemorrhagic rever virus in domestic animals in Iraq: a seroepidemiological survey. Int J Zoonoses 8:115-120. (Tantawi et al. 1981)*
Kosovo	*Sheep, cattle*	*Serological surveillance*	*1977*	*Avsic-Zupanc. 2007. Epidemiology of CCHF in the Balkans. In: "Crimean Congo Haemorrhagic Fever-A global prespective" (Ergonul O, and Whitehouse CA, eds.) Springer 75-88.(Avsic-Zupanc 2007)*
Kosovo	*Cattle, sheep*	*Serological surveillance*	*1995-2009*	*Humolli I, Dedushaj I, Zupanac TA, and Muçaj S, 2010.Epidemiological, serological and herd immunity of Crimean-Congo haemorrhagic fever in Kosovo. Med. Arh.,64, 91-93*
Oman	*Cattle, sheep, goat, camels*	*Serological surveillance*	*1995-1996*	*Williams RJ, Al-Busaidy S, Mehta FR, Maupin GO, Wagoner KD, Al-Awaidy S, Suleiman AJ, Khan AS, Peters CJ, Ksiazek TG, 2000. Crimean-Congo haemorrhagic fever: a seroepidemiological and tick survey in the Sultanate of OmanTropical Medicine and International Health, 5, 99-106*

LOCATION	*SPECIES*	*SEROLOGY*	*YEAR*	*REFERENCE*
Niger	*Cattle, sheep, goats, camels*	*Serological surveillance*	*1984-88*	*Mariner JC, Morrill J, Ksiazek TG. 1995.Antibodies to hemorrhagic fever viruses in domestic livestock in Niger: Rift Valley fever and Crimean-Congo hemorrhagic fever. Am J Trop Med Hyg. Sep;53(3):217-21. (Mariner et al. 1995)*
Saudi Arabia	*Sheep and goat*	*Serological surveillance*	*1993-1994*	*Agag, AE, 1998, Rapid detection of Congo-Crimean haemorrhagic fever antibodies. Assiut Veterinary Medical Journal, 39, 280-291*
Senegal	*Birds*	*Serological surveillance*	*1991-92*	*Zeller HG, Cornet J.P and Camicas JL.1994. Crimean-Congo haemorrhagic fever virus infection in birds: field investigations in Senegal. Res. Virol. 145, 105-109. (Zeller et al. 1994b)*
Senegal	*Sheep*	*Serological surveillance*	*1986-87*	*Wilson ML, LeGuenno B, Guillaud M, Desoutter D, Gonzalez JP, Camicas JL. 1990. Distribution of Crimean-Congo hemorrhagic fever viral antibody in Senegal: environmental and vectorial correlates. Am J Trop Med Hyg. Nov;43(5):557-66. (Wilson et al. 1990)*
South Africa	*Sheep and cattle, wild vertebrates*	*Serological surveillance*	*1993*	*Burt FJ, Swanepoel R, and Braack LEO, 1993. Enzyme-linked immunosorbent assays for the detection of antibody to Crimean-Congo haemorrhagic fever virus in the sera of livestock and wild vertebrates. Epidemiology and infection, 111, 547-558*
South Africa	*Cattle*	*Serological surveillance*	*1981-84*	*Swanepoel R, Shepherd AJ, Leman PA, Shepherd SP, McGillivray GM, Erasmus MJ, Searle LA, Gill DE. 1987.Epidemiologic and clinical features of Crimean-Congo hemorrhagic fever in southern Africa. Am J Trop Med Hyg. Jan;36(1):120-32. (Swanepoel et al. 1987)*
South Africa	*Ostriches*	*Serological surveillance*	*1984*	*Shepherd AJ, Swanepoel R, Leman PA, Shepherd SP. 1987. Field and laboratory investigation of Crimean-Congo haemorrhagic fever virus (Nairovirus, family Bunyaviridae) infection in birds. Trans R Soc Trop Med Hyg. 81(6):1004-7. (Shepherd et al. 1987)*
Sudan	*Ruminats*	*Serological surveillance*	*1997*	*Hassanein KM, El-Azazy OM, Yousef HM. 1997. Detection of Crimean-Congo haemorrhagic fever virus antibodies in humans and imported livestock in Saudia Arabia. Trans R SOC Trop Med Hyg 91:356-537. (Hassanein et al. 1997)*
United Arab Emirates	*Cattle sheep and goats*	*Serological surveillance*	*1994-1995*	*Khan AS, Maupin GO, Rollin PE, Noor AM, Shurie HH, Shalabi AG, Wasef S, Haddad YM,nSadek R, Ijaz K, Peters CJ, Ksiazek TG.1997. An outbreak of Crimean-Congo hemorrhagic fever in the United Arab Emirates, 1994-1995. Am J Trop Med Hyg 57:519-525. (Khan et al. 1997)*
Zimbabwe	*Cattle*	*Serological surveillance*	*1981-84*	*Swanepoel R, Shepherd AJ, Leman PA, Shepherd SP, McGillivray GM, Erasmus MJ, Searle LA, Gill DE. 1987.Epidemiologic and clinical features of Crimean-Congo hemorrhagic fever in southern Africa. Am J Trop Med Hyg. Jan;36(1):120-32. (Swanepoel et al. 1987)*

