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Molecular Ecology of Crimean-Congo Haemorrhagic Fever Virus in Western Palaearctic

PH.D. DISSERTATION THESIS

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Declaration

I, Seyma Celina, hereby declare that I have written the enclosed PhD thesis entitled "Molecular Ecology of Crimean-Congo Haemorrhagic Fever Virus in Western Palaearctic" independently and in collaboration with co-authors in the respective scientific articles related to this work. All the texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to the Citation rules of the FTA. I state that the work has not been submitted for any other degree to this or any other university within and outside the Czech Republic.

In Prague, 2nd of September, 2025

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Seyma Celina

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"Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less."

Marie Curie

This dissertation would not have been possible without the support, guidance, and encouragement of many incredible people, to whom I am deeply grateful. Throughout this journey, I have been lucky to be surrounded by those who have supported me both professionally and personally—especially during the challenges, moments of self-doubt, and the balancing of multiple responsibilities.

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To Hera - this work is dedicated to you..

Abstract

This thesis focuses on fundamental research in the rapidly evolving field of ecology, particularly molecular and computational ecology, and the coevolution between vectors and pathogens. Research on the ecology of *Hyalomma marginatum* remains a neglected topic in tick biology compared to other tick species, despite its significance as a primary vector of Crimean-Congo Haemorrhagic Fever Virus (CCHFV). Understanding the ecological, genetic, and environmental factors driving *H. marginatum* distribution and its ability to transmit pathogens is essential for mitigating emerging health risks.

Through six interrelated studies, this thesis investigates the distribution, genetic adaptation, and host-vector-virus interactions of *H. marginatum* and CCHFV across the Western Palaearctic. By integrating molecular, serological, ecological, and computational approaches, the thesis provides insights into how environmental and evolutionary factors shape vector-pathogen dynamics. In the context of ongoing climate change and increasing global animal movements, these findings contribute to a broader understanding of how ecological shifts may influence the emergence and spread of *Hyalomma*-borne diseases.

The results emphasize the importance of a "One Health" approach that integrates environmental, veterinary, and public health perspectives to improve surveillance, predictive modeling, and vector control strategies. This research enhances our knowledge of *H. marginatum* as a vector and the mechanisms driving its role in pathogen transmission, offering critical insights for the prevention and management of *Hyalomma*-borne diseases in a changing world.

Keywords: *Hyalomma marginatum*; Crimean-Congo Haemorrhagic Fever Virus; molecular ecology, genetic adaptation; ecological niche modeling; One Health.

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List of Abbreaviations

AA: Amino Acid

AHFV: Alkhurma Haemorrhagic Fever Virus

BSL-4: Biosafety Level 4

CAI: Codon Adaptation Index

CCHF: Crimean-Congo Haemorrhagic Fever

CCHFV: Crimean-Congo Haemorrhagic Fever Virus

COA: Correspondence Analysis

CTFV: Colorado Tick Fever Virus

dN/dS: Ratio of Non-Synonymous to Synonymous Substitutions

DNA: Deoxyribonucleic Acid

ECDC: European Centre for Disease Prevention and Control

ELISA: Enzyme-Linked Immunosorbent Assay

ENC: Effective Number of Codons

ENM: Ecological Niche Modeling

FIR: Filial Infection Rate

GIS: Geographic Information System

Gn: Glycoprotein N

Gc: Glycoprotein C

HGA: Human Granulocytic Anaplasmosis

HME: Human Monocytic Ehrlichiosis

JMTV: Jingmen Tick Virus

KFDV: Kyasanur Forest Disease Virus

LIV: Louping Ill Virus

MCMC: Markov Chain Monte Carlo

MOP: Mobility-Oriented Parity

MSF: Mediterranean Spotted Fever

OHFV: Omsk Haemorrhagic Fever Virus

PCR: Polymerase Chain Reaction

POWV: Powassan Virus

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

qRT-PCR: Quantitative Reverse Transcription Polymerase Chain Reaction

RSCU: Relative Synonymous Codon Usage

RdRp: RNA-Dependent RNA Polymerase

RNA: Ribonucleic Acid

TBEV: Tick-Borne Encephalitis Virus

TEM: Transmission Electron Micrograph

THOV: Thogoto Virus

TOIR: Transovarial Infection Rate

tRNA: Transfer RNA

WHO: World Health Organization

CHAPTER 1

General Introduction

1. Introduction

Crimean-Congo Hemorrhagic Fever Virus (CCHFV), now taxonomically classified as *Orthonairovirus haemorrhagiae*, belongs to the genus *Orthonairovirus* within the family *Nairoviridae* (Postler & Kuhn, 2021). CCHFV is responsible for Crimean-Congo Haemorrhagic Fever (CCHF), a disease characterized by high fatality rates and severe socioeconomic impacts. Despite extensive research efforts aimed at understanding the virus, many aspects of its ecology, and evolution remain underexplored. Recent decades have witnessed substantial advancements in understanding CCHFV's genomic diversity, viral-host interactions, and the ecology of its primary tick vector, *Hyalomma marginatum* (Bente et al., 2013; Ergönül, 2006).

Nonetheless, the molecular mechanisms underlying the coevolution between CCHFV and its tick vectors remain underemphasized. This knowledge gap hinders the development of targeted control strategies and predictive models for the virus's geographic spread. Understanding these evolutionary processes is crucial for mitigating the risk of emerging zoonotic outbreaks and for advancing public health preparedness. Factors such as climate change, migratory bird pathways, and human-mediated livestock movements further complicate the dynamics of CCHFV transmission and necessitate a multidisciplinary approach.

This work is centered on the ecological and molecular evolution of CCHFV and its primary tick vector, *H. marginatum*. It integrates original research findings within the broader context of current knowledge, aiming to uncover critical insights into viral adaptation, vector-host interactions, and potential future distribution patterns of the virus and its vector.

1.1. Ticks

Ticks are obligate blood-feeding ectoparasites belonging to the phylum *Arthropoda*, class *Arachnida*, and order *Acarina*. They are grouped into three families according to evolutionary history: Ixodidae (hard ticks, comprising 722 species), Argasidae (soft ticks, comprising 208 species), and the monotypic family Nuttalliellidae, which includes a single species, *Nuttalliella namaqua* (Nava et al., 2017). These arthropods are widely distributed across diverse climatic zones, from tropical regions to subarctic areas (Anderson & Magnarelli, 2008). The highest species diversity is observed in tropical and subtropical regions, where favorable environmental conditions support their development and host availability. Ticks parasitize a wide range of hosts, including mammals, birds, reptiles, and amphibians (Sonenshine & Roe, 2015).

The family Ixodidae (hard ticks) is distinguished by the presence of a hard dorsal shield, known as the scutum, and represents the most ecologically significant and diverse group. It includes several important genera such as *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, and *Rhipicephalus* (Horak et al., 2002a). In contrast, the family Argasidae (soft ticks) lacks a scutum and encompasses four primary genera: *Argas*, *Carios*, *Ornithodoros*, and *Otobius*. These species are often nidicolous, inhabiting sheltered environments such as burrows or nests. Finally, the family Nuttalliellidae is represented by a single species, *Nuttalliella namaqua*, which exhibits a combination of traits from both Ixodidae and Argasidae, making it a unique and taxonomically distinct group.

1.2. Life Cycle of Ticks

The tick life cycle is characterized by three primary developmental stages: larva, nymph, and adult. The duration of each stage, host preference, and feeding behavior vary significantly among species. Larvae hatch as six-legged forms from eggs laid by females, and after feeding on a host's blood, they molt into eight-legged nymphs. Following another blood meal, nymphs develop into adult males or females. Female ticks require a final blood meal for reproductive success, after which they detach and lay eggs in suitable environments such as soil or vegetation. The number of eggs laid varies widely depending on the species and blood intake, ranging from 200 to 15,000 eggs. Unlike hard ticks (Ixodidae), which die after oviposition, soft ticks (Argasidae) can survive and reproduce multiple times, laying 12–70 eggs per blood meal.

The developmental duration of each stage is influenced by species-specific factors and environmental conditions, such as temperature and humidity. Larvae typically become active and seek hosts within 7–22 days after hatching. While Argasidae species feed multiple times during each life stage, Ixodidae species feed only once per stage, reflecting differences in their feeding strategies and ecological roles (Anderson & Magnarelli, 2008).

The life cycle duration varies among species and is strongly influenced by climate. Species in temperate regions may complete their life cycle within a year, while those in colder climates may require 3–4 years. Temperature plays a crucial role in regulating the developmental process; larvae and nymphs feed for 3–6 days, while adults may feed for up to two weeks under optimal conditions (Estrada-Peña & de la Fuente, 2014).

Ticks are classified into three categories based on their host relationships:

- One-host ticks: Complete all developmental stages on a single host (e.g., *Dermacentor nitens, Rhipicephalus annulatus, Rhipicephalus microplus*).
- Two-host ticks: Larvae and nymphs feed on one host, while adults feed on another (e.g., *Hyalomma dromedarii, Hyalomma marginatum, Hyalomma rufipes, Hyalomma truncatum, Rhipicephalus evertsi evertsi*).
- Three-host ticks: Each stage requires a different host (e.g., *Amblyomma americanum, Amblyomma variegatum, Dermacentor andersoni, Dermacentor variabilis, Ixodes ricinus, Ixodes scapularis).*

Hard ticks can feed on their hosts for up to 10 days, although the feeding duration is shorter for larvae (2–3 days) and longer for some adult species (Sonenshine & Roe, 2015).

Ticks primarily reproduce sexually, although parthenogenesis has been observed in some Ixodidae species. Mating in hard ticks occurs during blood feeding, whereas in soft ticks, it occurs post-feeding.

1.3. Ecology of Ticks

The ecology of ticks is primarily influenced by two critical factors: the physical environment and host availability. These factors determine the habitats where ticks can thrive and sustain their life cycles. During key life stages such as molting and host-seeking, ticks face significant mortality risks, including desiccation, starvation, and freezing. Additionally, they are susceptible to predation by small mammals, and other arthropods, as well as infections from pathogens such as fungi. These environmental pressures limit the range of habitats suitable for their survival. Many tick species have developed specialized adaptations to suppress the immune responses of their hosts, facilitating prolonged feeding. Consequently, the habitats preferred by their hosts play a pivotal role in shaping the distribution and abundance of tick populations (Estrada-Peña et al., 2014).

Ticks exhibit two distinct ecological behaviors: nidicolous (nest-dependent) and non-nidicolous (free-ranging). Nidicolous ticks, also referred to as "domestic ticks," reside in sheltered habitats such as rodent burrows, bird nests, poultry coops, or crevices in barn walls. These species are highly sensitive to environmental fluctuations and depend heavily on the microhabitat conditions of their hosts' shelters. In contrast, non-nidicolous ticks, or "pasture ticks," occupy open environments, including forests, shrublands, grasslands, steppes, and deserts, where they actively seek hosts. Most members of the Ixodidae family fall into this category and have adapted to a wide range of climatic conditions (Estrada-Peña & de la Fuente, 2014).

The abundance and seasonal activity of ticks are shaped by factors such as microclimatic conditions, host availability, and host suitability (Belozerov, 1982; Tälleklint-Eisen & Lane, 2000). The temperature and humidity gradients between the ground and vegetation layers, where tick-host interactions occur, are especially critical. In temperate regions, prolonged high temperatures during summer can increase mortality during molting and host-seeking stages. Similarly, subzero temperatures in winter can elevate mortality rates. However, persistent snow cover during winter provides insulation, protecting ticks from extreme cold and enhancing their overwintering survival (Estrada-Peña & de la Fuente, 2014).

Host-seeking behavior in ticks typically involves climbing vegetation to either ambush or actively pursue potential hosts. This process begins with locating a strategic position on vegetation, often termed an ambush site, to maximize host encounters (Estrada-Peña, Ruiz-Fons, et al., 2013). In habitats with high densities of both hosts and ticks, the probability of successful host contact increases, reducing mortality rates (Barnard, 1991). Numerous experimental studies have demonstrated that climatic conditions, particularly temperature and humidity, significantly influence tick host-seeking behavior (Gray, 2008; Ogden et al., 2006; Perret et al., 2000, 2003, 2004).

Additionally, photoperiod serves as a critical ecological cue, regulating a wide range of biological activities in ticks, including host-seeking, feeding, molting, and reproductive development (Estrada-Peña, Gray, et al., 2013). It governs seasonal rhythms by triggering transitions such as diapause onset in autumn and reactivation of questing in spring (Belozerov, 1982; Gray et al., 2016). In species like *I. ricinus*, decreasing daylight induces behavioral diapause in immature stages, while increasing daylight in spring stimulates host-seeking activity (Lees & Milne, 1951; Perret et al., 2004). These circadian and seasonal responses ensure synchronization with favorable environmental conditions and host availability. Questing behavior also follows a circadian pattern entrained by the light–dark cycle. For instance, adult *I. scapularis* display a bimodal questing rhythm in fall under short-day conditions, with peaks occurring shortly after lights-on and lights-off, whereas winter and early spring individuals exhibit predominantly nocturnal activity with minimal movement during daylight hours (Madden & Madden, 2005). Similarly, field studies of *I. ricinus* in Hungary revealed a seasonal shift in daily questing timing—from post-sunrise activity peaks in spring and early summer, to primarily nocturnal activity in late summer, and an equal distribution of day and night questing in October (Zöldi et al., 2013). These patterns suggest that both seasonal photoperiod and host availability (e.g., nocturnal rodents) help shape daily activity rhythms. Although many tick species retreat from vegetation at night to reduce desiccation risk, nocturnal host-seeking remains ecologically significant (Perret et al., 2004). For example, adult *I. scapularis* have been collected in comparable numbers during nighttime, potentially explaining infestations on nocturnal or resting hosts such as lizards and rodents (Durden et al., 1996). Thus, photoperiod provides a reliable environmental signal that coordinates both seasonal and diel patterns in tick behavior, enhancing their survival and host contact opportunities across varying ecological contexts (Erguler et al., 2025).

Ticks and their hosts share a coevolutionary relationship that spans approximately 400 million years. This prolonged association has driven host adaptation mechanisms, enabling ticks to exploit specific host characteristics. While some species exhibit strict host specificity, most are opportunistic feeders with broad host ranges. Host specificity tends to be more pronounced in adult stages compared to larvae and nymphs. Nidicolous ticks typically remain near the shelters of their hosts (e.g., nests or burrows), while non-nidicolous ticks must actively seek hosts in open environments to survive.

Host-seeking strategies vary between species. Passive host-seeking, employed by genera such as *Ixodes*, *Dermacentor*, and *Rhipicephalus*, involves a behavior known as questing—ticks climb vegetation and extend their front legs to detect passing hosts. In contrast, active host-seeking behavior, notably observed in *Hyalomma* species, is more dynamic and involves purposeful movement toward a potential host over longer distances.

Hyalomma marginatum, in particular, is renowned for its robust active host-seeking behavior. This species utilizes a sophisticated integration of thermal, chemical, mechanical, and possibly visual cues to locate hosts. Ticks detect thermal radiation emitted by warm-blooded hosts using specialized sensilla located in the Haller's organ on the first pair of legs. This organ contains infrared-sensitive receptors capable of detecting minute temperature gradients, allowing *H. marginatum* to perceive hosts from several meters away (Leonovich, 2004; Valcárcel et al., 2020). Chemically, ticks respond to host-emitted volatiles such as carbon dioxide (CO_2), ammonia, and short-chain carboxylic acids, including lactic acid and various fatty acids (Bezerra-Santos et al., 2024). These compounds are commonly found in human sweat and skin secretions, where they may be further modified by skin-dwelling bacteria into volatile substances that enhance host detection. CO_2 in particular serves as a long-distance attractant (Städele, 2024) and may trigger an upwind walking response in *Hyalomma* species (Valcárcel et al., 2020). Mechanical cues—such as ground vibrations generated by host movement—can also be sensed, possibly via subgenual organs in the legs, although this has been more clearly demonstrated in insects (Virant-Doberlet et al., 2023). There is also emerging evidence that some tick species, including *Hyalomma*, may employ rudimentary visual cues to detect host contrast or movement, particularly in open habitats with high light levels (Leonovich, 1986; Kaltenrieder et al., 1989; Faraone, 2022). Regarding detection distance, while most ticks respond to hosts within a range of 1–3 meters, *H. marginatum* has been observed to initiate pursuit from distances up to 9 meters under optimal field conditions (Latif & Walker, 2004; Romanenko, 2005). This distance may vary depending on environmental factors such as vegetation structure, temperature, wind, and host size (ECDC).

Habitat fragmentation significantly impacts tick populations, host availability, and pathogen transmission dynamics. Such fragmentation may result from gradual natural processes that alter the physical environment or rapid anthropogenic land-use changes. These disruptions can influence the abundance and spatial distribution of ticks and their associated pathogens, ultimately affecting disease transmission rates (Rosenzweig, 1995).

1.4. Ticks as Vectors

Ticks are recognized as one of the most effective vectors for transmitting a wide array of pathogens, including bacteria, helminths, protozoa, and viruses, making them a significant concern for public and veterinary health worldwide (Jongejan & Uilenberg, 2004). For a tick species to qualify as a vector, it must meet the following criteria (Gargili et al., 2017):

- Feed on an infected vertebrate host.
- Acquire the pathogen during feeding.
- Retain the pathogen through molting stages.
- Transmit the pathogen to uninfected hosts.

Simply detecting pathogen nucleic acids or serological markers in ticks or hosts does not confirm vector competence or reservoir status. Laboratory-based studies are necessary to establish whether a tick species can facilitate pathogen transmission. Epidemiological metrics, such as vector capacity (the potential to carry pathogens) and vector competence (the ability to transmit pathogens), are crucial for assessing the role of ticks in disease ecology (Gargili et al., 2017). These metrics are influenced by temporal and spatial factors, highlighting the complexity of vector-host interactions.

Ticks serve as vectors for numerous infectious agents, including:

Bacterial Pathogens:

- *Anaplasma marginale*: The causative agent of bovine anaplasmosis, which results in severe anemia and significant economic losses in livestock (Kocan et al., 2004). It is transmitted by *Dermacentor* and *Rhipicephalus* ticks and through mechanical means like contaminated instruments (de la Fuente et al., 2006).
- *Anaplasma phagocytophilum*: An obligate intracellular bacterium responsible for human granulocytic anaplasmosis (HGA), a zoonotic disease that primarily affects humans. It can also cause anaplasmosis in domestic animals and wildlife species. The pathogen is mainly transmitted by ticks of the *Ixodes* genus in North America and Europe (Dumler et al., 2005; Stuen et al., 2013).

- *Anaplasma platys*: The agent of canine cyclic thrombocytopenia, causing periodic decreases in platelet count in dogs. It is transmitted by *Rhipicephalus sanguineus* (Dantas-Torres, 2010).
- *Bartonella* spp.*:* Includes pathogens associated with febrile illnesses, endocarditis, and vasculitis in humans and animals (Breitschwerdt, 2014). Though ticks like *Ixodes* and *Rhipicephalus* are suspected vectors, transmission pathways remain under investigation (Chomel & Kasten, 2010).
- *Borrelia burgdorferi:* The primary agent of borreliosis, a multisystemic disease complex often referred to as Lyme disease. It is transmitted by *Ixodes ricinus* in Europe and *Ixodes scapularis* in North America (Steere et al., 2004; Stanek et al., 2012).
- *Borrelia miyamotoi*: A relapsing fever spirochete transmitted by *Ixodes* ticks, causing recurrent fever due to immune evasion (Platonov et al., 2011). Symptoms include fever, headache, fatigue, and potential neurological involvement (Krause et al., 2013). Other *Borrelia* species, such as *B. hermsii* and *B. turicatae*, also cause relapsing fever and pose a health risk in tick-endemic regions (Dworkin et al., 2002).
- *Coxiella burnetii:* The causative agent of Q fever, which can infect humans and livestock. It is transmitted by ticks such as *Dermacentor* and *Rhipicephalus* or via aerosolized environmental exposure (Celina & Cerny, 2022).
- *Ehrlichia chaffeensis*: Causes human monocytic ehrlichiosis (HME) and is primarily transmitted by *Amblyomma americanum* (Ewing et al., 1995).
- *Ehrlichia ewingii*: Infects humans and dogs, causing febrile illness, and is also spread by *Amblyomma americanum* (Bullock et al., 1999).
- *Ehrlichia canis*: Causes canine monocytic ehrlichiosis, transmitted by *Rhipicephalus sanguineus* (Dantas-Torres, 2008).
- *Francisella tularensis:* The pathogen behind tularemia, a zoonotic disease affecting both humans and animals (Ellis et al., 2002). It is transmitted by ticks like *Dermacentor* and *Amblyomma* species, as well as through direct contact with infected animals (Sykes et al., 2021).

- *Rickettsia aeschlimannii:* A spotted fever group pathogen primarily associated with *Hyalomma* ticks, known to cause febrile illnesses in humans (Beati et al., 1997; Parola et al. 2013).
- *Rickettsia conorii:* The causative agent of Mediterranean spotted fever, predominantly transmitted by *Rhipicephalus sanguineus* in the Mediterranean Basin (Raoult & Roux, 1997).
- *Rickettsia sibirica* subsp. *mongolitimonae*: A subspecies of *R. sibirica*, known to cause lymphangitis-associated rickettsiosis, often transmitted by *Dermacentor* ticks (Fournier et al., 2006; Faccini-Martínez et al., 2014).
- *Rickettsia raoultii:* A spotted fever group pathogen linked to tick-borne lymphadenopathy and transmitted by *Dermacentor* species (Mediannikov et al., 2004; Mediannikov et al., 2008).
- *Rickettsia rickettsii:* The etiological agent of Rocky Mountain spotted fever, primarily transmitted by *Dermacentor variabilis* and *Rhipicephalus sanguineus* (Demma et al., 2007; Harris et el., 2017).

Protozoan Pathogens:

- *Babesia bigemina*: Causes bovine babesiosis, also known as Texas cattle fever, and is transmitted by *Rhipicephalus annulatus* and *Rhipicephalus microplus* (de Castro, 1997; Bock et al., 2004).
- *Babesia bovis:* Another causative agent of bovine babesiosis, it induces severe anemia and high mortality in cattle. Transmission occurs via *Rhipicephalus microplus* (Delano et al., 2002; Bock et al., 2004).
- *Babesia divergens:* Affects humans in Europe, causing severe hemolytic anemia. It is transmitted by *I. ricinus* and is particularly dangerous for splenectomized individuals (Gray et al., 2013).
- *Babesia microti:* The agent of human babesiosis, a malaria-like illness presenting with fever, hemolytic anemia, and fatigue. It is transmitted by *I. scapularis* in the United States (Homer et al., 2000; Vannier & Krause, 2012).

- *Cytauxzoon felis:* A protozoan pathogen causing cytauxzoonosis in domestic cats. It is a rapidly fatal disease characterized by fever, anorexia, and jaundice. The vectors include *Amblyomma americanum* and *Dermacentor variabilis* in the United States (Meinkoth & Kocan, 2005; Reichard et al., 2009).
- *Theileria annulata:* Causes tropical theileriosis in cattle, leading to anemia and lymphoid hyperplasia. It is transmitted by *Hyalomma anatolicum* and is prevalent in North Africa, the Middle East, and parts of Asia (Darghouth et al., 1996; Gharbi et al., 2006).
- *Theileria equi:* A protozoan pathogen responsible for equine piroplasmosis, which causes fever, anemia, and jaundice in horses. It is transmitted by *Dermacentor* and *Rhipicephalus* species (Wise et al., 2013; Secorun Borges et al., 2014).
- *Theileria parva:* The causative agent of East Coast fever, a highly fatal disease in cattle, transmitted by *Rhipicephalus appendiculatus* in sub-Saharan Africa (Morrison et al., 2020).

Viral Pathogens:

- *Alkhurma Haemorrhagic Fever Virus (AHFV):* A member of the *Flavivirus* genus, this virus causes haemorrhagic fever in humans, with symptoms ranging from mild fever to severe bleeding and organ failure. It is transmitted by *Hyalomma* species and is primarily found in the Arabian Peninsula (Srivastava et al., 2024).
- *Crimean-Congo Haemorrhagic Fever Virus (CCHFV):* Belonging to the *Orthonairovirus* genus, this virus causes a severe haemorrhagic fever with high mortality rates. It is transmitted by *Hyalomma marginatum* and other *Hyalomma* species across Africa, Asia, and Europe (Celina et al., 2025).
- *Colorado Tick Fever Virus (CTFV):* A member of the *Coltivirus* genus, CTFV causes a self-limiting febrile illness in humans, characterized by fever, headache, and myalgia. It is transmitted by *Dermacentor andersoni* in the western United States and Canada (Meagher & Decker, 2012).
- *Jingmen Tick Virus (JMTV):* A novel segmented RNA virus genetically related to flaviviruses, identified in various tick genera, including *Amblyomma*, *Dermacentor*,

Haemaphysalis, *Hyalomma*, *Ixodes*, and *Rhipicephalus*. Strains such as JMTV and Alongshan virus have been linked to human febrile illnesses, indicating potential zoonotic risks (Wang et al., 2019; Wu et al., 2023).

- *Kyasanur Forest Disease Virus (KFDV):* A *Flavivirus* causing high fever, severe headache, haemorrhages, and neurological symptoms. It is transmitted by *Haemaphysalis spinigera* in South Asia, primarily in India (Odend'hal, 1983).
- *Louping Ill Virus (LIV):* A *Flavivirus* affecting sheep and occasionally humans, LIV causes neurological symptoms, ataxia, and encephalitis. It is transmitted by *I. ricinus* in Europe, particularly the British Isles (Jeffries et al., 2014).
- *Omsk Haemorrhagic Fever Virus (OHFV):* A *Flavivirus* closely related to tick-borne encephalitis virus (TBEV), causing haemorrhagic fever with neurological complications. It is transmitted by *Dermacentor* and *Ixodes* ticks in Siberia (Diani et al., 2025).
- *Powassan Virus (POWV):* Another member of the *Flavivirus* genus, POWV causes encephalitis with symptoms such as fever, headache, and neurological deficits. It is transmitted by *I. scapularis* and *Ixodes cookei* in North America (Hicar et al., 2011).
- *Tick-Borne Encephalitis Virus (TBEV):* A *Flavivirus* that causes tick-borne encephalitis (TBE), a severe neurological disease presenting with fever, meningitis, or encephalitis. It is transmitted by *I. ricinus* in Europe and *Ixodes persulcatus* in Asia (Michelitsch et al., 2019).
- *Thogoto Virus (THOV):* A member of the *Orthomyxoviridae* family, THOV causes febrile illnesses in humans and animals. It is transmitted by *Rhipicephalus* and *Amblyomma* ticks in Africa and parts of Europe.

Pathogen transmission within ticks occurs through several internal mechanisms:

- Transstadial transmission (stage-to-stage): Pathogens persist across developmental stages (larva, nymph, and adult) within the tick, ensuring their continuity and eventual transmission to a new host during subsequent feedings.
- Transovarial transmission (female-to-egg, also called vertical): Infected female ticks pass the pathogen to their offspring through eggs, ensuring its persistence within tick

populations across generations. This vertical transmission facilitates the survival and spread of pathogens even in the absence of infected vertebrate hosts.

- Venereal transmission (tick-to-tick): Transmission can occur between mating ticks, allowing the spread of pathogens without the involvement of a vertebrate host.
- Co-feeding transmission: Infected and uninfected ticks feeding simultaneously on a host (even without systemic infection in the host) can exchange pathogens through local skin interactions.

The predominance of transmission routes among tick-borne pathogens varies considerably by tick species, pathogen clade, and geographic region. Transstadial transmission is the primary mode for many pathogens, particularly among *Ixodes* species. For instance, *Borrelia burgdorferi* and *B. miyamotoi* are maintained via transstadial transmission in *I. ricinus* and *I. scapularis* in Europe and North America, respectively (Humair & Gern, 2000; Han et al., 2019). Likewise, *Coxiella burnetii* shows transstadial persistence in *Hyalomma lusitanicum* in Spain (González et al., 2020).

In contrast, transovarial transmission dominates in some *Rhipicephalus* and *Dermacentor* species. Notably, *Babesia* spp. of the sensu stricto clade (clade VI), including *B. bovis*, *B. bigemina*, and *B. caballi*, are vertically transmitted by *Rhipicephalus microplus* and *R. annulatus* in Asia, Africa, and South America (Battsetseg et al., 2002; Hembram et al., 2022). Similarly, *Babesia canis* is maintained transovarially in *D. reticulatus*, with reports from central and eastern Europe. However, both the transovarial infection rate (TOIR) and filial infection rate (FIR) can vary significantly, even within the same tick-pathogen system across regions. Several examples highlight regional variation in transmission dynamics for the same vector-pathogen pair. For instance, *Theileria orientalis* is transmitted by different tick species in different countries—*Haemaphysalis longicornis* in Asia and Oceania, *Rhipicephalus microplus* in Vietnam and India, and *Ha. punctata* in France—with transovarial transmission reported only in *R. microplus* in India (Kakati et al., 2015). Similarly, *Theileria equi* is typically transmitted transstadially but has been shown to undergo transovarial transmission in *R. microplus* in Brazil and *Ha. longicornis* in Japan (Battsetseg et al., 2002; Ikadai et al., 2007), indicating regional differences in vertical competence. *Anaplasma marginale*, another widespread pathogen, is predominantly transmitted transstadially and intrastadially by *Rhipicephalus* and *Dermacentor* species, but vertical transmission has been confirmed only in *R. microplus* and *R. annulatus* populations in India and Mexico, and not in other regions where these ticks are prevalent (Kumar et al., 2019; Amaro Estrada et al., 2020; Hembram et al., 2022). In the case of *H. marginatum*, transstadial transmission is the dominant mechanism for maintaining CCHFV (Bente et al., 2013; Celina et al., 2025). Although experimental studies have demonstrated transovarial transmission in *Hyalomma* spp., its efficiency appears lower compared to transstadial persistence (Gargili et al., 2017). Such transmission mode may vary with ecological and host density conditions, but comparative field data on regional variation are limited.

Lastly, co-feeding transmission—the exchange of pathogens among ticks feeding simultaneously on the same host without systemic infection—has been observed in various tick-borne viruses and bacteria, especially in nymphs and larvae, and is more likely under high tick densities, suggesting ecological modulation of this mechanism.

1.5. *Hyalomma marginatum* Koch, 1844

1.5.1. Taxonomy of *Hyalomma marginatum*

The taxonomy of *Hyalomma marginatum* Koch, 1844, has undergone significant revisions and presents challenges due to the complexity of identifying species within the subgenus *Euhyalomma* Filippova, 1944. This subgenus comprises approximately 20 species, which are grouped based on their phylogenetic relationships. Among these, the *Hyalomma (Euhyalomma) marginatum* complex is considered the most taxonomically problematic.

Initially, *H. marginatum* was classified as a complex of four subspecies: *Hyalomma marginatum marginatum*, *Hyalomma marginatum rufipes*, *Hyalomma marginatum turanicum*, and *Hyalomma marginatum isaaci*, based on morphological and geographic distinctions within the group. In 2008, Apanaskevich and Horak re-evaluated this classification and elevated *H. rufipes*, *H. turanicum*, and *H. isaaci* to full species status (Apanaskevich & Horak, 2008). These revisions redefined the *Hyalomma marginatum* sensu lato (s.l.) complex, which now comprises five species: *Hyalomma marginatum* (sensu stricto), *Hyalomma rufipes*, *Hyalomma turanicum*, *Hyalomma isaaci*, and *Hyalomma glabrum*.

Among these species, *H. marginatum* exhibits significant polymorphism and the broadest distribution, encompassing much of the genus's geographic range. Its presence spans Southern

Europe, Anatolia, the Arabian Peninsula, Central and Southeast Asia, and Africa (Apanaskevich & Horak, 2008). The initial description of *H. marginatum* was based on a male specimen collected in Italy (Koch, 1844); however, this type specimen was not referenced again until 1901 (Neumann, 1896). Subsequent studies by Schulze and Schlottke (1930) described numerous subspecies (Schulze & Schlottke, 1930), many of which were later synonymized with *H. marginatum*, including *Hyalomma transcaucasicum*, *Hyalomma steineri codinai*, and *Hyalomma marginatum balconicum* (Apanaskevich & Horak, 2008).

