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The role of ticks in the maintenance and transmission of Crimean-Congo hemorrhagic fever virus: A review of published field and laboratory studies

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Abstract

This manuscript is part of a series of reviews that aim to cover published research on Crimean-Congo hemorrhagic fever (CCHF) and its etiological agent, CCHF virus (CCHFV). The virus is maintained and transmitted in a vertical and horizontal transmission cycle involving a variety of wild and domestic vertebrate species that act as amplification hosts, without showing signs of illness. These vertebrates have traditionally been considered reservoirs of CCHFV, but in fact they develop only a transient viremia, while the virus can persist in ticks for their entire lifespan, and can also be transmitted vertically to the next generation. As a result, ticks are now considered to be both the vector and the reservoir for the virus. CCHFV has been detected in a wide range of tick species, but only a few have been proven to be vectors and reservoirs, mainly because most published studies have been performed under a broad variety of conditions, precluding definitive characterization. This article reviews the published literature, summarizes current knowledge of the role of ticks in CCHFV maintenance and transmission and provides guidance for how to fill the knowledge gaps. Special focus is given to existing data on tick species in which vertical passage has been demonstrated under natural or experimental conditions. At the same time, we identify earlier reports that used unreliable methods and perceptions to ascribe a vector role to some species of ticks, and have contributed to confusion regarding viral transmission. We also

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examine epidemiological pathways of CCHFV circulation and discuss priority areas for future research.

1. Scope of this review

This manuscript is part of a series of reviews that aims to cover all published research on Crimean-Congo hemorrhagic fever (CCHF), a frequently severe disease produced by Crimean-Congo hemorrhagic fever virus (CCHFV; family *Bunyaviridae*, genus *Nairovirus*). Previous reviews have focused on epidemiology, molecular characterization, seroepidemiological studies, and the role of wild and domestic vertebrate animals in CCHFV maintenance and amplification of the infection (Bente et al., 2013; Spengler et al., 2016a, b; Zivcec et al., 2016).

CCHFV has been detected in a wide range of tick species, but only a few have been definitively identified as vectors and reservoirs. In this paper, we discuss four main research questions:

1. the role of ticks in the maintenance and transmission of CCHFV in nature (e.g., vector competence and viral persistence in ticks);
2. the role of tick factors (e.g., gut barrier and saliva-assisted transmission) on CCHFV transmission and evolution;
3. vector-vertebrate host dynamics (e.g., host pattern, host species, host selection in experimental studies); and
4. the geographic distribution of tick vectors able to maintain CCHFV, potential introduction of CCHFV into new areas and the emergence of new disease foci.

We address these questions by providing a collective, comprehensive compilation of data on the role of ticks in the ecology of CCHFV, including those species that have been conclusively shown to play a role in CCHFV maintenance and transmission. We also establish an epidemiological context for the reported data, providing definitions and terms regarding the role of both ticks and vertebrates in CCHFV circulation. We identify studies that have reported the detection of CCHFV in feeding ticks, a method which is suitable to detect the presence of the virus in a territory, but not for discerning the vectorial capacity of the tick, and discuss the consequences of using unreliable methods for either the identification of the tick or the construction of unsubstantiated conclusions based solely on detection of viral RNA.

The data compiled in this review have been obtained by a search in both PubMed and Web of Science. A deliberately relaxed query was done in both datasets, to capture a larger than required set of papers. The query included the terms “CCHF”, “CHF, “CCHFV”, CHFV, “Congo virus” or “Crimean-Congo”, plus the terms “tick”, “Ixodidae”, “Argasidae” or the name of every genus of tick. We purposely avoided the inclusion of search terms in the query regarding countries and common or scientific names of vertebrates, so as not to restrict the search to a geographic region or group of hosts. This query produced a large number of papers including clinical manifestations or treatment of the disease in humans,

that we removed after reading the abstract and/or the body of the text. We also included data from references that are not recorded by these datasets, the majority of which are Russian reports translated into English (e.g., NAMRU-3 translations).

2. Background

2.1. Epidemiology of Crimean-Congo hemorrhagic fever

Among the tick-borne viruses, CCHFV is the most important cause of severe and fatal human hemorrhagic disease. CCHF has a variable case fatality rate of 3% to over 50%. However, based on improved diagnostic capabilities and data from cases in Turkey in the last decade, it is likely that the range is much smaller and the higher rates reflect a failure to recognize less severe infections in smaller outbreaks (Bente et al., 2013). No evidence of disease has been detected in animals naturally infected with CCHFV; humans are the notable exception. The range of clinical cases and reports of CCHFV in ticks extends over large regions of Africa and Eurasia (including the Mediterranean region), and from the Middle East to India (Ergönül and Whitehouse, 2007). Hoogstraal (1979) and Watts et al. (1988) reviewed in detail the history of CCHF outbreaks. More recent reports include comprehensive reviews of the recent history of outbreaks (Ergönül and Whitehouse, 2007; Bente et al., 2013), and a partial compendium of the global distribution of more than 1700 reports of human cases (Messina et al., 2015a).

Ticks of the family Ixodidae are the acknowledged vectors of CCHFV transmission to humans. *Hyalomma marginatum* has the most prominent role globally in the natural history of CCHF in the Mediterranean basin and Middle Asia. Dramatic increases in CCHFV circulation occur when *H. marginatum* populations dramatically increase as a result of optimal weather conditions and anthropogenic ecological changes [e.g, Crimea in 1944 and Turkey since ~2000 (Hoogstraal, 1979; Ergönül and Whitehouse, 2007)] are associated with an increase in *H. marginatum* populations. Besides transmission through tick bites, CCHFV infection can also occur through contact with patients during the acute phase of illness, or with blood or tissues of viremic animals. As with other tick-borne diseases, human cases are seasonal. Typically, a few dozen confirmed cases are reported annually in affected countries; sporadically, annual case counts are three to four times higher.

The incidence of CCHF has increased over the past decade, especially in Turkey and Central Asia (Gray et al., 2009; Estrada-Peña et al., 2010b, 2012a, b; EFSA, 2010), presumably due to a combination of biological and environmental factors that may trigger regional spikes (Burt and Swanepoel, 2005; Ergönül, 2006). In addition, enhanced awareness and diagnostic capability likely play a role. Such spikes in case numbers are a striking feature of CCHF incidence rates, because synchrony in human incidence between neighboring countries has never been detected, even if areas of outbreaks share similar bio-geographical features and are thus subjected to the same general climatic trends, which impact ticks, their hosts, and the rates of contact between humans and viral foci. This is why the trends in human incidence rates are believed also to be associated with social factors that modify the epidemiological background (ticks, hosts, and climate) in which the virus circulates.

Models predicting the spatial probability of CCHF outbreaks have limitations. For example, the persistent focus of CCHFV in the western Mediterranean is not predicted in current risk maps of the zoonotic niche of CCHF in the western Mediterranean region (Estrada-Peña et al., 2010a; Messina et al., 2015b). The report of the first autochthonous case of clinical CCHF in humans in Spain in 2016 (ECDC, 2016), near this persistent focus, demonstrates that our current knowledge of the factors affecting the circulation of the virus needs to be reexamined. European health authorities have emphasized the need for data regarding the possible importation routes of CCHFV into territories where it is currently unknown (Maltezou et al., 2010). In particular, there is concern that climate change, transportation of immature ticks by birds, and anthropogenic factors such as changes in land use may act together to alter the distribution of competent tick vectors, introducing CCHFV into new geographic regions (Gale et al., 2012).

2.2. Ticks

Ticks are hematophagous arthropods, with about 900 species divided into two large families of medical interest, the Argasidae (soft ticks) and the Ixodidae (hard ticks) (Guglielmone et al., 2010). Ixodid ticks develop through three active stages (larva, nymph, and adult), while argasid ticks have up to nine nymphal stages in addition to larva and adult stages. Whereas Ixodidae may feed for days or even weeks, most Argasidae feed for only 20–70 min (Estrada-Peña and de la Fuente, 2014). These radically different life cycles deeply impact the circulation of many micro-organisms, as the two families clearly differ in their ability to support active foci of pathogens (Mather and Ginsberg, 1994). The ecology and physiology of ticks make them second only to mosquitoes in the number of pathogens they vector (Hoogstraal, 1985), while ticks are the most important disease vector of moderate climates (Sonenshine and Mather, 1994). The discovery of tick involvement in transmission of *Borrelia burgdorferi* group bacteria (the etiological agent of Lyme borreliosis) and *Anaplasma phagocytophilum* to humans sparked a renewed interest in studies of the ecology of these arthropods (Sonenshine and Mather, 1994). Recent studies have been focused on defining the molecular interactions between ticks and the pathogens they transmit, and the roles ticks play in maintaining natural foci of infection (e.g., Busby et al., 2012; Francischetti et al., 2009; Pal et al., 2004; Nuttall, 2009; Korenberg et al., 2016).

The biology of hard ticks is especially suited to support their role as vectors and reservoirs of CCHFV. Fig. 1 summarizes the life cycle of ixodid ticks, and includes details of the involvement of ticks and vertebrates in CCHFV circulation; Table 1 provides the definition of several terms related to tick-borne viral transmission and maintenance. Readers can find additional details on tick biology, which are outside the focus of this review, in recent publications on the topic (e.g., Estrada-Peña and de la Fuente, 2014; Estrada-Peña et al., 2013a; Sonenshine and Roe, 2014).

2.2.1. Vector competence—By definition, a hematophagous arthropod species that transmits a pathogen during blood-feeding is known as a vector. In this context, vector competence is the innate ability of an arthropod to acquire, maintain, and transmit microbial agents (Kahl et al., 2002; Nuttall, 2009). A competent vector of a tick-borne virus is capable of being infected from feeding on an infected host, even if viremia is transient or

undetectable. Furthermore, a competent vector supports virus replication within its cells, and can then transmit the virus in its saliva as it feeds on a new host. In a competent tick vector species, the virus must be able to survive the molting process through to the subsequent developmental stage (transstadial survival). This is because the virus can only be transmitted after molting as ticks (unlike hematophagous insects) only feed once after each molt. It remains unclear in which organs tick-borne viruses (including CCHFV) survive during the molt. Some tick-borne viruses are also transmitted from one generation to the next (vertical transmission) by transovarial and/or trans-sexual (male to female) transmission. Both horizontal and vertical transmission contribute to the circulation of CCHFV. Ticks that are not competent vectors may acquire the virus as they feed on an infected host, but they cannot maintain an infection in their tissues and cannot transmit the virus horizontally.

The broader term “vectorial capacity” (see Table 1), comprises vector competence and any behavioral or environmental factors that may influence the spread of a pathogen by the vector, and thus denotes the relative importance that a tick species plays in the transmission of a pathogen. Vectorial capacity is a concept that considers both the ecological context of the vector (relative abundance, preferred hosts, climate tolerance) and its capacity to acquire and transmit the pathogen (Kahl et al., 2002; Rogers and Randolph, 2006; Estrada-Peña et al., 2013a). Ticks are especially well suited as pathogen vectors, first because of their hematophagous behavior (including the relatively long duration of blood-feeding), and second because of the wide variety of vertebrates they can utilize as hosts. Ticks commonly involved in the circulation of human pathogens often feed on small mammals and birds at their immature stages, while adult ticks usually feed on large herbivores and carnivores. This feeding strategy links phylogenetically diverse branches of vertebrates, like the Aves, Rodentia, Insectivora, Artiodactyla, and Carnivora. Therefore, ticks are able to collect pathogens from a very diverse range of potential vertebrate hosts and transmit them to humans, who are usually bitten by tick species with a generalist (non-specific or opportunistic) host behavior. Scientists recognize that the ecological relationships that exist between ticks and pathogens can profoundly influence the patterns of transmission of disease to humans, and that the association of ticks with pathogen reservoirs may be only an indirect result of the associations of ticks and vertebrates occupying the same environmental niche (Estrada-Peña et al., 2015; Pavlovsky, 1966).

Nothing overtly in the ecology, environmental requirements, or preferred hosts of *Hyalomma* ticks explains why they are found to be the principal species involved in CCHFV transmission. It can therefore only be assumed that ticks of the genus *Hyalomma* are necessary to support the circulation of the virus in natural foci, and while other species of ticks may be infected under laboratory or field conditions, species other than *Hyalomma* cannot maintain an active focus of CCHFV. Nevertheless, knowledge is lacking and studies addressing these questions are needed. Hoogstraal pointed out in his monumental review (1979) that ticks of the genus *Hyalomma* are especially important in causing epidemics and outbreaks of human CCHF due to their aggressiveness in seeking human hosts. Whether or not CCHFV infection of ticks increases host-seeking behavior has not been investigated. The virus has been repeatedly isolated and/or detected in *Hyalomma* ticks since it was first reported in these arthropods in 1967 (Hoogstraal, 1979). CCHFV has subsequently been detected in several other tick genera, including *Amblyomma*, *Rhipicephalus, Dermacentor*,

and *Ixodes* (Hoogstraal, 1979; Shepherd et al., 1989; Pak et al., 1974; González et al., 1991; Chumakov, 1965; Kondratenko et al., 1970), but the vectorial capacity and role in CCHFV maintenance of each tick genus is unclear (Hoogstraal, 1979; Turell, 2007).

2.2.2. Tick-borne CCHFV transmission and maintenance—Basic transmission principles include minimum duration of tick attachment prior to initial viral transmission, and amount of virus transmission during the different phases of the tick bite. Studies with *Borrelia* and *Ehrlichia* have indicated that the tick needs to be attached for about 24 h in order for the bacteria to become activated in the salivary glands (Peavey and Lane, 1995; Katavolos et al., 1998) In contrast, studies with Powassan virus, a flavivirus, have indicated that other pathogen transmission may occur much more rapidly, within 15 min (Ebel and Kramer, 2004). The kinetics for tick-to-host transmission is not known for CCHFV, but the aforementioned data support prompt and safe removal of ticks following established guidelines.

For a tick to transmit CCHFV to the next host, virus taken up in a bloodmeal needs to replicate in the midgut, spread to the hemocoel, and enter the salivary glands in order to be injected, in saliva, into the next host. Compared to mosquitoes, ticks feed for a long period of time on the host and take in a much greater volume of blood. Unlike mosquitoes that enzymatically digest blood rapidly in the gut lumen, blood digestion in ticks takes place in the acidic intracellular compartments of the gut epithelium (Sojka et al., 2013). Given the intracellular digestion of blood in ticks, it is conceivable that a virus does not need to bind to a receptor in the tick's midgut in order to infect and replicate in the midgut cells.

During the process of viral replication and spread, the virus has to overcome several barriers to infection within the tick; only if these barriers are overcome can a tick species be considered a competent vector of the virus. However, there have been few investigations of the CCHFV receptor and extrinsic incubation period in ticks. Only two studies have addressed CCHFV tissue localization and multiplication dynamics. Dickson and Turell (1992) evaluated the replication dynamics of CCHFV in *Hyalomma truncatum* after intracoelomic inoculation and found that titers remain low for 2 days and then gradually increase in various tissues. Ticks that had taken a post-inoculation bloodmeal had significantly higher viral titers in ovaries, testes, and salivary glands than unfed infected ticks. Titers in other tissues such as midgut, nervous tissue, and Malpighian tubules remained the same. This might indicate that viral replication is stimulated by attachment and feeding. Gargili et al. (2013) fed adult *H. marginatum* ticks on STAT-1 KO mice challenged with 100 PFU of CCHFV IbAr 10200 and removed them 3 days post virus challenge. Ticks were dissected, RNA extracted from salivary glands, midgut and ovaries, and the extracts tested for CCHFV by RT-qPCR. CCHFV RNA was found in pairs of salivary glands (up to 10^9 genome equivalents), total midgut (up to 10^7 genome equivalents) and in the ovaries (up to 10^3 genome equivalents).