APPENDIX I: PHOTOGRAPHS OF HYALOMMA MARGINATUM

(Photographs kindly provided by Dr. M. Madder).

Hyalomma marginatum Koch, 1844

Male, ventral view

Male dorsal (left) and ventral (right)

Female dorsal (left) and ventral (right)

APPENDIX J: PHOTOGRAPHS OF ORNITHODOROS ERRATICUS

(Sample kindly provided by Dr. L. Vial. Photographs kindly provided by Dr. M. Madder)

Ornithodoros erraticus (Lucas, 1849)

Male dorsal (left) and ventral (right)

APPENDIX K: LIST OF SCIENTIFIC PAPERS THAT COULD NOT BE RETRIEVED.

A) List of the 6 scientific papers in English language that could not be retrieved.

ANDRAS, L. (2002) Tick-borne lymphadenopathy (TIBOLA). Wiener Klinische Wochenschrift, 114, 648-654.

DARGHOUTH, M. A. (2004) Piroplasmids of livestock in Tunisia. Arch Inst Pasteur Tunis, 81, 21-5.

DASGUPTA, B. (2006) On the cause of decline of red squirrels of England with a note on the identity of Hepatozoon sciuri vis-a-vis Hepatozoon griseisciuri. Journal of Natural History - Kalyani, 2, 1-6.

HIDALGO, V. M. M. (2008) Detection of Borrelia spp. in Ixodes ricinus in recreation areas in Hannover (Northern Germany). Tierartzliche Hochschule Hannover, Hannover, Germany, ix + 98 pp.

SANTINO, I., SESSA, R., DI PIETRO, M. & DEL PIANO, M. (2000) Lyme borreliosis in Central Italy (1995-1998). Microbiologica, 23, 261-269.

TREML, F., PIKULA, J., NOVAK, P., HOLESOVSKA, Z. & TOFANT, A. (2004) Tularaemia in hares (Lepus europaeus). Priopcenja 5. Znanstveno strucni skup iz DDD-a s meunarodnim sudjelovanjem. Pouzdan put do zdravlja zivotinja, ljudi i njihova okolisa, Mali Losinj, Hrvatska, 5-8. svibnja 2004, 171-175.

B) List of the 119 references of papers in non English languages that could not be retrieved.

(2001) New information on Bartonella, Ehrlichia (Anaplasma) and other tickborne diseases, with particular reference to the gene group Ehrlichia (Anaplasma) phagocytophila). Norsk Veterinartidsskrift, 113, 786-789.

(2007) Watch out: marsh ticks. AFZ/Der Wald, Allgemeine Forst Zeitschrift fur Waldwirtschaft und Umweltvorsorge, 62, unpaginated.

(2008) The winter break was only short. And the ticks are on the move again. MMW Fortschritte der Medizin, 150, 12-14.