1.5.2. Ecology and Distribution of *Hyalomma marginatum*

Hyalomma marginatum is ecologically versatile, thriving in warm, arid climates characterized by prolonged dry periods and moderate humidity. It predominantly inhabits Mediterranean climates but has expanded into Central and Northern Europe. Climate change and migratory birds transporting immature ticks have facilitated this expansion, with occurrence records reported in Hungary and Germany, and occasional sightings in the United Kingdom and Scandinavia (Chitimia-Dobler et al., 2019; Földvári et al., 2022; Grandi et al., 2020; McGinley et al., 2021).

In the Northern Hemisphere, *H. marginatum* typically becomes active in spring (April–May) as temperatures rise, with pre-adult stages active between May and September. Adults overwinter in an unfed state and become active when temperatures exceed 12 °C. Larvae and nymphs generally activate at 14–16 °C (Petney et al., 2011). Studies suggest that development is supported within a temperature range of 22–27 °C. Laboratory observations have demonstrated that larvae can tolerate moderate to high humidity levels, with 75% relative humidity frequently cited as optimal for development. Interestingly, even at 90% humidity, one study reported that 77.8% of larvae developed normally, indicating a broader humidity tolerance (Buczek, 2000). Despite this adaptability, *H. marginatum* is notably adapted to drier environments compared to other ixodid species (Estrada-Peña et al., 2004; Randolph and Rogers, 2000).

Unfed adults seeking hosts remain active on the ground at temperatures below 27°C. At higher temperatures (air >30°C, soil >45°C), they retreat to shade or burrow into the soil. Females that detach after feeding cannot oviposit if the daily temperature average is below 16°C. Nymphs exhibit resilience, molting under extreme conditions ranging from 7–42°C and 0–100% relative humidity (Ouhelli, 1994; Petney et al., 2011).

Among ticks, *Hyalomma* species are particularly drought-tolerant, thriving in steppes and deserts (Anderson & Magnarelli, 2008).

1.5.3. Life Cycle of *Hyalomma marginatum*

Hyalomma marginatum follows a two-host life cycle (***Figure 1***). Adults preferentially parasitize wild and domestic ungulates, while larvae and nymphs target ground-dwelling birds and small mammals.

Unlike most tick species that rely on passive host-seeking strategies, *Hyalomma* species actively track their hosts over distances of several hundred meters (Balashov, 1972). This behavior is thought to have evolved in arid environments, where passive strategies are less effective (Hoogstraal, 1979). Active movement significantly increases the likelihood of unfed ticks locating a host.

Records of immature *H. marginatum* in regions such as western Africa (Nigeria) and northern Europe (e.g., Finland, Norway, Sweden) are associated with migratory birds transporting ticks across large distances (Apanaskevich & Horak, 2008; Jaenson et al., 1994).

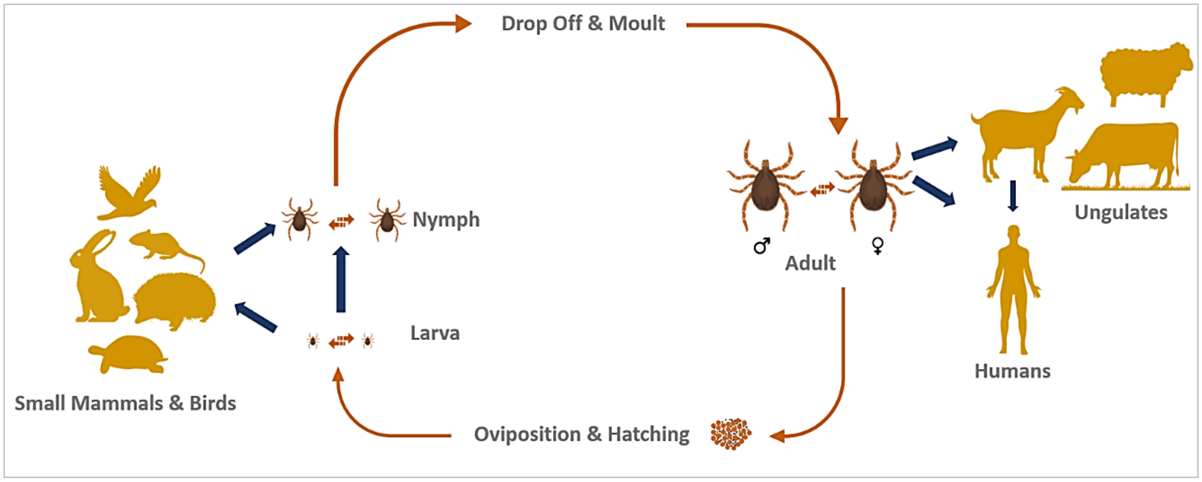


Figure 1. Life cycle of *Hyalomma* spp. ticks as two-host ticks.

1.5.4. Pathogen Transmission by *Hyalomma marginatum*

Hyalomma marginatum is widely distributed across the Mediterranean (southern Europe and North Africa), the Balkans, and continues eastward into West and Central Asia—including southern Russia, Ukraine, Turkey, Pakistan, and Turkmenistan—and serves as the primary vector of CCHFV within its distribution range. Additionally, it transmits a variety of pathogens responsible for diseases such as *Rickettsia aeschlimannii* (spotted fever) (Demoncheaux et al., 2012; Pereira et al., 2018; Rumer et al., 2011a), *Rickettsia sibirica* subsp. *mongolitimonae* (mongolicosis) (Fernández de Mera et al., 2013; Keskin et al., 2016), *Rickettsia raoultii* (tick-borne lymphadenopathy) (Pereira et al., 2018), *Coxiella burnetii* (Q fever) (Mancini et al., 2019; Psaroulaki et al., 2006), *Ehrlichia* spp. (ehrlichiosis) (Cicculli et al., 2020; Cicculli, Masse, Capai, de Lamballerie, et al., 2019; Mancini et al., 2019), *Anaplasma marginale* (bovine anaplasmosis) (De La Fuente et al., 2004), *Babesia* spp. (babesiosis) (Ionita et al., 2013; Iori et al., 2010), *Theileria annulata* and *Theileria equi* (tropical theileriosis and equine piroplasmosis, respectively) (Georges et al., 2001; Iori et al., 2010), and *Borrelia* spp. (relapsing fever) (Baptista et al., 2004; Rollins et al., 2021; Toma et al., 2014).

1.5.5. Crimean-Congo Haemorrhagic Fever Orthonairovirus (CCHFV)

The CCHFV has spherical shape with a diameter of approximately 80-100 nm (***Figure 2***). It is an enveloped, negative-sense, single-stranded RNA virus belonging to the genus *Orthonairovirus* within the family *Nairoviridae*. It is one of the most geographically widespread tick-borne viruses, with a range extending from China and South Asia through the Middle East, southeastern Europe, and Africa (Bente et al., 2013). CCHFV has three-segmented genome denoted by their size with different functions: S (small) segment encodes nucleoprotein (N), M (medium) segment the glycoproteins (Gn and Gc), and L (large) segment RNA-dependent polymerases (RdRp).

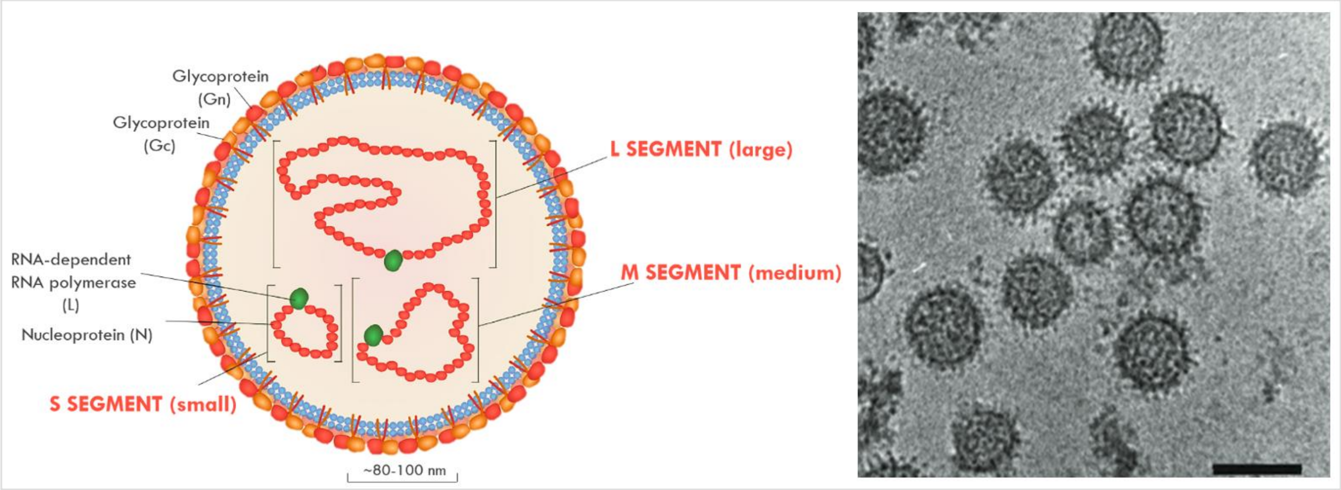


Figure 2. CCHFV virion structure. Diagrammatic representation of CCHFV virion in cross-section. The surface spikes comprise two glycoproteins termed Gn and Gc. CCHFV genome is composed of three RNA segments (L, large; M, medium; S, small) encapsidated by N protein and associated with RNA-dependent polymerases (left) (Bente et al. 2013). Transmission electron micrograph (TEM) of CCHFV particles is shown (right). Scale bar represents 100 nm (International Committee on Taxonomy of Viruses 2011).

Phylogenetic studies identified eight distinct clades of CCHFV: Africa 1–3, Europe 1–3, and Asia 1–2. These clades are characterized by their geographic distribution, genetic sequence variations, and differences in pathogenic potential (Anagnostou & Papa, 2009). Furthermore, genome segment reassortment and the migration of viral lineages across vast distances demonstrate the dynamic and complex evolutionary processes driving CCHFV's genetic diversity (Hewson et al., 2004a).

1.5.6. Geographical Distribution and Epidemiology of CCHF

CCHFV causes Crimean-Congo Haemorrhagic Fever (CCHF), a zoonotic disease with high fatality rates (10%–40%) (Mertens et al., 2013). While the virus circulates asymptomatically in ticks and various vertebrate hosts, human infections often lead to severe haemorrhagic symptoms.

CCHF has a predominantly Old World distribution, spanning Africa, Asia, and Europe (***Figure 3***). The virus has been reported across a wide range of regions, including Africa (e.g., Burkina Faso, Democratic Republic of the Congo, Kenya, Mauritania, Republic of the Congo, Senegal, South Africa, Sudan, Tanzania, and Uganda), Asia (e.g., Afghanistan, Armenia, China, Georgia, India, Iran, Iraq, Kazakhstan, Kuwait, Kyrgyzstan, Oman, Pakistan, Russia, Saudi Arabia, Tajikistan, Türkiye, Turkmenistan, United Arab Emirates, and Uzbekistan), and Europe (e.g., Albania, Bulgaria, Greece, Hungary, Kosovo, North Macedonia, Portugal, Russia, Serbia, Spain, Türkiye, and Ukraine) (Al-Abri et al., 2017; Ergönül, 2006; Hoogstraal, 1979; Pigott et al., 2017; Zé-Zé et al., 2024).

Despite its extensive geographical presence, the exact annual incidence and frequency of CCHF cases remain uncertain due to the absence of efficient surveillance systems and the limited availability of reliable diagnostic assays in many regions. As a result, the virus may circulate undetected within animal reservoirs and tick vectors, while sporadic human infections may go unreported or misdiagnosed, particularly in areas with limited clinical awareness or overlapping symptoms with other febrile illnesses.

The actual number of CCHF cases is likely underestimated, as outbreaks often occur in remote areas with limited healthcare access (Saijo et al., 2010). In the Northern Hemisphere, infections peak during spring and early summer, aligning with heightened tick activity and viral amplification in animal reservoirs.

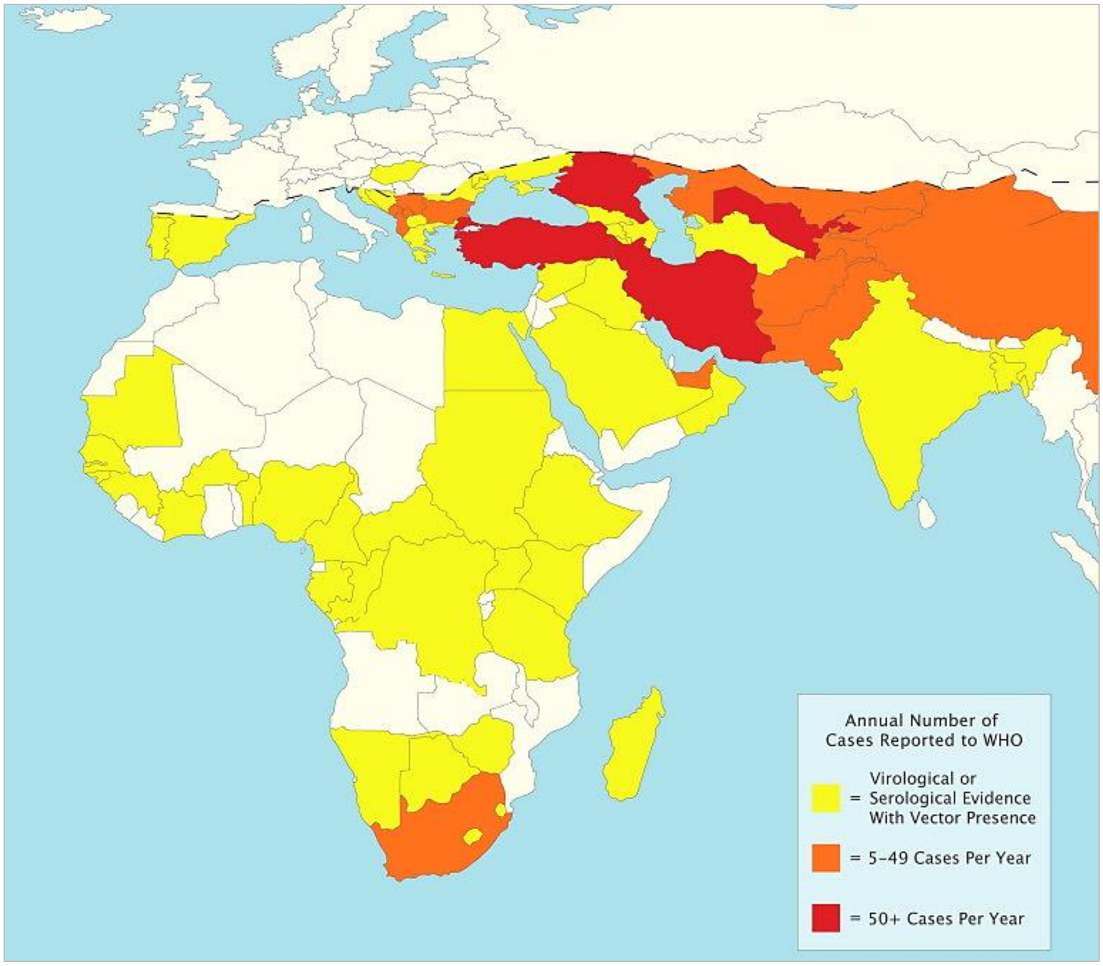


Figure 3. Geographic distribution of CCHF and annual number of reported cases of CCHF by country. Countries in red report more than 50 human cases annually to the WHO. Countries in orange report fewer than 50 cases. Countries in yellow have not reported human cases but there is serological or virological evidence of CCHFV with its vector presence (Bente et al., 2013).

Outbreak intensity in CCHF-endemic regions is influenced by host community composition, particularly the relative abundance of large mammals. *Hyalomma marginatum* exhibits a marked preference for large-bodied hosts, especially in its adult stage, with domestic livestock such as cattle, sheep, and goats serving as primary blood sources (Hoogstraal, 1979; Estrada-Peña et al., 2013). When large hosts are abundant, ticks preferentially feed on them, thereby reducing human exposure. However, in scenarios where livestock populations decline—as historically observed during the 1944 Crimea outbreak—ticks may increasingly target alternative hosts, including humans, particularly in landscapes with elevated densities of small mammals (e.g., hares, rabbits, hedgehogs) that support immature tick stages (Hoogstraal, 1979; Bente et al., 2013). This host-switching dynamic results in higher human-tick contact rates.

The increased risk to humans in such contexts is closely linked to the active host-tracking behavior of *H. marginatum*. Unlike nidicolous or passive questing ticks, *H. marginatum* adults are non-nidicolous and exhibit a highly mobile, target-oriented strategy. This includes the ability to detect and pursue warm-blooded hosts using a combination of thermal, chemical, mechanical, and possibly visual cues over distances up to 9 meters (Leonovich, 2004; Valcárcel et al., 2020; Latif & Walker, 2004). Thermal gradients sensed via Haller's organ, along with CO_2 and volatile organic compounds from host secretions, guide long-range orientation (Carr & Salgado; 2019; Bezerra-Santos et al., 2024), while visual and mechanical cues enhance precision tracking, particularly in open habitats. In the absence of preferred large hosts, this tracking flexibility enables ticks to locate and feed on suboptimal hosts—including humans—thereby amplifying transmission risk (ECDC).

1.5.7. Transmission of CCHF

Transmission of CCHFV occurs through the bite of ticks belonging to the *Hyalomma* genus. These ticks feed on a variety of domestic ruminants, including sheep, goats, and cattle, as well as wild animals such as hares, rabbits, hedgehogs, certain rodents, and ostriches. Unlike other tick species, *Hyalomma* ticks are natural reservoirs of CCHFV and can remain infected for several years, enabling the sustained transmission of the virus. In contrast, mammals serve as transient hosts, developing short-term viremia that does not contribute significantly to long-term viral maintenance.

In humans, who are considered dead-end hosts, infection primarily occurs through tick bites or direct contact with blood or tissues of infected livestock or individuals. Additional possible transmission routes include mother-to-child transfer, sexual contact, blood transfusion, and exposure to body fluids from infected patients or animals (Bente et al., 2013; Pshenichnaya et al., 2017).

1.5.8. Clinical Manifestations, Diagnosis and Prevention

Infection with CCHFV progresses through four distinct phases: the incubation phase (non-symptomatic), followed by the pre-haemorrhagic, haemorrhagic, and convalescent phases (symptomatic) (***Figure 4***). The incubation period typically spans 3–7 days post-infection and remains asymptomatic (Ergönül, 2006). Subsequently, the disease enters the pre-haemorrhagic phase, which usually lasts 4–5 days and is marked by an acute onset of symptoms. Common manifestations during this phase include high fever, severe headaches, abdominal pain, myalgia, hypotension, and facial flushing (Ergönül, 2006).

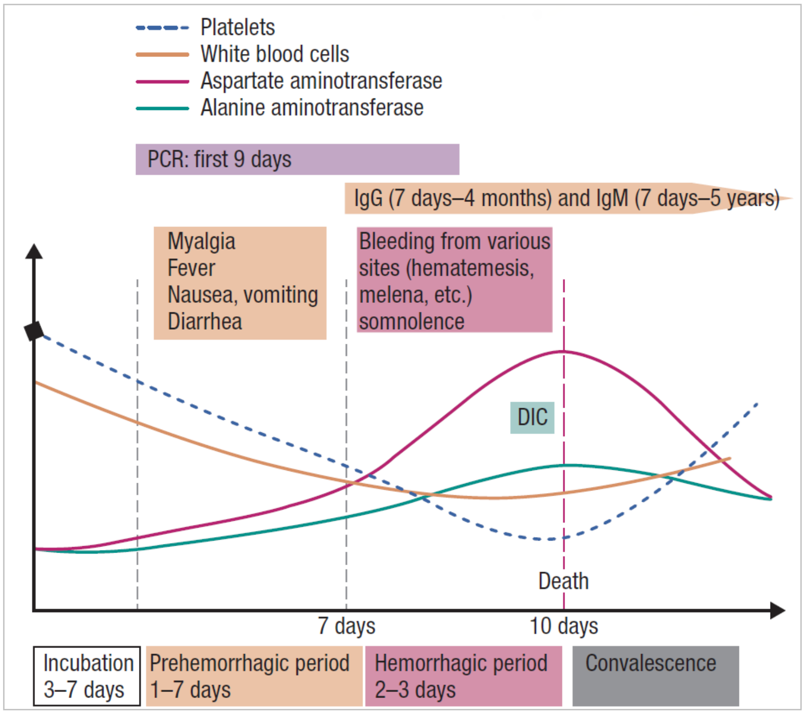


Figure 4. The clinical course of CCHF. The starting point is the entrance of the CCHFV to the human through tick bite or a contact with infected material such as body fluids (Endy 2020).

As the disease advances to the haemorrhagic phase, more severe clinical symptoms emerge, such as petechiae (small red spots on the skin caused by bleeding), ecchymosis (bruising due to blood extravasation), epistaxis (nosebleeds), gum bleeding, and emesis (Ergönül, 2006). Other potential complications include gastrointestinal disturbances, such as nausea and diarrhea, as well as neuropsychiatric and cardiovascular abnormalities. In the absence of effective medical intervention, the disease can lead to multiorgan failure, often resulting in fatal outcomes.

The classification of CCHFV as a World Health Organization (WHO) Risk Group 4 pathogen significantly complicates laboratory diagnostics due to the stringent biosafety requirements involved. Current diagnostic approaches include enzyme-linked immunosorbent assay (ELISA), real-time polymerase chain reaction (qPCR), antigen detection, serum neutralization assays, and viral isolation through cell culture (Vanhomwegen et al., 2012). Among these, qPCR has emerged as a sensitive and specific method for CCHFV detection. However, its application is limited in many rural and resource-constrained regions where the virus is endemic, creating diagnostic bottlenecks in such areas.

Preventing CCHFV infection relies on avoiding tick habitats, using repellents, wearing protective clothing, and adopting safety measures in workplaces like hospitals and slaughterhouses. Public awareness campaigns are crucial for educating at-risk populations on transmission modes and effective prevention strategies.

1.6. Coevolution between *Hyalomma marginatum* and CCHFV

Viruses are obligate intracellular parasites that depend entirely on the host's translational machinery, including ribosomes and transfer RNAs (tRNAs), for replication and protein synthesis. This dependency imposes strong evolutionary pressures on viruses to adopt codon preferences aligned with those of their hosts, ensuring efficient interaction with host cellular machinery. These host-virus interactions play an important role in shaping viral evolution and adaptation.

The CCHFV genome consists of three negative-sense, single-stranded RNA segments categorized by length as small (S), medium (M), and large (L). These segments encode essential viral proteins: the nucleocapsid protein (N), glycoproteins (Gn and Gc), and RNA-dependent RNA polymerase, respectively. Primarily transmitted by *Hyalomma* ticks, other genera like *Dermacentor* and *Rhipicephalus* also play a role. Understanding CCHFV's coevolution with its vectors is essential to uncovering its genetic adaptations for survival and transmission.

Due to the degeneracy of the genetic code, most amino acids are encoded by multiple codons, known as synonymous codons. Each species exhibits unique preferences for specific synonymous codons, leading to variation in codon frequencies across genes (Plotkin et al., 2006; Plotkin & Kudla, 2011). This phenomenon, termed codon usage bias, reflects the differential usage of synonymous codons and is influenced by natural selection and mutational pressures. Key factors associated with codon usage bias include gene expression levels, guanine-cytosine (GC) content, GC skew, amino acid conservation, protein hydropathy, and transcriptional selection (Chen & Chen, 2014; Ermolaeva, 2001; Knight et al., 2001; Quax et al., 2015).

Codon usage bias provides valuable insights into the evolutionary processes driving viral adaptation to host environments. The extent of this bias reveals patterns of selection and mutational pressures, shedding light on how viral-host coevolution influences replication efficiency, virulence, protein synthesis, and viral survival. For CCHFV, codon usage analysis unravels the complex interplay between the virus and its arthropod vectors, highlighting the evolutionary strategies employed by the virus to maintain fitness in diverse hosts.

2. The Aims of the Thesis

The geographic expansion of *H. marginatum* and its role in transmitting CCHFV present significant challenges to public and veterinary health. This thesis addresses critical knowledge gaps by focusing on the ecological, genetic, and epidemiological dynamics of *H. marginatum* and CCHFV, and aims to support improved monitoring, control measures, and public awareness. To achieve this aim, the following objectives were included:

1. To review the geographical distribution and pathogen diversity of *H. marginatum* in Europe to identify regions at risk for *Hyalomma*-borne diseases and associated public health risks.
2. To develop a precise habitat suitability model using current climatic data to identify regions in Transalpine Europe capable of supporting stable populations of *H. marginatum*.

3. To extend the habitat suitability model to incorporate future climatic data, predicting potential regions for *H. marginatum* establishment under various future climate scenarios.
4. To evaluate the prevalence of CCHFV in *H. marginatum* ticks collected from livestock and to assess cattle exposure in Kosovo, a CCHFV-endemic country.
5. To investigate the genetic drivers of coevolution between *H. marginatum* and CCHFV to gain insights into their evolutionary interactions.
6. To compile and analyze data on CCHFV tick vectors and vertebrate hosts to clarify their roles in virus transmission and provide insights into CCHFV ecology and its impact on animal health.

3. Chapter Overview

This thesis includes six research studies, of which four have been published, one is under review, and one is currently in preparation. The research outputs are interconnected and aim to enhance our understanding of CCHFV ecology, its transmission dynamics, and the role of *H. marginatum* in facilitating its spread, as presented in Chapters 2–6.

- **Chapter 2** provides a comprehensive review of the geographical distribution of *H. marginatum* and its associated pathogen diversity in Europe. This chapter consolidates current knowledge on the tick's prevalence, identifies regions at risk for *Hyalomma*-borne diseases, and highlights the public health risks associated with this species.
- **Chapter 3** develops a habitat suitability model for *H. marginatum* in Transalpine Europe, using current and future climatic data to assess its distribution and predict shifts under climate change. The findings highlight environmental factors driving its spread and the implications for CCHFV transmission.
- **Chapter 4** presents molecular and serological surveillance of CCHFV in Kosovo, a known CCHFV-endemic region, based on tick testing and cattle serology. The detection of viral RNA in ticks and antibodies in cattle highlights ongoing virus exposure and reinforces the need for integrated surveillance strategies that encompass

both animal and vector populations within a One Health framework.

- **Chapter 5** explores the coevolution between *H. marginatum* and CCHFV through codon usage analysis, highlighting the virus's genomic adaptations to its tick vectors. By analyzing codon usage bias, this chapter reveals the molecular processes driving CCHFV evolution and its ability to optimize replication and protein synthesis within tick host environments. These findings contribute to our understanding of host-virus interactions.

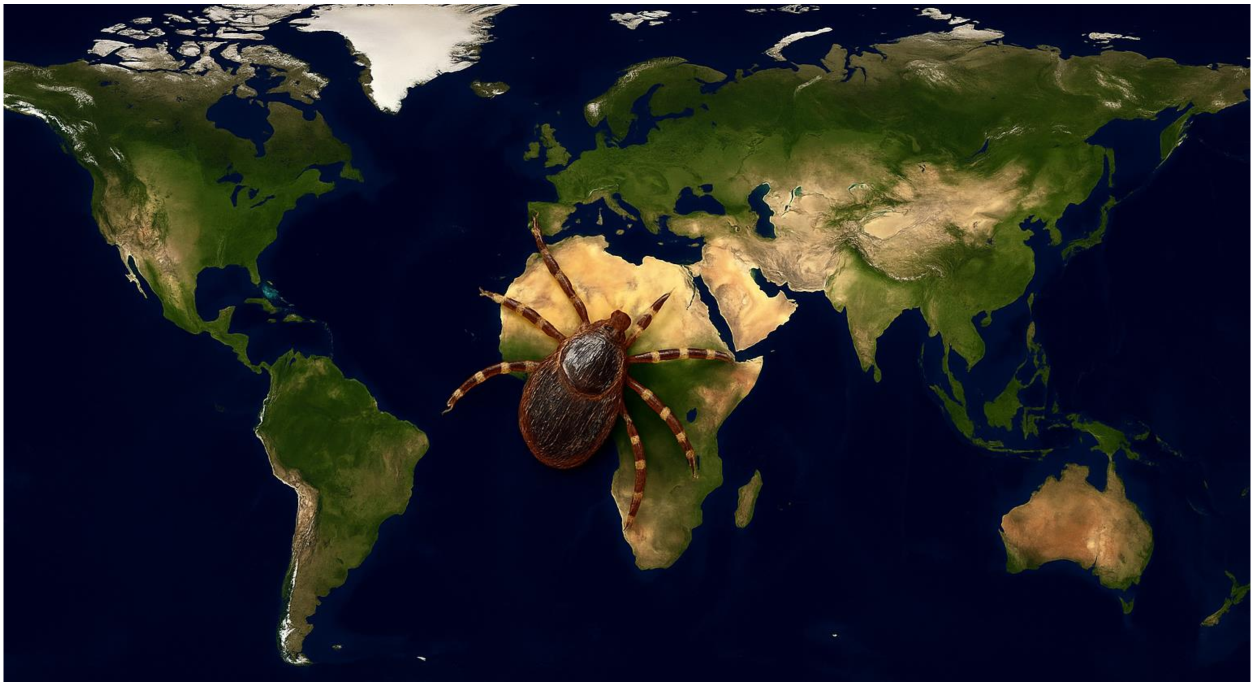
- **Chapter 6** compiles and analyzes data on CCHFV tick vectors and vertebrate hosts, clarifying their roles in virus transmission. It provides insights into the ecological dynamics of CCHFV and its impact on animal health, supporting improved surveillance and public health strategies.

- Finally, **Chapters 7** and **8** present the overall discussion and conclusions, synthesizing the research findings, highlighting their implications for CCHFV surveillance, vector control, and public health strategies in the Western Palearctic, and providing recommendations for future research to address the evolving risks posed by climate change, vector expansion, and zoonotic disease emergence.

CHAPTER 2:

This chapter is a literal copy of the published article.

Hyalomma marginatum in Europe: The Past, Current Status and Future Challenges – A Systematic Review



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Systematic Review

Hyalomma marginatum in Europe: The Past, Current Status and Future Challenges – A Systematic Review

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Abstract

Hyalomma marginatum is a prominent tick vector responsible for transmitting various pathogens, including the Crimean-Congo Haemorrhagic Fever Virus (CCHFV), across Europe. This systematic review consolidates findings from 144 publications regarding the geographical distribution of *H. marginatum* and its associated pathogens. Significant populations have been identified primarily in southern Europe and Balkan peninsula, indicating a concerning trend. Additionally, climate change and migratory bird movements may facilitate its further dispersal, potentially leading to the establishment of *H. marginatum* in transalpine regions. Consequently, robust monitoring and surveillance strategies are essential to mitigate the public health and livestock threats posed by *Hyalomma*-borne diseases. Raising awareness and implementing preventive measures will be crucial in addressing the challenges associated with this tick vector.

4. Introduction

Ticks (Acari: Ixodidae) are the most significant vectors of arthropod-borne diseases in Europe, transmitting numerous pathogens to humans, livestock, and wildlife (Jongejan & Uilenberg, 2004; Magnarelli, 2009). The genus *Hyalomma* are two-host species, where both subadult developmental stages feed on the same host, typically small mammals such as rodents, hares, or ground-feeding birds. In contrast, adult ticks prefer to feed on larger mammals including humans (Sonenshine & Roe, 2015). However, some species, such as *H. aegyptium*, exhibit three-host cycles, with larvae, nymphs, and adults utilizing different hosts at each stage. Large domestic mammals play a crucial role in maintaining high tick loads and increase the risk of exposure to *Hyalomma*-borne pathogens due to their proximity to agricultural workers by crushing of engorged ticks through or via contact with infected blood during slaughter (Chinikar et al., 2010; Mustafa et al., 2011; Sargianou et al., 2013). Migratory birds serve both as a blood meal source for unadult *Hyalomma* ticks and plus the introduce these ticks into new geographic regions (Hoogstraal, 1979).