Tick-borne viruses can be associated with the vector for a long period of time by persistent infection through different life stages and by passage to the next generation. Epidemiologically this long-term survival is very important especially when short-lived small mammal or bird hosts turn over quickly in an ecosystem, duration of host viremia is

short, or host antibody responses develop rapidly. Studies have demonstrated that CCHFV infection persists throughout the tick's life cycle, with no known detrimental effects to the tick; CCHFV can persist in its tick vector species transstadially and vertically, during all life stages and into the next generation (Lee and Kemp, 1970; Zeller et al., 1994; Zgurskaya et al., 1971). However, the frequency of transstadial transmission, the numbers of generations in which the virus persists in the tick population and the percentage of eggs infected are unknown. Because ticks can survive long periods without feeding, they enable CCHFV to over-winter. Thus, tick vectors of CCFHV provide reservoirs of infection even in the absence of vertebrate hosts. In *H. marginatum* maintained at 4 °C, CCHFV was detectable for up to 700 days after an infectious bloodmeal, and the ticks transmitted virus to vertebrates by biting even after storage at 4 °C for up to 10 months (Turell, 2007). Additional data on transovarial transmission and transstadial survival are necessary to evaluate their epidemiological relevance.

2.3. Role of vertebrate hosts

Larval and nymphal stages of ixodid ticks must take a bloodmeal to then molt to the next stage; adults require a bloodmeal to produce eggs. Ixodid species are categorized by the total number of hosts on which they feed to complete their life cycle: one-host ticks (all parasitic stages feed on the same host), two-host ticks (immatures and adults feed on different hosts), and three-host ticks (all parasitic stages feed on different hosts). The numbers of hosts parasitized by a tick during its lifetime and the host specificity, critical factors in the epidemiology of tick-borne disease, are well described in Hoogstraal (1979).

Ticks with a one-host pattern are restricted to a few ixodid species such as *Rhipicephalus* (*Boophilus*) spp. parasitizing mobile, hoofed domestic and wild herbivores with relatively extensive home ranges; they rarely feed on humans. Their epidemiological role is probably to maintain virus interaction between the tick species and a single host. During this time, uninfected ticks of other genera parasitizing the same host may acquire the virus, and subsequently transmit the virus to other hosts, although no data are available supporting this hypothesis.

A two-host pattern is very important in the ecology of CCHFV. It is common in *Hyalomma* spp. and a few other species inhabiting steppe or savanna environments with low to moderate rainfall and long dry seasons. In this pattern, the larvae molt to the nymphal stage on the host and both stages feed on the same host; adults feed on a second host. Infected nymphs feeding on an individual host can produce a transient viremia, and uninfected larvae feeding on the same host can then become infected. Notably, in this scenario, a single infected nymph could infect dozens or even hundreds of larvae. Also, after these larvae molt, the now infected nymphs feed on a second host, a ruminant, therefore producing another transient viremia that could infect other adult ticks feeding simultaneously on the same animal. The two-host pattern can be separated into two sub-categories (Table 2).

For tick-transmitted pathogens, a "reservoir" is commonly considered to be a vertebrate that supports the circulation of a pathogen in its blood for a period of time sufficient to allow feeding ticks to acquire the pathogen (Kahl et al., 2002). However, if the infected vertebrate becomes immune to the pathogen and is no longer a source of infection, this reservoir status

may be short-lived. For this reason, the term “maintenance host” is a more appropriate description of a vertebrate that contributes to maintaining the transmission cycle. Not every species of vertebrate can support CCHFV infection and replication, even for short periods of time. However, these non-competent species may still be important if they support the tick vector population.

While ticks are considered to be both vectors and reservoirs of CCHFV, due to the short-term viremia reported in vertebrates (Spengler et al., 2016b) and long-term survival in ticks, vertebrates are critical in viral ecology, serving as the bridges that the virus uses to pass from one tick to another and as viral amplifying hosts (Flick and Whitehouse, 2005; Shepherd et al., 1991; Swanepoel, 1998; Wilson et al., 1991). This concept is not new, and can be traced back to the pioneering studies of active foci in the Crimean Peninsula (Chumakov, 1947, 1948). CCHFV is thus maintained in a silent vertical and horizontal transmission cycle involving ticks as vectors and reservoirs, and a variety of vertebrate hosts for the tick (Swanepoel, 1998; Burt and Swanepoel, 2005), such as birds, hares, hedgehogs, and large ungulates, which transiently amplify the infection and allow CCHFV to be ingested by other ticks feeding on the viremic vertebrates. Ungulates can support a large tick load and may, even in a brief period, infect a considerable number of ticks, amplifying the infection to a significant fraction of the active tick population.

2.3.1. Dynamics of tick-vertebrate-tick transmission—Following tick inoculation, CCHFV enters the skin site of tick feeding. Here it most likely infects dendritic cells, the immune surveillance cells, and possibly other cell types, as little is known of the first steps of vertebrate host infection (Connolly-Andersen et al., 2009; Ergönül, 2012). Viremia probably occurs as a result of spillover into the blood of virus replicating in target organs such as liver and spleen (Bente et al., 2010). Ticks may acquire CCHFV in a bloodmeal from a viremic vertebrate host; typically, the viremia is brief hence there is only a limited opportunity for ticks to acquire the virus. Alternatively, ticks may acquire CCHFV in a bloodmeal while co-feeding with infected ticks together on a non-viremic host (see sections 2.3.2 and 4.2.3.). Such a non-viremic mechanism may be facilitated by the infection of dendritic cells (Nuttall and Labuda, 2003).

2.3.2. Vertebrate host contribution to geographic dispersion of CCHFV—While most avian species may be refractory to CCHFV infection (EFSA, 2010; Swanepoel, 1998; Shepherd et al., 1987; Spengler et al., 2016a, b), migrating birds serve as sources of bloodmeals for immature ticks, and therefore provide a mode of dispersal of infected ticks, contributing to the spread and subsequent emergence of disease foci (Gale et al., 2012; Vorou, 2009). Long-distance movement of livestock may also contribute to dispersal of CCHFV-infected ticks because of the long feeding time of ixodid ticks (Rodriguez et al., 1997).

Vertebrates also impact the circulation of CCHFV through co-feeding (non-systemic) transmission (Table 1). In this case, naïve ticks can acquire CCHFV while feeding in close proximity to infected ticks, even if the vertebrate host is not viremic (González et al., 1992; Gordon et al., 1993; Jones et al., 1987; Logan et al., 1989). Acquisition of the virus by co-feeding may enhance infection rates in co-feeding larvae hatched from eggs laid by infected

female ticks in which the prevalence of infected eggs is low, as postulated for tick-borne encephalitis virus (Labuda et al., 1993). Co-feeding transmission appears to represent an important mechanism for the survival of tick-borne viruses, in nature; however, the specific contribution to the maintenance and transmission of CCHFV has not been determined (González et al., 1992; Hartemink et al., 2008; Nuttall et al., 1994).

3. Studies on the role of ticks in the maintenance and transmission of CCHFV

A variety of studies have investigated the role of ticks in the ecology of CCHFV. Data from these studies fall into three categories:

- studies of unengorged ticks collected during questing, or using eggs laid by engorged females;
- studies of ticks collected feeding on hosts; and
- experimental tick infection studies in the laboratory.

Each approach presents limitations in our ability to interpret the data for formulating conclusions on reservoir and/or vector status of a tick species. Specifically, data from unengorged ticks can identify a tick species involved in virus maintenance, but does not prove its ability to transmit to naïve hosts. Data from ticks collected on hosts therefore indicate the presence of CCHFV in a region, but do not confirm the tick's role as a vector or reservoir. Data from experimental infection studies should ideally avoid "artificial" infection methods such as intracoelomic inoculation of virus, which does not take into account the gut barrier and the role of salivary gland secretions in virus transmission. Below we detail general considerations for these studies, including common errors in data interpretation, and draw conclusions from the three categories of published reports to date.

3.1. Confounding factors in studies of the role of ticks

Two factors confound our understanding of the epidemiological relationships between ticks and CCHFV. The first is the large number of published reports in which ticks have been described as "vectors" of CCHFV, which have relied on the detection of the virus in feeding ticks (see section 3.3). The isolation of infectious CCHFV from feeding or engorged ticks may simply represent virus in the bloodmeal, and does not prove that the tick is a competent vector of CCHFV. Moreover, RT-PCR may detect viral RNA that was already present in the tick or recently obtained through a bloodmeal, and not necessarily infectious virus. Finding virus in a tick also does not automatically mean that the tick can transmit the virus to new hosts.

The same reasoning can be applied to the inoculation of filtered extracts of fed ticks into laboratory animals aimed to assess the presence of CCHFV: infection of an animal with CCHFV through inoculation of a tick extract demonstrates that the virus was in the tick, but does not provide evidence of its presence in salivary glands (a *sine quae non* for transmission). These methods cannot reliably ascertain whether the virus was already present in the tick, was gained through a bloodmeal from a viremic host or was acquired by

co-feeding. Furthermore, the presence of a given tick species in a natural focus of infection is not evidence that it is a competent vector. Thus, these reports (see Tables 4–6 for a complete list of references) are not a demonstration of the vectorial capacity of the tick species. However, based on these reports, 29 species of ticks have been erroneously presented as “new vectors,” creating unnecessary alarm and distorting the understanding of CCHFV ecology. We firmly support the use of ticks as a probe for the presence of the virus in a surveyed region, but vectorial ability can only be claimed after adhering to well-established laboratory protocols (see section 5).

The second factor that has hampered a thorough identification of CCHFV vectors is the misidentification of tick species. Identification can be quite challenging, and requires strict adherence to taxonomic keys or comparison with adequate fragments of DNA sequences available in public repositories obtained from reliably identified specimens. For example, the 16 reports published between the years 2009 and 2015 that concern the association between CCHFV and ticks either used outdated keys or did not mention the taxonomic criteria used for tick identification. This unreliable identification, without voucher specimens, can introduce considerable uncertainty about the identity of the ticks involved in a focus of viral circulation.

3.2. Data on CCHFV from unengorged ticks

When CCHF was first described in 1944 in the agricultural steppe areas of Crimea, a vector-borne origin was immediately suspected (Hoogstraal, 1979). Epidemiological and ecological investigations supported a tick-borne origin as local inhabitants described unusually high tick numbers as compared with the previous years (Grobov, 1946; Petrova-Piontkovskaya, 1947), and *H. marginatum* was the only dominant blood-sucking parasite found in the Crimean steppe areas where cases were recorded (Grashchenkov, 1945). The role of ticks in CCHFV transmission was first confirmed in 1945 by development of human disease following inoculation of tick extracts (Chumakov, 1974) and subsequently by the inoculation of tick suspensions into newborn mice (Chumakov, 1973, 1974). Outbreaks in Bulgaria, China, Yugoslavia, Pakistan, United Arab Emirates, Iraq, and areas of Russia in the following decades reaffirmed that the disease was transmitted by ticks and that species of the genus *Hyalomma* were predominant vectors (thoroughly reviewed by Hoogstraal, 1979).

To date, the maintenance of CCHFV has been demonstrated in 6 species of unfed ticks (see Table 2). Unfed specimens result from the molt of a previously fed stage, or are newly hatched from eggs. Finding the virus in unfed ticks unequivocally demonstrates that the virus survived tick molting or was passed transovarially from the engorged female to the larvae via the eggs. CCHFV has been reliably demonstrated in unfed specimens of *Hyalomma anatolicum, H. marginatum, Hyalomma rufipes,* and *Hyalomma truncatum*; virus detected in the eggs of *Dermacentor marginatus* is probably not sufficient evidence for this species to maintain the virus (Table 3). Figs. 2–4 show the reported geographic distribution of these species. The presence of the virus was evaluated by inoculating newborn mice with homogenates from newly molted ticks or their eggs, therefore indicating either transstadial survival or transovarial passage. The above species of ticks are the only ones so far reported to acquire the virus in nature and to pass the virus to the eggs or to the next life cycle stage.

It is necessary to stress that finding CCHFV in unfed ticks does not prove the vectorial abilities of these ticks, but only demonstrates the passage of the virus to the next stage or the next generation of ticks. This, however, obviously strongly supports the role of these tick species as vectors in CCHFV circulation, which must be confirmed by additional laboratory protocols (see Section 6.1).

3.3. Data from ticks collected feeding on hosts

Most studies reporting CCHFV in ticks have relied on collecting ticks feeding on vertebrate hosts and then detecting the virus (Tables 4–6). Thus, CCHFV has been reported so far in 28 tick species collected from hosts; these include one species of *Amblyomma*, one of *Dermacentor*, 15 species of *Hyalomma*, three of *Haemaphysalis*, one of *Ixodes*, 10 of *Rhipicephalus*, and three species of Argasidae (in the genera *Argas* and *Ornithodoros*). More recent studies involve RT-PCR, while pre-PCR reports are based on inoculating filtered tick extracts into newborn mice. These reports commonly referred to ticks collected on domestic animals in a relatively small region and assess the presence of the virus in engorged ticks feeding on different species of ungulates or livestock, without further indication of the ecological conditions, the viremic status of the host, or the possibility of other tick species cofeeding on the same host. Most studies have focused on domestic ungulates, such as *Bos taurus* (44% of total reports), *Ovis aries* (19%), and *Capra hircus* (12%). The only studies dealing with wild ungulates were on *H. rufipes* feeding on *Taurotragus oryx* in South Africa (Swanepoel et al., 1983), and *Hyalomma lusitanicum* feeding on *Cervus elaphus* in Spain (Estrada-Peña et al., 2010a). A few studies reported ticks collected on other wild animals, like hedgehogs (*Erinaceus europeaus* and *Hemiechinus auritus*) and birds (*Corvus frugilegus* and *Tockus* sp.). Most studies, too, are focused on a large area covering Central Asia and Eastern Europe, with fewer reports from Africa. Most studies focused on *Hyalomma* and *Rhipicephalus* spp. ticks, which commonly occur together. Some tick species in these reports may have been unreliably classified, and it is difficult to ascertain the reliability of identifications without the availability of voucher specimens.

As mentioned above, ticks of the family Ixodidae are considered the only vectors of CCHFV. This is why reports concerning ticks of the family Argasidae are important to note. In 2010, Tahmasebi et al. reported detection of CCHFV RNA in *Argas reflexus*. However, despite recognizing that *A. reflexus* is a tick “which occasionally infests mammals”, these studies concluded that *A. reflexus* is important in maintaining CCHFV as a persistently infected reservoir for transmission to livestock and hard ticks, and as the origin of CCHF outbreaks. Based on these reports, it was concluded that *A. reflexus* is a competent vector of CCHFV, and the association was used to highlight a potential risk for CCHFV spread through *A. reflexus* (Tahmasebi et al., 2010; Telmadarraiy et al., 2009). However, it is well known that *A. reflexus* does not feed on mammals (Hoogstraal, 1956), and in controlled laboratory infection studies, the virus has never been detected in the body of a soft tick after one day of infection (Shepherd et al., 1989; see section 3.4.3.).

3.4. Data from experimental tick infection in the laboratory

3.4.1. Inoculation route and tick factors—To date, studies of CCHFV transmission by ticks under laboratory conditions have investigated 15 species of Ixodidae and four species

of Argasidae (see Tables 6–8). These studies varied greatly in the inoculation route of the virus, the host species the ticks fed on, and the method of virus detection in the subsequent life stages or generations of ticks. Results are also highly variable, and likely reflect our lack of knowledge of the factors regulating the circulation of the virus, including: (1) the ability of the vertebrate to support the circulation of the virus; (2) the importance of the gut barrier; and (3) the factors in the salivary glands that could enhance CCHFV circulation. The gut barrier is a cellular interface that tick-transmitted pathogens must cross in order to disseminate into the body. Some pathogens exploit molecules in gut cells to enter the body. For example, the bacterium *Anaplasma marginale* exploits the protein MSP1-a (Estrada-Peña et al., 2009), and *B. burgdorferi* s.l. uses the ospA protein (Ma and Weis, 1993; Pal et al., 2004). However, experimental CCHFV infection studies that use intracoelomic inoculation directly inject the pathogen into the body cavity, passing over the natural regulatory mechanisms and interactions between the pathogen and the gut.