ACICI, M. (2002) Field study in the Black Sea Region on cattle vaccinated with attenuated Theileria annulata schizonts. Turkiye Parazitoloji Dergisi, 26, 257-265.

AKTAS, M. & DUMANL, N. (2000) Subclinical Babesia equi (Laveran, 1901) and Babesia caballi (Nuttall, 1910) infections in horses in the Sultansuyu Agriculture Unit in Malatya. Acta Parasitologica Turcica, 24, 55-56.

AKYAZ, R. & ECEVIT, O. (2006) Ticks and Crimean Congo Haemorrhagic Fever. Ondokuz Mays Universitesi, Ziraat Fakultesi Dergisi, 21, 340-349.

ALP, H. G. & GUVEREN, A. R. (2001) Determination of the seroprevalence of Theileria annulata and Babesia bovis. Pendik Veteriner Mikrobiyoloji Dergisi, 32, 15-19.

ANDRIC, B., LAUSEVIC, D., LAKO, B., VUCINIC, N., DUPANOVIC, B., TERZIC, D., DRASKOVIC, N. & ECEVIC, J. (2005) Q fever in human and veterinary pathology in Montenegro. Veterinaria (Sarajevo), 54, 191-198.

ANDRZEJEWSKI, A., WOZNIAKOWSKA-GESICKA, T. & WISNIEWSKA-LIGIER, M. (2006) [Specific features of Borrelia burgdorferi infection in children]. Przegl Epidemiol, 60 Suppl 1, 16-22.

APANASKEVICH, D. A. (2003) Differentiation of closely related species Hyalomma anatolicum and Hyalomma excavatum (Acari, Ixodidae) based on a study of all life cycle stages, throughout entire geographical range. Parazitologiya (St. Petersburg), 37, 259-280.

APANSKEVICH, D. A. & FILIPPOVA, N. A. (2007) Larval identification of species and subspecies of the genus Hyalomma (Acari: Ixodidae) from Russia and neighbouring territories. Parazitologiya (St. Petersburg), 41, 268-282.

AYDN, L. (2000) Distribution and species of ticks on ruminants in the southern Marmara Region. Turkiye Parazitoloji Dergisi, 24, 194-200.

BANDOUCHOVA, H., PIKULA, J., HORAKOVA, D. & TREML, F. (2007) Tularaemia in a small animals veterinary practice. Veterinarstvi, 57, 422-425.

BIADUN, W., CHYBOWSKI, J. & NAJDA, N. (2007) A new records of Dermacentor reticulatus (Fabricius, 1794) in Lublin region. Wiadomosci Parazytologiczne, 53, 29-32.

BOULOUIS, H. J., VAYSSIER-TAUSSAT, M., HADDAD, N. & MAILLARD, R. (2007) Bartonella infections in ruminants. Le Nouveau Praticien Veterinaire Elevages et Sante, 35-37, 90.

CHESLER, A. D., CODREANU, M. D., MITREA, L., BALBA, A. & CODREANU, I. (2008) Study about the incidence of canine babesiosis in UNIVET clinic Constanta. Lucrari Stiintifice - Universitatea de Stiinte Agronomice si Medicina Veterinara Bucuresti. Seria C, Medicina Veterinara, 54, 51-53.

CHITIMIA, L., COSOROABA, I. & SARBU, M. (2005) Ixodide ticks ecology in Bogda area - Timis County. Revista Romana de Medicina Veterinara, 15, 111-120.

CICEK, H., KARATEPE, M., CAKIR, M. & ESER, M. (2009) Blood parasites detected from Anatolian squirrel, Spermophilus xanthophrymnus (Rodentia:Sciuridae) in Nigde province, Turkey. Ankara Universitesi Veteriner Fakultesi Dergisi, 56, 147-148.

CISLAKOVA, L. (2003) Lyme borreliosis in people in Slovakia during 1999-2001. Slovensky Veterinarsky Casopis, 28, 45-46.

CSANGO, P. A., PEDERSEN, J. E. & STAMBERG, P. (2006) Serological studies on sheep in an area where tickborne encephalitis has been reported. Norsk Veterinartidsskrift, 118, 606-607.