Hyalomma marginatum, commonly known as the "Mediterranean *Hyalomma*," has historically been referred to as *Hyalomma plumbeum* in some Russian and Eastern European literature. However, *Hyalomma plumbeum* is not an exclusive synonym for *H. marginatum* but has also been used for other species, including *Hyalomma marginatum turanicum* (Hoogstraal & Kaiser, 1960), *Hyalomma rufipes* (Enigk & Grittner, 1953), *Hyalomma plumbeum turanicum* (Pomerantsev, 1959), *Hyalomma rufipes glabrum* (Delpy, 1949), and *Hyalomma marginatum turanicum* (Pomerantzev, 1946).

Historically, *Hyalomma* (Euhyalomma) *marginatum* Koch, 1844 was recognized as a taxonomic complex comprising four subspecies: *Hyalomma* (E.) *marginatum marginatum* Koch, 1844; *Hyalomma* (E.) *marginatum rufipes* Koch, 1844; *Hyalomma* (E.) *marginatum turanicum* Pomerantzev, 1946; and *Hyalomma* (E.) *marginatum isaaci* Sharif, 1928 (Horak et al., 2002). Over time, the taxonomy of the *H. marginatum* complex has undergone major revisions, leading to the reclassification of some of these taxa as distinct species. In 2008, Apanaskevich and Horak redefined *H. rufipes*, *H. turanicum*, and *H. isaaci* as distinct species (Apanaskevich & Horak, 2008). Currently, the *Hyalomma marginatum* sensu lato (s.l.) complex includes five distinct species: *Hyalomma marginatum* (sensu stricto), *Hyalomma rufipes*, *Hyalomma isaaci, Hyalomma turanicum* and *Hyalomma glabrum* which are distributed across the Afrotropical, Palearctic, and Oriental regions (Guglielmone et al., 2016).

Hyalomma marginatum ticks primarily inhabit steppe, savannah, and scrubland hill and valley biotypes in regions with a Mediterranean climate characterized by low to moderate levels of humidity and long, hot, dry summers (ECDC (Available at:

https://www.ecdc.europa.eu/en/disease-vectors/facts/tick-factsheets/hyalomma-marginatum), n.d.). They are notably absent from European deciduous and mixed forests, which are more commonly inhabited by *I. ricinus* (Hoogstraal, 1979).

Hyalomma marginatum is primarily found in North Africa, Southern Europe, and parts of Asia, whereas *H. rufipes* is mostly present in sub-Saharan Africa and the region around the Red Sea and has recently expanded its range into the Eastern Palearctic (Apanaskevich & Horak, 2008).

The distinction between *H. rufipes* and *H. marginatum* remains challenging due to their morphological similarities and overlapping ecological niches, as noted by the European Centre for Disease Prevention and Control (ECDC) (ECDC (Available at: https://www.ecdc.europa.eu/en/disease-vectors/facts/tick-factsheets/hyalomma-marginatum)). This ambiguity can introduce bias in literature-based research, including this review, and we address this issue by noting potential outdated or inaccurate taxonomic classifications when relevant.

Hyalomma marginatum is of significant veterinary and public health importance due to its role in transmitting various tick-borne pathogens. This review specifically focuses on *H. marginatum*, which is the primary vector of Crimean-Congo Haemorrhagic Fever Virus (CCHFV) in Europe, an emerging arbovirus with a human mortality rate of up to 40% (Bente et al., 2013; Ergönül, 2006; Yilmaz et al., 2009). *Hyalomma* ticks acquire the virus when feeding on the blood of infected mammals, although viremia in the mammalian host is transient. Unlike mammals, ticks act as natural reservoirs for CCHFV, remaining infected for several years. Humans, however, serve as dead-end hosts, with infections occurring through tick bites or direct contact with the blood or tissues of infected livestock or patients. The disease initially presents with non-specific symptoms such as headaches, muscle pain, and fever, which can progress to severe hemorrhagic manifestations, including skin hemorrhages.

The endemic range of CCHFV closely matches the geographical distribution of *H. marginatum*. In addition to CCHFV, *H. marginatum* also transmits a range of other pathogens, including bacterial and protozoal agents such as *Anaplasma*, *Rickettsia*, *Babesia*, and *Theileria* species, which can cause diseases in both humans and animals (Bakheit et al., 2012; EFSA, 2010; Tirosh-Levy et al., 2021).

Among *Rickettsia* spp. *Hyalomma* ticks are very capable vectors of the spotted fever group (SFG). The SFG *Rickettsia* are responsible for spotted fever, a condition marked by papular exanthems, eschars, and skin lesions, accompanied by high fever, muscle and joint pain, headaches, and photophobia. Several pathogens within this group, including *Rickettsia aeschlimannii*, *R. africae*, *R. raoultii*, and *R. sibirica mongolotimonae*, are associated with human diseases such as Spotted Fever Rickettsiosis, African Tick Bite Fever (ATBF), tick-borne lymphadenopathy (TIBOLA), and lymphangitis-associated rickettsiosis (LAR), all of them were detected in *H. marginatum*.

This review provides a comprehensive assessment of the distribution of *H. marginatum* and its associated pathogens across various regions of Europe. By integrating information from multiple sources, this study aims to inform vector surveillance programs and public health interventions in regions at risk for Crimean-Congo Haemorrhagic Fever (CCHF) and other *Hyalomma*-borne diseases**.**

5. Materials and Methods

5.1. Study Design and Search Strategy

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021). We reviewed published studies reporting occurrence records of *H. marginatum* and *H. rufipes* in Europe. The systematic searches were conducted on 16 June 2023 in two major databases: Scopus, a multidisciplinary database, and PubMed, a primary biomedical sciences database. The search was limited to English-language publications with no restrictions on the year of publication. We used the following Boolean operators and search terms: ["(*Hyalomma marginatum*) OR (*Hyalomma rufipes*)"]. Titles and abstracts were screened, and relevant full-text articles were retrieved via library resources and online platforms.

5.2. Selection Criteria and Outcome Measures

The inclusion criteria for articles involved the documented occurrences of *H. marginatum* across the European continent. Eligible studies described the collection of *H. marginatum* from vegetation, animals, or humans. Studies reporting the occurrence of *H. marginatum* in Europe, even if they lacked detailed information on host, location within the country, or tick counts, were also included. Additionally, studies that detected pathogens in *H. marginatum* ticks collected in Europe were considered. Given the complexity of *Hyalomma* species taxonomy and the challenges distinguishing *H. marginatum* from *H. rufipes*, studies that reported on either species or the *H. marginatum* s.l. complex in Europe were included to minimize potential bias; these results are presented separately. We excluded studies focused on laboratory-maintained tick populations, experimental investigations, tick behavior, systematics, insecticide resistance, or those on occurrences of *H. marginatum* or *H. rufipes* outside Europe. Publications in languages other than English, and those for which the full original article could not be obtained, were also excluded.

5.3. Data Extraction and Analysis

The extracted data were organized into the following categories (***Supplementary Table 1***):

- **Main Characteristics of Studies**: This included information such as article ID, publication year, authors, and journal of publication.
- **Tick-Related Information**: Details about the ticks, including species, sex, developmental stage, collection date, identification methods (morphological or molecular), total number of ticks collected, the number subjected to pathogen detection, the number of positive ticks, and pathogen prevalence.
- **Pathogen-Related Information**: This covered the pathogens under investigation, diagnostic tests used, and species detected. Information about the number of pathogen species was also recorded.
- **Host-Related Information**: Data on host species, including both common and scientific names, the number of hosts screened, and the number of hosts carrying ticks.
- **Geographical Context**: This included study location details, such as country, province, and, where available, GPS coordinates of the study sites.

The extracted data were used to generate maps illustrating the distribution and prevalence of *H. marginatum* and its associated pathogens across Europe. These maps were created using ArcGIS Pro 3.1.2.

6. General Aspects of *Hyalomma marginatum* Biology in Europe

6.1. Bibliometric Data

This systematic review analyzed a total of 144 publications (***Figure 5***). Of these, 128 articles specifically focused on the presence of *H. marginatum* in Europe. Among these, 15 publications also reported the co-occurrence of *H. marginatum* with other members of the *H. marginatum* s.l. complex, including *H. rufipes*, *H. marginatum turanicum*, and references to broader taxonomic labels such as the *H. marginatum* complex, *H. marginatum* s.l., and *Hyalomma* spp. In some cases, the term *H. marginatum* sensu stricto was used to differentiate it from other species in the complex. Additionally, 11 articles focused exclusively on *H. rufipes*, 2 on the *H. marginatum* complex, 2 on *H. marginatum* s.l., and 1 article reported only *Hyalomma* spp. Throughout this manuscript, unless otherwise indicated, *H. marginatum* refers to *H. marginatum* sensu stricto as a distinct species.

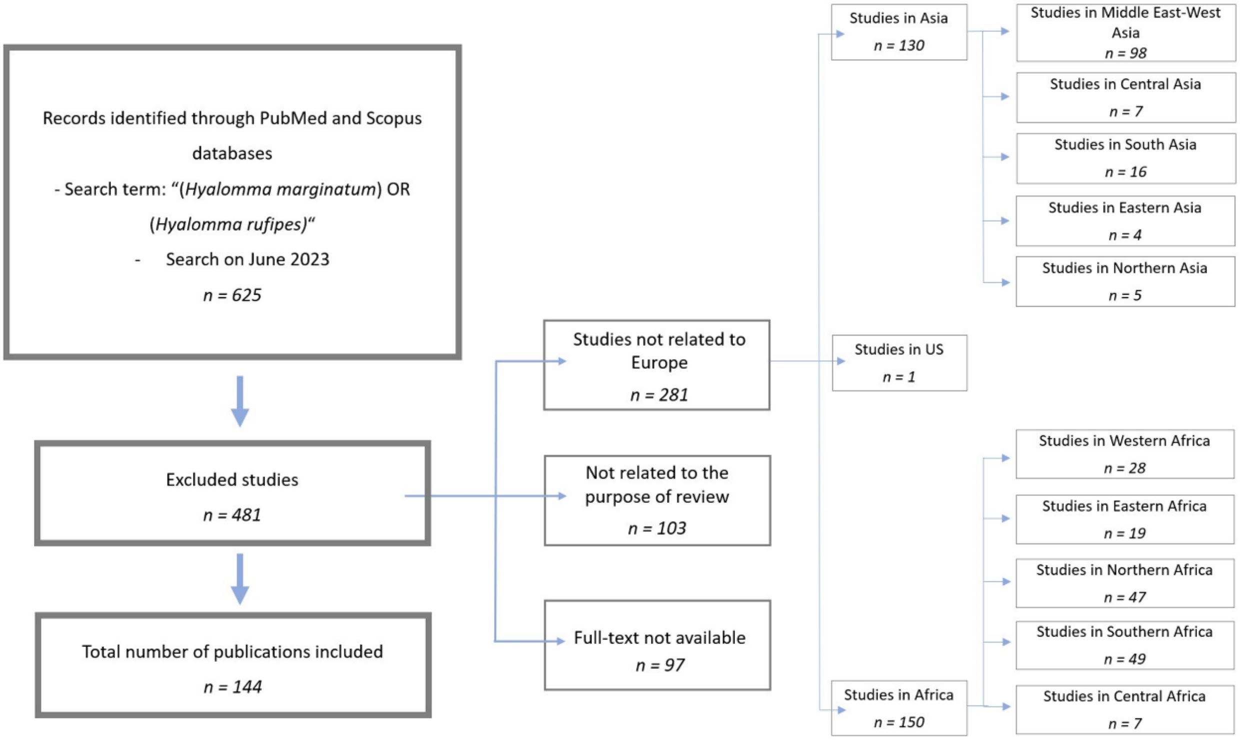

Figure 5. Systematic literature review methodology - PRISMA flow diagram.

6.2. Geographic Distribution

The continent was divided into six regions: the Balkan Peninsula, Central, Eastern, Northern, Southern, and Western Europe. Southern Europe recorded the highest number of *H. marginatum* occurrences, with Italy (n=36), Malta (n=1), Portugal (n=8), and Spain (n=17) contributing a total of 62 articles, representing 48.4% of the total publications reporting exclusively on *H. marginatum* occurrences (***Supplementary Table 1***). The results also indicated the presence of *H. marginatum* in the Balkans, with varying numbers of publications from each country: Albania (n=1), Bosnia (n=3), Bulgaria (n=4), Croatia (n=2), Greece (n=6), Kosovo (n=3), and Romania (n=8). In Western Europe, most reports came from France (n=14), particularly from its Mediterranean regions, with the UK (n=3) and the Netherlands (n=1) being the other countries in this region to report *Hyalomma* tick occurrences.

Central and Northern Europe had fewer publications on *H. marginatum*, with records from Austria (n=1), Czechia (n=1), Germany (n=4), Hungary (n=3), Poland (n=2), Denmark (n=1), Finland (n=1), Norway (n=1), and Sweden (n=2), being the only countries reporting its occurrence. Similarly, Eastern Europe had limited publications on *H. marginatum*, with reports from Moldova (n=1), Russia (n=3), and Ukraine (n=2); however, additional records from Russia were excluded from this analysis due to their availability only in Russian.

In contrast, 31 articles reported the presence of *Hyalomma* ticks without species-level identification, referring to them as *Hyalomma spp.*, the *H. marginatum* complex, or *H. marginatum s.l.* Additionally, some studies specifically identified *H. rufipes* and *H. marginatum turanicum*. These studies covered Czechia (n=4), France (n=1), Germany (n=2), Greece (n=7), Hungary (n=4), Italy (n=12), Malta (n=2), the Netherlands (n=1), Norway (n=1), Slovakia (n=1), Spain (n=3), Sweden (n=1), and the UK (n=1).

The findings of this review, based on the content of the analyzed studies rather than just the number of publications, indicate that *H. marginatum* is primarily established in the Mediterranean region of Europe. In contrast, sporadic occurrences have been documented in transalpine areas. Countries such as Austria (Duscher et al., 2018), Czechia (Lesiczka et al., 2022), Finland (Sormunen et al., 2022a), Germany (Chitimia-Dobler et al., 2019; Oehme et al., 2017; Rumer et al., 2011), Hungary (Földvári et al., 2022; Hornok et al., 2013a), the Netherlands (Uiterwijk et al., 2021a), Poland (Cuber, 2016; Nowak-Chmura, 2014), Sweden (Grandi et al., 2020), and the UK (Jameson, Morgan, et al., 2012a; Jameson & Medlock, 2011) have reported isolated cases, often linked to imported animals, humans, or migratory birds. However, there is no evidence of established populations in these regions.

It is important to note that the number of publications reporting *H. marginatum* presence does not necessarily reflect its true distribution but rather the intensity of research and surveillance efforts in each country. Some regions with few or no reports may still harbor populations of *H. marginatum* that remain undocumented due to limited surveillance or reporting biases. Therefore, caution is necessary when interpreting these numbers as direct indicators of species prevalence.

6.3. Preferred Hosts

The study also examined the primary sources of *H. marginatum* collection across Europe, identifying cattle and birds as the predominant hosts in all regions (***Figure 6***). In the Balkans, *H.*

marginatum was additionally collected from vegetation (in Bulgaria, Croatia, Kosovo, and Romania) , horses (in Greece and Romania), dogs (in Greece, Kosovo, and Romania), and, to a lesser extent, humans (in Bosnia, Croatia, Kosovo, and Romania). The tick species was also found on lizards and hedgehogs in Romania.

In Western Europe, horses were the primary hosts for *H. marginatum*, with cattle serving as secondary hosts. In the Netherlands, all reported occurrences of this tick species were limited to horses, with a single *H. rufipes* collected from a bird. In France, *H. marginatum* was found on a wider variety of hosts, including cattle, horses, birds, dogs, wild boars, humans, and vegetation. In the UK*,* *H. marginatum* were collected from birds, horses, and humans.

In Central and Northern Europe, *H. marginatum* was predominantly collected from birds, with other sources including horses (in Austria, Czechia, Germany, and Sweden), livestock (in Hungary, Germany, and Sweden), humans (in Germany and Sweden), and dogs and hedgehogs (in Hungary). In Eastern Europe, *H. marginatum* was primarily collected from birds (in Russia, Moldova, and Ukraine), but was also found on other hosts, including hares (in Russia), livestock (in Russia and Ukraine), humans (in Russia), and horses (in Russia), as well as from vegetation (in Russia and Ukraine).

In Southern Europe, *H. marginatum* was primarily collected from cattle and birds. Other hosts included vegetation (in Italy, Portugal, and Spain), deer (in Italy and Spain), wild boar (in Italy, Portugal, and Spain), humans (in Italy, Malta, and Spain), hares (in Italy and Portugal), rabbits (in Portugal), donkeys, mouflons, and horses (in Italy) (***Figure 6***).

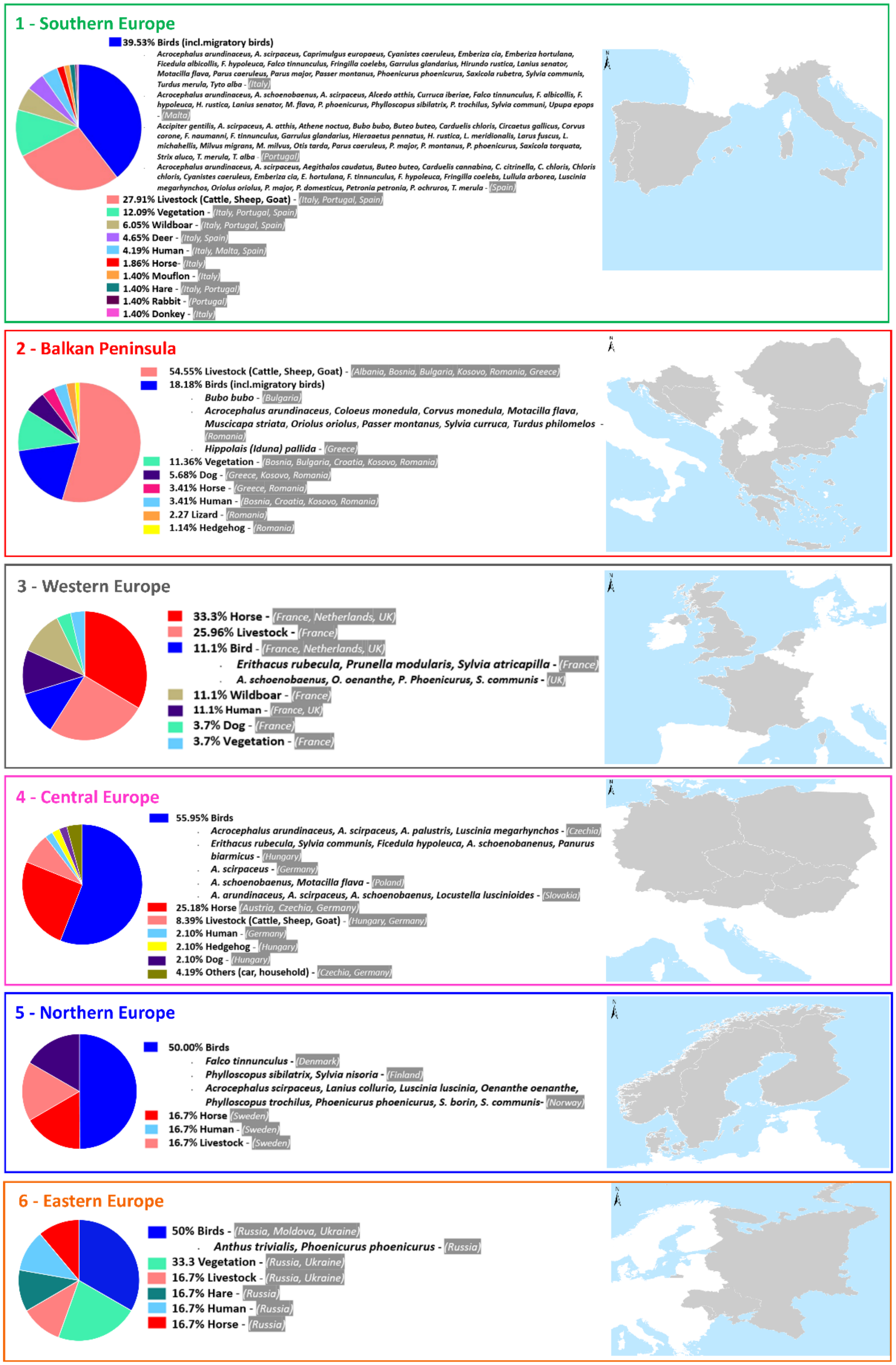

Figure 6. Distribution of sources of *Hyalomma marginatum* collection across Europe.

6.4. Vector Capacity

Of the 128 publications reviewed, 36.7% focused on the detection of bacterial pathogens in *H. marginatum* (***Figure 7***), 10.9% reported viral pathogens ***(Figure 8***), and 7% protozoan pathogens (***Figure 9***). In total, 24 pathogens were identified in *H. marginatum* specimens collected across Europe. These pathogens were classified into seven bacterial genera, four viral genera, and two protozoan genera (***Table 1***).

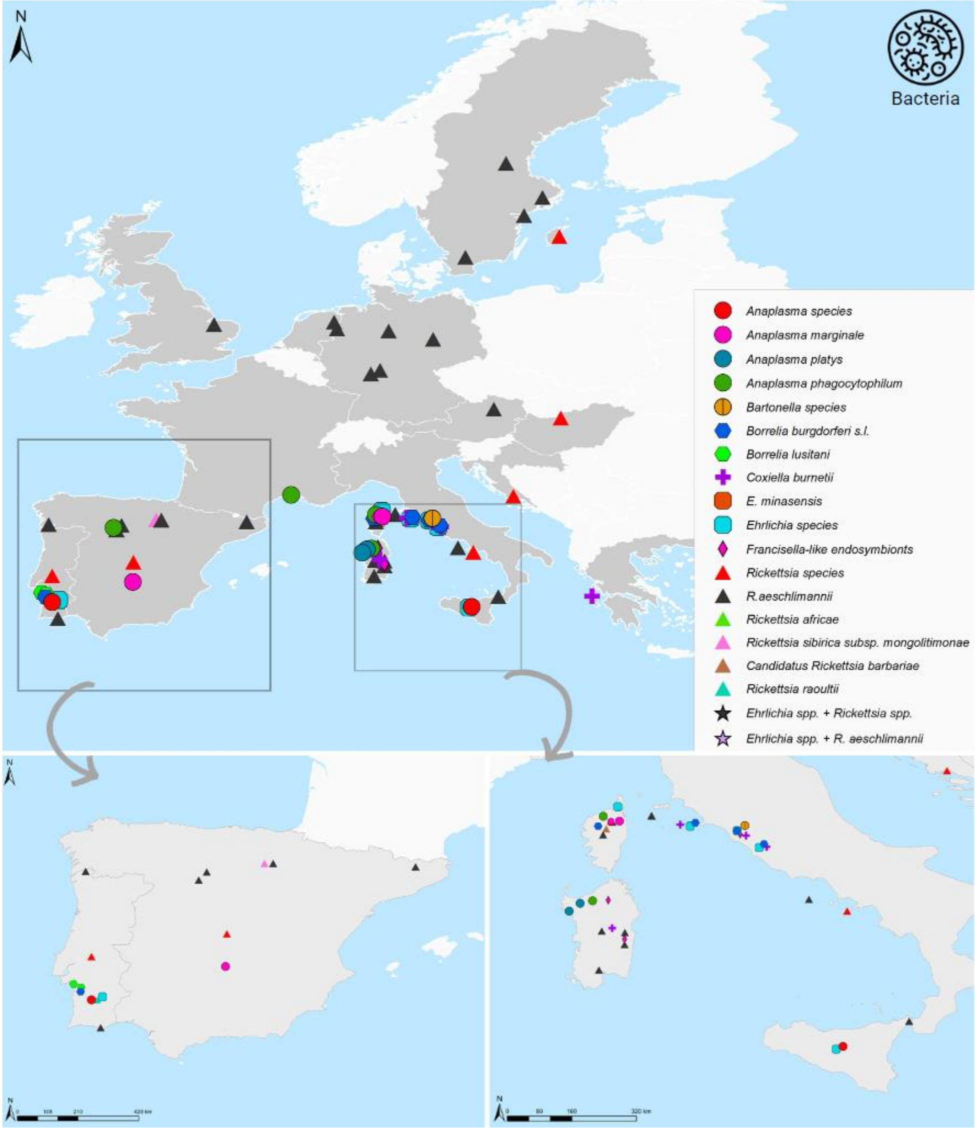

Figure 7. Distribution of bacteria detection in *Hyalomma marginatum* ticks across Europe, and close-ups of Southern Europe (Italy, Portugal and Spain), to provide additional details to bacteria species detected in *Hyalomma marginatum* ticks in the region.

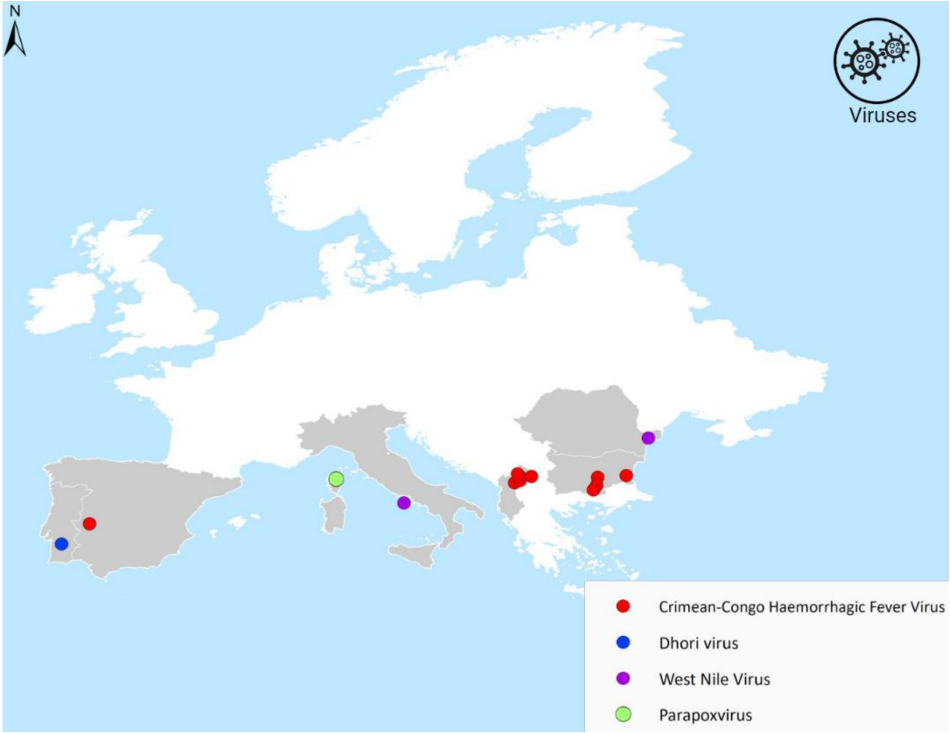

Figure 8. Distribution of virus detection in *Hyalomma marginatum* ticks across Europe.

Figure 9. Distribution of protozoa detection in *Hyalomma marginatum* ticks across Europe.

Table 1. Pathogens detected in *Hyalomma marginatum* in Europe and its associated diseases.

	Etiological Agent	**Disease**
Bacteria		
Anaplasma	*Anaplasma* spp.	Anaplasmosis
	Anaplasma. phagocytophilum	Human granulocytic anaplasmosis, Ovine anaplasmosis
	Anaplasma platys	Thrombocytic anaplasmosis
	Anaplasma marginale	Bovine anaplasmosis
Bartonella	*Bartonella* spp.	Bartonellosis
Borrelia	*Borrelia burgdorferi* sensu lato.	Lyme borreliosis
	Borrelia lusitaniae	Lyme borreliosis
Coxiella	*Coxiella burnetii*	Q fever
	Ehrlichia spp.	Ehrlichiosis
Ehrlichia	*Ehrlichia minasensis*	Ehrlichiosis
Francisella	*Francisella*-like	Francisellosis
Rickettsia	*Rickettsia* spp.	Tick-borne rickettsiosis
	Rickettsia sibirica mongolitimonae	Lymphagitis-associated rickettsiosis (LAR)
	Rickettsia aeschlimannii	Tick-borne lymphadeno-pathy (TIBOLA)
	Rickettsia africae	African tick-bite fever
	Rickettsia raoultii	Tick-borne lymphadeno-pathy (TIBOLA)
Protozoa		
Babesia	*Babesia bigemina*	Bovine babesiosis (Texas cattle fever)

	Babesia bovis	Bovine babesiosis
	Babesia caballi	Equine piroplasmosis
	Babesia occultans	Bovine piroplasmosis
Theileria	*Theileria* spp.	Theileriosis
	Theileria annulata	Tropical theileriosis (Mediterranean bovine theileriosis)
	Theileria equi	Equine piroplasmosis
	Theileria buffeli/sergentii/orientalis	Bovine theileriosis (Theileria-associated bovine anaemia)
Viruses		
	Crimean-Congo Haemorrhagic Fever Virus	Crimean-Congo Haemorrhagic Fever
	West Nile Virus	West Nile encephalitis
	Dhori virus	Dhori virus infection
	Parapoxvirus	Orf (Contagious ecthyma) Bovine papular stomatitis Pseudocowpox

Hyalomma marginatum is a recognized vector of multiple pathogens, including several viruses such as Thogoto, Dhori, and CCHFV, as well as a range of bacterial and protozoal agents from the genera *Rickettsia*, *Anaplasma*, *Babesia*, and *Theileria*. Although RNA from various arboviruses—including West Nile virus (WNV)—has been detected in *H. marginatum*, its role in WNV transmission remains uncertain, as there is currently no evidence supporting its competence as a biological vector for this virus.

In Europe, CCHFV and *Rickettsia* species are the most frequently reported pathogens. CCHF is a zoonotic disease transmitted by ticks, with outbreaks reported in regions of Africa, Asia, southeastern Europe, and the Middle East. The causative agent, CCHFV, belongs to the *Orthonairovirus* genus within the *Nairoviridae* family and is one of the most widespread tick-borne viruses affecting humans (Leblebicioglu, 2010). While *Hyalomma* ticks are the primary vectors of CCHFV, other tick species, such as *Rhipicephalus* and *Dermacentor*, may also contribute to the virus's life cycle (Gargili et al., 2011; Papa et al., 2011; O. Papadopoulos & Koptopoulos, 1978; Yesilbag et al., 2013).

Rickettsia species, particularly *R. aeschlimannii*, are frequently detected in *H. marginatum* populations. Spotted fever group (SFG) rickettsiae, transmitted by *Hyalomma* ticks, cause a range of febrile illnesses in humans, often characterized by rash, eschars, and systemic symptoms. The presence of *R. aeschlimannii, R. africae, R. raoultii,* and *R. sibirica mongolotimonae* in *H. marginatum* across Europe underscores its importance as a vector of emerging zoonotic diseases.

As further discussed in this review, several studies across Europe have highlighted that *H. marginatum* frequently harbors a variety of tick-borne pathogens at significant prevalence rates, posing serious risks to both human and animal health. To offer a comprehensive overview of the prevalence of CCHFV and *Rickettsia* species in *H. marginatum* across Europe, ***Tables 2*** and ***3*** summarize key data, including collection dates, locations, sources of tick collection, pathogen prevalence, and the detection methods employed, as reported in the literature.

Table 2. Summary of the key findings of published studies on the prevalence of Crimean-Congo Haemorrhagic Fever Virus (CCHFV) in *Hyalomma marginatum* ticks across Europe.