Additionally, certain molecules in tick saliva have been shown to promote pathogen transmission to the vertebrate host, a phenomenon termed saliva-assisted transmission (SAT). SAT is well documented in transmission of several tick-borne pathogens, both viral and bacterial (Francischetti et al., 2009; Nuttall and Labuda, 2003, 2004; Titus and Ribeiro, 1990), but has only been demonstrated for CCHFV in two studies and only through indirect evidence (Gordon et al., 1993; Zeller et al., 1994). SAT seems to be a finely-tuned evolutionary mechanism that depends both on the species of tick and the host on which it is feeding; the particular association would probably regulate the transcription of different molecules in tick saliva, indicating that tick-host partners must be carefully selected in experimental studies. At least in the case of *Anaplasma phagocytophilum*, a bacterial pathogen transmitted by ticks to a variety of vertebrates, there is empirical evidence for modulation by the bacterium of the tick biological pathways (de la Fuente et al., 2016). Our preliminary data with *H. marginatum* salivary gland extract (SGE) and human antigen presenting cells (APC) indicate that, rather than enhancing infectivity or viral output of CCHFV, SGE immunomodulates APC and masks CCHFV infection in these cells potentially giving the virus a head start at the feeding site (Bente, unpublished data). Additional studies are required to investigate this phenomenon.

Seventeen laboratory studies have attempted to infect ticks with CCHFV by letting them feed on experimentally infected vertebrates (Tables 7 and 8), while 13 studies inoculated the virus into the tick body at varying concentrations (Tables 9 and 10). Thirteen studies addressed the transstadial survival of the virus, and a further 7 examined transovarial passage. Some of these studies simultaneously addressed both questions. Collectively, studies based on feeding on infected hosts and intracoelomic inoculation of the virus resulted in transstadial survival of CCHFV in 39% and 100% of the ticks examined, respectively. Intracoelomic inoculation of the virus provides precise dosing information (see Tables 9 and 10). However, results may be misleading because intracoelomic inoculation bypasses viral interactions with the midgut epithelium (Turell et al., 1997) and ignores the yet unknown effects of these interactions on tick salivary gland secretions, which are well known to impact transmission rates of other tick-borne pathogens. We must conclude that, because such inoculations bypass key biological factors, they preclude conclusive

interpretations about the relative importance of each tick species in supporting permanent foci of infection.

The conclusion is that intracoelomic and intra-anal perfusion of CCHFV into ticks produces a detectable amount of viable virus after the tick molt, even if the virus did not enter the gut and colonize tick cells through natural mechanisms. This is significant, because it demonstrates that CCHFV can persist or even replicate in the tick body during tissue histolysis and regeneration caused by molting, a pre-requisite of transmission. However, even if the virus is detected in the salivary glands after inoculation and molting, there may be mechanisms that prevent the release of the virus into saliva, as demonstrated for some mosquito-borne viruses. Hence demonstration of vector competence requires infection by feeding on an infected bloodmeal (overcoming potential gut barriers to infection), survival through molting, and then virus transmission during feeding (overcoming potential salivary gland barriers and tick immune mechanisms) (Nuttall, 2009).

3.4.2. Studies confirming CCHFV vector status of ticks—The vector status of several species of ticks, namely *H. rufipes* (Causey et al., 1970; González et al., 1991; Lee and Kemp, 1970; Okorie, 1991; Zeller et al., 1994), *H. marginatum* (Hoogstraal, 1979; Zgurskaya et al., 1971; Kondratenko, 1976), and *D. marginatus* (Hoogstraal, 1979; Kondratenko, 1976; Zarubinsky et al., 1976) has been confirmed using protocols under controlled laboratory conditions, involving feeding ticks of various stages on infected animals (including cattle, the long-eared hedgehog (*Hemiechinus auritus*), and the European hare (*Lepus europaeus*). The virus was detected by various protocols in the engorged ticks, after passage to the next stage in the life cycle, or to the eggs and the next generation of ticks. Importantly, laboratory studies also confirmed the lack of detection of CCHFV in some tick species that are reported as relatively common in active foci of the virus, such as *H. dromedarii*, *H. impeltatum* (Logan et al., 1990), *Rhipicephalus appendiculatus* (Logan et al., 1990; González et al., 1991), *R. simus*, *R. e. evertsi*, *R. decoloratus*, and *A. variegatum* (Shepherd et al., 1991).

However, some reports present conflicting results (Tables 8 and 9). For example, some laboratory studies did not detect CCHFV in tick species that have been already reported by other studies to be efficient vectors, such as *H. marginatum* (Blagoveshchenskaya et al., 1975) and *H. rufipes* (González et al., 1991; Shepherd et al., 1991), adding uncertainty about the suitability of the methods employed. In addition, some studies detected the virus in *Rhipicephalus rossicus* (Hoogstraal, 1979; Kondratenko, 1976; Zarubinsky et al., 1976), *R. e. evertsi*, *R. e. mimeticus* (Shepherd et al., 1991), *R. appendiculatus* (Logan et al., 1990), *Amblyomma hebraeum* (Shepherd et al., 1991), and *A. variegatum* (González et al., 1991) ticks, while others did not.

3.4.3. Importance of host selection for experimental studies—Most reports that did not detect transstadial survival or transovarial passage of CCHFV in ticks feeding on infected hosts used domestic ungulates (most notably sheep), while most reports that detected the virus used small or medium-sized vertebrates, such as hares (*L. europaeus*), the European suslik (*Spermophilus citellus*) or several species of wild birds. The ticks in which virus was not detected after feeding were, in most cases, non-*Hyalomma* species,

underscoring that specific tick/host interactions affect the efficiency of CCHFV transmission. These results further stress the importance of choosing appropriate ticks and hosts for laboratory studies. For example, studies using the same tick species, viral strain, and amount of inoculum reported (Shepherd et al., 1991) that scrub hares are able to transmit the virus to feeding immature ticks, but guinea pigs (*Cavia porcellus*) or white-tailed rats (*Uromys caudimaculatus*) are not. The same study reported transmission by feeding on infected cattle, but not on infected sheep. It seems that some vertebrates have a higher ability to transmit CCHFV to feeding ticks, perhaps because of evolutionary adaptations between hosts and ticks, which may regulate salivary gland secretions.

More than 90% of studies investigating the circulation of CCHFV between ticks and vertebrates under laboratory conditions used variously infected ticks feeding on naive domestic ruminants, and fewer than 10% of these studies examined the transmission to rabbits (*O. cuniculus*), hares (*L. europaeus*), or susliks (*S. citellus*). The large number of negative results of viral transmission obtained by feeding potentially infected ticks on vertebrates support the previous comments on the need of adequately pairing ticks and hosts to engage essential molecular mechanisms that allow CCHFV to disseminate into tick salivary glands and transmit to other hosts.

3.4.4. Experimental studies on Argasidae ticks—Shepherd et al. (1989) demonstrated that CCHFV could not be detected later than one day after inoculation into specimens of *Argas walkerae*, *Ornithodoros savignyi*, and *Ornithodoros p. porcinus*, all members of the family Argasidae. These experiments used intracoelomic inoculation of the virus into tick bodies, a method that commonly yielded CCHFV-positive ixodid ticks even after molting. CCHFV also failed to replicate in *Ornithodoros sonrai* after these ticks fed on viremic suckling mice; thus, this species may not be a CCHFV vector (Durden et al., 1993). These findings suggest that CCHFV isolated from soft ticks in nature (Hoogstraal, 1979; Tahmasebi et al., 2010; Watts et al., 1988) may be from virus present in an infected bloodmeal or from contaminated tick mouth parts, and CCHFV is unlikely to persist in these ticks in nature.

4. Distribution, emergence and evolution of foci: tick and host factors

4.1. Relative roles of tick species in maintenance and transmission

The geographic distribution of CCHFV overlaps well with the known distribution of the ticks of the genus *Hyalomma*, and suggests that several species of the genus are naturally involved in its circulation. While CCHFV has been detected and/or isolated in non-*Hyalomma* species, such as *Rhipicephalus*, *Dermacentor*, and *Ixodes*, there is still little evidence that these ticks circulate the virus in nature or support an active focus. Rather, non-*Hyalomma* species may feed on the same host(s) as CCHFV vector species, thereby acquiring the virus. However, such an infection likely represents a spillover event and is not significant in maintenance or transmission in nature.

For some tick-borne viruses, primary and secondary vectors have been distinguished (Labuda and Nuttall, 2004). This concept defines primary vectors as those required for the persistence of the virus in a natural focus and secondary vectors as those that further

contribute to the circulation of the pathogen. This idea was originally formulated based on studies with mosquitoes, which have radically different life cycles and feeding patterns compared to ticks. While the concept fits well with some tick-transmitted pathogens, such as the complex of *B. burgdorferi* species (Estrada-Peña et al., 2016), we believe that it does not apply to CCHFV as no data substantiate that some *Hyalomma* species are better vectors than others. While we know that *Hyalomma* spp. are vectors of CCHFV, limited data exist to aid in characterizing *Hyalomma* species-specific CCHFV transmission efficiency. Although *Hyalomma* spp. appear to have varying vector competence according to the geographical region, nothing in the ecology, host range, or distribution of ticks indicates which species could be or could not be vectors, because all of them have comparable ecological and host features. For example, the immatures of all *Hyalomma* species that have been confirmed to be involved in CCHFV circulation parasitize small mammals and birds, and the adults parasitize ungulates (Apanaskevich et al., 2008). The relative density of different coexisting species of *Hyalomma* in a region is likely a matter of environmental suitability and not an effect of the presence of potentially key hosts (Cumming, 1999).

Even if a potential “secondary” vector (i.e., a non-*Hyalomma* species) feeds on a viremic vertebrate or co-feeds with the hypothetical “primary” vectors and becomes infected, there is no evidence *to date* that these ticks alone could support the circulation of the virus, and no field studies have demonstrated that permanent foci of the virus exist in zones where only non-*Hyalomma* species are present. Data outlined in Tables 4–8 suggest multiple lines of evidence for the possible role of other tick species in the maintenance of active foci of CCHFV. However, the virus could not be recovered from most non-*Hyalomma* species tested, and when it was, the persistence of the virus in the next generation was not evaluated, and therefore no conclusions can be drawn. This seems to be a *sine quae non* for persistence of active foci, because vertebrates cannot be considered the main candidates to support the persistence of CCHFV in nature.

It should be noted that no studies have been performed to test the ability of New World non-Hyalomma ticks (e.g., the Nearctic and Neotropical *Amblyomma* and *Dermacentor*) to transmit or maintain CCHFV. These species therefore cannot be excluded as possible vectors CCHFV maintenance and transmission, and highlights the lack of fundamental data on the ability of the virus to establish permanent foci in the New World.

4.2. How and why foci persist or emerge

Natural foci of tick-borne virus infections persist because of a combination of factors including the presence of suitable vector species and an adequate density of suitable hosts. This concept was developed by Evgeniy Pavlovsky as the natural nidality of transmissible diseases (Pavlovsky, 1966). In the context of CCHFV, the term “suitable hosts” refers to vertebrates that are both adequate sources of a bloodmeal for ticks and that can support a transient viremia and/or co-feeding transmission.

We hypothesize that whether or not a permanent natural focus of CCHFV becomes established is related to the densities of both *Hyalomma* ticks and vertebrate hosts. For vertebrate hosts, this concept is based on the faunal composition of vertebrates, their ability to support a transient viremia, and their relative importance as tick hosts. Below unknown

threshold densities of both ticks and hosts, CCHFV would not persist, but could be re-introduced resulting in sporadic epidemics. Such re-introductions could result from the movement of wild vertebrates, bird transport of adequate loads of infected ticks, or the introduction by humans of viremic livestock. In contrast, above these threshold densities, tick and vertebrate host abundance would permit maintenance of endemic foci: large numbers of infected ticks would feed on a large population of vertebrates, and transmission rates to humans (and CCHF incidence) would depend on human contact rates with active foci. The following sections discuss the importance of host immunity, vertebrate host tick load and co-feeding in this cycle.

4.2.1. Vertebrate host immunity—Many species of *Hyalomma* are two-host ticks, which may have special significance in the self-amplifying transmission loop of CCHFV. Two-host ticks can feed for around 20 days, which gives ample time for new, uninfected larvae to attach to the host. However, the impact of host immunity in the ecology of CCHFV is not known: if infected larvae feed on a naïve vertebrate, does the host develop neutralizing antibodies? If so, how does this affect subsequent transmission?

Jones et al. (1997) demonstrated, in studies on tick-borne viruses transmitted by three-host ticks, that co-feeding virus transmission can occur on hosts that have neutralizing antibodies to the virus, though co-feeding transmission rates are reduced on immune hosts (0–12.5% infected ticks) compared to those on naïve hosts (0–69% infected ticks). In theory, host immunity to a tickborne virus vectored by a two-host tick will have a greater impact than for viruses transmitted by three-host ticks. Preexisting host immunity to CCHFV has been investigated in one study of intra-peritoneally inoculated sheep with varying levels of pre-existing immunity, and higher levels of antibody levels corresponded with decreased levels of viremia (Wilson et al., 1991). However, in previously exposed sheep with no detectable viremia, virus was transmitted to a subset of feeding ticks, suggesting that antibody does not completely prohibit viral transmission to ticks. This study highlights the need for additional investigations into the dynamics of host immunity and transmission.

4.2.2. Vertebrate host tick load—In a two-host infection cycle, engorged infected nymphs drop to the ground and molt to adults, still retaining the virus. Infected females (tick males do not feed to repletion like females) that resulted from the immatures feeding on the previous host now feed on large animals (often ruminants). Large mammals can support massive numbers of ticks feeding simultaneously, often in the same region of the body. Infected females inoculate the virus into the host, and the resulting transient viremia allows infection of naive adult ticks. A new generation of tick larvae would thus be infected by transovarial passage of the virus from infected females. Even if the percentage of infected eggs is low (in the range of 1%–10%, Gargili, unpublished data), the massive numbers of infected females would result in large numbers of infected larvae in the focus. Each female of *Hyalomma* may lay around 8000–10,000 eggs and dozens to hundreds of ticks may feed on one cow, most of them tightly attached in the same region of the body, which also favors co-feeding infection between adults.

4.2.3. Co-feeding transmission—Studies on other tick-borne viruses, such as TBEV, have demonstrated the importance of co-feeding transmission in the amplification and

maintenance of foci of infection (Randolph et al., 1996). The basic reproduction number R_0 is a tool to assess the probability of a pathogen spreading or becoming extinct (Heesterbeek, 2002). Hartemink et al. (2008) proposed a next-generation matrix to compute R_0 for tick-borne diseases, which resulted in a greater understanding of the transmission dynamics of TBEV. Matser et al. (2009) further developed the methods for CCHFV. Estrada-Peña et al. (2013b) elaborated risk maps for CCHF based on spatial estimations of R_0 to highlight factors driving the possible expansion of the disease. These studies demonstrated that both non-systemic transmission (by co-feeding ticks) and transovarial routes are critical factors allowing the virus to circulate. As in the case of TBEV, Estrada-Peña et al. (2013b) identified that the non-systemic route or transmission by co-feeding is one of the most important factors regarding the probable persistence of active foci: under conditions of low co-feeding rates, the transovarial route acquired highest importance. It is however of interest to stress that these results were obtained without a specific assessment of the importance of the hosts in supporting the permanent foci, because they were obtained for a large territory for which adequate data are unavailable.

The co-feeding route may be of special importance in the case of CCHFV, since demonstrated tick vectors behave as two-hosts ticks. It is however important to consider co-feeding transmission among adult ticks feeding on the same host. In this scenario, co-feeding transmission would also promote an increase in transovarial transmission, because uninfected females would feed close to infected ones, therefore enhancing the transovarial route. Both hypotheses remain to be tested experimentally. Furthermore, the role of co-feeding has yet to be evaluated in active foci of CCHFV, or with particular attention to investigating how pre-existing immunity in the host may influence this process.