CSIKOS, K., VARGA, J., HADHAZY, A. & BANDY, P. (2001) Changes of epidemiology and clinical pattern in Szekszard between 1992 and 1999. Magyar Allatorvosok Lapja, 123, 259-264.

CZAPLICKI, G., HOUTAIN, J. Y., MULLENDER, C., MANTECA, C. & SAEGERMAN, C. (2009) Bulk tank milk, reliable tool for diagnosing Q Fever in dairy herds? Epidemiologie et Sante Animale, 117-127.

DAUTOVIC-KRKIC, S., CAVALJUGA, S., FERHATOVIC, M., MOSTARAC, N., GOJAK, R., HADZOVIC, M. & HADZIC, A. (2008) [Lyme borreliosis in Bosnia and Herzegovina--clinical, laboratory and epidemiological research]. Med Arh, 62, 107-10.

DEDEOGLU KILINC, G., GURCAN, S., ESKIOCAK, M., KILIC, H. & KUNDURACILAR, H. (2007) [Investigation of tularemia seroprevalence in the rural area of Thrace region in Turkey]. Mikrobiyol Bul, 41, 411-8.

DEMIRCI, M., YORGANCGIL, B., TAHAN, V. & ARDA, M. (2001) Lyme disease seropositivity in people with history of tick bite in the Isparta Region of Turkey. Infeksiyon Dergisi = Turkish Journal of Infection, 15, 17-20.

DEN BOON, S., SCHELLEKENS, J. F., SCHOULS, L. M., SUIJKERBUIJK, A. W., DOCTERS VAN LEEUWEN, B. & VAN PELT, W. (2004) [Doubling of the number of cases of tick bites and lyme borreliosis seen by general practitioners in the Netherlands]. Ned Tijdschr Geneeskd, 148, 665-70.

DOBRACKI, W., DOBRACKA, B., PACZOSA, W., ZIEBA, J. & BERES, P. (2007) [Epidemiology of borreliosis in workers of the district forestry offices in Lower Silesia]. Przegl Epidemiol, 61, 385-91.

DORN, W., SUNDER, U., STEIL, B. & FLUGEL, C. (2000) On the correlation of seasonal density and infection rate with Borrelia burgdorferi sensu lato in field collected ticks of the species Ixodes ricinus L. 1758 (Acari: Ixodidae) - a case study in the Free State of Thuringia (Germany). Mitteilungen der Deutschen Gesellschaft fuer Allgemeine und Angewandte Entomologie, 12, 187-191.

DYBOWSKA, D., KOZIELEWICZ, D., ABDULGATER, A., BORELIOZY, R., LASOW, W. P. & KUJAWSKO-POMORSKIEGO, W. (2007) [Prevalence of borreliosis among forestry workers in Kujawsko-Pomorskie voivodeship]. Przegl Epidemiol, 61, 67-71.

DZIERZECKA, M. (2002) Correlation between the presence of antibodies against B. burgdorferi and-the clinical signs of Lyme disease. Medycyna Weterynaryjna, 58, 523-526.

FARKAS, R. & FOLDVARI, G. (2001) Examination of dogs' and cats' tick infestation in Hungary. Magyar Allatorvosok Lapja, 123, 534-539.

FLISIAK, R., ZALEZNY, W. & PROKOPOWICZ, D. (2000) Evaluation of the relationship between humoral immunological response against Borrelia burgdorferi and exposure to ticks. Medycyna Weterynaryjna, 56, 579-581.

FLISIAK, R., ZALEZNY, W. & PROKOPOWICZ, D. (2004) [Clinical course of Lyme borreliosis with respect to the initial disease manifestation in Bialowieza residents]. Przegl Epidemiol, 58, 445-50.

FOLDVARI, G. & FARKAS, R. (2005) Review of literature relating to Dermacentor reticulatus (Acari : Ixodidae) and newer data on the occurrence in Hungary. Magyar Allatorvosok Lapja, 127, 289-298.