Country	**Province**	**Collection Date**	**Number of tested/collected *H. marginatum* ticks**	**Prevalence of CCHFV**	**Stage of CCHFV positive tick**	**Source**	**Method of detection**	**Reference**
Albania	Kukes	2007-2014		4.7% (16/341)	A	Livestock	Nested RT-PCR	(Papa et al., 2017)
Bosnia		2019-2021	122/122	2.5% (3/122)	A	Livestock	qRT-PCR	(Goletic et al., 2022)
Bulgaria	Region of Stara Zagora, Central Bulgaria	May-Jun, 2006-2010		3.7% (6/161)	A	Livestock	IFHA, RT-PCR, RT-Nested-PCR	(Gergova et al., 2012)
	Region of Haskovo, Southeastern Bulgaria	May-Jun, 2006-2010		13.9% (5/36)	A	Livestock	IFHA, RT-PCR, RT-Nested-PCR	
	Region of Kardzhali, Southeastern Bulgaria	May-Jun, 2006-2010		3.4% (3/87)	A	Livestock	IFHA, RT-PCR, RT-Nested-PCR	
	Kardzhali	Jun-Jul, 2014	233/389	8.6% (20/233)	A	Livestock	qRT-PCR	(Panayotova et al., 2016)
	Burgas	Jun-Jul, 2014	217/388	8.8% (19/217)	A	Livestock	qRT-PCR	

Kosovo	Prizren	Apr-Jun, Sep-Oct, 2014-2018		11% (1/9)	A (M)	Livestock	qRT-PCR	(Sherifi et al., 2018)
	Malishevë	May-Jun, 2012	148/244	14.2% (21/148)	A	Cattle, Goat	qRT-PCR	(Sherifi et al., 2014)
	Klinë	May-Jun, 2012	119/167	5.9% (7/119)	A	Cattle, Goat	qRT-PCR	
	Gjilan	May-Jun, 2012	30/156	3.3% (1/30)	A	Cattle	qRT-PCR	
Russia	Stavropol, Volgograd, Astrakhan and Rostov	2000	4787/4787	10.2% (46 of 449 pools)	na	na	ELISA	(Yashina et al., 2003)
	Stavropol, Rostov, Krasnodar, Dagestan,Karachay-Cherkess Republic, Crimea	2012-2019	2045/10,257A; 285/15,536L-N	7.2% (148/2045A); 21%(60/285L-N)	L/N/A	Cattle, Sheep, Horse, Human, European hare, Hedgehog, Rook, Crested lark	Nested-PCR	(Tsapko et al., 2022a)
Spain	Cáceres	2015		20% (1/5)	A (F)	Cattle	RT-PCR	(Negredo et al., 2019)

*A = adult tick; M = adult male tick; F = female adult tick.

Table 3. Summary of the key findings of published studies on the prevalence of *Rickettsia* species in *Hyalomma marginatum* ticks across Europe.

Country	**Province**	**Collection Date**	**Species of Rickettsia**	**Prevalence of *Rickettsia* species**	**Stage of *Rickettsia* positive ticks**	**Source**	**Method of detection**	**Reference**
Austria	Melk, Lower Austria	2 Oct, 2018	*R. aeschlimannii*	%100 (1/1)	A (M)	Horse	qPCR	(Duscher et al., 2018)
Croatia	Kastela Bay, Split, Dalmatia County	Oct, 2000	*R. aeschlimannii*	64.7% (11/17)	na	Cattle	PCR	(Punda-Polic et al., 2002)
France	Corsica	May-May, 2014-2015	*R. aeschlimannii*	100% (89 pools/89 pools - 362 ticks)	A		PCR, qPCR	(Grech-Angelini et al., 2020)
	Ponte-Leccia slaughterhouse, Corsica	May-Sep, 2016	*Rickettsia* spp.	59% (16 pools/27 pools)	A	Cattle	PCR, qPCR	(Cicculli, Capai, Quilichini, Masse, Fernández-Alvarez, et al., 2019)
	Ponte-Leccia slaughterhouse, Corsica	May-Aug, 2017; Jul-Dec, 2018	*R. aeschlimannii*	83.5% (81 pools/97 pools - 216 ticks)	na	Cattle	PCR, qPCR	
	Corsica	Aug-Jan, 2018-2019	*R. aeschlimannii*	100% (1/1)	A (F)	Wildboar	PCR, qPCR	
	Corsica	2018-2020	*R. aeschlimannii*	50% (1/2)	A	Wildboar	PCR, qPCR, Nested PCR	(Defaye et al., 2021)
	Corsica	2019-2021	*R. aeschlimannii*	100% (23/23)	na	Cattle	PCR, qPCR	(Maitre et al., 2023)
Germany	Wächtersbach, Hesse	26 Jun, 2018	*R. aeschlimannii*	%100 (1/1)	A (F)	Sheep	PCR, qPCR, qRT-PCR	(Chitimia-Dobler et al., 2019)
	Wächtersbach, Hesse	5 Aug, 2018	*R. aeschlimannii*	%100 (1/1)	A (M)	Horse	PCR, qPCR, qRT-PCR	
	Hannover, Hesse	24 Aug, 2018	*R. aeschlimannii*	%100 (1/1)	A (M)	Car	PCR, qPCR, qRT-PCR	
	Neuenhaus, Lower Saxony	22 Aug, 2018	*R. aeschlimannii*	%100 (1/1)	A (F)	Horse	PCR, qPCR, qRT-PCR	
	Fechenheimer Aue, Hesse	4 Sep, 2018	*R. aeschlimannii*	%100 (1/1)	A (F)	House	PCR, qPCR, qRT-PCR	
	Saxony-Anhalt	May, 2007	*R. aeschlimannii*	%100 (3/3)	A	Eurasian reed warbler	PCR	(Rumer et al., 2011b)
Greece	Northern Greece	Jun, Jul, Sep, Dec, 2013	*Rickettsia* spp.	100% (1/1)	N	Eastern olivaceous warbler	Nested-PCR	(Diakou et al., 2016)

Hungary	Ocsa Ringing Station, Duna-Ipoly National Park	2011	*Rickettsia* spp.	66.6% (2/3)	L/N	European robin	PCR	(Hornok et al., 2013)
Italy	Ogliastra and Sassari, Sardinia	Jun-Jul, 2007	*R. aeschlimannii*	100% (11/11)	A	Horse	PCR	(Mura et al., 2008)
	Corleone, Palermo		*R. aeschlimannii*	4.2% (2/48)	na	Livestock	PCR	(Beninati et al., 2005)
	Corleone, Palermo		*R. africae*	2.1% (1/48)	na	Livestock	PCR	
	Sicily		*Rickettsia* spp.	4.5% (3/67)	na	Cattle	PCR, Reverse lineblot	(Torina et al., 2010)
	Pianosa	2004-2007	*R. aeschlimannii*	18.2 (2/11)	na	Ground	PCR	(Tomassone et al., 2013)
	Urzulei, Ogliastra	10 Jun, 2010	*R. aeschlimannii*	8.3% (1/12)	A	Horse	PCR	(Chisu et al., 2014)
	Castel di Guido and Rome	Jul-Sept, 2010-2011	*R. aeschlimannii*	21.1% (8/38)	N	Migratory birds	PCR, qPCR	(Toma et al., 2014)
	Ponzian Islands and Rome	Apr-May, 2010-2011	*R. aeschlimannii*	50.7% (36/71)	N	Migratory birds	PCR, qPCR	
	Livorno, Pianosa Island	2012-2013	*R. aeschlimannii*	10% (1/10)	na	Ground	Nested PCR	(Scarpulla et al., 2016)
	Messina, Sicily	2012-2013	*R. aeschlimannii*	100% (2/2)	na	Human	PCR	(Blanda et al., 2017)
	Gairo, Sardinia	May-Sept, 2014	*R. aeschlimannii*	50% (1/2)	na	Mouflon	PCR	(Chisu et al., 2017)
	Sardinia	2010-2015	*Rickettsia* spp.	33% (15/45)	10F/3M/2N	Bird, Goat, Hedhehog, Horse, Human, Sheep	PCR	(Chisu, Foxi, Mannu, et al., 2018)
	Uta, Sardinia	Mar-Nov, 2017	*R. aeschlimannii*	28.6 (2/7)	N/A	Cattle		
	Monte Romano, Viterbo	2012-2013	*R. aeschlimannii*	66% (8/12)	na	Ground	PCR, qPCR	(Mancini et al., 2019)
	Ventotene island	Apr-May, 2013	*R. aeschlimannii*	50% (5/10)	N	Migratory birds	qPCR	(Pascucci et al., 2019)
Italy - Greece	Capri (Italy), Antikythira (Greece)	2009-2010	*Rickettsia* spp.	2.4% (16/658)	7L/9N	Migratory birds	qPCR	(Wallménius et al., 2014)
	Capri (Italy), Antikythira (Greece)	2009-2010	*R. aeschlimannii*	45.7% (301/658)	73L/228N	Migratory birds	qPCR	

	Capri (Italy), Antikythira (Greece)	2009-2010	*R. africae*	2.3% (15/658)	11L/4N	Migratory birds	qPCR	
Netherlands	Odoorn, Drenthe	17 Jul, 2019	*R. aeschlimannii*	100% (1/1)	A (F)	Horse	PCR, qPCR	(Uiterwijk et al., 2021)
Portugal	Bird Rehabilitation Centers of Monsanto Forest Park; Quercus Santo Andre	2002-2004	*R. aeschlimannii*	6.7% (1/15)	N	Common kingfisher	PCR	(Santos-Silva et al., 2006)
	Bird Rehabilitation Centers of Monsanto Forest Park; Quercus Santo Andre	2002-2004	*R. aeschlimannii*	33% (1/3)	N	Little owl	PCR	
	Bird Rehabilitation Centers of Monsanto Forest Park; Quercus Santo Andre	2002-2004	*R. aeschlimannii*	25% (4/3)	N	Eurasian eagle-owl	PCR	
	Beja	Dec-Sep, 2013-2015	*R. aeschlimannii*	12.1% (13/107)	10M/3F	Cattle	PCR, Nested PCR	(Pereira et al., 2018)
	Beja	Dec-Sep, 2013-2015	*R. raoultii*	5.6% (6/107)	1M/5F	Cattle	PCR, Nested PCR	
	Faro and Beja	Dec-Sep, 2013-2015	*R. aeschlimannii*	5.3% (1/19)	N	Little owl	PCR, Nested PCR	
Russia	Stavropol	2004	*R. aeschlimannii*	20%(8/40)	20F/20M	Cattle, Vegetation	PCR	(Shpynov et al., 2009)
	Kaliningrad	2009	*R. aeschlimannii*	20% (1/5)	1L/4N	Tree pipit, Common redstart, Common blackbird	PCR	(Movila et al., 2013)
Spain	La Rioja	2001-2005	*R. aeschlimannii*	40% (2/5)	na	Human	PCR, semi-nested PCR	(Oteo et al., 2006)
	La Rioja	2001-2005	*R. aeschlimannii*	0.9% (1/110)	na	Cattle	PCR, semi-nested PCR	
	Central Spain, Madrid and Toledo	Mar-Dec, 1999-2002	*R. aeschlimannii*	61.5% (8/13)	na	Livestock, Birds	PCR	(Toledo et al., 2009)
	La Rioja	2012-2015	*R. aeschlimannii*	16.7% (2/12)	A	Human	PCR, qPCR	(Palomar et al., 2016)
	La Rioja	2009-2015	*R. aeschlimannii*	100% (7/7)	N	Great reed warbler	PCR, qPCR	
	La Rioja	2009-2015	*R. aeschlimannii*	40% (4/10)	N	Eurasian reed warbler	PCR, qPCR	

	La Rioja	2009-2015	*R. aeschlimannii*	16.7% (1/6)	N	Eurasian blue tit	PCR, qPCR	
	La Rioja	2009-2015	*R. aeschlimannii*	33.3% (6/18)	2L/4N	Ortolan bunting	PCR, qPCR	
	La Rioja	2009-2015	*R. aeschlimannii*	75% (6/8)	L	Woodlark	PCR, qPCR	
	La Rioja	2009-2015	*R. sibirica mongolitimonae*	10% (1/10)	A (F)	Common nightingale	PCR, qPCR	
	La Rioja	2009-2015	*R. aeschlimannii*	30% (3/10)	3N	Common nightingale	PCR, qPCR	
	La Rioja	2009-2015	*R. aeschlimannii*	50% (1/2)	N	Eurasian golden oriole	PCR, qPCR	
	La Rioja	2009-2015	*R. sibirica mongolitimonae*	12.5% (1/8)	A (M)	Great tit	PCR, qPCR	
	La Rioja	2009-2015	*R. aeschlimannii*	25% (2/8)	1L/1M	Great tit	PCR, qPCR	
	La Rioja	2009-2015	*R. aeschlimannii*	50% (1/2)	L	Rock sparrow	PCR, qPCR	
	La Rioja	2009-2015	*R. aeschlimannii*	6.5% (2/31)	1L/1N	Common blackbird	PCR, qPCR	
	Palencia	2013	*R. aeschlimannii*	54% (13/24)	N	Common buzzard	PCR, qPCR	
	Castilla y León	na	*R. aeschlimannii*	5.9% (19/324)	na	Human	PCR	(Fernández-Soto et al., 2003)
	Castilla y León	1997-2003	*R. aeschlimannii*	6.1% (26/426)	na	Human	PCR	(Fernández-Soto et al., 2009)
	Castilla y León	2004-2007	*R. aeschlimannii*	14.2% (73/515)	46M/26F/1N	Human	PCR	
Sweden	Nyköping, Södermanland	21 Aug, 2018	*R. aeschlimannii*	%100 (1/1)	A (F)	Horse	qRT-PCR	(Grandi et al., 2020)
	Upplands Väsby, Stockholm	5 Sep, 2018	*R. aeschlimannii*	%100 (1/1)	A (F)	Horse	qRT-PCR	
	Funnarp, Hästveda, Hässleholm, Skåne	25 Sep, 2018	*R. aeschlimannii*	%100 (1/1)	A (M)	Cattle	qRT-PCR	
	Katthammarsvik, Gotland	11 Oct, 2018	*Rickettsia* spp.	%100 (1/1)	A (F)	Horse	qRT-PCR	
	Ovanåker, Hälsingland	18 Sep, 2018	*R. aeschlimannii*	%100 (1/1)	A (M)	Horse	qRT-PCR	
UK	East Anglia, England	21 Jun, 2018	*R. aeschlimannii*	%100 (1/1)	A	Human	qPCR	(McGinley et al., 2021)

Prevalence of Rickettsia species in non-Hyalomma marginatum ticks across Europe

Italy, Greece	(**) (***)	Capri and Antikythira	2009-2010	*R. aeschlimannii*	50.3% (331/658) %100 (1/1)	L/N N	Migratory birds	qPCR	(Wallménius et al., 2014)
Italy	(*)	Ponzian Islands and Rome	2010-2011	*R. aeschlimannii*	50.7%(36/71)	N	Migratory birds	PCR, qPCR	(Toma et al., 2014)
Italy	(*)	Ponza Island	March - May, 2019	*R. aeschlimannii*	%100 (1/1)	N	Common whitethroat Northern wheatear Whinchat	qPCR	(Rollins et al., 2021)
Germany	(*)	Hannover, Lower Saxony Saulheim, Rhineland-Palatinate Neuenkirchen, Lower Saxony Mörsdorf, Rhineland-Palatinate	Aug, 2018 Oct, 2018	*R. aeschlimannii*	%100 (1/1)	A	Horse	PCR, qPCR, qRT-PCR	(Chitimia-Dobler et al., 2019)
Sweden	(*)	Klintehamn Örebro Älvnäs, Vålberg Sölvesborg Vreta kloster Örserum, Gränna Bjurbäcken	2018	*R. aeschlimannii*	%100 (1/1)	A (M) A (F) A (M) A (F) A (M)	Horse	qRT-PCR	(Grandi et al., 2020)
UK	(*)	Dorset, England	Sep, 2018	*R. aeschlimannii*	%100 (1/1)	A	Horse	PCR, qPCR	(Hansford et al., 2019)

*A = adult tick; M = adult male tick; F = female adult tick; N = nymph; L = larva;

(*) = *Hyalomma rufipes*; (**) = *Hyalomma marginatum* sensu lato; (***) = *Hyalomma marginatum* sensu stricto.

7. Overview of *Hyalomma marginatum* distribution and its role as a vector for pathogens in main European regions

The following subsections present a structured overview of *H. marginatum* distribution across various European regions, organized as follows:

- **Regions** are presented in order of the number of publications and their significance regarding *H. marginatum* distribution.
- Within each region, **countries** are listed alphabetically to enhance readability.
- For some countries (France, Italy, and Spain), information is organized by **sub-regions**.
- Information in all countries and sub-regions are presented chronologically.

7.1. *Hyalomma marginatum* Distribution in Southern Europe

Italy: Italy lies along a major migratory route for birds carrying *H. marginatum* larvae and nymphs. The tick was first recorded in Italy near Naples in 1973, discovered on a racehorse transported from northern Italy (Converse et al., 1974). Since then, the tick has been increasingly reported across various habitats and hosts, highlighting its ecological plasticity and expanding role as a vector.

Sicily, with its Mediterranean climate—dry summers and mild winters—is one of the primary hotspots for *H. marginatum* in Europe. The island consistently reports high burdens of tick-borne diseases in livestock. A survey conducted 1998-1999 found *H. marginatum* to be the most common tick species on cattle, accounting for 27.1% of the total ticks, with some specimens testing positive for *Theileria annulata* and *T. buffeli/sergentii/orientalis* (Georges et al., 2001). In a 2001-2002 survey, *Rickettsia aeschlimannii* was detected in 2 out of 48 *H. marginatum* ticks, and *R. africae* was identified in one specimen (Beninati et al., 2005). A further survey conducted between 2002 and 2003 found that 207 out of 6,208 ticks (3.3%) collected from cattle, sheep, and vegetation were *H. marginatum*. Among these, 9.9% tested positive for pathogens, including *Babesia/Theileria* (16.4%), *Rickettsia* spp. (4.5%), *Ehrlichia* spp. (3%), and *Anaplasma* spp. (1.5%) (Torina et al., 2006).

In southern Italy, a retrospective study conducted on samples collected in 2000 found 24 *H. marginatum* (out of 744 ticks tested) to be positive for *Babesia*, *Theileria*, and *Anaplasma*, although no positive results were detected for certain pathogens (Ceci Luigi et al., 2014). *Hyalomma marginatum* has been identified on both wild and domesticated species, including dogs, livestock, and wildlife. In 2009, *H. marginatum* was found on vegetation and hares in a wildlife reserve, with 51 adults and 165 nymphs collected (Dantas-Torres et al., 2011), as well as one additional adult female from a road-killed hare (Lorusso et al., 2011). A separate survey recorded 109 *H. marginatum* specimens among 10,795 ticks collected from vegetation (Dantas-Torres & Otranto, 2013). Another investigation in the Basilicata region identified 15 *H. marginatum* specimens (Ramos et al., 2015). In a study testing 123 ticks from mammals and vegetation for *Rickettsia*, five ticks (4.1%) were identified as *H. marginatum*, including three from Corsican red deer and two from mouflon, with one mouflon tick testing positive for *R. aeschlimannii* (Chisu et al., 2017). A broader survey of 50,325 birds across 13 ringing sites in southern and central Italy (2013–2014) found that 0.22% were infested with *Hyalomma* ticks, suggesting the potential for dissemination of this tick via migratory birds (de Liberato et al., 2018). Additionally, a study in Italy and Spain documented *H. marginatum* with dual symbiosis involving *Francisella*-like and *Midichloria* endosymbionts, marking the first such finding in ticks (Buysse et al., 2021). On Capri Island, located off the southwestern coast of Italy, a separate study conducted in 2014–2015 collected 575 ticks from migrating birds during migration, identifying *H. rufipes* (77.7%) and *H. marginatum* (4.2%) (Hoffman et al., 2021). *Hyalomma marginatum* was detected on a variety of bird species, with *H. rufipes* being prevalent across multiple species. Pathogen screening revealed *Francisella* spp. in 76.7% (343/447) of *H. rufipes* and 75% (18/24) of *H. marginatum*, and spotted fever group rickettsiae (SFGR) in 61.5% (275/447) of *H. rufipes* and 50% (12/24) of *H. marginatum*. Further analysis confirmed the presence of *Francisella*, *Rickettsia*, and *Midichloria* in two *H. rufipes* ticks, reinforcing the health risks associated with *Hyalomma* ticks in Southern Europe (Hoffman et al., 2022).

Sardinia has also emerged as a key focus for *H. marginatum* surveillance. In 2007, 83 *H. marginatum marginatum* ticks were collected from horses, with 11 testing positive for *R. aeschlimannii* (Mura et al., 2008). A separate study conducted during the same period reported 15 (1%) *H. marginatum* ticks on cattle, although no pathogens were detected (Satta et al., 2011). Subsequent surveys have identified *H. marginatum* on cattle, dogs, and wild animals, with 8.3%

testing positive for *R. aeschlimannii* in one instance (Chisu et al., 2014). *Hyalomma marginatum* was also found on wild donkeys in Sardinia, with 12 (1.9%) of 256 ticks identified on this host (Zanzani et al., 2019). Chisu et al. (2018) reported 14 *H. marginatum* out of 115 ticks collected from domestic and wild animals, with all ticks collected from common whitethroats (*Sylvia communis*) testing positive for *Anaplasma phagocytophilum* and *A. platys*, (100%), while one specimen from a wild boar was positive for *A. platys* (Chisu, Zobba, et al., 2018). Between 2010 and 2015, a large-scale survey on 1,619 animal ticks in Sardinia identified 45 *H. marginatum*, with 33.3% testing positive for *Rickettsia* spp. and 4.4% for *Coxiella burnetii* (Chisu, Foxi, Mannu, et al., 2018). Among humans, a survey of 42 ticks collected from 2012 to 2013 found two *H. marginatum* specimens, both testing positive for *R. aeschlimannii* (100%) (Blanda et al., 2017). Another survey identified 16 *H. marginatum* ticks among 185 ixodid ticks, with two specimens (12.5%) testing positive for *R. aeschlimannii* (Chisu, Foxi, & Masala, 2018). Additional pathogens detected in *H. marginatum* included *Francisella* spp. DNA, *T. buffeli/sergentii/orientalis*, and *C. burnetii*, emphasizing the zoonotic potential of *H. marginatum* ticks in the region as potential vectors of zoonotic pathogens (Chisu, Alberti, et al., 2019; Chisu, Foxi, et al., 2019).

In western Italy, on Ventotene Island, a stopover site for migratory birds, the presence of exotic tick species and tick-borne pathogens was investigated by trapping birds (Pascucci et al., 2019). *Hyalomma marginatum* was confirmed as the second most abundant tick species (2.3%, 10/433), following *H. rufipes* (82.6%, 366/443). Five *H. marginatum* ticks tested positive for *R. aeschlimannii*; one nymph collected from a European nightjar (*Caprimulgus europaeus*) and another from a western yellow wagtail (*Motacilla flava*) were positive for Flavivirus, with West Nile Virus (WNV) RNA identified in the latter (Pascucci et al., 2019). Between 2017 and 2019, studies of migratory birds and vegetation reported *H. marginatum* in 4.3% of 2,681 collected ticks (Toma et al., 2021). Further, a survey of wild ungulates and their ectoparasites found a single *H. marginatum* on a wild boar, though no *Anaplasma* DNA was detected (Grassi et al., 2021). In a separate study conducted between 2017 and 2019, 2,344 ticks were collected from 1,079 birds. Two ticks identified as *H. marginatum* and *Hyalomma* spp. tested positive for WNV, while CCHFV RNA was detected in two immature *H. rufipes* ticks from Ventotene Island. Although *H. marginatum* has tested positive for WNV RNA, there is no evidence that it acts as a biological vector. WNV transmission primarily occurs via *Culex* mosquitoes, and the presence of WNV in

Hyalomma ticks is likely due to ingestion from infected hosts rather than active replication. Further studies are needed to confirm any potential vector competence.

Hyalomma ticks were prevalent on various migratory birds, with notable counts of 28 from golden orioles (*Oriolus oriolus*), 83 from wood warblers (*Phylloscopus* spp.), and 65 *H. rufipes* from common redstarts (*Phoenicurus phoenicurus*) and whinchats (*Saxicola rubetra*), among others from species like the icterine warbler (*Hippolais icterina*) and woodchat shrike (*Lanius senator*). These findings emphasize the role of migratory birds in introducing African ticks and zoonotic pathogens into Europe (Mancuso et al., 2022). On Pianosa Island, 11 *H. marginatum* were collected from vegetation in 2006-2007, with 2 (18.2%) testing positive for *R. aeschlimannii* (Tomassone et al., 2013).

In central and northern Italy, *H. marginatum* has frequently been found on livestock. A 2005 survey reported infection rates of 4.5% for *Babesia bovis* and 2.7% for *B. bigemina* in *H. marginatum* ticks from cattle, while 1.8% of ticks collected from horses tested positive for *T. equi* (Iori et al., 2010). Further studies revealed that 22.8% of *H. marginatum* ticks from cattle were infected with *Babesia*, with 27.6% exhibiting dual infections with *B. bigemina* and *B. bovis* (Cassini et al., 2012). In the spring of 2009, 386 ticks were collected from migratory birds on Capri and Antikythera islands, 369 of which were identified as *Hyalomma*, with nine identified as *H. rufipes* and one as *H. marginatum* (Molin et al., 2011). Ornithological surveys in central Italy in 2010-2011 collected 137 ticks from 41 birds across 17 species, all identified as nymphs within the *Hyalomma* genus (Mancini et al., 2013). Surveys conducted in Lazio in 2010–2011 found *H. marginatum* nymphs on migratory birds, with pathogens such as *R. aeschlimannii* (21.6%), *Ehrlichia* spp. (16.2%), C. *burnetii* (27.0%), and *Borrelia burgdorferi* sensu lato (s.l.) (73.0%) detected (Toma et al., 2014). In the Maremma region of Lazio, central Italy, a 2011–2012 study collected 154 ticks, identifying four as adult *H. marginatum* (Toma et al., 2015). Between 2012 and 2013, additional surveys in Lazio and Tuscany found 10 *H. marginatum* ticks (8.8%), with one testing positive for *R. aeschlimannii* (10%) (Scarpulla et al., 2016). Another survey in Lazio collected 96 ticks from vegetation, 49 of which were identified as *H. marginatum*, with one testing positive for *Babesia caballi* (2%) (Toma et al., 2017). Molecular screening of 255 tick samples, including 12 *H. marginatum* (5%), revealed positivity for *R. aeschlimannii* (66.7%), *Francisella*-like endosymbionts (66.7%), *B. burgdorferi* s.l. (50%), *Bartonella* spp. (25%), *C. burnetii* (33%), and *Ehrlichia* spp. (16%) (Mancini et al., 2019). In 2019, a study on Ponza Island collected 14 *H.*

rufipes ticks from various birds, detecting *R. aeschlimannii* in six specimens, *Francisella*-like endosymbionts in all, and *B. burgdorferi* s.l. in four ticks (Rollins et al., 2021). In summary, Italy represents a critical zone for the establishment and spread of *H. marginatum* in Europe, due to its favorable Mediterranean climate, high livestock density, and role as a major migratory bird corridor. The species is well-established in southern regions and islands like Sicily and Sardinia, with consistent reports of pathogen detections—including *Rickettsia spp.*, *C. burnetii*, *Babesia*, *Theileria*, and *Anaplasma*—from both domestic and wild hosts. These findings highlight Italy's importance in regional tick ecology and the need for sustained surveillance to monitor *Hyalomma*-borne disease risks.

Malta: Reports confirm the presence of *H. marginatum* in Malta. A recent study identified this tick species among 113 ixodid ticks collected from various bird species. Notably, one was found on a common kingfisher (*Alcedo atthis*), another on a common kestrel (*Falco tinnunculus*), and one on a common whitethroat. Additionally, two *H. marginatum* ticks were identified on sedge warblers (*Acrocephalus schoenobaenus*) and two on willow warblers (*Phylloscopus trochilus*) (Hornok et al., 2022). Furthermore, *H. rufipes* was identified on a broader range of birds, including 14 ticks from great reed warblers (*Acrocephalus arundinaceus*) and three from Eurasian reed warblers (*Acrocephalus scirpaceus*). Other species, such as barn swallows (*Hirundo rustica*), woodchat shrikes, and yellow wagtails, were also found to carry *H. rufipes*. A total of four *Hyalomma* spp. ticks were collected from sedge warblers, wood warblers, and common whitethroats. A 2021 study also reported the presence of *H. rufipes* on a migrant human, though no evidence of CCHFV or other pathogens were detected in the patient (Medialdea-Carrera et al., 2021). Findings from Malta highlight the island's role as an important stopover site for migratory birds carrying *H. marginatum* and *H. rufipes*. Although the number of ticks detected remains low and no pathogens have been found in humans, the wide range of avian hosts suggests ongoing passive introduction. These observations support the need for continued surveillance to monitor the potential establishment and spread of *Hyalomma* ticks in the region.

Portugal: *Hyalomma marginatum* is widely distributed across the westernmost country of continental Europe. It was first recorded in cattle in Vidigueira in 1971, with 177 adult male ticks collected; one pool of ticks tested positive for Dhori virus, with a 20% infection rate (Filipe & Casals, 1979). In 1985, two human cases of CCHFV seropositivity were reported in southern Portugal (Filipe et al., 1985). A 1998 study recorded 27 *H. marginatum* ticks among 82 ticks collected from vegetation, with two (7.4%) testing positive for *B. lusitaniae* (De Michelis et al., 2000) A nationwide survey in 2001 identified five *H. marginatum* ticks (6.6%) out of 76 total ticks collected, while in the Mafra and Grandola regions, 62 *H. marginatum* ticks (1.1%) were found among a total of 5,459 ticks, with two testing positive for *B. burgdorferi* s.l. and two for *B. lusitaniae* (Baptista et al., 2004).

Between 2002 and 2004, 118 *H. marginatum* ticks (77.6%) were collected from various bird species in Santo Andre Natural Reserve and Monsanto Forestal Park, with three testing positive for *R. aeschlimannii* (Santos-Silva et al., 2006). From 2006 to 2009, a study conducted in a Safari Park in Alentejo collected 677 ticks, identifying eight as *H. marginatum* (Milhano et al., 2010). In an updated study by Silva et al. (2011), *H. marginatum* was found to be the fourth most common tick in Portugal, with 6.1% of the total ticks collected, primarily from vegetation and ungulates. Immature stages were predominantly found on birds and hares (Santos-Silva et al., 2011). Between 2010 and 2011, four *H. marginatum* ticks were identified from a sample of 848 ticks collected in Tapada de Mafra National Park, and 22 *H. marginatum* were found among 1,122 ticks sampled from birds during the same period (Norte et al., 2012). Pereira et al. (2018) reported 176 *H. marginatum* ticks (29.7%) collected from various hosts, with positive results for *Anaplasma*, *Rickettsia* spp., *R. aeschlimannii*, *R. raoultii*, *Ehrlichia* spp., and mixed infections involving *Ehrlichia* and *Rickettsia* spp. (Pereira et al., 2018).

In Portugal, *H. marginatum* appears to be well-established across diverse habitats, with records spanning livestock, birds, wildlife, and vegetation. Pathogen detections over several decades—ranging from *Borrelia* spp. and *Rickettsia* spp. to *Anaplasma* and *Ehrlichia*—reflect both historical and ongoing public and veterinary health relevance. The presence of immature ticks on birds suggests continued passive dispersal, while detections in ungulates and vegetation confirm local maintenance.