4.3. Other variables: climate and land use

Vector-borne disease emergence and re-emergence result from a complex interplay of host, pathogen, vector, and environmental factors, including climate trends and social habits (Elliott, 2009). These factors may operate differentially over large heterogeneous regions; each may change independently while being indirectly linked, or they may synergistically or antagonistically affect transmission cycles.

Prevailing weather may impact the timing of activation and densities of ticks. As is typical for ectotherms, tick development and activity rates increase with temperature. Thus, warmer and shorter autumn-winter periods contribute to increased tick survival and probably to increased hatching rates of larvae earlier in the year (Estrada-Peña et al., 2011). For example, *Hyalomma* species are better adapted for surviving in drier conditions than many other species (Hoogstraal, 1979). Increasing temperatures in the spring, particularly in April or May, usually activate *H. marginatum*, and the immature stages are active from May to September. For example, the first adult *Hyalomma* ticks appear above a threshold of 5°–7°C of average daily temperatures in the Ukrainian steppes (Hoogstraal, 1979). However, tick populations are dramatically reduced by extremely cold winters, such as the severe 1968–1969 winter in the Astrakhan Oblast, in which temperatures fell below −30°C and there was no snow to insulate the ground surface where ticks overwinter. Consequently, such harsh winters are followed by lower numbers of human CCHF cases.

In addition to the influence of weather, outbreaks can arise when environmental conditions permit the proliferation of massive numbers of vertebrate hosts feeding the ticks, including neglected agricultural land and changes in pasture utilization. For example, a reduction in agricultural activity as people migrate to towns may leave domestic ruminants (mainly cattle) to graze on increasingly bushy land where ticks thrive. The ticks, in turn, survive and reproduce better when more hosts are readily available. Such environmental changes have been identified as the main factors behind the outbreak of CCHF in the war-torn Crimean peninsula in 1941–1944 (Grobov, 1946; Petrova-Piontkovskaya, 1947; Hoogstraal, 1979). Prior to the recognition of increasing numbers of human infections in Turkey in 2002, the affected regions had been abandoned for hunting and pasture use between 1995 and 2001 because of terrorist activities in the region. During this period, both hares and wild boar proliferated (Z. Vatansever, personal communications).

Changes in climate or land-use are not always the explanation for emergence of CCHFV, as is the case with the first recognized human fatality by CCHF in Spain, in August 2016. The patterns of circulation of CCHFV in Spain are still unclear. In 2010, viral RNA was detected in ticks of the species *H. lusitanicum* collected while feeding on red deer (*Cervus elaphus)*. Notably, prior to this report, the virus had never been isolated inWestern Europe (Estrada-Peña et al., 2012a,b), a situation that could not be explained by an absence of competent vectors, as both *H. lusitanicum* and *H. marginatum* co-exist in large regions of the southwestern Iberian Peninsula. The epidemiological details of the case demonstrated that the case-patient was walking, and likely bitten by a tick, in a site about 200 km from the original focus of detection. In response to the case, active surveillance by human health authorities, that included testing of more than 9000 ticks for evidence of virus, demonstrated that the virus is circulating in a wide territory that covers most of central Spain; an area where *C. elaphus* is abundant and maintained as a game resource.

A large campaign began in the spring of this year to capture the actual distribution of the virus in a large territory of Spain by collecting and analyzing questing *H. marginatum*. The campaign is still ongoing, but data so far indicate that the virus most likely follows the distribution of the two species of *Hyalomma* ticks over the target region, which does not necessarily indicate a recent introduction or a re-emergence. If the virus has such a large distribution in Spain, the occurrence of only a single human case is striking and explains the unrecognized circulation of CCHFV in Spain. Given these findings, we urge the initiation of active surveillance campaigns in other countries of the western Mediterranean, where *Hyalomma* ticks are common, to further investigate the presence of CCHFV.

4.4. Evolutionary pressure: influence of the tick on CCHFV

RNA viruses such as CCHFV exist as complex viral populations (Brackney and Armstrong, 2016), because they encode an RNA-dependent RNA polymerase that lacks proofreading capability and have a short generation time. There is substantial genetic diversity between CCHFV strains, with divergence of up to 20%, 31%, and 22% among the S, M, and L segments, respectively (Deyde et al., 2006; Carroll et al., 2010). Phylogenetic analyses provide evidence for multiple reassortment events in field-isolated CCHFV strains (Hewson

et al., 2004; Chamberlain et al., 2005). However, such analyses cannot address the forces that shape the CCHFV genome separately within tick and vertebrate hosts.

Few studies have investigated CCHFV mutation and evolution during its replication in ticks (Dohm et al., 1996; Dickson and Turell, 1992; Gargili et al., 2013; Xia et al., 2016), skewing our current understanding of evolutionary pressure towards the influence of the vertebrate host. Furthermore, the “original” viral sequence when isolated from a tick is often not known as most CCHFV isolates studied in the laboratory have been generated by multiple mouse-brain passage or mammalian cell culture. Virus replication during transstadial and transovarial passage can result in selective pressure on the viral genome. Xia et al. (2016) recently investigated how the tick and the vertebrate host shape genome plasticity by studying the virus in a tick-mouse transmission model. Interestingly, a significant number of consensus-level mutations occurred within a single tick stage, but none were found in virus recovered from mice. Almost all mutations occurred in nucleotide sites with high variability. The results suggest that pressures encountered during replication in the tick, not the vertebrate, are driving the genetic diversity of CCHFV, particularly in the M segment. To investigate these important aspects of tick infection and evolution, more laboratory-based models must be established.

How the virus adapts to permit long-term association with the tick reservoir is a significant factor contributing to the support of permanent CCHFV foci. Thus, adaptation to the tick environment (saliva, gut) may play a major role in the emergence of novel, genetically and antigenically diverse CCHFV strains, and the genetic diversity between CCHFV isolates can be explained in part by the tick species involved in its circulation (Dohm et al., 1996). For example, a study in Russia and Central Asia found that the greatest genetic variability was among isolates of CCHFV from different tick species rather than from different geographic areas (Yashina et al., 2003). However, limited data from Turkey involving the CCHFV AP92 Greek strain failed to support these results; no genetic differences, based on S- and M-segment RNA sequences, were found between human and tick viral isolates whether *Rhiphicephalus* spp. or *Hyalomma* spp. (Ozkaya et al., 2010). While the genetic diversity of CCHFV is clearly linked with geographic location (Deyde et al., 2006), further studies are needed to better understand the mechanisms of emergence of distinct genotypes *within* a site.

While substantial genetic diversity can exist within a region, CCHFV strains from widely separated regions can have similar genotypes, even if the species of ticks supporting their circulation in these territories are different (i.e. Deyde et al., 2006; Lukashev et al., 2016). This is an unexpected finding, as the evolutionary pressure of a tick species on CCHFV should shape the necessary adaptations of CCHFV to its particular tick environment, a hypothesis not yet empirically supported. To further investigate this finding, the same analyses, described above, on probable dispersion routes and evolutionary pathways should be performed using strains of CCHFV isolated from the ticks in each region, which may present a different evolutionary picture. We propose that selective pressure on CCHFV is not based on geographical location itself, but on the climate of that location, and that circulating strains have adapted to several species of ticks with similar life-cycle strategies, driven by the prevailing climate. Therefore, geographically distant strains of the virus may be

genetically similar, even if associated with different species of ticks, because they share a common environment. This hypothesis would explain why most of the European isolates are more genetically conserved compared to those from Central Asia or Africa; colder conditions restricting the tick cycle would impose a different selective pressure than warmer conditions, independent of tick species.

5. Conclusions

During recent years, new foci of CCHFV have been recognized in several parts of the world, including the Balkan countries, southwest Russia, the Middle East, India, Turkey, and southwestern Europe. Furthermore, the distribution of known tick vectors and reservoirs is expanding. For example, *H. marginatum* has been introduced into the UK (Jameson et al., 2012), Germany (Kampen et al., 2007), and the Netherlands (Nijhof et al., 2007). Permanent populations of *H. marginatum* have yet to establish in these regions, confirmed by the absence of all life cycle stages detected within a period of around a year. However, permanent populations are now found in southern France (Vial et al., 2016) in contrast to the situation 25 years ago. Without doubt, globalization and ever-expanding international animal transport are permitting pathogens and their vectors to disseminate globally. It is therefore possible for introduced populations of infected ticks to find suitable environments to establish permanent populations, or, perhaps most importantly, to introduce CCHFV into territories where other species of ticks could engage in its active circulation.

The first conclusion of our review is that accurate identification of tick species is as important as the reliability of probes used to detect viral RNA. Reports built on the basis of incorrect species identification cannot substantially contribute to scientific knowledge. The second conclusion is that researchers must strictly adhere to the reformulated Koch's postulates that are adapted to the increasing reliance on sequence-based methods for microbial identification (Fredericks and Relman, 1996). The detection of CCHFV RNA in a tick collected from a host does not directly imply that it is a vector, and no implications for human health should be based on such findings. To date, CCHFV has been detected in questing ticks of five species of Ixodidae and the eggs of one more collected in nature. As discussed above, this does not mean that they are involved in viral circulation, but that the virus survives in these species through the molting process. Fifteen other species of Ixodidae have been investigated under laboratory conditions. Although there are caveats in the interpretation and correlation of laboratory studies, because they involve different protocols, virus detection methods, and routes of delivering the virus into the ticks, these studies have been instrumental in confirming the role of ixodid ticks. In addition, laboratory studies investigating four species of Argasidae were equally important in demonstrating that argasid ticks are not biologically significant in CCHFV circulation.

6. Recommendations for future investigations

6.1. Basic rules to demonstrate vectorial ability

Researchers must adhere to a set of basic procedures to demonstrate the vectorial ability of a species of tick:

1. ticks must be fed on infected natural hosts, and not exposed to virus through intracoelomic, immersion, or intra-anal inoculation;
2. following feeding, virus must be detected in molted ticks from the feeding batch;
3. infected ticks must then be allowed to feed on naive hosts that the particular tick species investigated would feed on in nature;
4. virus must then be found in those hosts and;
5. virus must be found in the new generation of ticks resulting from adults infected in the first step.

Only by adhering strictly to these procedures can we gain definitive knowledge of the vectorial abilities of a given tick species.

6.2. Studies of the vectorial ability of ticks in the New World

Adhering to the procedures listed above, the vectorial ability of ticks in the New World, where no natural foci of CCHFV infection exist, must be investigated. The results would provide new clues both about the relationships between the ticks and the virus, and the probability of CCHFV spreading in the Americas.

6.3. Studies of virus replication and spread in ticks

It is necessary to understand the factors that allow CCHFV to overcome the gut barrier of the ticks, the molecular mechanisms involved in this process, and the routes by which it colonizes the salivary glands for further transmission. Detecting virus in whole tick extracts is not a valid protocol to demonstrate the involvement of ticks in maintaining active CCHFV foci. Rather, these studies merely demonstrate that viable viral particles remain in the tick body; they do not show whether virus can be transmitted, which stresses the need for using animals in experimental studies to permit natural routes of transmission.

6.4. Long-term longitudinal field studies

An important conclusion of this review is that the biological and environmental factors that contribute to CCHFV circulation are incompletely understood. Reliable empirical data can explain the circumstances under which CCHFV circulates at a regional scale, but they are only circumstantial clues if extrapolated to the complete geographical range of the virus. There are dynamic interactions between the factors that influence the life cycle of a tick, such as their hosts and the climate, that vary by region. Modeling approaches should be based on a comprehensive assessment of these variables, of the distribution of ticks and natural hosts that contributes to the maintenance of active foci. Modeling approaches aimed to explain CCHFV incidence rates in humans (Messina et al., 2015b) therefore may not reflect the presence and abundance of vectors, the role of the different host species involved, the key molecular mechanisms of transmission, and the contact rates of humans with infected ticks. Field studies should not be restricted to months, but should be planned for years. We are aware of the logistical difficulties involved in such studies, but field data obtained for shorter periods are unreliable.

6.5. Studies of CCHFV selection pressure in ticks

Field and laboratory studies are needed to consider the possibility that genetic divergence is influenced by tick species or stage, and to characterize mutations that may impact viral maintenance and transmission rates. It is important to study the driving force behind the diversity amongst CCHFV strains, including how bottlenecks, within and between the tick and the host, affect replication and genetic diversification. Work with mosquito-borne viruses has shown that RNA interference (RNAi) is the major innate immune pathway controlling mutational diversity of mosquito-borne viruses. As very little is known about RNAi in ticks (Brackney and Armstrong, 2016), future studies should further investigate its potential significance in tick-borne viruses and in the diversity of CCHFV (Grubaugh et al., 2016).

Studies should also address the possibility that CCHFV strains introduced into a region could adapt to new tick species. For example, it still needs to be elucidated if the association of the AP92-like CCHFV strain with *R. bursa* ticks is driven by vector competence of the tick, or simply due to the prevalence of *R. bursa* in the region where AP92 happens to circulate. The overall goal should be to gain a better understanding of how tick factors may alter virus distribution and the establishment of new geographic foci.

6.6. Experimental studies of tick-host transmission

Only a few laboratory studies of tick-host transmission have employed the procedures required to demonstrate vector competence; and studies of the tick-virus-host interface, transmission dynamics, and associations between tick species and viral strains are also few in number. These studies are long-term studies (years) and require high-biocontainment, which presents unique challenges that, the majority of earlier published studies did not face. Furthermore, the development of tick-host transmission models requires expertise with tick colonies and vertebrate feeding (Thangamani and Bente, 2014). However, these studies are key in addressing questions of when and how much virus is transmitted to the host, the characteristics of the initial host immune response, and the role of tick saliva components. Furthermore, they can provide insight into how virus interactions at the molecular and cellular level in ticks impact the dynamics of virus growth, spread and ultimately the severity of disease in humans.

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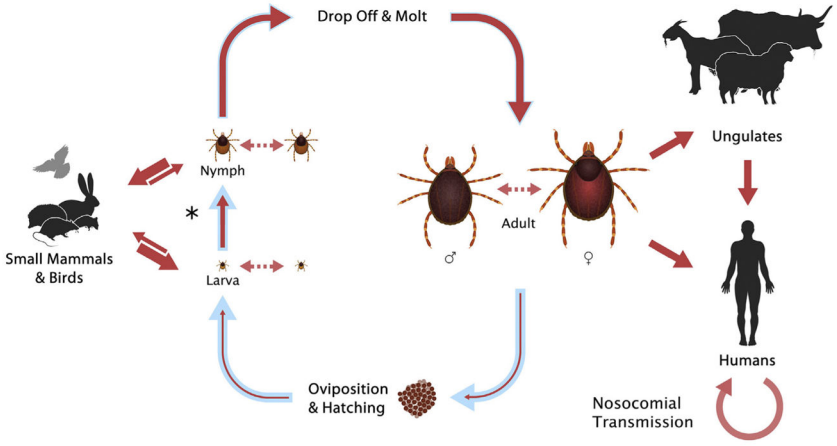

Fig. 1.