FOLDVARI, G. & RIGO, K. (2009) Epidemiology of Lyme borreliosis and the role of lizards in disease maintenance. Magyar Allatorvosok Lapja, 131, 494-502.

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APPENDIX L: LIST OF THE SCIENTIFIC PAPERS APPROPRIATE FOR THE SYSTEMATIC REVIEW.

List of the scientific papers that were considered appropriate for the systematic review and data extraction, sorted by their reference identificiation number.

0 "Estrada Peña A, Guglielmone AA, Bouattour A, Camicas JL, Horak I, Latif A, Pegram R, Preston P, Walker AR, Barros-Battesti D, Labruna M, Venzal JM and Nijhof A, " The distribution of ticks in the Mediterranean Region Part of the Virtual Tick Museum (www.icttd.nl) distributed in CD-ROM format

11 "Adaszek L and Winiarczyk S, 2007. " Epizootic situation of canine ehrlichiosis in the area of Lubelskie voivodship. "Annales Universitatis Mariae Curie-Skodowska. Section DD, Medicina Veterinaria, 62, 65-72"

18 "Aguirre E, Tesouro M A, Amusategui I, Rodriguez-Franco; F and Sainz A, 2004. " Assessment of feline ehrlichiosis in central Spain using serology and a polymerase chain reaction technique. "Impact of Ecological Changes on Tropical Animal Health and Disease Control, 1026, 103-105"

19 "Akalin H, Helvaci S and Gedikoglu S, 2009. " Re-emergence of tularemia in Turkey. "International Journal of Infectious Diseases 13, 547-551"

21 "Aktas M, Altay K and Dumanli N, 2006. " PCR-based detection of Theileria ovis in Rhipicephalus bursa adult ticks. "Veterinary Parasitology 140, 259-263"

22 "Aktas M, Altay K and Dumanli N, 2006. " A molecular survey of bovine Theileria parasites among apparently healthy cattle and with a note on the distribution of ticks in eastern Turkey. "Veterinary Parasitology 138, 179-185"

23 "Aktas M, Altay K and Dumanli N, 2007" Determination of prevalence and risk factors for infection with Babesia ovis in small ruminants from Turkey by polymerase chain reaction. "Parasitology Research 100, 797-802"

24 "Aktas M, Altay K, Dumanli N and Kalkan A, 2009. " Molecular detection and identification of Ehrlichia and Anaplasma species in ixodid ticks. "Parasitology Research 104, 1243-1248"

25 "Aktas M and Dumanli N, 2000. " "Subclinical Babesia equi (Laveran, 1901) and Babesia caballi (Nuttall, 1910) infections in horses in the Sultansuyu Agriculture Unit in Malatya." "Acta Parasitologica Turcica, 24, 55-56."

26 "Aktas M, Vatansever Z, Altay K, Aydin M F and Dumanli N." Molecular evidence for Anaplasma phagocytophilum in Ixodes ricinus from Turkey. "Transactions of Royal Society of Troprical Medicine Hygiene 104, 10-15"

28 "Alberti A, Zobba R, Chessa B, Addis M F, Sparagano O, Parpaglia M L P, Cubeddu T, Pintori G and Pittau M, 2005. " Equine and canine Anaplasma phagocytophilum strains isolated on the island of Sardinia (Italy) are phylogenetically related to pathogenic strains from the United States. "Applied and Environmental Microbiology, 71, 6418-6422"

31 "Aldea-Mansilla C, Nebreda T, Garcia de Cruz S, Dodero E, Escudero R, Anda P and Campos A. " Tularemia: A decade in the province of Soria (Spain). "Enfermedades Infecciosas y Microbiología Clínica, 28, 21-26."