Spain: *Hyalomma marginatum* is widely distributed across various regions in the country. ***In Northern Spain***, a survey conducted in 1998, *H. marginatum marginatum* was scarcely found among 12,832 ticks collected from sheep (Estrada-Pena et al., 2004). In Castilla y León, a survey conducted between 1996 and 2002 identified 324 *H. marginatum* ticks (10.6%) out of 3,059 ticks, with 5.9% testing positive for *R. aeschlimannii*, marking the first detection of this pathogen in Spain (Fernández-Soto et al., 2003). A study on Iberian red deer in the same region found that 558 of 582 ticks (96%) were adult *H. marginatum*, with 39% testing positive for *A. marginale* (De La Fuente et al., 2004). From 2001 to 2005, in La Rioja, 34.3% of 496 ticks collected were identified as *H. marginatum*, with a low prevalence (1.8%) of SFGR (Oteo et al., 2006). Later, Fernández-Soto et al. (2006) identified *H. marginatum marginatum* in 426 out of 4,049 ticks collected from humans in Castilla y León between 1997 and 2003, with 6.1% testing positive for *R. aeschlimannii* (Fernandez-Soto et al., 2006). Fernández-Soto et al. (2008) continued their work, identifying *R. aeschlimannii* in *H. marginatum marginatum* ticks from humans in northwestern Spain, collecting a total of 3,853 ticks from 2004 to 2007. Of these, 515 (13.4%) were identified as *H. marginatum marginatum*, with 73 testing positive for *R. aeschlimannii*. Additionally, two male ticks were co-infected with *A. phagocytophilum*, marking the first report of such dual infection in this tick species (Fernández-Soto et al., 2009). Further studies showed *H. marginatum* frequently parasitizing both wildlife and domestic animals. Ruiz-Fons et al. (2006) found *H. marginatum* to be the most abundant tick on red deer (63.7%) and wild boar (68.7%) (Ruiz-Fons et al., 2006). In La Rioja, a study conducted from 2009 to 2011 collected 336 ticks from 19 bird species, with 34 (10%) identified as *H. marginatum* (Palomar et al., 2015). Palomar et al. (2016) analyzed *Hyalomma* ticks from humans and birds in northern Spain between 2009 and 2015, identifying all 161 ticks as *H. marginatum*. Although no ticks tested positive for CCHFV, *R. aeschlimannii* was found in 53 ticks (24.9%), and *R. sibirica* subsp. *mongolitimonae* was found in 2 ticks (0.9%) (Palomar et al., 2016). Tick surveillance conducted in Castilla y León from 2014 to 2019 collected 734 *H. marginatum* ticks from humans, including 2 nymphs, 233 females, and 499 males (Vieira Lista et al., 2022).

In central Spain, a survey on tick-borne bacteria found *R. aeschlimannii* DNA in 8 of 13 *H. marginatum* ticks (61.5%) out of a total of 1,480 adult ticks (Toledo et al., 2009).

In eastern Spain, on Minorca Island, a survey conducted between 1999 and 2000 found that 26.8% (972 out of 3,624) of ticks collected from dairy cattle were adult *H. marginatum* (Castellà et al., 2001). In 2010, a study on Minorca Island tested 14 *H. marginatum* ticks from cattle farms, with one positive for *B. occultans* (14.3%) and two positives for *T. buffeli* (28.6%) (Ros-García et al., 2012). Between 2013 and 2015, 2,053 ticks were collected from livestock and vegetation, with 1,333 (64.9%) identified as *H. marginatum*, though none tested positive for CCHFV (Palomar et al., 2017). In 2017, Mateos-Hernández et al. reported 11 *H. marginatum* ticks from Minorca and Castilla-La Mancha, 10 from cattle and one from a human (Mateos-Hernández et al., 2017).

In western Spain, the first detection of CCHFV occurred in 2010, when *H. lusitanicum* ticks from red deer in Cáceres tested positive for the virus (Estrada-Peña, Palomar, et al., 2012). A survey conducted from 2011 to 2015 detected CCHFV in only one *H. marginatum* tick (0.4%) collected from cattle in Cáceres (Negredo et al., 2019). This positive finding was among a total of 238 *H. marginatum* ticks, which comprised 15.1% of the total sample, including 206 ticks from vegetation, 27 from red deer, and 5 from cattle. Data from Spain reveal a broad and long-standing presence of *H. marginatum*, with detections across northern, central, eastern, and western regions. The species has been recorded on a wide range of hosts and frequently carries pathogens. The involvement of both immature and adult ticks in pathogen transmission, along with evidence of coinfections and regional differences in prevalence, highlights the complex eco-epidemiology of *H. marginatum* in Spain and highlights the importance of continued, regionally tailored surveillance efforts.

Overall, Southern Europe remains the most important ecological and epidemiological hotspot for *H. marginatum* in Europe, with established populations, high host diversity, and frequent pathogen circulation highlighting its central role in tick-borne disease dynamics. Together, records from Italy, Malta, Portugal, and Spain confirm that *H. marginatum* is firmly established in the region, where warm Mediterranean climates support its full life cycle and reproduction. The species is consistently found on a wide range of hosts—including livestock, wildlife, and birds—and across varied habitats, demonstrating its strong adaptability. Importantly, migratory birds play a dual role: not only do they support local tick populations by hosting immature stages, but they also facilitate the species' northward dispersal. This bird-mediated spread introduces *H. marginatum* into new areas, potentially expanding its geographic range under changing environmental conditions.

7.2. *Hyalomma marginatum* Distribution in the Balkan Peninsula

The Balkan Peninsula ranks as the second most extensively studied region, after Southern Europe, in research on *H. marginatum*, with 28 studies documenting its presence, accounting for 23% of all publications reviewed. In addition, four studies have reported broader findings, including the presence of *H. marginatum* s.l., *H. marginatum* sensu stricto, the *H. marginatum* complex, and *Hyalomma* species across the region.

The Balkan Peninsula is particularly vulnerable to extensive colonization by *H. marginatum*. Since 1952, the region has experienced both ongoing outbreaks and sporadic cases of Crimean-Congo Hemorrhagic Fever (CCHF), with *H. marginatum* as the primary vector. However, the tick species *Rhipicephalus bursa* is also recognized as a significant vector for CCHFV after *H. marginatum*. Alongside CCHFV, various species of *Rickettsia* have been identified in *H. marginatum* ticks in this region, suggesting a potential for multiple pathogen transmission.

Albania: The presence of *H. marginatum* has been confirmed through recent surveillance efforts. In a study by (Papa et al., 2017), 341 *H. marginatum* ticks were collected from livestock, of which 4.7% tested positive for CCHFV. This finding is consistent with Albania's long-standing status as a CCHF-endemic country. The first human case was recorded in 1986, and the northeastern region bordering Kosovo continues to be considered a high-risk area for virus circulation (Papa et al., 2002). These data, though limited, point to active enzootic transmission cycles involving *H. marginatum* and its involvement in CCHFV transmission in high-endemic areas.

Bosnia: *Hyalomma marginatum* was first documented in 2008, representing 5.7% of the 10,050 ticks sampled nationwide, with specimens primarily collected from sheep (54.4%), cattle (22.2%), dogs (21.5%), and goats (1.9%) (Omeragic, 2011). In a follow-up survey, CCHFV RNA was detected in one pool of three male *H. marginatum* ticks. Another survey later reported that 2.2% of the 6,067 ticks collected were identified as *H. marginatum*, primarily found on cattle and sheep (Omeragić et al., 2022). The most recent survey in Bosnia detected CCHFV RNA in one pool of three male *H. marginatum* ticks among a sample of 760 ticks (Goletic et al., 2022). Although limited in scope, studies in Bosnia consistently report the presence of *H. marginatum* in livestock, with occasional detection of CCHFV RNA. These findings suggest an established population capable of pathogen transmission, warranting expanded surveillance in both livestock and environmental reservoirs.

Bulgaria: The first recorded presence of *H. marginatum* was reported in Carevo in 2006 (Cuber, 2016). Although the exact distribution of *H. marginatum* remains uncertain, Bulgaria has reported multiple cases of CCHF since its initial identification in 1952, with 1,105 cases documented between 1953 and 1974 and a fatality rate of approximately 17% (Gergova et al., 2012). Between 1975 and 2010, 450 additional cases were reported, with a reduced fatality rate of 5.48%, likely due to the introduction of a Bulgarian-developed CCHFV vaccine in 1974 (Gergova et al., 2012). Bulgaria is now considered endemic for CCHFV, posing an ongoing public health risk. From 2006 to 2010, *H. marginatum* was the most prevalent tick species in endemic regions, accounting for 31.2% of 911 adult ticks collected from livestock, and 14 of 284 *H. marginatum* specimens tested positive for CCHFV RNA (Gergova et al., 2012). In 2014, a survey across five districts found that 60.5% of 1,030 *H. marginatum* ticks tested positive for CCHFV RNA (Panayotova et al., 2016). Recent research has also identified *H. marginatum* on Eurasian eagle-owls (*Bubo bubo*) in southeastern Bulgaria, with 15 ticks collected from this host, further confirming the species' widespread presence in the country (Sándor et al., 2021). Bulgaria is one of the most extensively studied Balkan countries for both *H. marginatum* and CCHFV, with sustained surveillance confirming the tick's central role in virus transmission. Decades of data and control efforts highlight its critical role in the country's endemic transmission cycle.

Croatia: The presence of *H. marginatum* is well-established along the Mediterranean coast of Europe and is documented in Croatia. In a tick survey conducted in 2000 in Split, Dalmatia County, 17 out of 197 collected ticks (8.6%) were identified as *H. marginatum*, with 64.7% of these specimens (11 individuals) testing positive for *R. aeschlimannii* (Punda-Polic et al., 2002). Despite limited understanding of the epidemiology of rickettsial diseases in Croatia, two tick-borne rickettsial illnesses have been reported in the southern part of the country: Mediterranean spotted fever, caused by *R. conorii*, in coastal regions, and rickettsial pox, caused by *R. akari*, in northern areas (Punda-Polic et al., 2002). A separate study investigating rickettsiae in Croatian ticks found *R. conorii* present in 12.6% of *R. bursa* ticks, 10.6% of *R. sanguineus* ticks, and 7.3% of *Dermacentor marginatus* ticks; however, *H. marginatum* was not included in that survey (Crocquet-Valdes et al., 1994). The most recent data, covering tick collections from 2017 to 2020, documented three *H. marginatum* specimens collected from vegetation (Krčmar et al., 2022). Data from Croatia indicate a scattered presence of *H. marginatum*, mainly in coastal areas. Despite sparse records, the detection of *R. aeschlimannii* in local specimens signals a possible, though underreported, contribution to the region's tick-borne pathogen landscape.

Greece: *Hyalomma marginatum* and various other ixodid tick species have been identified in domestic animals and humans, with numerous studies investigating *H. marginatum* distribution and associated pathogens. The first and largest tick survey on domestic animals was conducted in

Macedonia between 1983 and 1986, with 11,620 ticks collected from cattle, sheep, goats, and dogs. *H. marginatum marginatum* comprised 3.5% of the total ticks collected, while *R. bursa* was the most abundant species, comprising 36.3% of the collection. Additionally, adult *H. marginatum rufipes* and *H. marginatum turanicum* were observed on cattle (B. Papadopoulos et al., 1996). A survey conducted in 1998–1999 on Cephalonia Island found 130 *H. marginatum* specimens (7% of the 1,848 ticks collected), with two specimens testing positive for *C. burnetii*, though no ticks tested positive for rickettsia (Psaroulaki et al., 2006).

In a survey conducted from 2003 to 2006 in Northern Greece, a total of 3,249 adult ticks were collected from goats and sheep, with 12.4% identified as *H. marginatum* (Pavlidou et al., 2008). This species was also commonly found in migratory birds, as shown by surveys from 2009 to 2010 at bird observatories in Capri, Italy, and Antikythira, Greece, where 88.2% (659 of 747) of ticks collected were *H. marginatum* (Hagman et al., 2014). In subsequent testing of 13,332 migratory birds, *H. marginatum* s.l. accounted for 90% of the ticks collected. Pathogen screening of *H. marginatum* s.l. ticks revealed positive results for *R. aeschlimannii* (45.6%), *Rickettsia* spp. (2.4%), and *R. africae* (2.3%) (Wallménius et al., 2014). Additionally, one *H. marginatum* sensu stricto specimen was identified that tested positive for *R. aeschlimannii*.

Recent surveys indicate a decline in *H. marginatum* presence, with only 0.2% of ticks collected from livestock identified as *H. marginatum* between 2012 and 2013 (Chaligiannis et al., 2016). Although CCHFV assessments in Greece reported no human cases until 2008, 2.8% of tick pools collected between 2012 and 2014 tested positive, predominantly in *R. sanguineus* and *R. bursa* samples, while all *H. marginatum* samples tested negative (Papa Anna et al., 2017). *Hyalomma marginatum* is widely distributed across Greece and has been detected on domestic hosts, migratory birds, and humans. Although recent studies show a decline in prevalence, the tick's frequent association with *Rickettsia* species and past CCHFV surveillance affirm its continued epidemiological relevance.

Kosovo: Tick collections conducted in between 2001 and 2014 confirmed *H. marginatum* presence across Kosovo (Fournier et al., 2003; Sherifi et al., 2014, 2018). The country experiences periodic CCHF outbreaks, with the first major outbreak documented in 1970 among shepherds near the border with North Macedonia (Ergönül, 2006). However, the earliest known case dates back to 1954. Since 1989, Kosovo has experienced regular CCHF outbreaks approximately every

4-5 years (Ahmeti et al., 2019; Vesenjak-Hirjan et al., 1991). Following the initial 1970 outbreak, another significant outbreak occurred between 1991 and 1992, with subsequent outbreaks in 1995 (65 patients, 7 fatalities) and during the period of 1996 to 2000 (33 sporadic cases, 7 fatalities). In 2001, shortly after the Kosovo war, the largest epidemic was reported with 155 suspected and 30 confirmed cases (Ergönül, 2006). According to Jameson et al. (2012), around 50% of Kosovo's municipalities are considered at risk for CCHF, with highly endemic areas identified in Skënderaj, Klinë, Malishevë, Rahovec, and Suharekë (Jameson, Ramadani, et al., 2012). There are two distinct genetic lineages of CCHFV in Kosovo and Albania. The Europe 1 (clade V) lineage, primarily vectored by *H. marginatum*, is highly pathogenic to humans. In contrast, the Europe 2 (clade VI) lineage, vectored by *R. bursa*, exhibits mild or non-pathogenic characteristics in humans (Papa et al., 2017). The frequent circulation of CCHFV in ticks, combined with repeated human outbreaks in southwestern Kosovo and northern Albania—where *H. marginatum* is prevalent—strongly suggests the presence of the highly pathogenic Europe 1 strain in these regions (Sherifi et al., 2018).
Kosovo remains a high-risk area for CCHFV, with *H. marginatum* playing a central role in the transmission of the Europe 1 strain. Recurring outbreaks and consistent tick positivity indicate the tick's well-established presence and epidemiological importance in endemic zones.

Romania: The first report of *H. marginatu*m dates back over 55 years, as documented in Feider's review of tick distribution (Mihalca, Dumitrache, et al., 2012). While *I. ricinus*, *D. marginatus*, and *Haemaphysalis punctata* are more prevalent nationwide, *H. marginatum* is primarily found in southern Romania. Between 1998 and 2004, a livestock surveillance study identified only 6.9% of 2,706 ticks as *H. marginatum* (Popa E & Teodorescu I, 2006). Subsequent studies have confirmed this species' presence on vegetation, mammals, and birds. For example, a survey reported just 0.02% (2 out of 13,771) of ticks collected from vegetation as *H. marginatum* and 2.4% (20 out of 840) from animals (Dumitrache et al., 2012; Mihalca, Dumitrache, et al., 2012). In 2010, 35.3% of 382 ticks collected in a study on tick-borne pathogens were identified as *H. marginatum*, with *Babesia* spp. and *Theileria* spp. DNA detected in 12 (8.9%) of these ticks, showing sequences similar to *T. equi*, *T. orientalis/sergenti/buffeli*-group, and *B. occultans* (Ionita et al., 2013). In 2013, three adult *H. marginatum* ticks were reported from dogs in southern Romania (Dumitrache et al., 2014). Additional findings in the Danube Delta Biosphere Reserve between 2012 and 2013 detected 32 ticks on birds, 31 of which were *H. marginatum marginatum*, with one specimen collected from a juvenile song thrush testing positive for WNV (Kolodziejek et al., 2014). A study on urban crows in 2013 also identified one *H. marginatum marginatum* nymph from a Eurasian jackdaw (*Corvus monedula*) (Sándor et al., 2017). In Romania, *H. marginatum* appears to be locally established, particularly in southern regions. Although less abundant than other tick species, it has been found to harbor several pathogens, including *Babesia*, *Theileria*, and WNV, highlighting its medical and veterinary relevance.

Other Balkan countries: Reports also confirm the presence of *H. marginatum* in North Macedonia, Montenegro, and Serbia, although specific data on its distribution and pathogen prevalence in these countries remain limited (EFSA, 2010).

The Balkan Peninsula is a critical region for understanding *H. marginatum* and its role in transmitting zoonotic pathogens like CCHFV and *Rickettsia* spp. While significant progress has been made in mapping its distribution and pathogen associations, some countries in the region remain underrepresented in the data, leaving critical gaps in knowledge. Targeted studies and improved diagnostics are essential for developing effective control strategies to mitigate the impact of *Hyalomma*-borne diseases in the region.

7.3. *Hyalomma marginatum* Distribution in Western Europe

A total of 18 publications were included from Western Europe, accounting for 14.1% of all the studies analyzed.

France: *Hyalomma marginatum* is widely distributed in the southern regions of the country, where the climate, high density of migratory birds, and the presence of ungulates, especially horses, create favorable conditions for its establishment.

In southern France, between 2007 and 2010, a survey identified five *H. marginatum* ticks (1.2%) out of 406 collected, with one tick infected with *A. phagocytophilum* (Chastagner et al., 2013). While historical records have sometimes been inconsistent, recent studies have confirmed the established presence of *H. marginatum* in southern France. Comprehensive tick collections conducted from 2007 to 2016 on horses and birds identified 84 *H. marginatum* ticks (7.1%) out of a total of 1,179 specimens (Vial et al., 2016).

In the Camargue region, a study conducted between 2015 and 2016 identified 47 *H. marginatum* out of 585 horse ticks, with 43% positive for *T. equi*, a significant pathogen in equine piroplasmosis (Rocafort-Ferrer et al., 2022). *Hyalomma marginatum* was less common in wet habitats of Camargue, aligning with its xerophilic nature, which favors drier environments like Corsica. These findings reinforce the expanding range of *H. marginatum* in France and its critical role in transmitting pathogens such as *Rickettsia*, *Theileria*, and *Ehrlichia* across diverse hosts. From 2016 to 2019, a survey of ticks collected from horses across 14 French departments in the Mediterranean corridor of southern France found that *H. marginatum* comprised 36% of the 2,588 ticks collected (Bah et al., 2022).

In Corsica, a favorable climate and extensive livestock farming have allowed *H. marginatum* to thrive. Initial records date back to 1959, with additional reports in 2004 and 2007 (Grech-Angelini et al., 2016). This survey collected 3,134 ticks, of which 571 (18.2%) were *H. marginatum*, predominantly on cattle (73.2%) and horses (24.5%). Pathogen analysis detected *R. aeschlimannii*, *Francisella*-like endosymbionts, *A. marginale*, and *A. phagocytophilum* in these ticks (Grech-Angelini et al., 2020). Since 2015, large-scale tick surveys in Corsica have significantly enhanced understanding of *H. marginatum* distribution and its role in pathogen transmission. In 2015, Dahmani et al. identified 5 *H. marginatum* (4.1%) out of 123 ticks collected (Dahmani et al., 2017). In 2016, 91 *H. marginatum* ticks (13.8%) were found among 660 collected from cattle, making it the second most abundant species after *R. bursa* (Cicculli, Masse, et al., 2019). Molecular analysis detected *Rickettsia* spp., *B. burgdorferi* (s.l.), *Anaplasma* spp., and *E. minasensis* DNA, marking the first identification of *E. minasensis* in *H. marginatum* in Corsica (Cicculli, Capai, et al., 2019; Cicculli, Masse, et al., 2019). From 2017 to 2019, surveys collected 216 *H. marginatum* ticks (19.3%) from cattle and wild boar out of 1,117 total ticks, all of which tested positive for *R. aeschlimannii* (Cicculli, Oscar, et al., 2019). Concurrently, in Palasca and Nessa, 216 *H. marginatum* ticks (9.8%) out of 820 samples tested positive for *A. phagocytophilum* and *E. minasensis* DNA, highlighting the species' widespread presence and its involvement in pathogen transmission (Cicculli et al., 2020). Recent studies across Corsica and France highlight the abundance of *H. marginatum* in livestock and wild animals. Between 2018 and 2019, 3,555 ticks were collected from sheep, cattle, wild boars, and horses in Corsica, with 1,454 identified as *H. marginatum* (Cicculli et al., 2022). Parapoxvirus DNA was detected in 8.2% of *H. marginatum* pools. In a separate study (2018-2020), 113 ticks were collected from wild boars, with two identified as *H. marginatum*, and one (50%) positive for *R. aeschlimannii* (Defaye et al., 2021).

From 2019 to 2021, 702 *H. marginatum* ticks from Corsican cattle tested positive for *R. aeschlimannii*, with 4.3% also positive for *Theileria* spp. and *E. minasensis* (Maitre et al., 2023).

France—especially its southern regions and Corsica—has seen a marked increase in *H. marginatum* detections across multiple ecosystems. The tick's adaptation to drier climates, strong presence on livestock and equids, and consistent detection of multiple pathogens in both mainland and island contexts suggest an expanding ecological niche and a rising risk of tick-borne disease transmission in Mediterranean France.

Netherlands: The first documented exotic *Hyalomma* species was *H. aegyptium*, reported by Bronswijk et al. in 1979 (Nijhof et al., 2007). Between 2012 and 2014, tick collections on birds in the Netherlands confirmed the presence of imported *Hyalomma* species in the country (Heylen et al., 2017). While immature stages have been sporadically found on migratory birds, adult stages have been reported less frequently in the Netherlands. According to Uiterwijk et al. (2021), three adult *Hyalomma* ticks were identified on horses between 2005 and 2009, one of which was identified *as H. marginatum rufipes* (Uiterwijk et al., 2021). No pathogens were detected in these specimens (Nijhof et al., 2007). Exotic tick species, such as *Hyalomma*, are occasionally introduced into the Netherlands via imported reptiles, pets, migratory birds, or companion animals returning from endemic areas (Uiterwijk et al., 2021). From 2019 to 2020, reports of *Hyalomma* ticks were documented through a citizen science project (Uiterwijk et al., 2021). One adult *Hyalomma* species was found in 2018 and reported in 2020, with 17 additional specimens identified as adult *Hyalomma* species ticks (one in 2018, eleven in 2019, and five in 2020). Based on morphological and molecular species identification, along with cluster analysis, 12 of these ticks were confirmed as adult *H. marginatum*. Nearly all of the specimens (11 out of 12) were discovered on horses, with one adult tick collected from a human. Pathogen detection analysis revealed that one adult female *H. marginatum*, reported from a horse in 2019, tested positive for *R. aeschlimannii*.

Hyalomma marginatum is not considered established in the Netherlands but is occasionally detected, predominantly through introductions via migratory birds and horses. Although these records are rare and mostly involve adult ticks, the occasional detection of pathogens such as *R. aeschlimannii* warrants ongoing surveillance. The role of citizen science has proven valuable in documenting these sporadic occurrences, reinforcing the importance of public engagement in early warning systems, particularly as climate change and animal movement patterns evolve.

United Kingdom: Sporadic *H. marginatum* records date back to historical data compiled by the Biological Records Centre (1860–2001) (Jameson & Medlock, 2011). Between 2005 and 2009, one *H. marginatum* specimen was found among 4,172 ticks, marking the first adult record in the UK, likely transported from Portugal (Jameson & Medlock, 2011). In 2010 and 2011, 14 nymphal *H. marginatum* ticks (21%) were collected from bird species such as sedge warbler, northern wheatear (*Oenanthe oenanthe*), common redstart, and whitethroat, though none tested positive for CCHFV (Grech-Angelini et al., 2020). Over the past three decades, *H. marginatum* has been intermittently introduced to the UK through passive transport on migratory birds flying northward, yet no permanent populations of this species have been established. This is likely due to the unsuitable climatic conditions in the UK, where the summers are typically too wet or too cold for the tick's full development (Estrada-Peña et al., 2011; Gray et al., 2009). A recent 2021 report documented the first human exposure to an adult *H. marginatum* in England, identified as *H. marginatum* and positive for *R. aeschlimannii* (McGinley et al., 2021). Additionally, a 2018 finding in England involved a male *H. rufipes* on a horse with no travel history, also testing positive for *R. aeschlimannii*, potentially indicating a successful molt within the UK (Hansford et al., 2019). Despite repeated introductions of *H. marginatum* into the UK, primarily through migratory birds and occasional detections on horses and humans, environmental conditions currently remain unsuitable for stable establishment. The findings of pathogen-positive specimens emphasize potential health risks, reinforcing the importance of continuous surveillance amid changing climate conditions and animal movement patterns.

Western Europe reflects a gradient—from established foci in the south to passive introduction zones in the north—highlighting the need for region-specific monitoring strategies. In this region, *H. marginatum* is well-established in southern France and Corsica but remains sporadically introduced into the UK and the Netherlands. Its repeated detection alongside pathogens highlights potential health risks, necessitating continued surveillance under changing climate conditions.

7.4. *Hyalomma marginatum* Distribution in Central Europe

A total of 13 studies in Central Europe (10.7% of all publications) report the presence of *H. marginatum* across various countries in this region; however, no permanent populations have been established.

Austria: The first recorded occurrence of *H. marginatum* was in 2018, when a male tick was found on a horse in Lower Austria (Duscher et al., 2018). Likely introduced by migratory birds, the tick tested negative for CCHFV but positive for *R. aeschlimannii*. The detection of *H. marginatum* on domestic animals highlights the potential for tick introductions linked to migratory birds and animal transport. Ongoing surveillance and pathogen monitoring are therefore essential to detect potential establishment and associated disease risks.

Czechia: According to Capek et al. (2014), immature stages of *H. marginatum* complex ticks were detected on migratory birds in the former Czechoslovakia over 60 years ago. Three nymphs were collected from tree pipits (*Anthus trivialis*), and one nymph from a bluethroat (*Luscinia svecica*) in 1953 (Hubálek et al., 2020). Surveys from 2010 to 2012 also identified larval and nymphal stages of the *H. marginatum* complex on migratory birds in central Moravia and eastern Bohemia. Specifically, 12 ticks were collected in 2010 from great reed warblers and common nightingales (*Luscinia megarhynchos*); in 2011, one adult female was found on an Eurasian reedv warbler; and in 2012, two ticks were collected from marsh warblers (*Acrocephalus palustris*) and common nightingales (Capek et al., 2014). These host species are long-distance migratory birds that winter in sub-Saharan Africa and are likely responsible for introducing *H. marginatum* into Central Europe during their spring migration from North Africa or the Mediterranean basin.

Hyalomma rufipes has also been documented in Czechia. In 2019, a male *H. rufipes* tick was collected from a horse in South Moravia (Hubálek et al., 2020), followed by another adult male specimen in April 2020 from the same location (Rudolf et al., 2021). Testing of these specimens for flaviviruses, bunyaviruses, phleboviruses, and *Rickettsia* spp. yielded negative results. Recent studies also documented four adult *H. marginatum* ticks on horses and one from a household, totaling nine *Hyalomma* ticks, including one nymph on a ringed common nightingale (Lesiczka et al., 2022). Molecular analysis confirmed both *H. marginatum* and *H. rufipes*, marking the first molecular identification of these species in Czechia. Recent detections of immature *H. marginatum* and *H. rufipes* ticks on migratory birds in Czechia highlight the country's role as an important transit area for these species into Central Europe. Although consistent findings indicate repeated seasonal introductions, there remains no evidence to support the establishment of stable local populations.

Germany: Although *H. marginatum* is not part of the indigenous tick fauna in Germany, its presence has been sporadically reported in recent years. The first documented case occurred in 2006, when a questing female *H. marginatum marginatum* was found on a person's clothing after spending time in rural areas. Molecular testing for CCHFV and *R. aeschlimannii* yielded negative results (Kampen et al., 2007). Migratory birds regularly transport immature stages of *H. marginatum* into Germany during spring migration. In a study by Rumer et al. (2011), 294 ticks were collected from birds in the Berlin-Brandenburg region; molecular analysis identified three as *H. marginatum* complex, all positive for *R. aeschlimannii* (Rumer et al., 2011). Another occurrence was reported in 2016 when a female *H. marginatum* was found on a man's trousers in Tübingen, marking the first confirmed morphological and genetic identification of the species in Germany (Oehme et al., 2017). In a 2018 survey by Chitimia-Dobler et al., 10 out of 18 ticks collected across various districts were identified as *H. marginatum*, with 50% testing positive for *R. aeschlimannii* (Chitimia-Dobler et al., 2019). The remaining eight ticks were *H. rufipes*; three collected from horses and one from a horse's habitat tested positive for *R. aeschlimannii*. Additionally, in December 2015, *H. rufipes* was first reported in Germany on a horse in Rhineland-Palatinate, with negative tests for rickettsiae and CCHFV (Chitimia-Dobler et al., 2016). Germany's repeated detection of *H. marginatum*, particularly linked to migratory birds and sporadic human encounters, highlights its ongoing but transient presence. The country's climatic conditions currently limit permanent establishment, yet the potential for pathogen introductions necessitates sustained surveillance efforts.

Hungary: Sporadic records of *H. marginatum* have been noted from imported animals, humans, and migratory birds. In 2009, Földvári et al. (2011) identified a nymphal *H. marginatum* feeding on a hedgehog in a Budapest park (Földvári et al., 2011). A 2011 survey on cattle and wild ruminants found two adult *H. marginatum rufipes* males on cattle, marking the first record of this species on cattle in Central Europe (Hornok & Horváth, 2012). This area was previously identified by Estrada et al. (2011) and Celina et al. (2023) as high risk for *Hyalomma* establishment (Celina et al., 2023; Estrada-Peña et al., 2011). The first molecular evidence of *H. marginatum* in Hungary came from Hornok et al. (2013), who identified three immature ticks on a robin (*Erithacus rubecula*), with two testing positive for *R. aeschlimannii* (66.6%) (Hornok et al., 2013). These findings, combined with seropositivity rates among animals, suggest Hungary could be a new region for CCHFV distribution (Magyar et al., 2021). Magyar et al. (2021) found CCHFV exposure in 12 out of 2,700 human serum samples, although the limited presence of *H. marginatum* does not indicate stable populations in the country.

In 2021, a citizen-science project recorded two *Hyalomma* specimens: a male *H. marginatum* found in a dog and a male *H. rufipes* feeding on a cow (Földvári et al., 2022). These findings highlight the sporadic nature of *Hyalomma* ticks in Hungary and the need for ongoing surveillance. Between 2012 and 2014, a total of 3,339 ixodid ticks were collected from 1,167 passerine birds in Hungary, including three *H. rufipes* nymphs on a common whitethroat in May 2014, marking the first molecular evidence of avian transport of immature *H. rufipes* in Central Europe (Hornok et al., 2016). In 2022, 956 ixodid ticks were collected from birds in Hungary, including 12 *H. rufipes*, were found on various species: one nymph on a common whitethroat, one on a European pied flycatcher (*Ficedula hypoleuca*), three on sedge warblers, and eight on bearded reedlings (*Panurus biarmicus*), comprising five nymphs and one larva (Keve et al., 2023). Hungary's intermittent findings of *Hyalomma* ticks across diverse host species, alongside evidence of pathogen presence and human CCHFV exposure, reflect periodic introductions and underline the need for proactive tick and pathogen surveillance.