A schematic overview of the life cycle of ixodid ticks, with special focus on the implications for CCHFV circulation (Bente et al., 2013). The course of the tick life cycle is indicated by blue arrows, and specifically refers to species of the genus *Hyalomma* with two-host behavior, in which larvae and nymphs feed on the same host. Larvae hatch from eggs and, after feeding, molt to the nymph stage while remaining attached to the host. The nymphs feed again on the same host and return to the ground for molting. At this point (asterisk) nymphs may indirectly infect (via virus in saliva) simultaneously co-feeding larvae and nymphs. The resulting adults find a host, feed again and mate on the host, drop off, and the females lay thousands of eggs, which are left in decaying vegetation at protected sites with high relative humidity to ensure survival. At each bloodmeal, ticks can become integrated into the epidemiological chain of CCHFV transmission by means of transstadial (stage-to-stage) or transovarial (female-to-egg, also called vertical) transmission. Solid red arrows mark the possible transmission of CCHFV between ticks and mammals, or transmission between co-feeding ticks. For each form of virus transfer, the thickness of the red arrow indicates the efficiency from one stage of the tick to the next or to the eggs. Humans acquire infection through the bite of an infected tick or through exposure to body fluids of a viremic animal or a CCHF patient. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

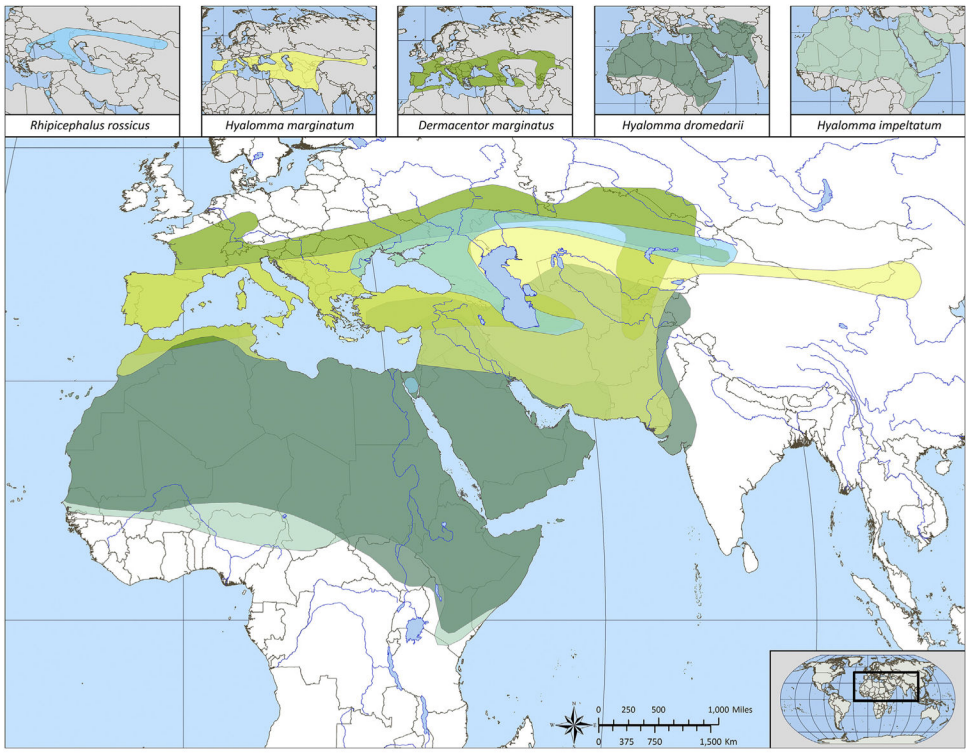

Fig. 2.

Distribution of competent tick vectors of CCHFV in the Palearctic region, in which virus has either been detected in questing specimens and/or its transmission has been demonstrated by feeding previously infected ticks on naïve hosts. These species include: *Dermacentor marginatus*, *Hyalomma marginatum*, *H. dromedarii*, and *H. impeltatum* and *Rhipicephalus rossicus*. Note that within the reported distribution of *H. marginatum* is also that of *H. turanicum*; the map should be regarded as the combination of known distributions of both species. Data is presented in this form as these species are frequently reported without a clear delineation of their identity, and have been historically considered closely related, both previously regarded as subspecies of *H. marginatum*.

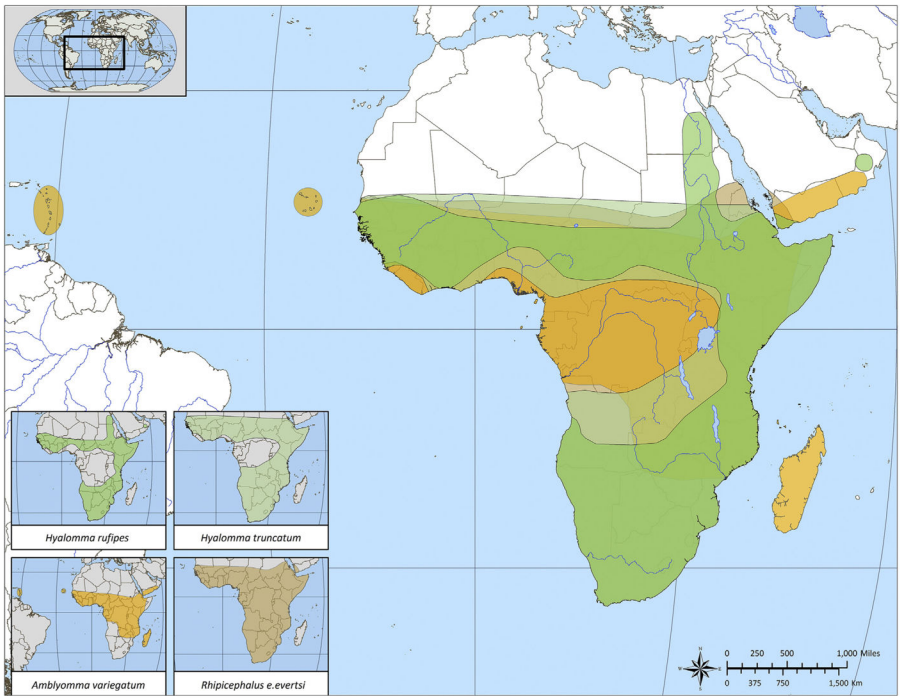

Fig. 3.

Distribution of competent tick vectors of CCHFV in the pan-African region, in which virus has either been detected in questing specimens and/or its transmission has been demonstrated by feeding previously infected ticks on naïve hosts. These species include: *Amblyomma variegatum*, *Hyalomma rufipes*, *H. truncatum*, and *Rhipicephalus e. evertsi*.

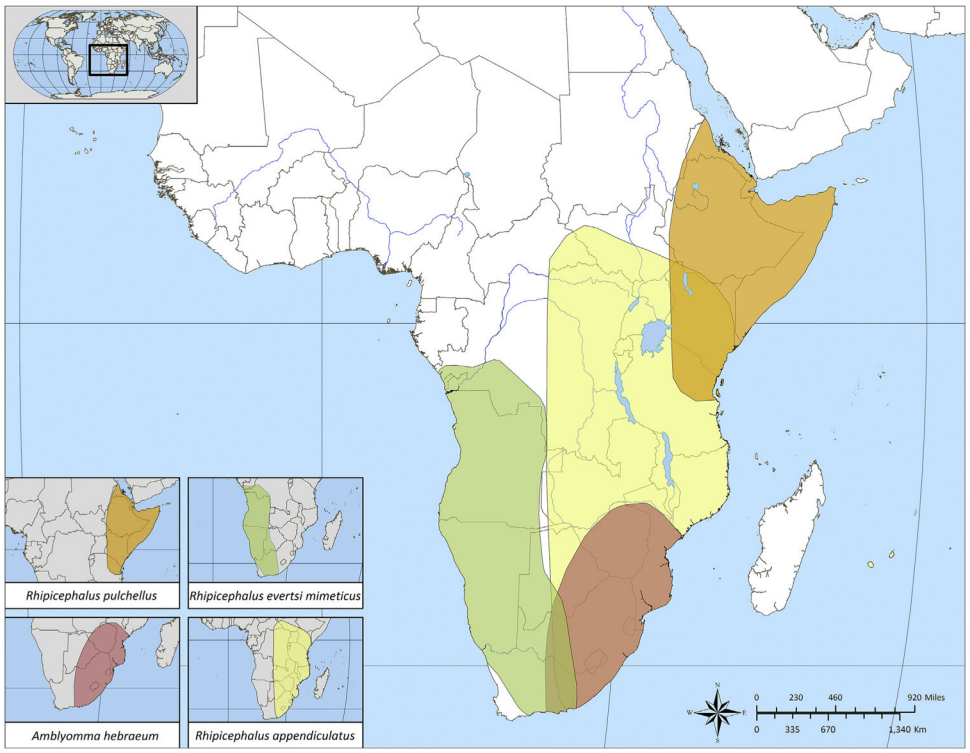

Fig. 4.

Distribution of competent tick vectors of CCHFV in southern and eastern Africa, in which virus has either been detected in questing specimens and/or its transmission has been demonstrated by feeding previously infected ticks on naïve hosts. These species include: *Amblyomma hebraeum*, *Hyalomma* spp., *Rhipicephalus pulchellus, R. appendiculatus and R. evertsi mimeticus*.

Table 1

Basic terms defining the ecological relationships between ticks and tick-borne viruses.

Term	Definition
Tick maintenance host	Vertebrate species on which ticks feed and which support the presence of permanent tick populations. Local and regional tick abundance largely depend on the abundance of these hosts. The lack of sufficient numbers of hosts or the absence of a key host species in a particular area may preclude the occurrence of a species of tick, even if climate and other abiotic features are permissive.
Virus maintenance host	Vertebrate host species commonly infested by tick vector species and supporting horizontal virus transmission during blood-feeding. Virus maintenance hosts are often referred to as reservoirs.
Reservoir	Vertebrate hosts or tick vector species that maintain the virus infection during conditions that preclude active virus transmission (e.g. winter in temperate regions).
Host infectivity	The efficiency with which a virus is transmitted from an infected vertebrate host to the ticks feeding on it. Usually measured by the percentage of ticks infected by feeding on an infected host.
Tick infectivity and specific tick infectivity	These terms refer to the efficiency with which an infection is transmitted from ticks to hosts, or from a given tick species to a particular species of host, respectively.
Extrinsic incubation period	The interval between the acquisition of an infectious agent by a vector and the vector's ability to transmit the agent to other susceptible vertebrate hosts.
Horizontal transmission	Virus transmission from an infected tick to an uninfected vertebrate host, or from an infected vertebrate host to an uninfected tick, during blood-feeding.
Vertical transmission	Virus transmission from adult ticks to their offspring. Usually involves transovarial transmission from an infected female tick to her eggs but may include transmission from an infected male to an uninfected female during mating.
Vectorial capacity	Quantitative term that defines the potential of tick species to transmit a virus to vertebrate hosts. Includes biotic (e.g. vector competence) and abiotic (e.g. climate) factors.
Vector competence	Qualitative term that defines the innate ability of an arthropod to acquire, maintain, and transmit microbial agents.
Co-feeding transmission	Transmission from an infected tick to an uninfected tick during feeding in close proximity on an uninfected vertebrate host. The saliva of infected ticks provides the viral load that contaminates the "feeding pool" where uninfected ticks also feed, probably through the dendritic cells.
Trans-stadial survival	Also called trans-stadial passage, is the ability of a virus to survive molting from one tick blood-feeding stage to the next developmental stage. "Trans-stadial transmission" is an inaccurate term because it implies transmission from one stage to another stage of different individual ticks.

Table 2

Sub-categories of two-host pattern tick feeding relevant to CCHFV ecology.

Two-host pattern subcategory	Vertebrate hosts	Species relevant for CCHFV transmission
Similar host species for immature and adult ticks Dissimilar host species for immature and adult ticks	Larvae/nymph AND adult: deer, wild pigs, cattle, horses, camels, or goats Larvae/nymph: ground-feeding birds, on hares, or on hedgehogs (very rarely rodents) Adult: all domestic mammals (e.g., goats, sheep, cattle)	*Rhipicephalus bursa* *Hyalomma scupense* *Hyalomma anatolicum* *Hyalomma marginatum* *Hyalomma turanicum* *Hyalomma rufipes*

Table 3

Tick species in which CCHFV has been detected in hatched larvae or newly molted ticks, providing evidence of trans-stadial or transovarial passage of the virus. Viral presence was confirmed by the inoculation of crushed and filtered ticks into newborn mice.

Tick species	Country/region	Context	Vector competence	Reference
Dermacentor marginatus	Crimea/Rostov	Eggs of females feeding on cattle were positive for CCHFV	Transovarial	Kondratenko et al., 1970
Hyalomma anatolicum	Tajikistan/Southern	Unfed adults collected from clay fences were positive for CCHFV	Trans-stadial	Pak et al., 1974
	Tajikistan/Southern	Adults molting from nymphs feeding on cattle were positive for CCHFV	Trans-stadial	Pak et al., 1974
Hyalomma marginatum	Crimea	Unfed adults collected from vegetation were positive for CCHFV	Trans-stadial	Chumakov, 1965
Hyalomma rufipes	South Africa/Lombard Nature reserve	Unfed adults collected from the vegetation were positive for CCHFV	Trans-stadial	Swanepoel et al., 1983
Hyalomma turanicum	Kirgizia/Osh	Adults molting from nymphs feeding on crested larks and tree sparrows were positive for CCHFV	Trans-stadial	In Hoogstraal (1979)
Hyalomma truncatum	South Africa/Lombard Nature reserve	Unfed adults collected from the vegetation were positive for CCHFV	Trans-stadial	Swanepoel et al., 1983

Table 4

Studies reporting the presence of CCHFV in *Hyalomma* ticks collected while feeding on hosts. All the ticks were tested after feeding either by inoculation of filtrates into newborn mice or by RT-PCR. Data in this table do not represent conclusive evidence of the vectorial abilities of the tested ticks, but simply serve as markers for the presence of CCHFV in the surveyed region and/or the potential of the vertebrates to circulate the virus to feeding ticks. When collection hosts are listed as livestock, it indicates that host species were not detailed further; when listed as domestic ruminants, it indicates that the data were not delineated by host species.

Tick species	Country (region)	Collected from	Reference
Hyalomma aegyptium	Iran (Ardabil)	*Bubalus bubalis*	Telmadarraiy et al., 2010
	Turkey-Syria border	*Testudo graeca*	Siroky et al., 2014
Hyalomma anatolicum	Armenia	*Bos taurus*	Matevosyan et al., 1974
	Armenia	Not indicated	Semashko et al., 1975
	India	*Bubalus bubalis*	Mourya et al., 2012
	India (Ahmedabad, Gujarat)	Not indicated	Yadav et al., 2013
	Iran (Fars)	Domestic ruminants	Farhadpour et al., 2016
	Iran (Hamadan)	*Ovis aries*	Tahmasebi et al., 2010
	Iran (Ilam)	Livestock	Sharifinia et al., 2015
	Iran (Khorasan)	*Camelus dromedarius*	Champour et al., 2016
	Iran (Lorestan)	Livestock, chicken	Kayedi et al., 2015
	Iran (Sarpole-Zahab)	*Bos taurus, Ovis aries, Capra hircus*	Mohammadian et al., 2016
	Iran (Yazd)	*Camelus bactrianus*	Salim-Abadi et al., 2011
	Kazakhstan, Tajikistan	*Bos taurus*	Onishchenko et al., 2005
	Oman	*Ovis aries*	Williams et al., 2000
	Tajikistan	*Bos taurus*	Pak et al., 1974
	Turkey (Northern)	*Bos taurus, Ovis aries, Capra hircus*	Albayrak et al., 2010a, b
	Turkey (Tokat)	*Bos taurus*	Tekin et al., 2012
	Turkmenistan (Murgab valley)	*Bos taurus*	Aristova et al., 1973
	Uzbekistan	*Bos taurus*	Chumakov et al., 1974
Hyalomma asiaticum	China (Yunnan)	*Bos taurus, Capra hircus*	Xia et al., 2016
	Iran (Ilam)	Livestock	Sharifinia et al., 2015
	Iran (Lorestan)	Livestock, chicken	Kayedi et al., 2015
	Iran (Sarpole-Zahab)	*Bos taurus, Ovis aries, Capra hircus*	Mohammadian et al., 2016
	Iran (Yazd)	*Bos taurus*	Salim-Abadi et al., 2011
	Kazakhstan	Not indicated	Chumakov, 1973
	Kazakhstan, Tajikistan	*Bos taurus*	Onishchenko et al., 2005
	Kirgizia	*Bos taurus, Ovis aries, Capra hircus*	Karas et al., 1976
	Turkmenistan (Ashkabad)	*Camelus bactrianus*	Smirnova et al., 1974
	Turkmenistan (Geok-tepe)	*Ovis aries*	Smirnova et al., 1978
	Turkmenistan (Karakum)	*Bos taurus*	Kurbanov et al., 1974
	Uzbekistan	*Bos taurus*	Chumakov et al., 1974