32 "Aldemir O S, 2007. " Epidemiological study of ectoparasites in dogs from Erzurum region in Turkey. "Revue de Medecine Veterinaire, 158, 148-151"

34 "Alekseev A N, Dubinina H V, Jaaskelainen A E, Vapalahti O and Vaheri A, 2007. " "First report on tick-borne pathogens and exoskeleton anomalies in Ixodes persulcatus Schulze (Acari : Ixodidae) collected in Kokkola Coastal Region, Finland." "International Journal of Acarology 33, 253-258"

35 "Alekseev A N, Dubinina H V, Van De Pol I and Schouls L M, 2001. " "Identification of Ehrlichia spp, and Borrelia burgdorferi in Ixodes ticks in the Baltic regions of Russia." "Journal of Clinical Microbiology, 39, 2237-2242"

37 "Alexandre N, Santos A S, Nuncio M S, de Sousa R, Boinas F and Bacellar F, 2009. " Detection of Ehrlichia canis by polymerase chain reaction in dogs from Portugal. "Veterinary Journal 181, 343-344"

39 "Allue M, Ruiz Sopena C, Gallardo MT, Mateos L, Vian E, García MJ, Ramos J, Berjon A C, Vina MC, García MP, Yáñez; J, González LC, Muñoz T, Andrés C, Tamames S, Ruiz C, Gómez Iglesias LA and Castrodeza J, 2008. " "Tularaemia outbreak in Castilla y Leon, Spain, 2007: an update." "Eurosurveillance, 13, 18-28"

40 "Almeria S, Castellá J, Ferrer D, Gutierrez J F, Estrada-Peña A and Sparagano O, 2002. " Reverse line blot hybridization used to identify hemoprotozoa in Minorcan cattle. "Domestic Animal/Wildlife Interface: Issue for Disease Control, Conservation, Sustainable Food Production, and Emerging Diseases 969, 78-82"

41 "Almeria S, Castellá J, Ferrer D, Ortuño A, Estrada-Peña A and Gutierrez J F, 2001. " "Bovine piroplasms in Minorca (Balearic Islands, Spain): a comparison of PCR-based and light microscopy detection." "Veterinary Parasitology 99, 249-259"

43 "Alp H G and Guveren A R, 2001. " Determination of the seroprevalence of Theileria annulata and Babesia bovis. "Pendik Veteriner Mikrobiyoloji Dergisi 32, 15-19"

44 "Altay K, Aktas M and Dumanli N, 2007. " Theileria infections in small ruminants in the East and Southeast Anatolia. "Turkiye Parazitoloji Dergisi 31, 268-271"

45 "Altay K, Aktas M and Dumanli N, 2008" Detection of Babesia ovis by PCR in Rhipicephalus bursa collected from naturally infested sheep and goats. "Research in Veterinary Science 85, 116-119."

46 "Altay K, Aydin M F, Dumanli N and Aktas M, 2008. " Molecular detection of Theileria and Babesia infections in cattle. "Veterinary Parasitology 158, 295-301"

49 "Altobelli A, Boemo B, Mignozzi K, Bandi M, Floris R, Menardt G and Cinco M, 2008." Spatial Lyme borreliosis risk assessment in north-eastern Italy. "International Journal of Medical Microbiology, 298, 125-128"

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GLOSSARY AND ABBREVIATIONS

GLOSSARY

Argasid ticks: soft ticks

Biocenose: biological community. All the interacting organisms living together in a specific habitat. .

Co-feeding. A phenomenum in which ticks become infected with a pathogen during feeding adjacent to infectious ticks on the same vertebrate host, even when the vertebrate host has not developed a systemic infection.

Conscutum: The hard, sclerotized protective shield (plate) which covers most of the dorsal surface of Ixodidae males

Competence: the ability of a vector to transmit a pathogen to a susceptible host, in a way that the host becomes infected.

Cumulative degree-days: the total number of degree days that have accumulated since a designated starting-date (usually January 1st)

Degree-day (heat unit, thermal unit): Insect development occurs only between an upper and lower temperature threshold. A degree-day is a measure of the amount of the heat that accumulates above a specified base temperature during a 24-hour period.

Ditropic: When adult ticks feed on a different type of host, e.g. ruminants, compared to the host of immature ticks, e.g., rodents.

Enamel is often called ornamentation. It is most conspicuous on the conscutum, of males. The colour is mainly pink, orange or red; the enamel looks like paint on the surface of the integument.