Poland: Historical records indicate the presence of *H. marginatum* in the 1930s and 1940s, with four unfed male specimens found in Bytom, Upper Silesia, preserved in the Bytom Museum (Cuber, 2016). More recent records are limited, with only two findings on migratory birds: one on a western yellow wagtail and another on a sedge warbler (Nowak-Chmura Magdalena & Solarz Wojciech, 2010). A recent epidemiological study of CCHFV in cattle in southeastern Poland did not detect *H. marginatum*, identifying only *D. reticulatus* and *I. ricinus* instead (Bażanów et al., 2017). Current evidence of *H. marginatum* in Poland is scarce, limited to occasional detections, suggesting transient occurrences rather than established populations. Continued monitoring remains necessary to clarify its status.

Slovakia: *Hyalomma marginatum* has also been recorded on migratory birds. Historical records show a nymph of *H. marginatum* complex collected from a robin in 1953 and two larvae and four nymphs from a marsh warbler in 1987 (Hubálek et al., 2020). Additionally, in 1981, a unique instance documented a female *H. marginatum* on a man's clothing (Hubálek et al., 2020). More recently, between 2008 and 2009, immature stages of the *H. marginatum* complex were found on migratory birds in southwestern Slovakia, including species similar to those in Czechia, such as the great reed warbler, Eurasian reed warbler, sedge warbler, and Savi's warbler (*Locustella luscinioides*) (Capek et al., 2014). Immature *Hyalomma* ticks have been sporadically detected on passerines in Slovakia; however, species-level confirmation is limited. Further research is needed to determine whether *H. marginatum* specifically occurs and whether conditions could support its establishment.

Overall, In Central Europe, *H. marginatum* is not yet established. Its presence is sporadic and primarily linked to passive introduction of immature stages via migratory birds during spring. These ticks rarely develop into adults, and there is no evidence of local reproduction or sustained life cycle completion. Most records involve larvae and nymphs on birds, while occasional adult ticks on livestock or humans likely reflect recent importation or local molting events, rather than true colonization. Importantly, despite repeated introductions, climatic conditions in the region are likely insufficient to support full tick development and overwintering. Despite this, some introduced ticks carry pathogens like *R. aeschlimannii*, posing a risk of pathogen introduction. As climate change alters environmental suitability, Central Europe may become increasingly vulnerable to colonization. The patterns observed emphasize the region's role as a sentinel front for tracking early signals of northward tick expansion under changing climatic and ecological pressures.

7.5. *Hyalomma marginatum* Distribution in Eastern Europe

Eastern Europe, with the fewest recorded publications on *H. marginatum* (only 4.7% of the total), likely harbors a significant population, especially in southern Ukraine and Russia. However, ongoing conflict in these areas has severely limited research capabilities, making it nearly impossible to study the actual abundance and distribution of *H. marginatum*. War-driven changes in land use, such as abandoned farms, displaced livestock, and altered human activities, may also increase the risk of CCHF outbreaks by creating favorable conditions for tick proliferation and pathogen transmission.

Moldova: Since the 1980s, the presence of *H. marginatum* ticks had not been recorded in the Republic of Moldova. However, during systematic surveillance of migratory birds in the spring seasons of 2012 and 2015, four *H. marginatum* were identified (Morozov et al., 2022).

Russia: *Hyalomma marginatum* is extensively distributed in southern Russia, where it poses a major public health concern. This tick species is prevalent in several Russian regions, including Stavropol, Rostov, Krasnodar, Dagestan, and the Karachay-Cherkess Republic, all recognized as CCHF-endemic areas where *H. marginatum* plays a dominant role in the virus's transmission (Tsapko et al., 2022). The tick was associated with periodic CCHF outbreaks in Russia, notably resurging in 1999, when 40 CCHF cases were reported (Yashina et al., 2003). In European Russia, sporadic cases of the CCHF are registered each year. During 2000 and 2001, 133 primary cases of CCHF were identified in European Russia, with a fatality rate of 9.0% (Onishchenko, 2001). In southern Russia, *H. marginatum* accounts for nearly 50% of ticks collected from domestic animals, with studies confirming the presence of pathogens in this species. For instance, Yashina et al. (2003) examined 4,787 *H. marginatum* ticks from European Russia, detecting CCHFV in 10.2% of 449 pools (Yashina et al., 2003). Similarly, Tsapko et al. (2022) found *H. marginatum* to be the dominant species among over 38,000 ticks, comprising 66% of the specimens, with 81% of the CCHFV-positive pools from this species (Tsapko et al., 2022). In May 2004, Shpynov et al. collected forty adult *H. marginatum* ticks (20 females, 20 males) from cattle and vegetation in Stavropol, with 20% testing positive for *R. aeschlimannii*. *Hyalomma marginatum* has also been documented outside southern Russia. Movila et al. (2012) recorded five *H. marginatum* on migratory birds in Kaliningrad Oblast, marking the first detection in Russia's Baltic region (Movila et al., 2013). In one nymph collected from a tree pipit, *R. aeschlimannii* was detected. The Stavropol and Rostov regions remain notable hotspots for CCHFV, largely due to the high density of *H. marginatum* populations.

Hyalomma marginatum is firmly established in southern Russia and remains a dominant vector of CCHFV in endemic regions. Its high abundance, coupled with consistent pathogen detection, highlights its central role in the country's tick-borne disease ecology. Sporadic findings beyond its core range point to a potential for further expansion.

Ukraine: The distribution of *H. marginatum* in Ukraine extends along northern Odesa, Mykolayiv, Kirovograd, Dnipropetrovsk, Zaporizhia, Donetsk, Luhansk Oblasts, and the Crimean peninsula, with widespread presence in the southern regions. Further, regular findings in atypical locations suggest potential for spreading zoonotic diseases to new areas, increasing epidemic risks (Akimov & Nebogatkin, 2011). The first reports of *H. marginatum* in former Soviet union date back to the 1940s, in the context of CCHFV outbreaks in the Crimean region.

From 1977 to 2009, researchers conducted comprehensive surveys across Crimea, 22 other Oblasts, and major cities, including Kyiv, Mariupol, and Sevastopol, to determine the range of *H. marginatum* in Ukraine (Akimov & Nebogatkin, 2011). Approximately 12,000 ticks were collected using flag-dragging and host examination methods, with 78% of adult *H. marginatum* collected from vegetation and 22% from animal hosts.
In Ukraine, *H. marginatum* is well-established across southern parts of the country. Historical associations with CCHFV highlight the country's relevance in the regional ecology of tick-borne diseases. Recent military conflicts and large-scale population displacements may disrupt local ecosystems, alter host movements, and reduce surveillance capacities, collectively increasing the risk of tick expansion and zoonotic pathogen transmission.

Eastern Europe, particularly southern Russia and Ukraine, harbors long-established populations of *H. marginatum*, playing a central role in CCHFV transmission. Historical outbreak data and high tick abundance confirm its vector importance in these endemic zones. Despite low publication numbers, surveillance shows the species is well adapted to dry steppe and agricultural habitats. However, ongoing conflicts and limited research capacity have hampered recent monitoring efforts. This region combines a strong ecological foundation for tick-borne diseases with an urgent need for renewed surveillance and control programs.

7.6. *Hyalomma marginatum* Distribution in Northern Europe

The recorded publications on *H. marginatum* in Northern Europe are limited, accounting for only 3.9% of the total analyzed studies. All reported occurrences of *H. marginatum* in this region have involved immature stages, which were likely transported via migratory birds during their spring migration northward.

Denmark: The first recorded instance of *H. marginatum marginatum* in Northern Europe dates back to June 1939, when it was found on the island of Bornholm, Denmark, likely carried as a nymph by a migratory bird from the Mediterranean or Africa (Johnsen, 1943). Similar evidence of bird-mediated tick dispersal was noted by Jaenson et al. (1994), who documented a nymph on a common kestrel in 1991 on the Danish island of Christiansø (Jaenson et al., 1994). *Hylomma marginatum* has been rarely detected in Denmark, with records limited to migratory birds. While establishment is unlikely under current climatic conditions, the country's northern position makes it relevant for monitoring future range shifts.

Finland: Research on ticks parasitizing birds remains limited, with only a few studies documenting tick species detected on avian hosts (Laakkonen et al., 2009; Nuorteva P & Hoogstraal H., 1963; Saikku et al., 1971; Ulmanen et al., 1977). These studies have reported the presence of four tick species: *I. ricinus*, *I. arboricola*, *I. lividus*, and *H. marginatum*. A historical record noted *H. marginatum* on a migratory bird (Nuorteva P & Hoogstraal H., 1963), and a recent survey (2018-2020) found *H. marginatum* in 0.7% of 434 ticks collected from 32 bird species (Sormunen et al., 2022). Specifically, two specimens were found on a barred warbler (*Curruca nisoria*), and one was collected on a tree warbler (*Iduna caligata*). Although research is limited, both historical and recent studies confirm low-frequency introductions of *H. marginatum* via migratory birds. The detection of the species in recent bird surveillance highlights the need for continued avian-based monitoring.

Norway: Similarly, *H. marginatum* has occasionally been detected on migratory birds in Norway. The first records of *H. marginatum* nymphs were reported from common redstart, Eurasian reed warbler, willow warbler, and red-backed shrike (*Lanius collurio*) (Jaenson et al., 1994). Since these early reports, no further scientific studies have confirmed the presence of *H. marginatum* in Norway, though occasional media reports have mentioned sightings. In addition to these records, a study conducted between 2003 and 2005 identified seven fully engorged nymphs of *H. rufipes* on six bird species, including the garden warbler (*Sylvia borin*), common redstart, reed warbler, wheatear, whitethroat, and two individuals of the thrush nightingale (*Luscinia luscinia*)—all of which are bird species that winter in Africa (Hasle et al., 2009). The absence of confirmed adult specimens and follow-up detections suggests minimal risk of establishment in Norway, though occasional monitoring remains warranted.

Sweden: The initial reports of *H. marginatum* nymphs on birds date back to the early 1990s (Jaenson et al., 1994). However, a more recent survey between 2018 and 2019 recorded over forty adult *Hyalomma* ticks found on horses, cattle, and humans across various provinces (Grandi et al., 2020). Among these, 11 specimens were morphologically identified as *H. marginatum*, marking the first documented presence of adult *H. marginatum* in Sweden. Although testing for CCHFV and piroplasms was negative, four specimens tested positive for *R. aeschlimannii*, indicating potential zoonotic risks (Grandi et al., 2020).

In Northern Europe, *H. marginatum* occurrences remain sporadic and largely limited to immature stages introduced by migratory birds. The climatic conditions currently inhibit molting, development, and overwintering, thereby preventing local establishment. However, rare reports of molted adult ticks, particularly in Sweden, suggest that under increasingly favorable microclimatic conditions, individual ticks may complete development after arrival. While the risk of establishment remains low, ongoing climate change and repeated introductions across multiple countries highlight the need for continued ornithological and passive surveillance to detect early ecological shifts and emerging tick-borne disease risks.

8. Overview of Regional Findings and Study Limitations

The findings from this review reveal that *H. marginatum* distribution is concentrated mainly in the Mediterranean and Balkan regions of Europe, with sporadic occurrences in Western, Central, Eastern, and Northern Europe. This distribution is consistent with studies showing that *H. marginatum* is well-suited to warm, arid climates characteristic of Mediterranean and sub-Mediterranean areas, where the tick has traditionally established stable populations (Apanaskevich & Horak, 2008; Estrada-Peña et al., 2011). Additionally, evidence of *H. marginatum* dispersal into more temperate regions through migratory birds supports the notion that these birds contribute to the seasonal transport of tick species, facilitating their introduction into non-endemic areas (Hillyard, 1996; Hoogstraal et al., 1961). However, the environmental constraints—particularly cooler temperatures and higher humidity—of Central and Northern Europe have so far hindered the establishment of stable *H. marginatum* populations in these regions. This pattern is also observed in studies of other tick species, which tend to expand their ranges in response to climate change, indicating that projected warming could make northern habitats more favorable for *H. marginatum* colonization in the future (Medlock et al., 2013; Pfäffle et al., 2013).

Beyond climatic constraints, geopolitical instability further complicates tick surveillance efforts, particularly in regions experiencing military conflicts. Armed conflicts disrupt health infrastructure, impede vector surveillance programs, and create ecological conditions that favor tick population growth and disease emergence. Despite the significant role that war-related environmental disturbances play in the epidemiology of tick-borne diseases, this factor remains largely overlooked in existing literature. Historical records indicate that conflict-induced ecological changes have directly contributed to *H. marginatum* population surges and increased human exposure to CCHFV. For instance, during World War II in Crimea, the widespread destruction of farmland and the sharp decline in livestock led to an overpopulation of wild hares—key hosts for immature *H. marginatum*—which in turn resulted in heightened tick exposure among soldiers and agricultural workers, triggering an outbreak of CCHF (Estrada-Peña & de la Fuente, 2014; Hoogstraal, 1979). A similar situation occurred in Bulgaria in the 1950s, when enforced collectivization of agriculture converted natural landscapes into farmland, increasing human exposure to *Hyalomma* ticks and their small vertebrate hosts, contributing to a surge in CCHF cases (Bente et al., 2013). In Turkey, a comparable pattern emerged when residents returned to farmlands that had been abandoned during civil strife, leading to increased human exposure to *H. marginatum* populations sustained by small vertebrate hosts (Bente et al., 2013). More recently, conflict-related surveillance gaps have been observed in Iraq, where war and civil unrest halted tick surveillance for nearly a decade, allowing CCHF cases to emerge undetected until recently (Majeed et al., 2012). Similarly, in Ukraine, a country currently experiencing military conflict, surveillance efforts have been deprioritized due to instability, potentially creating unmonitored transmission hotspots (FAO Animal Health Report, 2022). Environmental disruptions caused by war, including land abandonment and altered land use, can create new habitats favorable for tick proliferation (Estrada-Peña & de la Fuente, 2014). In regions where small mammals such as hares and hedgehogs thrive while large mammal populations decline, humans become the primary blood source for adult *H. marginatum*, exacerbating disease transmission risks, as observed in historical CCHF outbreaks (Bente et al., 2013).

A significant limitation of our review is the inconsistency in data collection methods and reporting standards across different studies. Variations in sampling techniques, host selection, and pathogen screening protocols creates difficulties in forming a directly comparable dataset, and this inconsistency limits the generalizability of findings. Further complicating the issue is the challenge of accurate taxonomic classification, especially in distinguishing between *H. marginatum* and morphologically similar species like *H. rufipes*. While this review includes studies on *H. rufipes* and other species within the *H. marginatum* complex to account for classification variability, studies that did not provide molecular confirmation of species may have led to potential underrepresentation of the species' distribution. Another limitation relates to the geographic focus of surveillance efforts. Much of the available data focuses on certain high-risk regions (e.g., Southern Europe), which may overlook sporadic occurrences in less-sampled areas. Additionally, tick collection is often concentrated on certain hosts (e.g., livestock or migratory birds), potentially missing data from other wildlife species. Furthermore, we acknowledge that our review may not be entirely comprehensive, as studies published in non-English languages were not included, which could have led to the omission of relevant research findings.

9. Future Challenges

Hyalomma marginatum, a principal vector of CCHFV in Europe, is expanding its geographic range under the combined pressures of climate change, anthropogenic land use changes, and animal movements. While the introduction of immature stages via migratory birds is well documented (Keve et al., 2022), the establishment of stable populations requires a confluence of favorable climatic conditions and the availability of suitable vertebrate hosts, particularly large mammals such as cattle, horses, goats, and sheep. Surveillance strategies must therefore go beyond sporadic tick collections and include coordinated monitoring of birds, livestock, land use, and environmental variables, particularly in regions currently considered CCHF-free but environmentally suitable for *Hyalomma* species.

One of the key challenges in CCHFV monitoring is the virus's "invisible" circulation in nature. Ticks—especially of the *Hyalomma* genus—are considered not only vectors but also potential reservoirs of CCHFV (Gargili et al., 2017). However, their vector competence can only be verified through complex and high-cost experiments in biosafety level-4 laboratories, which are not accessible in many regions. Consequently, the virus may remain undetected until human clinical manifestations appear, masking the broader epidemiological picture. This highlights the need for proactive, multi-level surveillance that includes both vector and vertebrate host populations to identify areas with silent virus presence.

Climate change is a critical driver of *H. marginatum* expansion. Rising temperatures may enhance tick survival and facilitate range extension into northern latitudes previously deemed unsuitable (EFSA, 2010). While warmer conditions support tick development, moderate humidity levels are essential to prevent desiccation during off-host stages. This species typically thrives in semi-arid environments characterized by hot, dry summers but cannot establish in regions that are either too humid or overly arid. Recent studies suggest that *H. marginatum* populations are sustained in areas where summer precipitation remains below approximately 32 mm and relative humidity is around 70% (Bah et al., 2022). However, excessively arid conditions with high evaporative stress can limit its colonization, even in regions experiencing temperature increases. Conversely, highly humid environments are also unsuitable. While climate warming opens new territories, only regions with sufficient but not excessive humidity may support permanent populations. Currently, southern Europe and northern Africa populations are regulated by summer rainfall, while eastern Europe and the Caucasus populations are controlled by autumn temperatures (EFSA, 2010). Warmer climates may lower tick mortality rates, aiding their spread into suitable areas (Gray et al., 2009). Climate projections suggest that warming trends could reduce tick mortality and enable further northward expansion, potentially surpassing the 47°N latitude boundary (Celina et al., 2023; Estrada-Peña et al., 2011; Fernández-Ruiz & Estrada-Peña, 2021).

Environmental events, such as the unusually hot and dry summer of 2018, have highlighted the potential for sudden shifts in tick distribution (Gillingham et al., 2023). That year, *H. marginatum* was reported in Austria (Duscher et al., 2018), western Germany (Chitimia-Dobler et al., 2019), and Sweden (Grandi et al., 2020), while *H. rufipes* was also detected in Germany and the UK (Chitimia-Dobler et al., 2019; Hansford et al., 2019). These findings likely resulted from the molting of nymphs introduced by migratory birds under favorable climatic conditions. Understanding the locations of migratory bird stopovers and resting sites is therefore critical for predicting new tick populations, and the use of citizen science and bird migration data may significantly enhance predictive model performance (Duscher et al., 2022).

In addition to climate and avian-mediated dispersal, the availability of suitable hosts is essential for population establishment. Adult *H. marginatum* preferentially feed on large mammals, and the absence of such hosts can limit population sustainability (Estrada-Peña, Ayllón, et al., 2012). Moreover, a critical density of molting individuals must be reached to establish a reproducing population capable of surviving seasonal environmental fluctuations (Estrada-Peña et al., 2021).

Socioeconomic conditions, agricultural practices, and human-induced land use changes also significantly influence tick distribution (Celina et al., 2023; Estrada-Peña, Jameson, et al., 2012). Globalization has accelerated the spread of exotic vectors and pathogens through increased trade and travel (Semenza et al., 2016). Armed conflicts and climate-related population displacement may further contribute to tick dispersal by disrupting land management practices and facilitating the movement of infested livestock. Unregulated trade and cross-border animal transport, including via personal vehicles, add another layer of complexity to the epidemiological landscape. Thus, preventive strategies should account for both climate and non-climate factors.

Predictive modeling is an essential tool for anticipating future range shifts of *H. marginatum* and guiding targeted surveillance and intervention strategies. To improve the accuracy and ecological relevance of these models, a suite of meaningful variables should be included, such as long-term temperature indices, relative humidity, seasonal precipitation, land cover, and land use changes. Additionally, data on livestock densities and the geographic distribution of domestic and wild hosts are important. Socioeconomic variables may also be important for modeling. While human population density is commonly used as a predictor in ecological models for many tick species, its relevance for *H. marginatum* specifically remains uncertain. To date, no published studies have shown whether human density enhances or detracts from model performance for this species. Therefore, both environmental and anthropogenic variables should be carefully assessed to develop accurate, evidence-based tools for surveillance and disease prevention.

This review highlights the need for strengthened tick surveillance and public health awareness, particularly in high-risk regions and during heatwave years that may favor tick survival and expansion. Integrated monitoring strategies should focus on migratory bird routes and livestock-dense areas. Future research must address gaps in our understanding of CCHFV transmission, including the vector competence of local ticks and the reservoir role of hosts. Developing affordable, sensitive diagnostics is essential for expanding surveillance in resource-limited settings. Finally, a standardized surveillance framework across Europe is crucial to improve data comparability, predictive modeling, and preparedness in the context of climate change and increasing animal movement.

10. Conclusion

Hyalomma marginatum has increasingly been detected beyond its native range, raising significant public health concerns. This review highlights that the spread of *H. marginatum* ticks into non-native regions is primarily facilitated by migratory birds and animal trade, with these imported ticks often testing positive for pathogens such as CCHFV and *Rickettsia* species. As a primary vector for CCHFV and a major vector of SFGR, the expansion of *H. marginatum* in Europe could substantially heighten public health risks, particularly under the influence of climate change.

To mitigate these risks, proactive monitoring of *H. marginatum* spread is essential, especially in areas where it has not been previously established. Monitoring should encompass surveillance of migratory bird routes, identification of favorable tick habitats, and analysis of environmental factors supporting tick establishment. These insights will be crucial for developing control strategies aimed at reducing the risk of CCHFV and other *Hyalomma*-borne pathogens impacting public health and livestock in Europe.

While climate change is expected to significantly affect *H. marginatum* distribution, additional factors such as socio-economic development, agricultural practices, and land use changes may also drive shifts in its range. Therefore, preventive measures should address both climate-related and non-climate factors to effectively limit the introduction and establishment of exotic ticks and associated pathogens in new regions.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CHAPTER 3:

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This chapter includes a published article and a manuscript currently under review.

Mapping the Potential Distribution of the Principal Vector of Crimean-Congo Haemorrhagic Fever Virus *Hyalomma marginatum* in the Old World

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Research Article

Mapping the Potential Distribution of the Principal Vector of Crimean-Congo Haemorrhagic Fever Virus *Hyalomma marginatum* in the Old World

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Abstract

Crimean-Congo Haemorrhagic Fever (CCHF) is the most widely distributed tick-borne viral disease in humans and is caused by the Crimean-Congo Haemorrhagic Fever Virus (CCHFV). The virus has a broader distribution, expanding from western China and South Asia to the Middle East, southeast Europe, and Africa. The historical known distribution of the CCHFV vector *Hyalomma marginatum* in Europe includes most of the Mediterranean and the Balkan countries, Ukraine, and southern Russia. Further expansion of its potential distribution may have occurred in and out of the Mediterranean region. This study updated the distributional map of the principal vector of CCHFV, *H. marginatum*, in the Old World using an ecological niche modeling approach based on occurrence records from the Global Biodiversity Information Facility (GBIF) and a set of covariates. The model predicted higher suitability of *H. marginatum* occurrences in diverse regions of Africa and Asia. Furthermore, the model estimated the environmental suitability of *H. marginatum* across Europe. On a continental scale, the model anticipated a widespread potential distribution encompassing the southern, western, central, and eastern parts of Europe, reaching as far north as the southern regions of Scandinavian countries. The distribution of *H. marginatum* also covered countries across Central Europe where the species is not autochthonous. All models were statistically robust and performed better than random expectations ($p < 0.001$). Based on the model results, climatic conditions could hamper the successful overwintering of *H. marginatum* and their survival as adults in many regions of the Old World. Regular updates of the models are still required to continually assess the areas at risk using up-to-date occurrence and climatic data in present-day and future conditions.

Keywords: *Hyalomma marginatum*; Crimean-Congo Haemorrhagic Fever Virus; ecological niche modeling; tick; tick mapping.

12. Introduction

Crimean-Congo Haemorrhagic Fever (CCHF) is the most widely distributed tick-borne viral disease in humans, extending from western China, South Asia, and the Middle East to southeastern Europe and Africa (Bente et al., 2013). CCHF is caused by the Crimean-Congo Haemorrhagic Fever Virus (CCHFV), an emerging arbovirus associated with high fatality rates, reaching up to 40% (Bente et al., 2013; Ergönül, 2006; Shayan et al., 2015; Yilmaz et al., 2009). CCHFV is also among the deadliest human pathogens in Africa and Eurasia (Shayan et al., 2015).

Hyalomma marginatum sensu lato is a complex species that includes *H. marginatum* sensu stricto, *Hyalomma rufipes*, and other closely related species (Apanaskevich & Horak, 2008). In Europe, *H. marginatum* remains the main vector of CCHFV. *Hyalomma marginatum* has veterinary and public health importance, particularly if this species can transmit various tick-borne pathogens in humans and animals other than CCHFV, such as *Spotted Fever rickettsia* to humans, *Anaplasma* species to animals, *Babesia caballi* and *Theileria equi* (piroplasmosis) to horses, and *Theileria annulata* (tropical theileriosis) to bovines (Bakheit et al., 2012; Wallménius et al., 2012; Tirosh-Levy et al., 2021; EFSA, 2010). This is one of the tick species whose distribution and expansion are closely monitored by the European Center for Disease Prevention and Control (ECDC) because of its major medical importance (ECDC, 2023).

Unlike *Ixodes* ticks, which adopt an ambush strategy, *H. marginatum* actively seeks hosts using an active locomotory hunting strategy (Sonenshine & Roe, 2013). This tick species has evolved these hunting skills due to the necessity of quickly locating hosts in the harsh and arid environments it inhabits. Upon spotting a host by sensing certain signals such as vibration, visual objects, carbon dioxide, ammonia, or body heat, *H. marginatum* can run rapidly several meters across the ground to attack the host. Therefore, they are known as "hunter ticks." *Hyalomma marginatum* demonstrates adaptability to diverse abiotic conditions although it prefers localities with high summer temperatures (Hoogstraal, 1979). As a two-host tick, immature stages feed on the same individual animal (e.g., a small mammal like hares, hedgehogs, and rodents) or a ground-dwelling bird, whereas adults prefer larger hosts such as cattle, horses, or occasionally humans (Hoogstraal, 1979). Large domestic mammals play an important role in the biology of *H. marginatum* and the transmission of Hyalomma-borne pathogens. These mammals serve as hosts, supporting a high tick load, and consequently bringing *H. marginatum* into close proximity with agricultural workers. In addition, livestock can directly expose humans to Hyalomma-borne pathogens via infected blood or crushing of engorged ticks on the animals during slaughter (Chinikar et al., 2010; Sargianou et al., 2013; Mustafa et al., 2011). Moreover, migratory birds may introduce infected *H. marginatum* to new regions by carrying immature ticks during feeding (Hoogstraal, 1979).

Hyalomma marginatum is widely distributed across several African countries, particularly in North Africa and the Sahel region. It can be found in Morocco, Algeria, Tunisia, Libya, Egypt, Sudan, Chad, Ethiopia, Niger, Mali, Mauritania, and Senegal. In addition to Africa, *H. marginatum* is prevalent in various parts of Asia, including the Middle East, India, and the Caucasus. The historically known distribution of *H. marginatum* in Europe includes most of the Mediterranean and Balkan countries, Ukraine, and southern Russia (Apanaskevich & Horak, 2008). Further expansion of its potential distribution may have occurred in and out of the Mediterranean region. The wide dispersal of *H. marginatum* reflects its tolerance to diverse environments, including savannah, steppe, and scrubland hill and valley biotypes (Hoogstraal, 1979).

Hyalomma marginatum was first detected in southern Germany in 2007 in Central Europe (Kampen et al., 2007). Subsequent observations have confirmed its presence in other Central European countries, including Hungary in 2009 (Földvári et al., 2011), Austria in 2018 (Duscher et al., 2018), and the Czech Republic in 2018–2019 (Lesiczka et al., 2022). Typically, permanent populations of *H. marginatum* are limited to warmer areas of the Mediterranean basin in Europe. Permanent populations of *H. marginatum* in Central Europe are likely to have occurred owing to ongoing climatic changes. The establishment of these populations and the northern spread of *H. marginatum* are possibly anticipated due to the passive transportation of immature stages by migratory birds flying to temperate Europe (Hoogstraal et al., 1961; Hillyard, 1996). During the spring migration of birds from the southern to the northern regions, *H. marginatum* is possibly introduced to Central Europe (Jaenson et al., 1994; Jameson et al., 2012; Hornok et al., 2016; Hubálek et al., 2020). The expansion of *H. marginatum* into new geographic areas raises concerns about the possible emergence of CCHFV in these newly invaded regions. The distribution of CCHFV is related to the distributional potential of its vector species. The presence of the virus, along with its vectors, reservoirs, and amplifying hosts, plays a crucial role in the emergence of CCHF under suitable environmental conditions (Portillo et al., 2021). Climate change, transportation of immature ticks through international animal trade, and migratory birds significantly impact the distributional potential of *H. marginatum* by allowing the emergence of CCHF in new geographic regions such as Central Europe. Additionally, socioeconomic factors such as human migration and settlement, as well as increasing human populations, may also influence the emergence of various zoonotic tick-borne diseases (Gray et al., 2009). Considering these factors, we hypothesize that several abiotic, biotic, and socioeconomic factors may affect the ability of *H. marginatum* to colonize European areas. Here, we assess the current potential distribution of *H. marginatum*, the principal vector of CCHFV, across the Old World, with a specific emphasis on Europe and a particular focus on Central Europe using an ecological niche modeling (ENM) approach. Thus, we aimed to derive detailed predictions to assess the potential invasion of new areas by *H. marginatum*.

13. Materials and Methods

13.1. Occurrence Records

The occurrence records of *H. marginatum* were obtained from the Global Biodiversity Information Facility (GBIF; www.gbif.org). We meticulously reviewed records for synonymous species of *H. marginatum*, including *Hyalomma plumbeum* Panzer, 1795, and *Hyalomma savignyi* Gervais, 1844, while carefully distinguishing them from *Hyalomma rufipes*. Only occurrence records corresponding to established populations of *H. marginatum* were considered in this study. These datasets were subjected to several data cleaning steps: (1) the dataset was cleaned by removing duplicate records to reduce possible biases in estimating ecological niche models (Barve et al., 2011), and (2) occurrence records were filtered based on a distance filter of ≤2.5′ (≈5 km) using SDMtoolbox 2.4 (Brown et al., 2017) in ArcGIS 10.7.1 (Environmental Systems Research Institute [ESRI], Redlands, CA). This distance-based thinning approach retains a single unique record within each pixel, preventing overprediction at specific locations or regions. The dataset was randomly partitioned into two portions using Hawth's Tools (Beyer, 2004) available in ArcGIS 10.7.1: 75% for model calibration, and 25% for internal evaluation of model predictions.