Tick species	Country (region)	Collected from	Reference
Hyalomma dromedarii	Egypt *	*Camelus bactrianus*	Chisholm et al., 2012
	Iran (Ilam)	Livestock	Sharifinia et al., 2015
	Iran (Khorasan)	*Camelus dromedarius*	Champour et al., 2016
	Iran (Sarpole-Zahab)	*Bos taurus, Ovis aries, Capra hircus*	Mohammadian et al., 2016
	Iran (Yazd)	*Bos taurus, Camelus bactrianus*	Salim-Abadi et al., 2011
	Turkmenistan	*Camelus bactrianus*	Smirnova et al., 1978
Hyalomma excavatum	Egypt *	*Camelus bactrianus*	Chisholm et al., 2012
	Ghana (Ashanti region)	Not indicated	Akuffo et al., 2016
	Nigeria	*Camelus bactrianus*	Causey et al., 1970
	Oman *	*Ovis aries, Capra hircus*	Williams et al., 2000
	Turkey (Central Anatolia)	*Bos taurus*	Orkun et al., 2017
	Turkey (Northern)	*Bos taurus, Ovis aries, Capra hircus*	Albayrak et al., 2010a, b
Hyalomma impeltatum	Ethiopia	*Bos taurus, Ovis aries, Capra hircus, Camelus bactrianus*	Wood et al., 1978
	Nigeria	*Bos taurus, Ovis aries*	Causey et al., 1970
	Senegal (Dakar)	*Bos taurus*	Robin, 1972, 1975 (in Hoogstraal, 1979)
Hyalomma impressum	Senegal (Dakar)	*Bos taurus*	Robin 1972, 1975 (in Hoogstraal, 1979)
Hyalomma lusitanicum	Spain (Extremadura)	*Cervus elaphus*	Estrada Peña et al., 2010a
Hyalomma nitidum	Central African Republic (Bangui)	*Bos taurus*	Robin 1975 (in Hoogstraal, 1979) Sureau 1974; Sureau et al., 1976 (in Hoogstraal, 1979)
Hyalomma marginatum	Armenia	*Bos taurus*	Matevosyan et al., 1974
	Armenia	Not indicated	Semashko et al., 1975
	Bulgaria (Blagoevgrad, Kardzhali, Yambol, Burgas)	Domestic ruminants	Panayotova et al., 2016
	Bulgaria (Central and Southeastern)	*Bos taurus*	Gergova et al., 2012
	Crimea (Astrakhan)	Birds (unspecified)	Berezin et al., 1971
	Crimea (Rostov)	*Bos taurus*, *Corvus frugilegus*	Kondratenko et al., 1970
	Crimea (Rostov)	*Corvus frugilegus*	Rabinovich et al., 1970
	Crimea (Sivastopol)	*Bos taurus*	Chumakov et al., 1974
	Iran (Ardabil)	*Ovis aries*	Telmadarraiy et al., 2010
	Iran (Fars)	Domestic ruminants	Farhadpour et al., 2016
	Iran (Ilam)	Livestock	Sharifinia et al., 2015
	Iran (Heris county)	*Ovis aries, Capra hircus*	Shafei et al., 2016
	Iran (Lorestan)	Livestock and chickens	Kayedi et al., 2015
	Iran (Sarpole-Zahab)	*Bos taurus, Ovis aries, Capra hircus*	Mohammadian et al., 2016
	Iran (Yazd)	*Ovis aries*	Salim-Abadi et al., 2011
	Kirgizia (Osh)	Birds (unspecified)	Tsirkin et al., 1972 (in Hoogstraal, 1979)
	Kosovo	*Bos taurus, Capra hircus*	Sherifi et al., 2014
	Turkey (Ankara)	*Ovis aries*	Hekimoglu et al., 2012

Tick species	Country (region)	Collected from	Reference
	Turkey (Central Anatolia)	*Bos taurus*, *Sus scrofa*	Orkun et al., 2017
	Turkey (Kutahya)	Domestic ruminants	Iça and Çetin, 2016
	Turkey (Northern-Central)	*Bos taurus*	Tonbak et al., 2016
	Turkey (Northern)	*Bos taurus, Ovis aries, Capra hircus*	Albayrak et al., 2010a, b
	Turkey (Northern)	*Ovis aries, Capra hircus*	Albayrak et al., 2012
	Turkey (South Marmara)	Domestic ruminants	Yesilbag et al., 2013
	Turkey (Thrace)	*Bos taurus*	Gargili et al., 2011
	Turkey (Tokat)	*Bos taurus*	Tekin et al., 2012
	Kenya	*Bos taurus*	Sang et al., 2011
	Mauritania	*Camelus bactrianus*	Saluzzo et al., 1985
Hyalomma rufipes	Nigeria	*Bos taurus*	Causey et al., 1970
	Senegal	*Tockus* sp.	Camicas et al., 1994
	Senegal (Bandia)	*Bos taurus*	Zeller et al., 1997
	Senegal (Dakar)	*Bos taurus*	Robin 1972, 1975 (in Hoogstraal, 1979)
	South Africa (Lombard nature reserve)	*Taurotragus oryx*	Swanepoel et al., 1983
	Iran (Ardabil)	*Bos taurus*	Telmadarraiy et al., 2010
	Iran (Hamadan)	*Ovis aries*	Tahmasebi et al., 2010
Hyalomma schulzei	Iran (Ilam)	Livestock	Sharifinia et al., 2015
Hyalomma scupense	Iran (Yazd)	*Bos taurus, Ovis aries*	Salim-Abadi et al., 2011
	Kazakhstan	Not indicated	Chumakov, 1973
	Tajikistan	*Bos taurus*	Pak et al., 1974
	Turkey (Northern)	*Bos taurus, Ovis aries, Capra hircus*	Albayrak et al., 2010a, b
	Turkey (Tokat)	*Bos taurus*	Tekin et al., 2012
	Uzbekistan	*Bos taurus*	Chumakov et al., 1974
	Kenya	*Bos taurus, Camelus bactrianus*	Sang et al., 2011
	Kirgizia	*Bos taurus, Ovis aries, Capra hircus*	Karas et al., 1976
Hyalomma truncatum	Kirgizia	*Ovis aries*	Chumakov et al., 1974
	Nigeria	*Bos taurus*	Causey et al., 1970
	Senegal (Bandia)	*Bos taurus*	Zeller et al., 1997
	Senegal (Dakar)	*Bos taurus*	Robin 1972, 1975 (in Hoogstraal, 1979)
	South Africa (Lombard nature reserve)	*Taurotragus oryx*	Swanepoel et al., 1983
	Tajikistan	*Bos taurus*	Tsilinsky et al., 1971
	Kirgizia	*Ovis aries*	Dandurov et al., 1975
	Turkey (Tokat)	*Homo sapiens*	Tekin et al., 2012
Hyalomma turanicum	Albania	*Bos taurus*	Papa et al., 2009
	Crimea (Sivastopol)	*Bos taurus*	Chumakov et al., 1974
Hyalomma spp. (not specified)	Iran (Ardabil)	*Bos taurus, Ovis aries, Capra hircus, Camelus bactrianus*	Telmadarraiy et al., 2010
	Iran (Heris county)	*Ovis aries, Capra hircus*	Shafei et al., 2016
	Iran (Zahedan)	*Ovis aries*	Mehravaran et al., 2013

Tick species	Country (region)	Collected from	Reference
	Mali (Kati livestock market)	*Bos taurus*	Zivcec et al., 2014
	Turkey (Amasya)	*Homo sapiens*	Bursali et al., 2011

* Positive ticks were collected from imported animals but the conditions under which animals were kept before collection are not available or described.

Table 5

Studies reporting the presence of CCHFV in non-*Hyalomma* ixodid ticks collected while feeding on hosts. All the ticks were tested after feeding either by inoculation of filtrates into newborn mice or by RT-PCR. Data do not represent conclusive evidence of the vectorial abilities of the tested ticks, but simply serve as markers for the presence of CCHFV in the surveyed region and/or the potential of the vertebrates to transmit the virus to feeding ticks. When collection hosts are listed as livestock, it indicates that host species were not detailed further; when listed as domestic ruminants, it indicates that the data were not delineated by host species.

Tick species	Country (region)	Collected from	Reference
Amblyomma variegatum	Ghana (Ashanti)	Not indicated	Akuffo et al., 2016
	Nigeria (savanna and Jos plateau)	*Bos taurus, Ovis aries*	Causey et al., 1970
	Senegal (Bandia)	*Bos taurus, Capra hircus*	Zeller et al., 1997
	Senegal (Dakar)	*Bos taurus*	Robin 1972, 1975, (in Hoogstraal, 1979)
	Uganda (Ankole)	*Bos taurus*	Kirya, 1972
Dermacentor marginatus (including *D. niveus* and *D. daghestanicus*)	Crimea (Rostov)	*Bos taurus*	Butenko et al., 1971
	Kazakhstan	Not indicated	Chumakov, 1973
	Kazakhstan, Tajikistan	*Bos taurus*	Onishchenko et al., 2005
	Moldova	Not indicated	Chumakov et al., 1974
	Turkey (Central Anatolia)	*Bos taurus, Capra hircus, Sus scrofa*	Orkun et al., 2017
	Turkey (Northern)	*Bos taurus, Ovis aries, Capra hircus*	Albayrak et al., 2010a, b
	Turkey (South Marmara)	Domestic ruminants	Yesilbag et al., 2013
	Uzbekistan	*Bos taurus*	Chumakov et al., 1974
Haemaphysalis concinna	Turkey (Tokat)	*Ovis aries*	Tekin et al., 2012
Haemaphysalis inermis	Iran (Zahedan)	*Ovis aries*	Mehravaran et al., 2013
Haemaphysalis parva	Caucasus (North)	*Bos taurus*	Shchelkanov et al., 2005
	Turkey (Ankara)	*Capra hircus*	Hekimoglu et al., 2012
	Turkey (Central Anatolia)	Hare (unspecified)	Orkun et al., 2017
Haemaphysalis punctata	Bulgaria (Central and southeastern)	*Bos taurus*	Gergova et al., 2012
	Crimea (Astrakhan)	Birds (unspecified)	Berezin et al., 1971
	Crimea (Rostov)	*Bos taurus, Corvus frugilegus*	Kondratenko et al., 1970
	Crimea (Rostov)	*Corvus frugilegus*	Rabinovich et al., 1970
	Crimea (Sivastopol)	*Bos taurus*	Chumakov et al., 1974
	Iran (Ardabil)	*Ovis aries*	Telmadarraiy et al., 2015
	Kenya	*Bos taurus, Camelus bactrianus*	Sang et al., 2011
	Kirgizia	*Bos taurus, Ovis aries, Capra hircus*	Karas et al., 1976
	Kirgizia (Osh)	Birds (unspecified)	Tsirkin et al., 1972 (in Hoogstraal, 1979)
	Moldavia	Not indicated	Chumakov et al., 1974
	South Africa (Lombard nature reserve)	*Taurotragus oryx*	Swanepoel et al., 1983
	Turkey (Ankara)	*Ovis aries*	Hekimoglu et al., 2012

Tick species	Country (region)	Collected from	Reference
	Turkey (Northern)	*Bos taurus, Ovis aries, Capra hircus*	Albayrak et al., 2010a, b
	Turkey (Northern)	*Ovis aries, Capra hircus*	Albayrak et al., 2012
	Turkey (Tokat)	*Bos taurus*	Tekin et al., 2012
	Bulgaria (Central and southeastern)	*Bos taurus*	Gergova et al., 2012
Ixodes ricinus	Crimea (Sivastopol)	*Bos taurus*	Chumakov et al., 1974
	Moldavia	Not indicated	Chumakov et al., 1974
	Turkey (Northern)	*Bos taurus, Ovis aries, Capra hircus*	Albayrak et al., 2010a, b
	Armenia	*Bos taurus*	Matevosyan et al., 1974
Rhipicephalus annulatus	Armenia	Not indicated	Semashko et al., 1975
	North Caucasus (Malgobeksky District, Republic of Ingushetia)	*Bos taurus*	Shchelkanov et al., 2005
	Turkey (Thrace)	*Bos taurus*	Gargili et al., 2011
	Uzbekistan	*Bos taurus*	Chumakov et al., 1974
	Senegal (Bandia)	*Ovis aries*	Zeller et al., 1997
Rhipicephalus decoloratus	Senegal (Dakar)	*Bos taurus*	Robin 1972, 1975 (in Hoogstraal, 1979)
	Uganda	*Bos taurus*	Kalunda and Mukwaya, 1978 (in Hoogstraal, 1979)
Rhipicephalus appendiculatus	Armenia	*Bos taurus*	In Hoogstraal, 1979
Rhipicephalus bursa	Armenia	Not indicated	Semashko et al., 1975
	Crimea (Sivastopol)	*Bos taurus*	Chumakov et al., 1974
	Greece (Northern)	*Capra* sp.	Papadopoulos and Koptopoulos, 1980
	Iran (Ardabil)	*Bos taurus, Ovis aries, Capra hircus, Camelus bactrianus*	Albayrak et al., 2010a, b
	Turkey (Ankara)	*Ovis aries, Capra hircus*	Telmadarraiy et al., 2009
	Turkey (Central Anatolia)	*Bos taurus*	Orkun et al., 2017
	Turkey (Northern-Central)	*Bos taurus*, *Capra hircus*	Tekin et al., 2012
	Turkey (Northern)	*Bos taurus, Ovis aries, Capra hircus*	Tonbak et al., 2016
	Turkey (South Marmara)	Domestic ruminants	Yesilbag et al., 2013
	Turkey (Thrace)	*Bos taurus*	Matevosyan et al., 1974
	Turkey (Tokat)	*Capra hircus*	Gargili et al., 2011
	Oman *	*Ovis aries*	Swanepoel et al., 1983
Rhipicehalus e. evertsi	Senegal (Bandia)	*Capra hircus*	Williams et al., 2000
	South Africa (Lombard nature reserve)	*Ovis aries*	Hekimoglu et al., 2012
	Senegal (Bandia)	*Capra hircus*	Zeller et al., 1997
Rhipicephalus guilhoni	Uzbekistan	*Bos taurus*	Zeller et al., 1997
Rhipicephalus pumilio	Armenia	Not indicated	Chumakov et al., 1974
Rhipicephalus rossicus	Crimea	*Bos taurus, Erinaceus europaeus, Lepus euroapeus*	Semashko et al., 1975
	Crimea (Rostov)	*Bos taurus, Erinaceus europaeus*	Kondratenko et al., 1970

Tick species	Country (region)	Collected from	Reference
	Bulgaria (Central and southeastern)	*Bos taurus*	Gergova et al., 2012
Rhipicephalus sanguineus	Bulgaria (Kardzhali, Haskovo)	*Bos taurus, Ovis aries*	Panayotova et al., 2016
	Crimea (Sivastopol)	*Bos taurus*	Chumakov et al., 1974
	Iran (Fars)	Domestic ruminants	Farhadpour et al., 2016
	Iran (Hamadan)	*Ovis aries*	Tahmasebi et al., 2010
	Iran (Lorestan)	Livestock and chickens	Kayedi et al., 2015
	Iran (Sarpole-Zahab)	*Ovis aries*	Mohammadian et al., 2016
	Kirgizia	*Bos taurus, Ovis aries, Capra hircus*	Karas et al., 1976
Rhipicephalus turanicus	Turkey (Central Anatolia)	*Bos taurus*	Orkun et al., 2017
	Turkey (Northern)	*Bos taurus, Ovis aries, Capra hircus*	Albayrak et al., 2010a, b
	Turkey (South Marmara)	Domestic ruminants	Yesilbag et al., 2013
	Turkey (Tokat province)	*Ovis aries*	Tekin et al., 2012

* Positive ticks were collected from imported animals but the conditions under which animals were kept before collection are not described.

Table 6

Studies reporting the presence of CCHFV in *Argasid* ticks collected while feeding on hosts. All the ticks were tested after feeding either by inoculation of filtrates into newborn mice or by RT-PCR. The data do not represent conclusive evidence of the vectorial abilities of the tested ticks, but simply serve as markers for the presence of CCHFV in the surveyed region and/or the potential of a vertebrate host to transmit the virus to feeding ticks.