Endophilic and Exophilic. When not feeding, endophilic (=nidiculous) ticks live in the nest, burrow or den of the host. Exophilic ticks live in the open environment away from the host's nest or burrow.

Extrinsic incubation period is the time (in hours or days) between the ingestion of the pathogen by the vector and the moment when the pathogen has become infective (infectious) to the hosts. The extrinsic incubation period is temperature dependent and thus shortened by increasing temperature.

Gonotrophic cycle: The time from feeding to egg-laying. The ixodid female feeds once and dies after a single oviposition (single gonotrophic cycle). The adult argasid female may feed and oviposit several times (multigonotrophic cycle).

Hunting. Host-seeking ticks, e.g., adult Hyalomma ticks which actively and rapidly run towards and onto a host.

Instar. The tick's stage: e.g. in hard ticks the egg, the larva, the nymph and the adult are different instars (stages) of the tick life cycle. In soft ticks there are two to eight nymphal instars.

Ixodid ticks: hard ticks

Kairomone: A kairomone is a compound emanating from a potential host, e.g. carbon dioxide in ox breath that may induce appetitive behaviour in blood feeding arthropods (ticks, mosquitoes, etc.)

Monotropic: ticks, which as immatures feed on the same type of host, e.g., ruminants as the adult ticks.

Nidiculous. Endophilic and nidiculous mean the same.

Oromediterranean.zone: subalpine Mediterranean zone between 1,900 m to about 2,700 m a s l. In this zone coniferous plants and sparse *Juniperus thurifera* and *J. communis* appear. The vegetation is composed of trees and bushes such as *Scots pine*, *Savin juniper* and other bushes (*Vella spinosa, Erinacea anthyllis, Bupleurum spinosum*).

Questing. Many exophilic ticks cling to plant stems or similar substrates and await passing animals with the tick's front legs, having chemosensillae, held out. Such questing ticks may be collected by flagging or dragging a cloth onto which questing ticks try to attach.

Reservoir: an animate or inanimate object on or in which an infectious agent usually lives, and which therefore is often a source of infection by the agent.

Scutum. The scutum is the hard plate on the anterior dorsal surface of the larva, nymph and adult female ixodid tick. Soft ticks (Argasidae) do not have a scutum

Semiochemical: Information-carrying compounds. Semiochemicals are defined by the type of behaviour they initiate, not the specific compound(s) affecting that behaviour. In ticks as in most animals, chemical stimuli guide behaviour. These chemical compounds are secreted external to the animal body, and when recognized by, for instance by a tick, direct a specific behavioural response such as host location or mate location.

Telomere. Either of the sections of DNA occurring at the extreme ends of each chromosome in a eukaryotic cell. Telomeres consist of highly repetitive sequences of DNA that do not code for proteins, but function as caps to keep chromosomes from fusing together. The length of the telomere influences the stability of genetic information just interior of the telomere, since the nucleotide sequences at the ends of a chromosome are not copied by DNA polymerase.

Telotropic. When the immature stages of a tick are able to feed on both different types of hosts and the same types of host as the adult ticks. For instance, both rodents and ruminants can support feeding immature stages of a certain telotropic tick.

Transmission of pathogens by

Transstadial transmission. The passage of microorganisms and viruses in arthropods from one stage (stadium or instar) to the next.

Transovarial transmission. The transmission of microorganisms including viruses from mother to offspring, via the ovaries.

*Based on:

Hillyard, P.D. 1996. Ticks of North-West Europe.Field Studies Council, Shrewsbury, U.K.

Walker et al. 2003. Ticks of domestic animals in Africa. A guide to identification of species. Bioscience Reports, Edinburgh, U.K.

ABBREVIATIONS

CSM: Climatic suitability models

BCA: bio-control agents

Ha.: *Haemaphysalis*

Hy.: *Hyalomma*

ICTTD: Integrated Consortium on Ticks and Tick-borne Diseases (European project)

NUTS: Statistical territorial units

TCC: Trans Caucasus Countries

RF: Russian Federation

RH: Relative Humidity

Rh.: *Rhipicephalus*