13.2. Covariate Variables

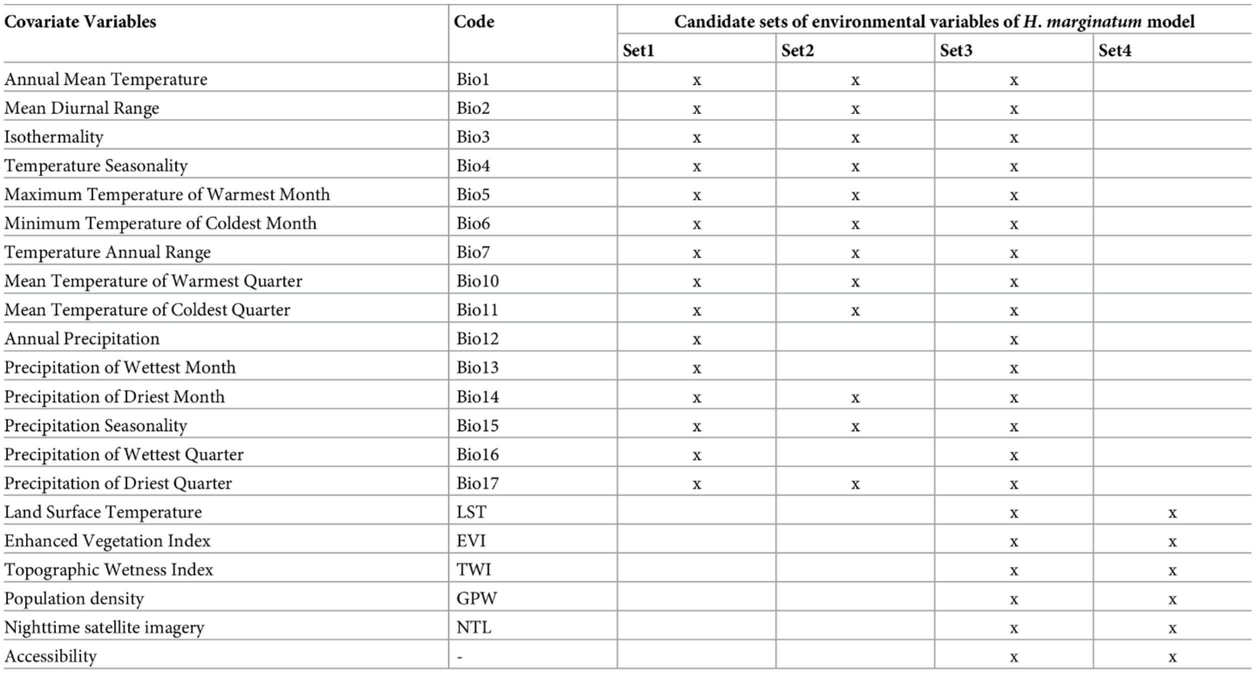
Several sets of environmental variables were used as independent variables in our model to characterize environmental variations across the calibration and projection areas. These variables were defined as potential drivers that limit the distributional potential of the vector *H. marginatum* (Estrada-Peña et al., 2016). These variables included satellite data from WorldGrids (WorldGrids.org, n.d.), which represented daytime and nighttime land surface temperature (LST), enhanced vegetation index (EVI), and topographic wetness index (TWI). EVI data was included due to its significant role in shaping the ecological niches of tick vectors, particularly immature stages, which live on vegetation (Estrada-Peña et al., 2016). EVI is also considered an important factor in reflecting soil moisture's availability for larvae and nymphs (Randolph, 2000; Guerra, 2002). Humidity and aridity are also important factors, considering their major roles in completing the life cycle of *H. marginatum* ticks. Effects of abiotic factors such as temperature and humidity on the distribution of *H. marginatum* have been previously described and used in modeling diverse tick vector species (Alkishe et al., 2021; Estrada-Peña et al., 2015). We also used data summarizing climate variables from the CHELSA database (Karger et al., 2021). CHELSA data includes 19 bioclimatic variables originally derived from monthly temperature and rainfall values collected from weather stations between 1981 and 2010 (Karger et al., 2021). Bioclimatic variables 8–9 and 18–19 were excluded from the analysis to avoid problems deriving from odd spatial artifacts (Williams et al., 2015).

Climatic and socioeconomic factors influence tick populations; socioeconomic factors exert indirect effects through human activities and land-use practices. It is noteworthy that tick vectors are not confined to natural habitats alone but have successfully adapted to urban environments. In recent years, urban areas have emerged as significant sites for the expansion of tick-borne diseases within regions where these diseases are endemic (VanAcker et al., 2019). Urban areas have characteristic dense human populations and offer suitable hosts and favorable conditions for tick survival. Several studies conducted in urbanized regions, including Finland, Hungary, Slovakia, Poland, and France, have revealed well-established tick populations and a comparable or even higher prevalence of tick-borne pathogens compared with endemic areas (Földvári et al., 2011; Klemola et al., 2019; Paul et al., 2016; Pangrácová et al., 2013; Kowalec et al., 2017). Human population, migration, and transportation play significant roles in shaping tick distribution worldwide (Gray et al., 2009). Higher human density increases the availability of tick hosts, whereas migration introduces ticks and diseases to new regions. Transportation networks additionally facilitate tick movement and allow their invasion into novel areas.

To comprehensively examine the interplay among climate, socioeconomic factors, and tick vectors, our model included anthropogenic data. Anthropogenic data consisted of data on human population density from 2015 to 2020, nighttime lights, and accessibility via transportation that may play a role in the distribution of *H. marginatum* ticks. Population density grids were obtained from the Gridded Population of the World, version 4 (GPWv4) (Warszawski et al., 2017), available at http://beta.sedac.ciesin.columbia.edu/data/collection/gpw-v4. Nighttime satellite imagery was obtained from the NOAA-Defense Meteorological Satellite Program (NOAA, 2008) and was used as a proxy for poverty estimates (Noor et al., 2008; Wang et al., 2012). Accessibility was summarized in terms of travel time by land or sea (Nelson, 2008), as connectivity between population sites is an important variable in estimating the potential distributions of disease vectors and emerging diseases (Barve et al., 2011; Cliff, 2004). This layer was developed by the European Commission and World Bank (European Commission, n.d.) and is available at http://forobs.jrc.ec.europa.eu/products/gam/download.php. All environmental and socioeconomic variables were resampled to a spatial resolution of 5 km.

The importance of the variables was evaluated using the Jackknife function in Maxent. The final sets of variables used in our analysis were as follows: Set 1 (15 bioclimatic variables from CHELSA); Set 2 (12 variables selected based on the Jackknife test in Maxent); Set 3 (a combination of 15 bioclimatic variables and satellite data summarizing daytime and nighttime LST, EVI, TWI, population density, nighttime satellite imagery, and accessibility); and Set 4 (only satellite data summarizing daytime and nighttime LST, EVI, TWI, population density, nighttime satellite imagery, and accessibility) (***Table 4***).

Table 4. Settings and variables used for the construction of the ecological niche modeling for *Hyalomma marginatum*.



Covariate Variables	**Code**	**Candidate sets of environmental variables of *H. marginatum* model**			
		Set1	**Set2**	**Set3**	**Set4**
Annual Mean Temperature	Bio1	x	x	x	
Mean Diurnal Range	Bio2	x	x	x	
Isothermality	Bio3	x	x	x	
Temperature Seasonality	Bio4	x	x	x	
Maximum Temperature of Warmest Month	Bio5	x	x	x	
Minimum Temperature of Coldest Month	Bio6	x	x	x	
Temperature Annual Range	Bio7	x	x	x	
Mean Temperature of Warmest Quarter	Bio10	x	x	x	
Mean Temperature of Coldest Quarter	Bio11	x	x	x	
Annual Precipitation	Bio12	x		x	
Precipitation of Wettest Month	Bio13	x		x	
Precipitation of Driest Month	Bio14	x	x	x	
Precipitation Seasonality	Bio15	x	x	x	
Precipitation of Wettest Quarter	Bio16	x		x	
Precipitation of Driest Quarter	Bio17	x	x	x	
Land Surface Temperature	LST			x	x
Enhanced Vegetation Index	EVI			x	x
Topographic Wetness Index	TWI			x	x
Population density	GPW			x	x
Nighttime satellite imagery	NTL			x	x
Accessibility	-			x	x

13.3. Accessible Area ("*M*")

The accessible area ***"M"*** is a crucial component of the biotic, abiotic, and movement (BAM) diagram (Soberón & Peterson, 2005). It defines the key parameters for constructing an ecological niche model for the species in question. Accessible area ***"M"*** indicates the areas that the species explored and had access to over relevant periods of the species' history (Barve et al., 2011). The delimitation of the *H. marginatum* accessible area ***"M"*** was estimated using the grinnell package in R (Machado-Stredel et al., 2021). This method simulates dispersal and accessibility based on niche estimations. The *grinnell* package uses a combination of clean occurrence records of the target species and a set of covariates as inputs to estimate the niche.

13.4. Ecological Niche Modeling

We constructed the ENM using the maximum entropy algorithm implemented in the *kuenm* R package (Cobos et al., 2019). While there are various modeling algorithms available for estimating niche models, such as generalized linear models (GLM), generalized additive models (GAM), and boosted regression trees (BRT), each designed for different types of distribution data and modeling purposes (Samy et al., 2022), we selected Maxent because of its efficiency in handling complex interactions between response and predictor variables (Elith et al., 2011). A total of 1,972 candidate models (i.e., these models refer to the different model configurations or combinations of predictor variables considered during modeling) were built with four distinct sets of environmental and socioeconomic variables. These models were additionally constructed based on parameters reflecting all combinations of 17 regularization multiplier settings (0.1–1 with intervals of 0.1, 2–6 with intervals of 1, and 8 and 10; i.e., these settings determine the level of complexity or smoothness of the model), and 29 possible combinations of 5 feature classes (linear = l, quadratic = q, product = p, threshold = t, and hinge = h).

The best candidate model was selected based on three different criteria: (1) significance, (2) performance, and (3) the Akaike information criteria (AIC): AICc, delta AICc, and AICc weights. Performance was measured using the omission rate, which is a threshold that considers an estimate of the likely amount of error among occurrence data and thus removes 5% of occurrences with the lowest suitability values (E = 5%) (Peterson et al., 2011). Models were selected with delta AICc ≤ 2 from those that were statistically significant and had omission rates below 5%. We followed the criteria from the original *kuenm* study (Cobos et al., 2019) to select and evaluate the final model. We created the final model of *H. marginatum* using 10 replicates by bootstrap, with logistic, product, and hinge outputs. These models were finally transferred from the accessible area ***"M"*** to the projection area ***"G"***.

13.5. Extrapolation Risk of *H. marginatum*

This analysis identified the areas with extrapolation risk based on a mobility-oriented parity (MOP) approach to compare the environmental breadth of predictors within ***"M"*** (10% reference points sampled) with that in the projection area. Mobility-oriented parity analysis was performed using the MOP function (Owens et al., 2013) available in the *kuenm* package in R. The risk of extrapolation analysis calculates multivariate environmental distances between projection area ***"G"*** and the nearest portion of the calibration region to identify areas that have a condition of strict or combinational extrapolation.

13.6. Risk Independent Evaluation

We used a set of additional independent records for the final model evaluation. These records were retrieved from a previous study (Estrada-Peña et al., 2016) for additional evaluation of model performance to assess its ability to anticipate risk in unsampled areas. We cleaned the dataset to keep only unique records and double-checked the records to remove any source of overlap with the data used in the model training and testing (i.e., the occurrence records employed in this evaluation differ from those used for model calibration, specifically referring to occurrences distinct from the records obtained from GBIF for model calibration). These records were tested for successful predictions based on a binary model indicating areas where the species was present (suitable) or absent (unsuitable). The continuous model was converted to a binary map based on a threshold value. The threshold value was determined based on a maximum allowable omission error rate of 5%, accounting for potential errors in covariate values within 5% of the occurrences. Subsequently, a one-tailed cumulative binomial probability test was used to assess the probability of obtaining the observed level of correct predictions by chance alone, given the background expectation of correct predictions determined by the proportional coverage of the study area by regions of predicted suitability.

14. Results

The final dataset used for calibration, derived from GBIF, comprised 95 unique occurrence records after removing duplicated and redundant occurrence records and undergoing diverse cleaning steps (***Figure 10***). Of the 1,972 candidate models, 1,846 models were statistically significant. After applying the three selection criteria, a single model successfully met all requirements and was designated as the best candidate model, as evidenced by its performance (***Table 5***). The optimal model used Set 4 of the satellite data, summarizing daytime and nighttime land surface temperature, EVI, TWI, population density, nighttime light, and accessibility.

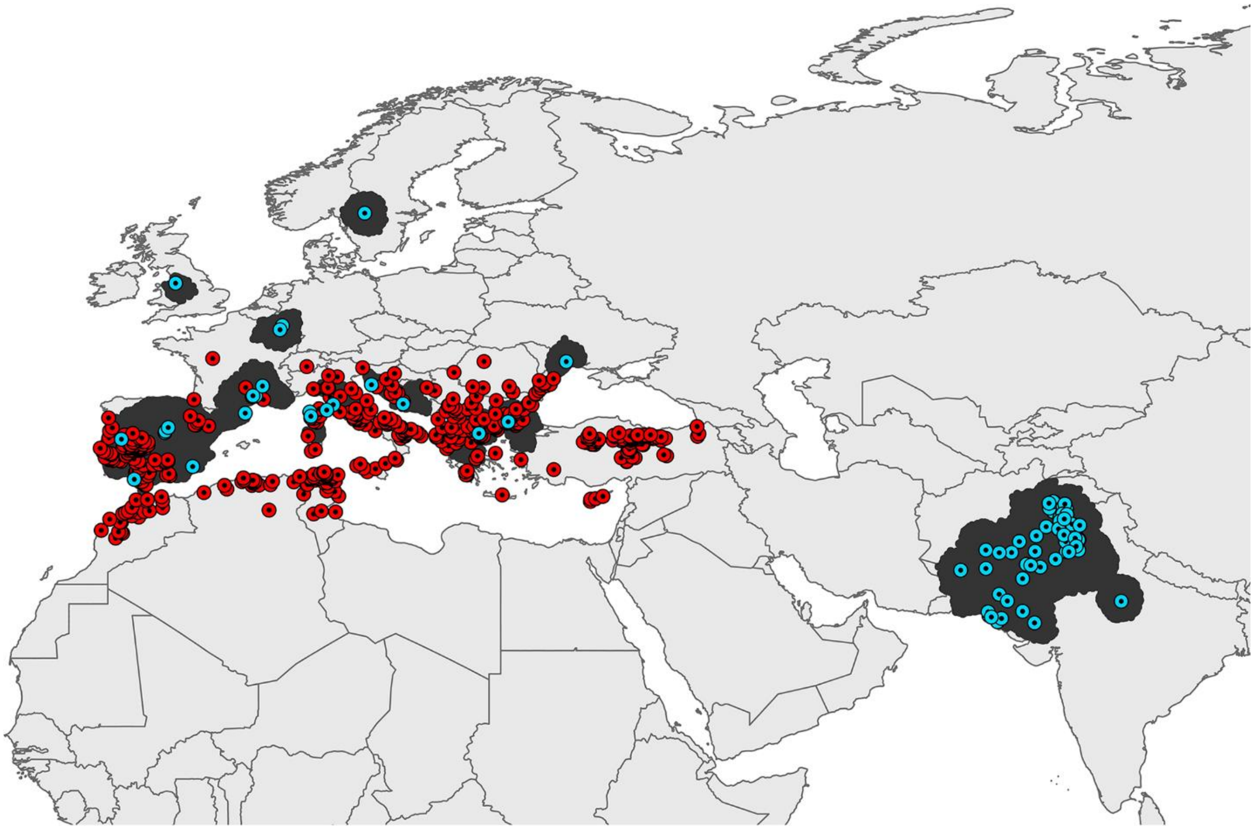


Figure 10. *Hyalomma marginatum* occurrence records used in model calibration and final model evaluation across Europe, North Africa, Western and South-Central Asia. The blue dotted circles represent the occurrence records of *H. marginatum* collected from GBIF used for model calibration across the ***"M"*** area. The red dotted circles represent the retrieved occurrence records of *H. marginatum* from the literature used for the final model evaluation. Dark grey polygons represent the accessible areas (***"M"***) where the *H. marginatum* model was calibrated.

Table 5. The best candidate model for the construction of the ecological niche modeling for *Hyalomma marginatum*. Model performance under optimal parameters using sets of environmental predictors (SEP), statistically significant models (SSM), best candidate models (BCM), regularization multiplier (RM), features classes (FC), mean Area Under the Curve ratio (AUC.r), partial Receiver Operating Characteristic (p.ROC), omission rate 5% (O.rate 5%), Akaike information criterion corrected (AICc), delta Akaike information criterion corrected (ΔAICc), Akaike information criterion corrected weight (AICc.W), number of parameters (#; summarizes the combination of environmental variables, multiple regularizations, and features other than 0 that provide information for the construction of the model based on lambdas—lambda refers to counting all parameters with a nonzero weight in a Maxent-generated text file), and environmental variables of Set4 tested during calibration of *Hyalomma marginatum* model.

SEP	**SSM**	**BCM**	**RM**	**FC**	**AUC.r**	**p.ROC**	**O.rate 5%**	**AICc**	**ΔAICc**	**AICc.W**	**#**
Set4	**1846**	**1**	**3.0**	**lph**	**1.21**	**0.00**	**0.05**	**1960.75**	**0.00**	**1.00**	**15**
Set4											
		Daytime and Nighttime Land Surface Temperature, Enhanced Vegetation Index, Topographic Wetness Index, Population density, Nighttime satellite imagery, and Accessibility									

* q = quadratic; t = threshold; h = hinge; p = product.

High to very high suitability of *Hyalomma marginatum* occurrence was observed in various regions of Africa, including the northern parts of North Africa (Morocco, Tunisia, and Egypt), West and East Africa, central parts of Central Africa, and eastern parts of Southern Africa (Fig. 2). In Asia, the model indicated medium to high suitability in expansive areas of the continent, encompassing India, Pakistan, Bangladesh, and parts of China (***Figure 11***).

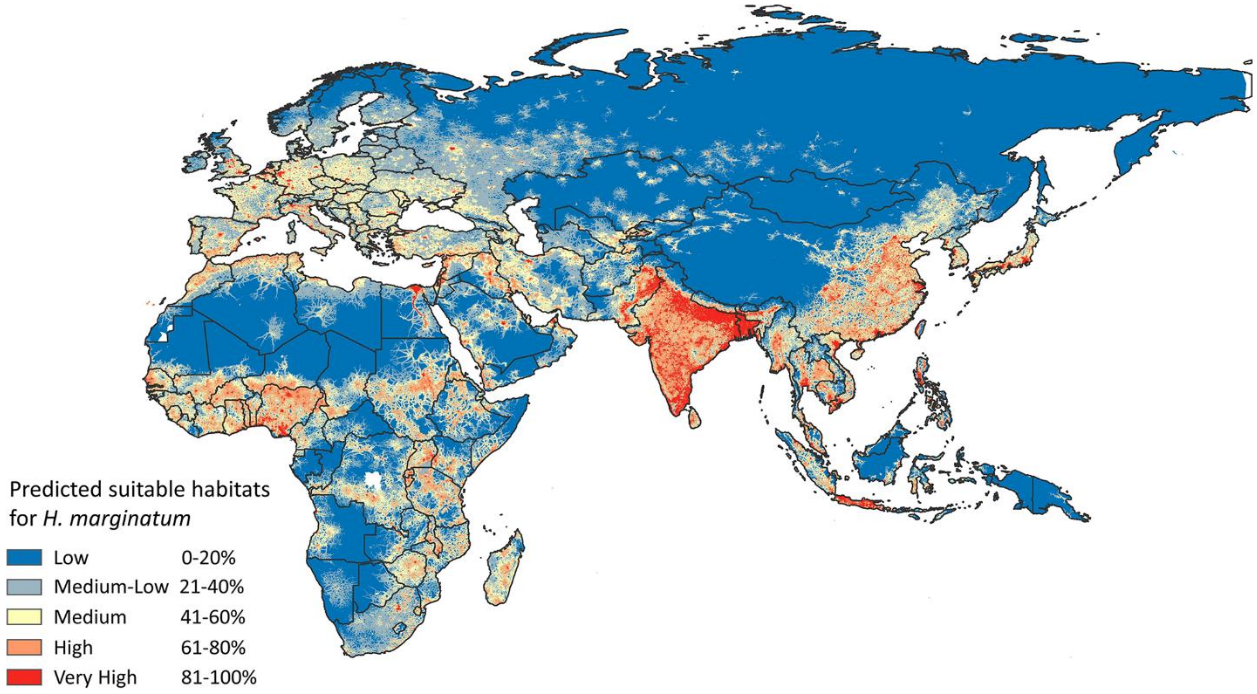


Figure 11. Predicted potential geographic distribution of Crimean-Congo Haemorrhagic Fever vector *Hyalomma marginatum* on a global scale. Red colors indicate highest habitat suitability and blue lowest suitability.

On a continental European scale, the model projected a widespread potential distribution of *H. marginatum* (***Figure 12***). Thus, the model anticipated the occurrence of *H. marginatum* in Southern, Western, Central, and Eastern Europe, as far north as the southern parts of Scandinavian countries (***Figure 12A***). A high probability of suitable conditions for *H. marginatum* in Southern Europe, especially in the Mediterranean parts of Spain, Portugal, Italy, and Greece and along the Adriatic shore, was presented. In the Balkan Peninsula, the southeastern part of the continent, wide areas of all countries were identified as highly suitable areas for *H. marginatum*. In Western Europe, large areas of northern and southern France, as well as the Benelux states, were predicted to be highly suitable for the distribution of the tick species. Although the Scandinavian countries generally presented low suitability, the model predicted medium to high suitability in the southern regions of most Northern European countries (Sweden, Norway, Denmark, and Finland, except Iceland), as well as high suitability in scattered areas in the southern parts of Northern European countries. The northeastern region of Europe primarily demonstrated low and low–medium suitability for *H. marginatum* distribution, with a few scattered points indicating medium to high suitability in the urban areas of the Baltic countries (Estonia, Latvia, and Lithuania). In northwestern Europe, the model depicted medium suitability across all areas in the UK, with very high suitability in specific regions such as southeast and southwest England, the East Midlands, the West Midlands, Yorkshire, London, and the southern parts of the northwest and central parts of northeast England. In Scotland, Ireland, and Northern Ireland, low and low–medium suitability for *H. marginatum* distribution predominated. Eastern Europe, including Romania, Ukraine, and Moldova, was identified as a highly suitable area, characterized by medium to very high suitability.

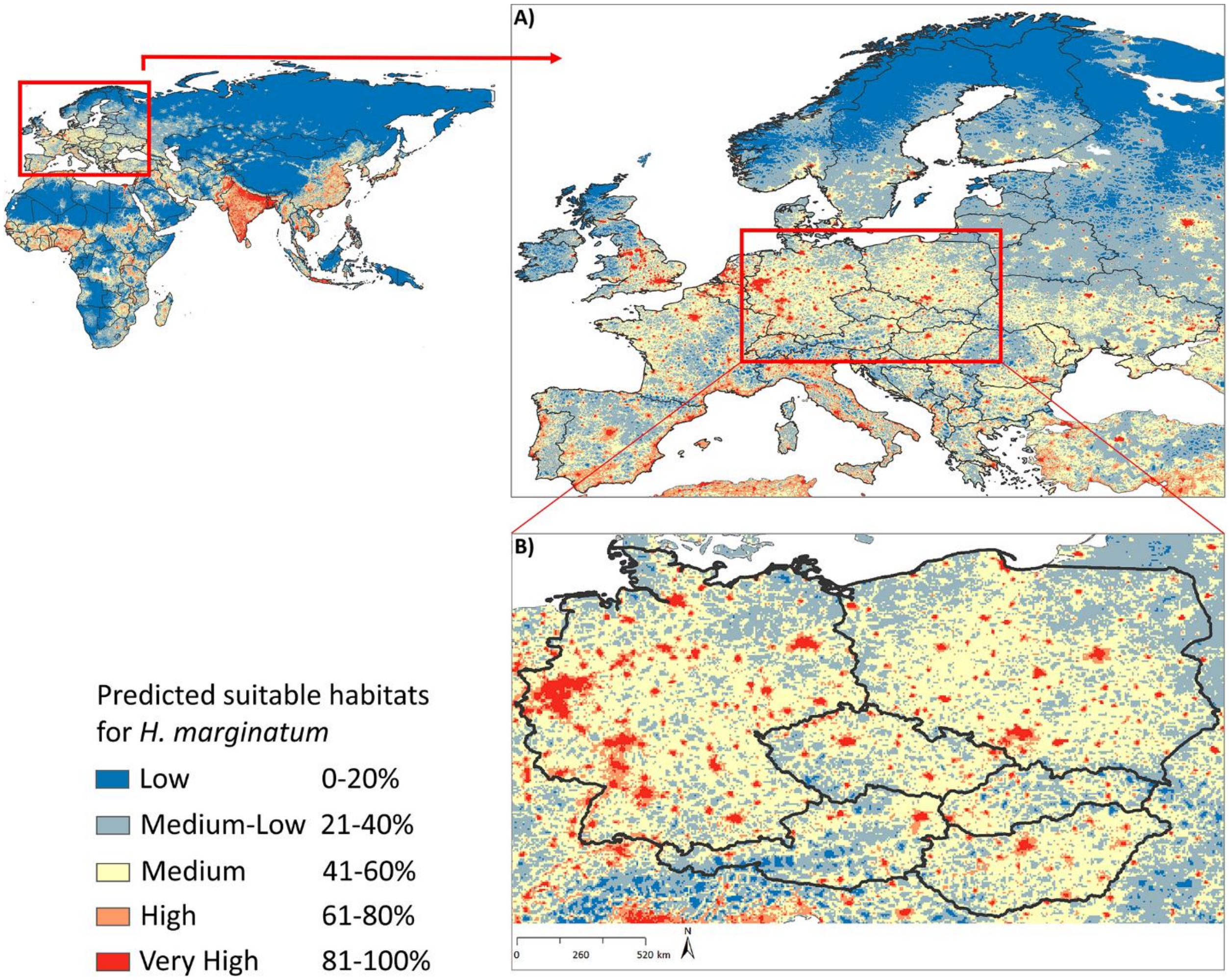


Figure 12. Predicted potential distribution of Crimean-Congo Haemorrhagic Fever vector *Hyalomma marginatum* on a global scale (left top), and close-ups of Europe (A) and Central Europe (B), to provide additional detail to predictions in the region. Red areas indicate modeled highest suitable conditions, and blue areas are lowest suitable conditions.

The ENM of *H. marginatum* in Central Europe provided insights into its distribution across all countries in the region (***Figure 17B***). The model indicated broader environmental suitability in Germany, Poland, Hungary, and the Czech Republic, with Austria and Slovakia following suit. Germany exhibited medium to high suitability, with the highest suitability observed in North Rhine-Westphalia, Hesse, Saarland, Baden-Württemberg, Bremen, Berlin, Hamburg, and scattered areas in Bavaria, Thuringia, Saxony, and Lower Saxony. The same is true for large parts of Poland and Hungary, particularly the entire city of Budapest, which was identified as a very highly suitable habitat for *H. marginatum* occurrence. High-risk areas occurred in eastern Austria and adjoining areas and in the western and northern parts of Slovakia. While large areas of the Czech Republic have medium suitability, very high suitability of *H. marginatum* also occurred in several scattered areas.

The final model was evaluated using independent occurrence data (Estrada-Peña et al., 2016), comprising 621 points after eliminating duplicated and redundant records through various cleaning steps. The independent set of *H. marginatum* occurrence records was related to the *H. marginatum* model prediction. The model successfully predicted 588 of 621 (94.68%) independent data points (***Figure 13***).

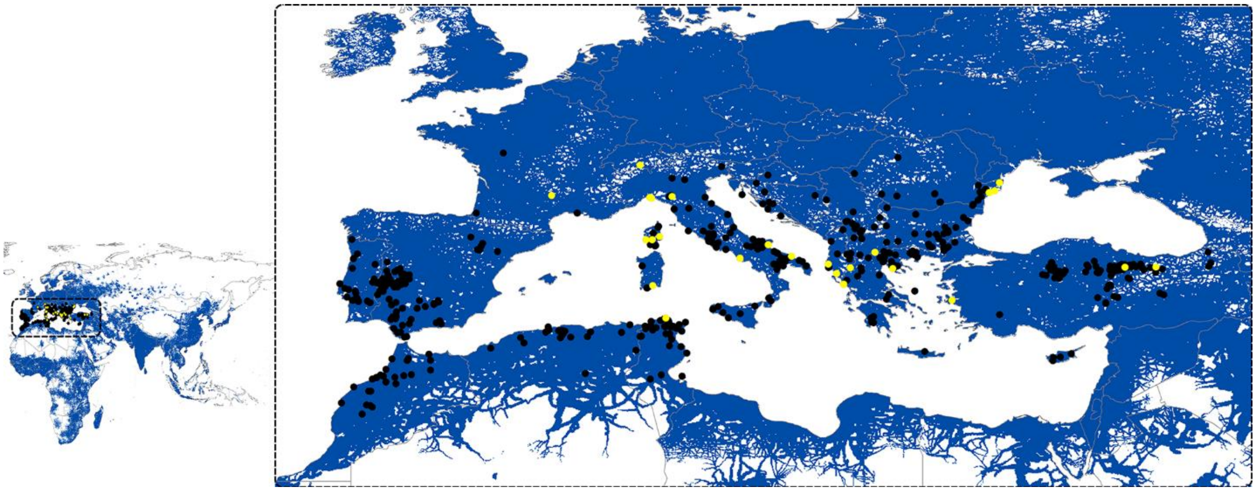


Figure 13. Relationship of *Hyalomma marginatum* ecological niche modeling prediction to the distribution of the independent set of *Hyalomma marginatum* occurrence records. Blue shading shows areas predicted suitable for *Hyalomma marginatum* occurrences. Black and yellow dots represent the independent records of *Hyalomma marginatum* used for the final model evaluation;

black dots are records with successful prediction and yellow dots are records where the prediction is not captured by the model.

The MOP results indicated high levels of environmental similarities in all areas under question, except Southwest China (e.g., northern parts of Tibet province), Northwest China (e.g., southern parts of Xinjiang province), and some areas in East Africa (e.g., Ethiopia, Kenya, Somalia, Sudan) where strict extrapolation occurred (***Figure 14***). Therefore, predictions in these areas should be taken with caution because they were consistently detected as areas with high extrapolation risk.

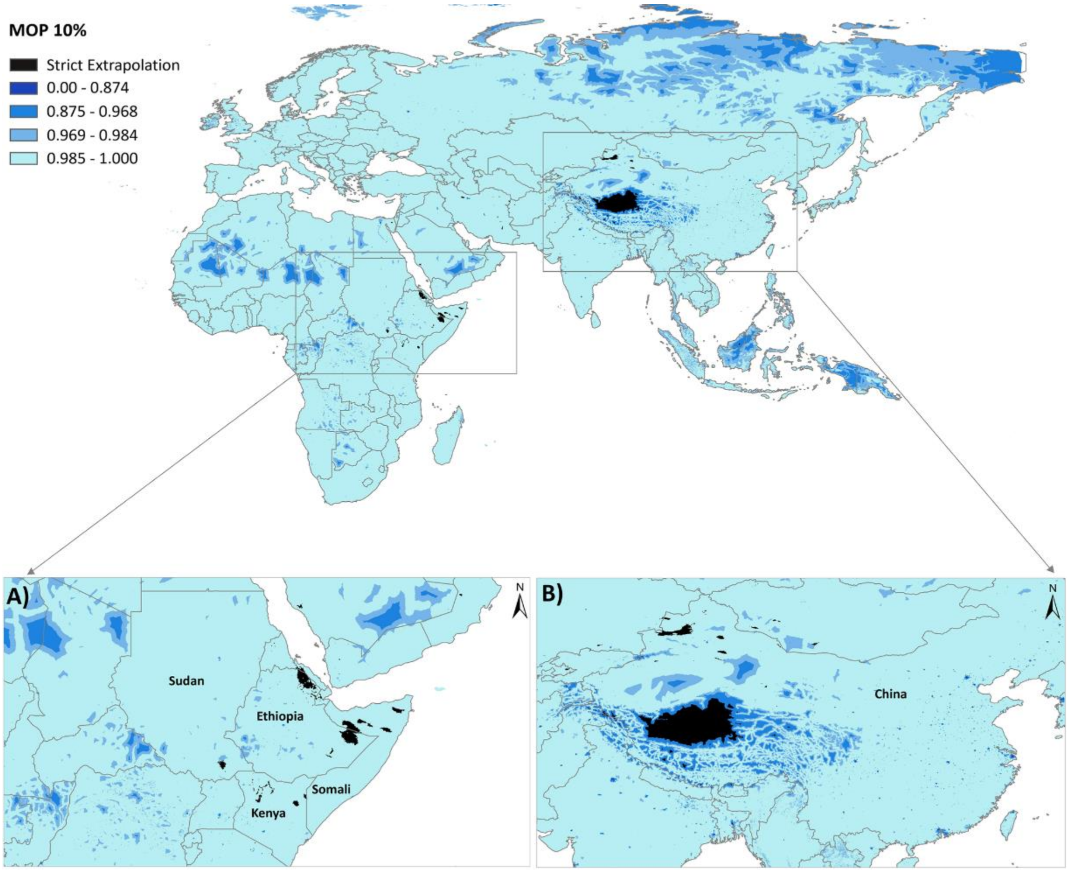


Figure 14. Mobility-oriented parity (MOP) 10% extrapolation risk analysis for the ecological niche model of *Hyalomma marginatum* from the calibration area ***("M")*** to a projection area **("*G*")**

(top), and close-ups of East Africa (A) and Eastern Asia (B), to provide additional detail to strict extrapolations occurred in the areas.