Tick species	Country (region)	Collected from	Reference
Argas persicus	Uzbekistan	*Gallus domesticus*	Chumakov et al., 1974
Argas reflexus	Iran (Hamadan)	*Ovis aries*	Tahmasebi et al., 2010
Ornithodoros	Iran (Ardabil)	*Bos taurus, Ovis aries*	Tahmasebi et al., 2010
lahorensis	Iran (Hamadan)	*Ovis aries*	Tahmasebi et al., 2010

Table 7

Laboratory studies evaluating vector competence of *Hyalomma* ticks by feeding ticks on vertebrates experimentally inoculated with CCHFV.

Tick species	Stage Infected	Vertebrate host	Viral strain/passage	Infection of host: route/dose (resultant host viremia)‡	CCHFV detected in fed ticks/ method of detection		TS	Trans-stadial transmission checked on/to and method of detection		TO	Virus in the next generation	Reference
Hyalomma impeltatum	Larvae	*Mus musculus*	*1* IbAr10200	IP, ≈1.0 × 10^2 PFU	NA	NA	+	Feeding nymphs on *Cavia porcellus*	Serology	−	−	Dohm et al., 1996
Hyalomma marginatum	Larvae	*Erinaceus euroapeus*	*2* Sudarkina	SC, 10% SMB suspension	−	NBM	NA	NA	NA	NA	NA	Blagoveshchenskaya et al., 1975
	Larvae	Rabbits (Belgian Giant breed)	Russian strains	IV, 3.16 × 10^4 −3.16 × 10^5 LD_{50} (source: plasma and liver suspensions from infected animals)	+	NBM	+	Crushed nymphs and adults.	NBM	+	+	Levi and Vasilenko, 1972
	Larvae	*Hemiechinus auritus*	*2* Sudarkina	SC, 10% SMB suspension	+	NBM	+	Crushed nymphs *	NBM	NA	NA	Blagoveshchenskaya et al., 1975
	Larvae	*Lepus euroapeus* or *Hemiechinus auritus*	Russian strains	IV/IM, 5% SMB suspension (viremia confirmed in host)	+	NBM	+	Feeding nymphs, adults and F1 larvae on *Oryctolagus cuniculus, Cavia porcellus*	NBM	+	+	Zgurskaya et al., 1971
	Larvae	*Spermophilus citellus* or *Oryctolagus cuniculus*	*3* Sudarkina	SC, 10% SMB suspension	+	NBM	+	Feeding nymphs and adults on *Spermophilus citellus Oryctolagus cuniculus*	NBM	+	NA	Kondratenko, 1976
	Nymphs	*Mus musculus* (STAT1 KO mice)	*4* IbAr10200	IP, 100 PFU	+	RT-PCR	+	Crushed adults	RT-PCR	−	−	Gargili et al., 2013
	Nymphs	*Mus musculus* (STAT1 KO mice)	*4* IbAr10200	IP, 100 PFU	+	RT-PCR	+	Crushed adults	RT-PCR	−	+	Xia et al., 2016
Hyalomma rufipes	Larvae	*Tockus erythrorhynchus* (Red-billed hornbill)	*5* HD49199	IP, 3.2 × 10^1 LD_{50}	+	NBM Cell cultures	+	Feeding nymphs, adults and F1 larvae on	NBM Cell cultures ELISA	+	+	Zeller et al., 1994

Tick species	Stage Infected	Vertebrate host	Viral strain/passage	Infection of host: route/dose (resultant host viremia)‡	CCHFV detected in fed ticks/ method of detection		TS	Trans-stadial transmission checked on/to and method of detection		TO	Virus in the next generation	Reference
								Oryctolagus cuniculus				
	Larvae	*Lamprotornis nitens* (Glossy starling)	*5* HD49199	IP, 3.2 × 10^1 LD_{50}	−	NBM Cell cultures ELISA	+	Feeding nymphs on *Oryctolagus cuniculus*	NBM Cell cultures ELISA	NA	NA	Zeller et al., 1994
	Larvae	*Gallus domesticus* (domestic chicken)	*5* HD49199	IP, 3.2 × 10^3 LD_{50}	−	NBM Cell cultures ELISA	NA	NA	NA	NA	NA	Zeller et al., 1994
	Larvae	*Lepus saxatilis*	*6* SPU 4/81	SC, 1.0 × 10^6 −5.0 × 10^6 LD_{50} (>1.6 × 10^4 LD_{50}/mL)	+	NBM	−	Crushed nymphs	NBM IFA RPHI	NA	NA	Shepherd et al., 1991
	Nymphs	*Lepus saxatilis*	*6* SPU 4/81	SC, 1.0 × 10^6 −5.0 × 10^6 LD_{50} (>4.0 × 10^2 −5.0 × 10^2 LD_{50}/mL)	+	NBM	−	Crushed adults	NBM IFA RPHI	NA	NA	Shepherd et al., 1991
	Adult	*Bos taurus*	IbAn7620	SC or SC/ID, ≥ 2 × 10^8 LD_{50}	+	NBM	NA	NA	NA	NA	NA	Causey et al., 1970
	Adults	*Bos taurus*	*6* SPU 4/81	SC, 1.0 × 10^6 −5.0 × 10^6 LD_{50} (3.2 × 10^1 −5.0 × 10^2 LD_{50}/mL)	−	NBM IFA RPHI	NA	NA	NA	NA	NA	Shepherd et al., 1991
	Adults	*Ovis aries*	*6* SPU 4/81	SC, 1.0 × 10^6 −5.0 × 10^6 LD_{50} (3.2 × 10^2 −1.6 × 10^3 LD_{50}/mL)	−	NBM IFA RPHI	NA	NA	NA	NA	NA	Shepherd et al., 1991
Hyalomma truncatum	Larvae	*Cavia porcellus*	*6* SPU 4/81	IV, 1.0 × 10^6 −5.0 × 10^6 LD_{50} (7.9 × 10^1 −1.0 × 10^2 LD_{50}/mL)	−	NBM IFA RPHI	NA	NA	NA	NA	NA	Shepherd et al., 1991

Tick species	Stage Infected	Vertebrate host	Viral strain/passage	Infection of host: route/dose (resultant host viremia)‡	CCHFV detected in fed ticks/ method of detection		TS	Trans-stadial transmission checked on/to and method of detection		TO	Virus in the next generation	Reference
	Larvae	*Lepus saxatilis*	*6* SPU 4/81	SC, 1.0 × 10^6 −5.0 × 10^6 LD_{50} (>1.6 × 10^4 LD_{50}/mL)	+	NBM IFA RPHI	−	Crushed nymphs	NBM IFA RPHI	NA	NA	Shepherd et al., 1991
	Larvae	*Mystromys albicaudatus*	*6* SPU 4/81	SC, 1.0 × 10^6 −5.0 × 10^6 LD_{50} (7.9 × 10^1 −1.0 × 10^2 LD_{50}/mL)	−	NBM IFA RPHI	−	NA	NA	NA	NA	Shepherd et al., 1991
	Larvae	NBM	*7* IbAr10200	IP, 3.16 × 10^3 PFU (at 5 DPI: 2.0 × 10^2 −4.0 × 10^3 PFU/mL)	+	PRNT IFA	+	Feeding nymphs and adults on *Cavia porcellus*	PRNT IFA	−	NA	Logan et al., 1989 **
	Nymphs	*Lepus saxatilis*	*6* SPU 4/81	SC, 1.0 × 10^6 −5.0 × 10^6 LD_{50} (>4.0 × 10^2 −5.0 × 10^2 LD_{50}/mL)	+	NBM IFA RPHI	−	Adults, *Ovis aries*	NBM IFA RPHI	NA	NA	Shepherd et al., 1991
	Adults	*Bos taurus*	*6* SPU 4/81	SC, 1.0 × 10^6 −5.0 × 10^6 LD_{50} (3.2 × 10^1 −5.0 × 10^2 LD_{50}/mL)	+	NBM IFA RPHI	NA	NA	NA	NA	NA	Shepherd et al., 1991
	Adults	*Ovis aries*	*5* HD49199	IP, 3.2 × 10^5 LD_{50}	+	NBM Cell culture ELISA	NA	NA	NA	+	NA	Wilson et al., 1991

ELISA, enzyme linked immunosorbent assay; IFA, immunofluorescence assay; IM, intramuscularly; IP, intraperitoneal; IV, intravenous; LD_{50}, 50% lethal dose in NBM; NA, not applicable or not tested; NBM, newborn mice/virus isolation in NBM; PFU, plaque-forming unit; RPHI, reverse passive hemagglutination inhibition; RT-PCR, reverse transcription polymerase chain reaction; SC, subcutaneously; SMB, suckling mouse brain; TS, trans-stadial transmission; TO, transovarial transmission (eggs of the engorged females were tested for CCHFV presence, some studies also tested virus presence in the next generation of ticks); +, tested and found positive; −, tested and found negative.

Passage history:

1 SMB ×15;

[2] passage ×56–58;

[3] passage ×56;

[4] SMB ×13, Vero E6 ×1, SW-13 ×2;

[5] SMB ×3;

[6] cell culture ×2, SMB ×3–4;

[7] SMB ×4.

‡ Dose/viremia conversions were performed to the best of our ability for standardization based on reported data;

* This reference studied the infection acquired by feeding larvae and detected in nymphs that did not detach (two-hosts tick). It is unclear if the authors detected a true TS or a continued ingestion of the virus by the feeding nymphs;

** This is the only reference regarding *Hyalomma* ticks to address co-feeding transmission while feeding on infected animals; co-feeding transmission was detected.

Table 8

Summary of laboratory studies evaluating vector competence of non-*Hyalomma* ticks by feeding ticks on vertebrates experimentally inoculated with CCHFV.

Tick species	Stage Infected	Vertebrate host	Viral strain	Infection of host: route/dose (resultant viremia in host)‡	CCHFV detected in fed t icks/ method of detection	TS	Trans-stadial transmission tested on/to and method of detection	TO	Virus in the next generation	Reference
Amblyomma hebraeum	Larvae	*Cavia porcellus*	*1* SPU 4/81	IV, 1.0 × 10^6–a5.0 × 10^6 LD_{50} (7.9 × 10^1–1.0 × 10^2 LD_{50}/mL)	− NBM IFA RPHI	−	NA	NA	NA	NA	Shepherd et al., 1991
	Larvae	*Mystromys albicaudatus*	*1* SPU 4/81	SC, 1.0 × 10^6–5.0 × 10^6 LD_{50} (7.9 × 10^1–1.0 × 10^2 LD_{50}/mL)	− NBM IFA RPHI	−	NA	NA	NA	NA	Shepherd et al., 1991
	Larvae	*Ovis aries*	*1* SPU 4/81	SC, 1.0 × 10^6–5.0 × 10^6 LD_{50} (3.2 × 10^2–1.6 × 10^3 LD_{50}/mL)	− NBM IFA RPHI	−	NA	NA	NA	−	Shepherd et al., 1991
	Nymphs	*Ovis aries*	*1* SPU 4/81	SC, 1.0 × 10^6–5.0 × 10^6 LD_{50} (3.2 × 10^2–1.6 × 10^3 LD_{50}/mL)	− NBM IFA RPHI	−	NA	NA	NA	−	Shepherd et al., 1991
	Adults	*Bos taurus*	*1* SPU 4/81	SC, 1.0 × 10^6–5.0 × 10^6 LD_{50} (3.2 × 10^1–5.0 × 10^2 LD_{50}/mL)	+ NBM IFA RPHI	NA	NA	NA	NA	−	Shepherd et al., 1991

Tick species	Stage Infected	Vertebrate host	Viral strain	Infection of host: route/dose (resultant viremia in host)‡	CCHFV detected in fed t icks/ method of detection	TS	Trans-stadial transmission tested on/to and method of detection		TO	Virus in the next generation	Reference
	Adults	*Ovis aries*	*1* SPU 4/81	SC, 1.0 × 10^6–5.0 × 10^6 LD_{50} (3.2 × 10^2–1.6 × 10^3 LD_{50}/mL)	− NBM IFA RPHI	NA	NA	NA	−	−	Shepherd et al., 1991
Dermacentor marginatus	Larvae	*Erinaceus euroapeus*	*2* Sudarkina	SC, 10% SMB suspension	− NBM	−	Crushed nymphs	NBM	NA	NA	Blagoveshchenskaya et al., 1975
	Larvae	*Hemiechinus auritus*	*2* Sudarkina	SC, 10% SMB suspension	− NBM	−	Crushed nymphs	NBM	NA	NA	Blagoveshchenskaya et al., 1975
	Nymphs	*Spermophilus citellus* or *Oryctolagus cuniculus*	*3* Sudarkina	SC, 10% SMB suspension	+ NBM	+	Feeding adults on *Spermophilus citellus, Oryctolagus cuniculus*	NBM	+	NA	Kondratenko, 1976
	Adults	*Bos taurus*	*4* Sudarkina	SC, IM or SC/ID, × 1.9 × 10^7 LD_{50}	− NBM	NA	NA	NA	NA	NA	Zarubinsky et al., 1976
Ornithodoros sonrai	Nymphs	*Mus musculus*	*5* IbAr10200	IP, ~3.2 × 10^3 PFU (1.6 × 10^3 PFU/mL)	− NBM	NA	NA	NA	NA	NA	Durden et al., 1993
	Adults	*Mus musculus*	*5* IbAr10200	IP, ~3.2 × 10^3 PFU (1.6 × 10^3 PFU/mL)	− NBM	NA	NA	NA	−	−	Durden et al., 1993
Rhipicephalus decoloratus	Adults	*Bos taurus*	*1* SPU 4/81	SC, 1.0 × 10^6–5.0 × 10^6 LD_{50} (3.2 × 10^1–5.0 × 10^2 LD_{50}/mL)	+ NBM IFA RPHI	NA	NA	NA	NA	−	Shepherd et al., 1991
Rhipicephalus appendiculatus	Adults	*Bos taurus*	*1* SPU 4/81	SC, 1.0 × 10^6–5.0 ×	+ NBM IFA RPHI	NA	NA	NA	NA	−	Shepherd et al., 1991

Tick species	Stage Infected	Vertebrate host	Viral strain	Infection of host: route/dose (resultant viremia in host)‡	CCHFV detected in fed t icks/ method of detection	TS	Trans-stadial transmission tested on/to and method of detection	TO	Virus in the next generation	Reference
				10^6 LD_{50} (3.2×10^1–5.0×10^2 LD_{50}/mL)						
	Adults	*Ovis aries*	*1* SPU 4/81	SC, 1.0×10^6–5.0×10^6 LD_{50} (3.2×10^2–1.6×10^3 LD_{50}/mL)	− NBM IFA RPHI	NA	NA	NA	NA	−	Shepherd et al., 1991
Rhipicephalus e. evertsi	Larvae	*Lepus saxatilis*	*1* SPU 4/81	SC, 1.0×10^6–5.0×10^6 LD_{50}	− NBM IFA RPHI	−	Feeding nymphs on *Ovis aries*	NBM IFA RPHI	NA	−	Shepherd et al., 1991
	Larvae	*Ovis aries*	*1* SPU 4/81	SC, 1.0×10^6–5.0×10^6 LD_{50} (3.2×10^2–1.6×10^3 LD_{50}/mL)	− NBM IFA RPHI	−	Feeding nymphs on *Ovis aries*	NBM IFA RPHI	NA	−	Shepherd et al., 1991
	Nymphs	*Capra hircus*	*6* HD49199	IP, 1.58×10^8 LD_{50}	+ NBM IFA RPHI	+	Feeding adults on *Oryctolagus cuniculus*	NBM IFA RPHI	+	+	Faye et al., 1999b
	Nymphs	*Lepus saxatilis*	*1* SPU 4/81	SC, 1.0×10^6–5.0×10^6 LD_{50} (4.0×10^2–5.0×10^2 LD_{50}/mL)	− NBM IFA RPHI	−	Feeding nymphs on *Ovis aries*	NBM IFA RPHI	NA	−	Shepherd et al., 1991
	Nymphs	*Ovis aries*	*1* SPU 4/81	SC, 1.0×10^6–5.0×10^6 LD_{50} (3.2×10^2–1.6×10^3 LD_{50}/mL)	− NBM IFA RPHI	−	Feeding nymphs on *Ovis aries*	NBM IFA RPHI	NA	−	Shepherd et al., 1991

Tick species	Stage Infected	Vertebrate host	Viral strain	Infection of host: route/dose (resultant viremia in host)‡	CCHFV detected in fed t icks/ method of detection	TS	Trans-stadial transmission tested on/to and method of detection		TO	Virus in the next generation	Reference
	Adults	*Bos taurus*	*1* SPU 4/81	SC, 1.0 × 10^6–5.0 × 10^6 LD_{50} (3.2 × 10^1–5.0 × 10^2 LD_{50}/mL)	+ NBM IFA RPHI	NA	NA	NA	NA	−	Shepherd et al., 1991
	Adults	*Ovis aries*	*1* SPU 4/81	SC, 1.0 × 10^6–5.0 × 10^6 LD_{50} (3.2 × 10^2–1.6 × 10^3 LD_{50}/mL)	− NBM IFA RPHI	NA	NA	NA	NA	−	Shepherd et al., 1991
Rhipicephalus e. mimeticus	Larvae	*Cavia porcellus*	*1* SPU 4/81	IV, 1.0 × 10^6–5.0 × 10^6 LD_{50} (7.9 × 10^1–1.0 × 10^2 LD_{50}/mL)	− NBM IFA RPHI	−	Feeding nymphs on *Ovis aries*	NBM IFA RPHI	NA	−	Shepherd et al., 1991
	Larvae	*Mystromys albicaudatus*	*1* SPU 4/81	SC, 1.0 × 10^6–5.0 × 10^6 LD_{50} (7.9 × 10^1–1.0 × 10^2 LD_{50}/mL)	− NBM IFA RPHI	−	Feeding nymphs on *Ovis aries*	NBM IFA RPHI	NA	−	Shepherd et al., 1991
Rhipicephalus rossicus	Larvae	*Erinaceus euroapeus*	*2* Sudarkina	SC, 10% SMB suspension	− NBM	−	Crushed nymphs	NBM	NA	NA	Blagoveshchenskaya et al., 1975
	Larvae	*Hemiechinus auritus*	*2* Sudarkina	SC, 10% SMB suspension	− NBM	−	Crushed nymphs	NBM	NA	NA	Blagoveshchenskaya et al., 1975
	Nymphs	*Spermophilus citellus* or *Oryctolagus cuniculus*	*3* Sudarkina	SC, 10% SMB suspension	+ NBM	+	Feeding adults on *Spermophilus citellus, Oryctolagus cuniculus*	NBM	+	NA	Kondratenko, 1976

Tick species	Stage Infected	Vertebrate host	Viral strain	Infection of host: route/dose (resultant viremia in host)‡	CCHFV detected in fed t icks/ method of detection	TS	Trans-stadial transmission tested on/to and method of detection	TO	Virus in the next generation	Reference
	Adults	*Bos taurus*	[4] Sudarkina	SC, IM or SC/ID, × 1.9×10^7 LD_{50}	− NBM	NA	NA	NBM	NA	NA	Zarubinsky et al., 1976
Rhipicephalus simus	Larvae	*Ovis aries*	[1] SPU4/81	SC, 1.0×10^6–5.0×10^6 LD_{50} (3.2×10^2–1.6×10^3 LD_{50}/mL)	− NBM IFA RPHI	−	Feeding nymphs on *Ovis aries*	NBM IFA RPHI	NA	−	Shepherd et al., 1991
	Nymphs	*Ovis aries*	[1] SPU4/81	SC, 1.0×10^6–5.0×10^6 LD_{50} (3.2×10^2–1.6×10^3 LD_{50}/mL)	− NBM IFA RPHI	−	Feeding nymphs on *Ovis aries*	NBM IFA RPHI	NA	−	Shepherd et al., 1991
	Adults	*Ovis aries*	[1] SPU4/81	SC, 1.0×10^6–5.0×10^6 LD_{50} (3.2×10^2–1.6×10^3 LD_{50}/mL)	− NBM IFA RPHI	NA	NA	NBM IFA RPHI	NA	−	Shepherd et al., 1991

ELISA, enzyme linked immunosorbent assay; IFA, immunofluorescence assay; IM, intramuscularly; IP, intraperitoneal; IV, intravenous; LD_{50}, 50% lethal dose in NBM; NA, not applicable or not tested; NBM, newborn mice/virus isolation in NBM; PFU, plaque-forming unit; RPHI, reverse passive hemagglutination inhibition; RT-PCR, reverse transcription polymerase chain reaction; SC, subcutaneous; SMB, suckling mouse brain; TS, transstadial transmission; TO, transovarial transmission (eggs of the engorged females were tested for CCHFV presence). Some studies also tested the next generation of ticks for virus presence); +, tested and found positive; −, tested and found negative.

Passage history:

[1] cell culture ×2, SMB ×3–4;

[2] passage ×56–58;

[3] passage ×56;

[4] SMB ×42–49;

[5] SMB ×4;

[6] SMB ×4.

[‡] Dose/viremia conversions were performed to the best of our ability for standardization based on reported data.

Table 9

Laboratory studies evaluating vector competence of *Hyalomma* ticks for CCHFV using intracoelomic (IC) or intra-anal (IA) inoculation.

Tick species	Stage Infected	Viral strain/passage	Route/Dose‡	CCHFV in inoculated ticks/ method of detection		TS transmission/ method of detection		TO transmission/ method of detection		Virus in the next generation	Authors
Hyalomma dromedarii	Adults	[1] IbAr10200	IC, 4.0×10^2 PFU	+	PRNT IFAT	NA	NA	−	Feeding larvae on *Cavia porcellus*	−	Logan et al., 1990
Hyalomma impeltatum	Larvae	[2] IbAr10200	8.5×10^2 PFU (co-feeding adults) *	+	Serology PRNT	+	Feeding nymphs on *Cavia porcellus*	NA	NA	NA	Gordon et al., 1993 *
	Nymphs	[2] IbAr10200	8.5×10^2 PFU (co-feeding adults) *	+	Serology PRNT	+	Feeding adults on *Cavia porcellus*	NA	NA	NA	Gordon et al., 1993 *
	Adults	[1] IbAr10200	IC, 4.0×10^2 PFU	+	PRNT FAT	NA	NA	−	Feeding larvae on *Cavia porcellus*	−	Logan et al., 1990
Hyalomma rufipes	Larvae	[4] IbAn7620	IC, 3.2×10^0 -3.2×10^3 LD_{50}	+	NBM	+	Feeding nymphs on *Oryctolagus cuniculus*	NA	NA	NA	Okorie, 1991
	Nymphs	[3] IbAn7620	IC, 3.5 LD_{50}	NA	NA	+	NBM	NA	NA	NA	Okorie and Fabiyi, 1980
	Nymphs	IbAn7620	IC, 1.6×10^5 -5.0×10^5 LD_{50}	+	NBM	+	Feeding adults on *Bos taurus*	+	Feeding F1 larvae on *Bos taurus*	+	Lee and Kemp, 1970
	Nymphs	[4] IbAn7620	IC, 3.2×10^0 -3.2×10^3 LD_{50}	+	NBM	+	Feeding nymphs on *Oryctolagus cuniculus*	NA	NA	NA	Okorie, 1991
	Nymphs (engorged)	[5] SPU 4/81	IC, 5.0 LD_{50}	+	NBM Cell culture	+	Feeding adults on *Ovis aries*	−	Feeding F1 larvae on *Cavia porcellus*	−	Shepherd et al., 1989
	Adults	[4] IbAn7620	IC, 3.5 LD_{50}	+	NBM	−	NA	NA	NA	NA	Okorie and Fabiyi, 1980
	Adults	[4] IbAn7620	IC, 3.2×10^0 -3.2×10^3 LD_{50}	+	CF	NA	NA	NA	NA	NA	Okorie, 1991
	Adults	[3] HD49199	IC, 8.0×10^2 -1.1×10^3 LD_{50}	NA	NBM PCR IFA ELISA	+	Feeding F1 larvae, nymphs and adults on	+	Feeding larvae, nymphs and adults of the F1 on	+	Faye et al., 1999a

Tick species	Stage Infected	Viral strain/passage	Route/Dose‡	CCHFV in inoculated ticks/ method of detection		TS transmission/ method of detection		TO transmission/ method of detection		Virus in the next generation	Authors
							Oryctolagus cuniculus		*Oryctolagus cuniculus*		
Hyalomma truncatum	Larvae	[2] IbAr10200	8.5×10^2 PFU (co-feeding adults) *	+	Serology PRNT	+	Feeding nymphs on *Cavia porcellus*	NA	NA	NA	Gordon et al., 1993 *
	Nymphs	[2] IbAr10200	8.5×10^2 PFU (co-feeding adults) *	+	Serology PRNT	+	Feeding adults on *Cavia porcellus*	NA	NA	NA	Gordon et al., 1993 *
	Nymphs (engorged)	[5] SPU 4/81	IC, 5 LD_{50}	+	NBM Cell culture	+	Feeding adults on *Ovis aries*	−	Feeding F1 larvae on *Cavia porcellus*	−	Shepherd et al., 1989
	Adults	IbAr10200	IC, ~3.2×10^1 PFU	+	Plaque assay PRNT	NA	NA	NA	NA	NA	Dickson and Turell, 1992
	Adults	[4] HD49199	IA, 2.21×10^3 LD_{50}	+	NBM AC-ELISA IFA	NA	NA	NA	NA	NA	González et al., 1991
	Adults	[4] HD49199	IA, 6.32×10^2 LD_{50}	+	NBM ELISA	NA	NA	+	Feeding F1 larvae on *Oryctolagus cuniculus*	NA	González et al., 1992 *
	Adults	[6] IbAr10200	IC, 1 PFU	+	PRNT IFAT	NA	NA	−	Feeding F1 larvae on *Cavia porcellus*	−	Logan et al., 1990
	Adults	[5] HD49199	IC, 8.0×10^2 -1.1×10^3 LD_{50}	NA	NBM PCR IFA ELISA	+	Feeding larvae, nymohs and adults of the F1 on *Oryctolagus cuniculus*	+	Feeding F1 larvae, nymphs and adults on *Oryctolagus cuniculus*	+	Faye et al., 1999a

ELISA, enzyme linked immunosorbent assay; AC-ELISA, antigen-capture ELISA; IFA, immunofluorescence assay; IFAT, immunofluorescence antibody test; LD_{50}, 50% lethal dose in NBM; NA, not applicable or not tested; NBM, newborn mice/virus isolation in NBM; PRNT, plaque-reduction neutralization test; SMB, suckling mouse brain; TS, transstadial; TO, transovarial (tested for virus in eggs from engorged females); +, tested and found positive; −, tested and found negative.

Passage history:

[1] SMB ×14;

[2] SMB ×18;

[3] Local isolate from cattle, SMB ×8–9;

[4] SMB ×3;

[5] cell culture ×2, SMB ×3;

[6] SMB ×14, duck cell ×1;

[*] Co-feeding on host (with non-detectable viremia) with adults that had been infected by intracoelomic or intra-anal inoculation with reported dose;

[‡] Dose conversions were performed to the best of our ability for standardization based on reported data.

Table 10

Laboratory studies evaluating vector competence of non-*Hyalomma* ticks for CCHFV using intracoelomic (IC) or intra-anal (IA) inoculation. The reference marked with an asterisk corresponds to a compilation of "unpublished results" reported by Linthicum et al. without date or further details about co-authors.

Tick species	Stage Infected	Viral strain/passage Route/dose‡		CCHFV in inoculated ticks/method of detection		TS transmission/ method of detection		TO transmission/ method of detection		Virus in the next generation	Authors
Amblyomma hebraeum	Nymphs (engorged)	*1* SPU 4/81	IC, 5.0 LD_{50}	+	NBM Cell culture	+	Feeding adults on *Ovis aries*	−	Feeding F1 larvae on *Cavia porcellus*	NA	Shepherd et al., 1989
Amblyomma variegatum	Larvae	*2* IbAn7620	IC, 3.2×10^0–3.2×10^3 LD_{50}	+	NBM	+	Feeding nymphs on *Oryctolagus cuniculus*	NA	NA	NA	Okorie, 1991
	Nymphs	*2* IbAn7620	IC, 3.2×10^0–3.2×10^3 LD_{50}	+	NBM	+	Feeding adults on *Oryctolagus cuniculus*	NA	NA	NA	Okorie, 1991
	Adults	*2* IbAn7620	IC, 3.2×10^0–3.2×10^3 LD_{50}	+	CF	NA	NA	NA	NA	NA	Okorie, 1991
	Adults	*3* HD49199	IC, 8.0×10^2–1.1×10^3 LD_{50}	NA	NBM PCR IFA ELISA	+	Feeding F1 larvae, nymphs and adults on *Oryctolagus cuniculus*	+	Feeding F1 larvae, nymphs and adults on *Oryctolagus cuniculus*	+	Faye et al., 1999a
Amblyomma walkerae	Adults	*1* SPU 4/81	IC, 5.0 LD_{50}	1 day after feeding	NBM Cell culture	NA	NA	NA	NA	NA	Shepherd et al., 1989
Ornithodoros p. porcinus	Nymphs	*1* SPU 4/81	IC, 5.0 LD_{50}	−	NBM Cell culture	NA	NA	NA	NA	NA	Shepherd et al., 1989
	Adults	*1* SPU 4/81	IC, 5.0 LD_{50}	−	NBM Cell culture	NA	NA	NA	NA	NA	Shepherd et al., 1989
Ornithodoros savignyi	Adults	*1* SPU 4/81	IC, 5.0 LD_{50}	1 day after feeding	NBM Cell culture	NA	NA	NA	NA	NA	Shepherd et al., 1989
Rhipicephalus appendiculatus	Adults	*4* IbAr10200	IC, 1 PFU	+	PRNT IFAT	NA	NA	−	Feeding F1 larvae on *Cavia porcellus*	−	Logan et al., 1990

Tick species	Stage Infected	Viral strain/passage Route/dose‡	CCHFV in inoculated ticks/method of detection		TS transmission/ method of detection		TO transmission/ method of detection		Virus in the next generation	Authors
Rhipicephalus e. evertsi	Adults	[5] HD49199 IC or IA, ≤1.1 × 10^3 LD_{50}	NA	NBM PCR IFA ELISA	+	Feeding F1 larvae, nymphs and adults on *Oryctolagus cuniculus*	+	Feeding F1 larvae, nymphs and adults on *Oryctolagus cuniculus*	+	Faye et al., 1999b
Rhipicephalus e. mimeticus	Nymphs (engorged)	[1] SPU 4/81 IC, 5.0 LD_{50}	+	NBM Cell culture	+	Feeding adults on *Ovis aries*	−	Feeding F1 larvae on *Cavia porcellus*	−	Shepherd et al., 1989
Rhipicephalus pulchellus	Adults	NR IC, dose not reported	+	NR	NR	NR	NR	NR	NR	Linthicum and Bailey, 1994*

ELISA, enzyme linked immunosorbent assay; AC-ELISA, antigen-capture ELISA; IFA, immunofluorescence assay; IFAT, immunofluorescence antibody test; LD_{50}, 50% lethal dose in NBM; NA, not applicable or not tested; NBM, newborn mice/virus isolation in NBM; NR, not reported; PRNT, plaque-reduction neutralization test; TS, transstadial; TO, transovarial (tested for virus in eggs from engorged females); +, tested and found positive; −, tested and found negative.

Passage history:

[1] cell culture ×2, SMB ×3;

[2] Local isolate from cattle, SMB ×8–9;

[3] SMB ×3;

[4] SMB ×14, duck cell ×1;

[5] SMB ×4.

‡ Dose conversions were performed to the best of our ability for standardization based on reported data.