The MOP analysis indicated that areas with the most dissimilar variables conditions (i.e., where one or more covariate variables are outside the range present in the training data) were found beyond the potential distributional areas predicted by the model in the ***"G"*** area. Areas with the most dissimilar variables conditions display strict extrapolative areas and are represented by zero value. Other values represent levels of similarity between the calibration area and the ***"G"*** transfer area. The MOP raster output was reclassified into five categories; the first category represented a strict extrapolation (i.e., zero value), and the fifth category represented the highest environmental similarities between calibration and projection areas.

15. Discussion

Ecological niche modeling has been widely implemented to predict the distribution of numerous vector-borne diseases (Estrada-Peña et al., 2016; Gray et al., 2009; Adjemian et al., 2006; Samy et al., 2016; Machado-Stredel et al., 2021; Cobos et al., 2019; Elith et al., 2011). These modeling studies play a crucial role in enhancing the efficiency of vector surveillance and control programs.

In this study, we provided detailed maps to identify the potential distribution of *H. marginatum*, the principal vector of CCHFV, in the Old World, particularly in Europe, with a specific emphasis on Central Europe. This study also addresses model uncertainty under current environmental and socioeconomic conditions. These conditions highlight significant factors influencing the spread of ticks and tick-borne diseases, including climate change, vegetation patterns, human population density, poverty levels, and increased movement of animals and humans due to improved accessibility. The introduction of *Hyalomma* species to new geographical areas is likely facilitated by infested hosts resulting from human activities, the movement of domesticated and wild mammals, and migratory birds (Bakheit et al., 2012; Valcárcel et al., 2020).

In Europe, *H. marginatum* is mostly endemic to Mediterranean countries, but isolated populations also exist in Transalpine Europe. Our study revealed extensive areas of high suitability for *H. marginatum* occurrence across the entire European continent. Specifically, high suitability was observed in the Mediterranean regions of Spain, Portugal, Italy, Greece, and along the Adriatic shore. Furthermore, southern and northern France, large areas in the Netherlands, Belgium, Germany, Balkan countries, Ukraine, Crimea, scattered areas in Central Europe, and even extending into the United Kingdom remained favorable for the tick species. A previous study anticipated high environmental suitability of *H. marginatum* in southern, southeast, and Central Europe, as well as regions in North Africa (Tunisia, Morocco, and Algeria), the Arabian Peninsula, south-central Asia, and China (Okely et al., 2020). Our model improved the prediction and provided a more reliable and detailed map of habitat suitability for *H. marginatum*. Similar to the previous ecological niche model (Okely et al., 2020), the highest environmental suitability for *H. marginatum* was predicted across Southern Europe. Additionally, we observed high suitability across all Balkan countries in southeast Europe. Interestingly, our predictions differed from those of the previous model in several aspects. We identified broader areas of high and very high suitability, particularly in Balkan countries (Albania, Croatia, Bosnia and Herzegovina, Kosovo, Montenegro, Romania, and Slovenia), Western Europe, the United Kingdom, and southern parts of Scandinavia, which were underestimated by Okely et al. (2020). This previous study ignored important model settings that can lead to errors in model estimation. In addition to the prediction of Okely et al. (2020), previous studies have mapped the geographic distribution of *H. marginatum* at regional and global scales (Estrada-Peña et al., 2015; Williams et al., 2015). In comparison with these studies, our model anticipated higher suitability for the occurrence of *H. marginatum* in Central, Western, Eastern, and Northern Europe, contradicting their predictions of low suitability for this tick species in the same areas.

Hyalomma marginatum was first reported in Central Europe in Germany in 2007 (Kampen et al., 2007). Although it was recorded in other countries of Central Europe, including Hungary (Földvári et al., 2011), the Czech Republic (Lesiczka et al., 2022), and Austria (Duscher et al., 2018), these observations did not represent stable populations of *H. marginatum* in Central Europe. *H. marginatum* ticks have ecological plasticity that supports tolerance to a range of temperature and humidity conditions (Bouattour et al., 1999). This tick species prefers dry and warm regions and is most abundant during summer. In the northern hemisphere, it becomes active in spring when temperatures rise, typically around April (Hillyard, 1996). Immature stages are active from June to October, peaking in numbers during July and August. After a blood meal, immature ticks either drop in early summer, molt into adults during the same season, and overwinter as adults, or drop in late summer, overwinter as nymphs, and molt into adults the following spring (Hillyard, 1996). Stable populations of *H. marginatum* in Europe are restricted to the warm areas of the Mediterranean basin and are absent in Central Europe, likely because of unsuitable environmental conditions.

However, our model predicted its distribution across all countries in Central Europe and provided strong evidence for extensive medium to very high suitability in Germany, Poland, Hungary, and the Czech Republic, followed by Austria and Slovakia. A recent study conducted in Hungary revealed seropositivity for CCHF, indicating that Hungary could be a novel geographical region for the distribution of CCHFV (Magyar et al., 2021). Sporadic records of *H. marginatum* in other Central European countries, apart from Hungary, indicate potential breeding habitats for this tick vector. In Austria, the first recorded occurrence of *H. marginatum* was an adult male tick found on a horse in 2018 (Duscher et al., 2018). Recent studies in the Czech Republic and Slovakia reported *H. marginatum* complex species exclusively on migratory birds (Capek et al., 2014). Additionally, a very recent study reported the collection of five adult *H. marginatum* ticks from horses and households in the Czech Republic (Lesiczka et al., 2022). Although *H. marginatum* does not belong to the endemic tick fauna in Germany, sporadic findings have been reported recently (e.g., 2007, 2011, 2017, and 2018) (Chitimia-Dobler et al., 2019).

In Poland, records of *H. marginatum* date back to earlier times, with unfed male ticks reported in 1935 and 1943, which are currently archived in the Bytom Museum collection (Siuda & Dutkiewicz, 1979; Nowak-Chmura & Solarz, 2010). The introduction of these adult ticks to Central Europe is highly probable because they likely originated as nymphs feeding on migratory birds and molted into adults after becoming fully engorged. The environmental conditions in Central Europe offer suitable habitats for *Hyalomma* spp. to develop and locate appropriate vertebrate hosts for subsequent blood feeding. While it has been proposed that *H. rufipes*, another significant vector of CCHFV, overwinters in Central Europe (Rudolf et al., 2021), there is currently no speculation regarding the establishment of *H. marginatum* populations in the region.

Furthermore, our results showed medium to high environmental suitability for *H. marginatum* in the south of Scandinavia. In Northern Europe, documented records of *H. marginatum* consist solely of transported immature stages found on migratory birds during their northward spring migration. These records include instances in Denmark in 1939 and 1991

(Jaenson et al., 1994; Johnsen, 1943), Finland in 1962 and between 2018 and 2020 (Nuorteva & Hoogstraal, 1963; Sormunen et al., 2022), Norway in 1994 (Jaenson et al., 1994), and Sweden between 1990 and 1991 (Jaenson et al., 1994). Although no scientific study has reported the occurrence of *H. marginatum* in Norway since 1994, Hasle et al. (2009) reported the presence of seven fully engorged nymphs of *Hyalomma rufipes* in migratory birds captured during 2003–2005. Recently, the first sightings of adult *H. marginatum* ticks have been documented on horses, cattle, and humans in Sweden (Grandi et al., 2020). The rapidly changing climate in Northern Europe appears to create favorable conditions for the development of immature stages of this tick species, carried by migratory birds, to reach the adult stage and potentially attach and feed on large mammals, including humans.

Appropriate climatic and biotic conditions in the regions depicted in our maps may provide a suitable environment for introduced *H. marginatum* ticks. A warming climate trend could enhance the persistence of infected *H. marginatum* ticks. The expansion of suitable areas has been documented in Central Europe (Fernández-Ruiz & Estrada-Peña, 2021). Thus, it is possible to hypothesize that climate change may potentially lead to an increase in winter temperatures, leading to an increase in the probability of *H. marginatum* overwintering, and subsequently raising the risk of its establishment in various parts of Central European countries. Therefore, modeling studies based on future scenarios should be implemented to better understand the impact of climate change on the dispersion of *H. marginatum*.

ENM results are valuable for understanding species tolerance to biotic and abiotic factors. When combined with knowledge of ecology, behavior, and life history, they assist in selecting the most realistic predictions (Escobar & Craft, 2016). Our current global potential distribution prediction for this tick vector can provide insights into disease risk areas associated with this vector. However, it is essential to assess the uncertainty in model projections, particularly for decision-makers in vector and disease surveillance and public health. CCHFV, a vector-borne pathogen, requires a “One Health” approach that incorporates a multidisciplinary perspective encompassing animal, human, and environmental health (Mackenzie & Jeggo, 2019). Therefore, it is crucial to develop effective control strategies that address emerging *Hyalomma*-borne diseases within the framework of a “One Health” perspective, where a rapidly changing environment and animal health may impact human health.

In this study, we used a novel improved methodology and allowed different modeling settings to construct the habitat suitability model for *Hyalomma marginatum* based on calibration and evaluation of our models using several updated variables that previously were not used. Although our continental-scale predictions provide valuable insights into the distribution of *H. marginatum*, it is crucial to investigate the species' interactions with microclimate and the influence of host availability on its response to prevailing climatic conditions (McCoy et al., 2013).

The model effectively identified known areas where the disease is prevalent, but it has a few limitations. Notably, the abundance of occurrence records in Europe and the Indian subcontinent has probably led to wider predictions, particularly in urban areas and transportation routes. It is noteworthy to mention that human-modified landscapes, such as urban areas, have gained attention as potential habitats for ticks, contrary to the belief that they are restricted to natural habitats. Studies conducted across Europe have revealed the successful establishment of tick populations in urban areas, and the spread of tick-borne diseases has been linked to these urban hotspots (Földvári et al., 2011; Klemola et al., 2019; Paul et al., 2016; Pangrácová et al., 2013; Kowalec et al., 2017). The ability of ticks to thrive in urban environments emphasizes the significance of considering urban areas as potential hubs for tick populations.

Furthermore, areas with a low number of occurrence records driven by accessibility bias should be further investigated, especially if the model predicts a high-suitability habitat for *H. marginatum*. However, it is essential to distinguish between two key aspects: (1) occurrence records, which represent sampling efforts in specific areas influenced by factors such as tick identification expertise or the need for intensive fieldwork to study tick-borne disease circulation, and (2) model predictions generated by applying algorithms to thinned occurrence records and covariate data. Occurrence records indicated the presence or absence of the species within the sampled areas, whereas the model provided probability values indicating pixel suitability for species presence. This pattern enables the assessment of tick suitability in unsampled areas based on available occurrence records and covariate information, which poses a significant challenge in ecological niche modeling. To ensure reliable predictions, our model underwent careful calibration, and necessary adjustments were implemented to avoid over-prediction. Host parameters were not incorporated into the model because the species' broad host range encompassed numerous vertebrate species. Incorporating host species data poses challenges because obtaining reliable occurrence information for multiple hosts is difficult, and the optimal approach to their inclusion remains unclear (Alkishe et al., 2021). Nevertheless, although *H. marginatum* is a generalist species, certain vertebrate hosts may be preferred within its extensive range of potential hosts (Grech-Angelini et al., 2016). However, caution should be exercised when interpreting these host preferences as they may vary across different regions (McCoy et al., 2013). Finally, the Maxent algorithm used in ecological niche modeling has a few limitations, including reliance on presence-only data that can introduce biases, potential overfitting with complex predictor variables, the risk of unreliable results when extrapolating beyond calibrated conditions, limited mechanistic understanding and sensitivity to variable selection, and the impact of spatial autocorrelation and the assumption of independence on model performance. Considering these limitations is essential when using this model.

In summary, our future studies will consider further detailed mapping of CCHF disease and *H. marginatum* distribution under different climate change scenarios. In addition to mapping the distribution of this tick species under different future projections, our understanding of the dynamics of the host–tick vector–virus system will be improved by further modeling studies that incorporate the effects of vertebrate hosts (different types of hosts, seasonality, and movements) as well as life traits of the tick species.

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Climate Change Impacts on the Potential Geographic Distribution of *Hyalomma marginatum*

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Abstract

Hyalomma marginatum is a medically and veterinary significant tick species and the primary vector of Crimean-Congo Haemorrhagic Fever Virus (CCHFV). Climate change is expected to influence its geographic distribution, potentially increasing the risk of disease transmission. This study employs ecological niche modeling (ENM) to assess the current distribution of *H. marginatum* and project its future range shifts under different climate scenarios. Using an extensive dataset of occurrence records and bioclimatic variables, we applied MaxEnt modeling to predict habitat suitability for 2041–2060 and 2081–2100 under ssp245 and ssp585 scenarios. Our results indicate that *H. marginatum* is currently highly suitable in the Mediterranean Basin, North Africa, and western and central Asia. Future projections reveal a significant northward expansion into temperate and continental Europe, particularly under ssp585, while extreme heat stress may reduce suitability in parts of the Mediterranean region. These findings underscore the potential for *H. marginatum* to establish in new regions, necessitating enhanced vector surveillance and control strategies. Future research should integrate host dynamics and land-use changes to refine risk assessments and improve predictive accuracy.

Keywords: *Hyalomma marginatum*; Crimean-Congo Haemorrhagic Fever Virus; ecological niche modeling; climate change; future distribution.

17. Introduction

Climate change is reshaping ecosystems worldwide, altering the distribution and abundance of many species, including vectors of infectious diseases. Among these, *Hyalomma marginatum* stands out as a tick species of significant medical and veterinary importance, primarily due to its role as the main vector of Crimean-Congo Haemorrhagic Fever Virus (CCHFV) (Ergönül, 2006). This zoonotic virus causes Crimean-Congo Haemorrhagic Fever (CCHF), a severe disease with high fatality rates and potential for widespread outbreaks. Regions across Africa, Europe, and Asia where *H. marginatum* is prevalent are particularly at risk for outbreaks, as the tick's distribution is closely influenced by ecological and climatic factors. These dynamics highlight the pressing need to investigate the environmental drivers of the species' spread to support effective public health and disease control measures.

Hyalomma marginatum exhibits ditropic host preferences, with immature stages parasitizing small mammals and ground-dwelling birds, while adults primarily feed on larger mammals such as cattle, sheep, horses, and occasionally humans (Hoogstraal, 1979). The species' spread and survival are influenced by climatic variables, including temperature, humidity, precipitation, and water vapor deficit (VD), as well as non-climatic factors like host availability, migratory birds, and human activities (Gray et al. 2009; Estrada-Peña et al., 2011; Estrada-Peña et al., 2012). Optimal temperatures for adult tick activity range between 22°C and 27°C, while sufficient rainfall and humidity prevent desiccation, promoting survival in arid regions (Valcárcel et al. 2020). Non-climatic drivers, such as migratory birds acting as long-distance dispersers and livestock transportation, further facilitate the colonization of new areas (Hoogstraal, 1979; Mild et al., 2009). Together, these factors shape the geographic distribution of *H. marginatum*. The wide dispersal of *H. marginatum* reflects its adaptability to diverse environments, including savannahs, steppes, and scrubland hill and valley biotypes (Hoogstraal, 1979).

Recent studies reveal the northward expansion of *H. marginatum* into northern Europe, influenced by factors such as climate change, international livestock trade, and migratory bird movements (Vial et al., 2016; Fernández-Ruiz & Estrada-Peña, 2021; Bah et al., 2022). This trend raises significant concerns about the potential emergence of CCHF in regions previously unaffected, emphasizing the urgent need for predictive tools to forecast changes in tick distribution and inform targeted public health interventions.

In a prior ecological niche modeling (ENM) study, the distribution of *H.marginatum* was analyzed using a combination of climatic, socioeconomic, and satellite data to account for diverse environmental factors (Celina et al., 2023). In this study, however, the focus on future projections necessitates the use of bioclimatic variables exclusively, as they provide reliable predictions for climate-driven range shifts, unlike satellite data, which do not include future projections. The aim of this study is to evaluate the current distribution of *H. marginatum* and forecast its potential range expansions under future climate scenarios using ENM. By integrating species occurrence data with bioclimatic predictors, this study highlights regions at risk of colonization, providing critical insights for vector surveillance, public health strategies, and the management of *Hyalomma*-borne diseases.

18. Materials and Methods

18.1. *Hyalomma marginatum* Occurrence Data

Occurrence records for *H. marginatum* were sourced from multiple databases, including the European Centre for Disease Prevention and Control (ECDC), Global Biodiversity Information Facility (GBIF), VectorMap, iNaturalist, and peer-reviewed literature (Estrada et al., 2016). To ensure taxonomic accuracy, records associated with synonymous species, such as *Hyalomma plumbeum* and *Hyalomma savignyi*, were included, while misidentified records, particularly those confused with *Hyalomma rufipes*, were excluded to maintain dataset integrity.

To minimize spatial and sampling biases, the dataset underwent rigorous cleaning and filtering. Records lacking geographic coordinates, containing zero longitude or latitude values, or marked as absent or uncommon were excluded. Duplicate records were removed to avoid overrepresentation, and spatial thinning was performed using a 5 km (≤2.5′) distance filter via SDMtoolbox 2.4 in ArcGIS 10.8 (ESRI, Redlands, CA). This step ensured that only one unique occurrence point was retained per grid cell, reducing the risk of overprediction in highly sampled areas.

For modeling, the cleaned dataset was randomly divided into two subsets: 75% of the records were allocated for model calibration, and 25% were reserved for validation. The final dataset included 1886 records in total – 818 records from ECDC, 505 from literature, 276 from iNaturalist, 176 from GBIF, and 111 from VectorMap. After all cleaning, thinning, and validation procedures, a total of 1807 unique records were retained for modeling (***Figure 15***).

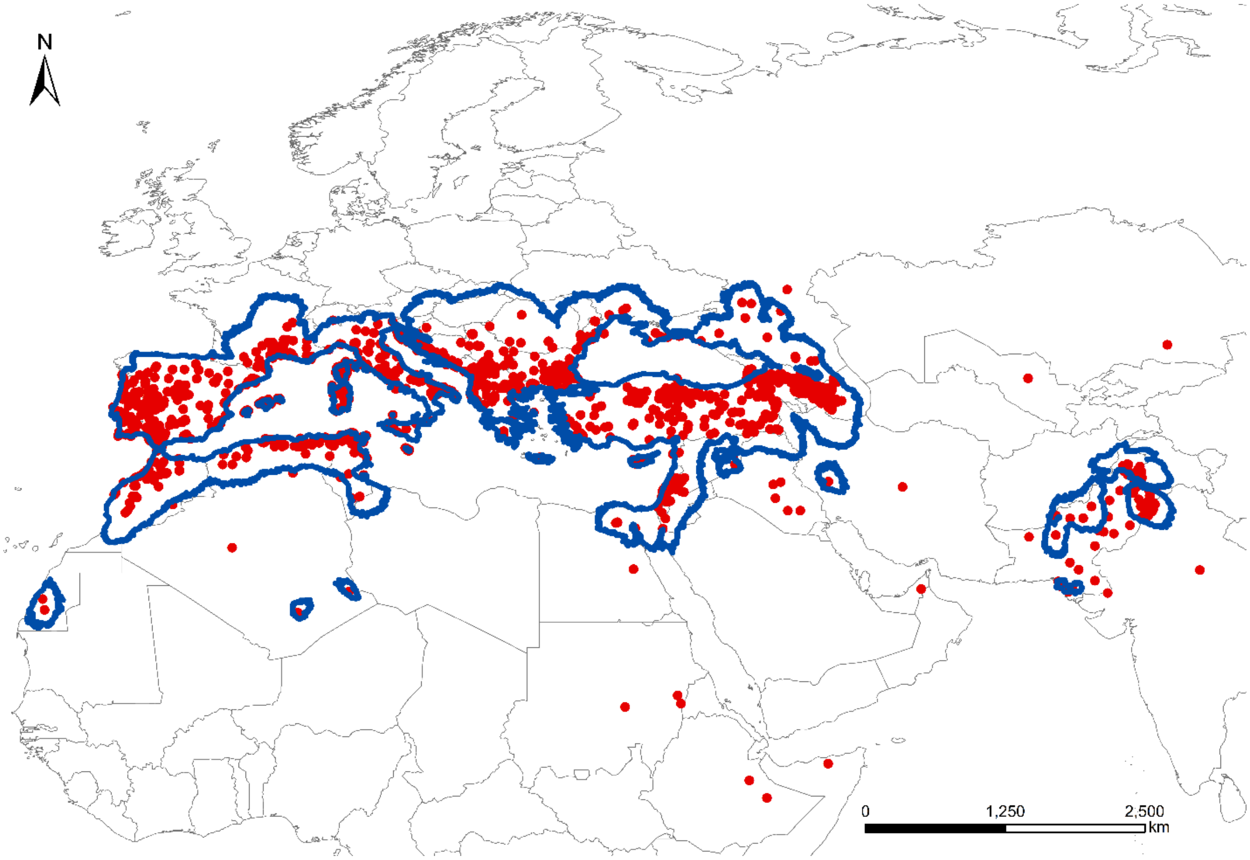

Figure 15. *Hyalomma marginatum* occurrence records used in model calibration. Blue polygons represent the accessible areas (***"M"***) where the model was calibrated.

18.2. Climate Data

Climate data for modeling the distribution of *H. marginatum* were obtained from the WorldClim database, which provides high-resolution (~5 km) bioclimatic variables for global climate analysis (Fick & Hijmans, 2017). These variables, derived from long-term averages of monthly temperature and precipitation data between 1981 and 2010, are widely used in ecological niche modeling due to their ability to represent key climatic patterns, including annual trends, seasonality, and extreme conditions.

From the 19 available bioclimatic variables, four (BIO8: mean temperature of the wettest quarter, BIO9: mean temperature of the driest quarter, BIO18: precipitation of the warmest quarter, and BIO19: precipitation of the coldest quarter) were excluded due to documented spatial artifacts that could lead to erroneous predictions (Samy & Peterson, 2016). These artifacts, which are known to cause abrupt discontinuities between adjacent pixels, can compromise the reliability of model outputs.The remaining 15 bioclimatic variables were used as environmental predictors and rasterized at a spatial resolution of ~5 km to ensure alignment with species occurrence data and the study area.

Future climate projections for modeling the potential distribution of *H. marginatum* were obtained from the WorldClim database, focusing on two future time periods: 2041–2060 and 2081–2100. Climate data were derived from two Shared Socioeconomic Pathways (SSPs): ssp245 (moderate emissions scenario) and ssp585 (high emissions scenario), representing intermediate and worst-case climate trajectories, respectively (Hausfather, 2020). These scenarios were selected to assess the potential impact of climate change on the geographic range of *H. marginatum* under varying levels of greenhouse gas emissions and anthropogenic influence. Bioclimatic variables for future projections were obtained from four Global Circulation Models (GCMs): BCC-CSM2-MR, IPSL-CM6A-LR, UKESM1-0-LL, and MPI-ESM1-2-HR. These projections were applied to the final ecological niche model to estimate potential range shifts, identifying regions that may become more suitable for *H. marginatum* establishment over time.

18.3. Ecological Niche Modeling

Ecological niche models (ENMs) were developed using the maximum entropy algorithm in MaxEnt 3.4.4, implemented within the *kuenm* R package (Cobos et al. 2019). MaxEnt was selected due to its effectiveness in modeling presence-only data and its ability to capture complex relationships between species occurrences and environmental factors (Elith et al., 2011). To optimize model performance, different combinations of feature classes—including linear, quadratic, product, threshold, and hinge—were tested along with a range of regularization multipliers. All environmental variables were resampled to a 5 km spatial resolution to ensure consistency with species occurrence data. To determine the most relevant environmental predictors, four distinct sets of bioclimatic variables were considered: Set 1 (all bioclimatic variables), Set 2 (bio1, bio2, bio4, bio5, bio6, bio7, bio10, bio11, bio12, bio13, and bio16), Set 3 (bio1, bio4, bio5, bio6, bio7, bio10, bio12, bio13, and bio16), and Set 4 (bio1, bio4, bio5, bio6, bio7, bio10, and bio13). The Jackknife function in MaxEnt was used to assess the importance of each bioclimatic variable by evaluating its contribution to model performance when included individually or excluded from the analysis.

Model selection was based on three key criteria: statistical significance using the partial receiver operating characteristic (pROC) test, omission rates below 5%, and model performance assessed with the Akaike information criterion corrected for small sample sizes (AICc). Models that met these criteria and had a delta AICc value of ≤2 were selected for further analysis. The final models were generated using 10 bootstrap replicates to improve robustness and reliability. These models were then projected across the full geographic area of interest (***"G"***) to estimate both the current and future distribution of *H. marginatum*.

To assess the extrapolation risk in regions where climatic conditions differ from the calibration area, a Mobility-Oriented Parity (MOP) analysis was conducted. This analysis identifies areas where model predictions may be less reliable due to novel environmental conditions. The MOP calculation was performed using the mop function in the *kuenm* package in R (Cobos et al., 2019), applying the nearest 10% reference approach (Owens et al., 2013). Although MOP was initially evaluated across multiple GCMs and climate scenarios, areas of strict extrapolation showed a high degree of consistency across all models. Therefore, a single representative MOP map was selected, using results from ssp245 for the 2061–2100 period, to illustrate regions of high extrapolation risk.

19. Results

A total of 2108 candidate models were constructed, combining 17 regularization multipliers, 31 feature class combinations, and four environmental predictor sets. Of these, 1063 models met the omission rate criterion of 5%, and 8 met the AICc threshold (ΔAICc ≤ 2). Two models fulfilled all three criteria: statistical significance (Partial ROC $p < 0.05$), omission rate ≤ 5%, and ΔAICc ≤ 2. The first model (LTH model) applied a linear-threshold-hinge feature class with a regularization multiplier of 0.1 and Set 1 predictors. It produced a mean AUC ratio of 1.271, an omission rate of 4.9%, and an AICc value of 42,034.92 (ΔAICc = 0, W_AICc = 1.0). The second mode (LQTH model) utilized a linear-quadratic-threshold-hinge feature class with the same regularization multiplier and predictors. It resulted in a mean AUC ratio of 1.270, an omission rate of 4.9%, and the same AICc value (W_AICc = 0.5). Both models were generated using 10 bootstrap replicates and included 186 parameters. The LTH model identified bio5 (maximum temperature of the warmest month) as the most influential variable, contributing 12.6%, followed by bio12 (annual precipitation, 11.5%) and bio4 (temperature seasonality, 10.2%) (***Table 6***). Other key predictors included bio6 (minimum temperature of the coldest month, 9.3%) and bio15 (precipitation seasonality, 8.3%). In the LQTH model, bio12 (annual precipitation) had the highest contribution at 15.7%, followed by bio5 (11.9%) and bio6 (9.7%). Similar to the LTH model, bio4 (temperature seasonality, 9%) and bio15 (precipitation seasonality, 8.4%) remained significant contributors.

Table 6. Percent contribution of environmental predictors to LTH and LQTH models.

Environmental Predictor	**LTH Model (%)**	**LQTH Model (%)**
bio5 (Max temp of warmest month)	12.6	11.9
bio12 (Annual precipitation)	11.5	15.7
bio4 (Temperature seasonality)	10.2	9
bio6 (Min temp of coldest month)	9.3	9.7
bio15 (Precipitation seasonality)	8.3	8.4
bio2 (Mean diurnal range)	7.9	6
bio1 (Annual mean temperature)	7.1	6.5
bio16 (Precipitation of wettest quarter)	6.7	5
bio3 (Isothermality)	5.1	5.5
bio11 (Mean temp of coldest quarter)	5.1	5.4
bio13 (Precipitation of wettest month)	4.9	4.5
bio14 (Precipitation of driest month)	3.6	3.6
bio10 (Mean temp of warmest quarter)	2.9	3.3
bio7 (Temperature annual range)	2.8	3.6
bio17 (Precipitation of driest quarter)	2	2

The modeled distribution of *H. marginatum* under current climatic conditions, as predicted by the LTH and LQTH models, highlights broad habitat suitability across southern Europe, northern and sub-Saharan Africa, and western and central Asia (***Figure 16***). Both models produced similar results, with the LQTH model predicting slightly broader suitable areas due to its higher sensitivity to precipitation.

In Europe, the highest suitability is found in the Mediterranean Basin, including southern Spain, Italy, Greece, and Turkey. High suitability extends into Portugal, southern France, and the Balkans. Central European regions such as southern Germany, Hungary, Romania, and Ukraine show moderate suitability, while northern Europe, including Scandinavia, the UK, and northern Germany, remains unsuitable.

In Africa, both models predict high suitability in North Africa (Morocco, Algeria, Tunisia, and Libya) and the Sahel region (Mauritania, Mali, Chad, and Sudan). Southern Africa remains largely unsuitable for *H. marginatum* due to arid conditions.

In Asia, the highest suitability is found in Saudi Arabia, Oman, Yemen, Iran, Iraq, Syria, and Pakistan, with moderate suitability in Kazakhstan and Uzbekistan. The LQTH model predicts additional suitability in Bangladesh and eastern India, while both models consistently indicate low suitability in Mongolia and northern China.

Future projections under ssp245 and ssp585 indicate substantial northward expansion of *H. marginatum*, particularly in Europe (***Figure 17***). By 2041–2060, increasing suitability is predicted for France, Germany, Switzerland, Austria, Hungary, and the Balkans, with new patches of moderate suitability emerging in southern Poland, the Czech Republic, and Slovakia. Under ssp585, the expansion is more pronounced, extending into southern England, the Netherlands, and western Ukraine, reflecting the species' response to warming temperatures.

By 2081–2100, *H. marginatum* suitability increases significantly across northern and central Europe, particularly under ssp585. France, Germany, and Poland exhibit widespread areas of moderate to high suitability, with further encroachment into Belgium, Denmark, and southern Scandinavia. The models indicate potential establishment in southern Sweden and Norway under extreme warming scenarios. While Italy, Spain, Greece, and the Balkans remain strongholds, some southern Mediterranean areas, particularly in southern Spain, Greece, and Turkey, may experience local declines due to extreme heat stress. In eastern Europe, moderate suitability gains are projected in Ukraine, Belarus, and western Russia, but with some fluctuations in habitat stability between mid-century (2041–2060) and late-century (2081–2100). The Baltic countries and southern Russia show slight declines in suitability in the long term, likely due to increasing aridity.

In Africa, North African regions (Morocco, Algeria, Tunisia, Libya, and Egypt) retain stable high suitability, while the Sahel region (Mauritania, Mali, Chad, and Sudan) shows a southward shift due to projected increases in precipitation.

In the Middle East and western Asia, high suitability remains stable in Turkey, Iraq, Iran, and the Arabian Peninsula, with minor declines in southern Saudi Arabia and Oman due to extreme heat stress. Expansion is evident in Pakistan and northern India where warming and precipitation changes create more favorable conditions. By 2081–2100, under ssp585, significant increases in suitability are projected for China, Bangladesh, and parts of Southeast Asia. However, some overly humid regions in South and Southeast Asia may experience declining suitability due to excessive moisture levels. Overall, both ssp scenarios project northward expansion, with ssp585 showing a more extensive shift. While the Mediterranean Basin and the Middle East remain key habitats, southern Europe and parts of North Africa may experience localized suitability declines due to extreme temperatures.

The MOP results indicate high environmental similarity across most of Europe, the Middle East, and Central Asia, ensuring reliable model predictions in these regions (***Figure 18***). However, strict extrapolation is observed in large parts of Africa, particularly in arid and tropical zones, and northern Eurasia, where climatic conditions differ significantly from the training data. Therefore, predictions in these areas should be interpreted with caution due to high extrapolation risk.

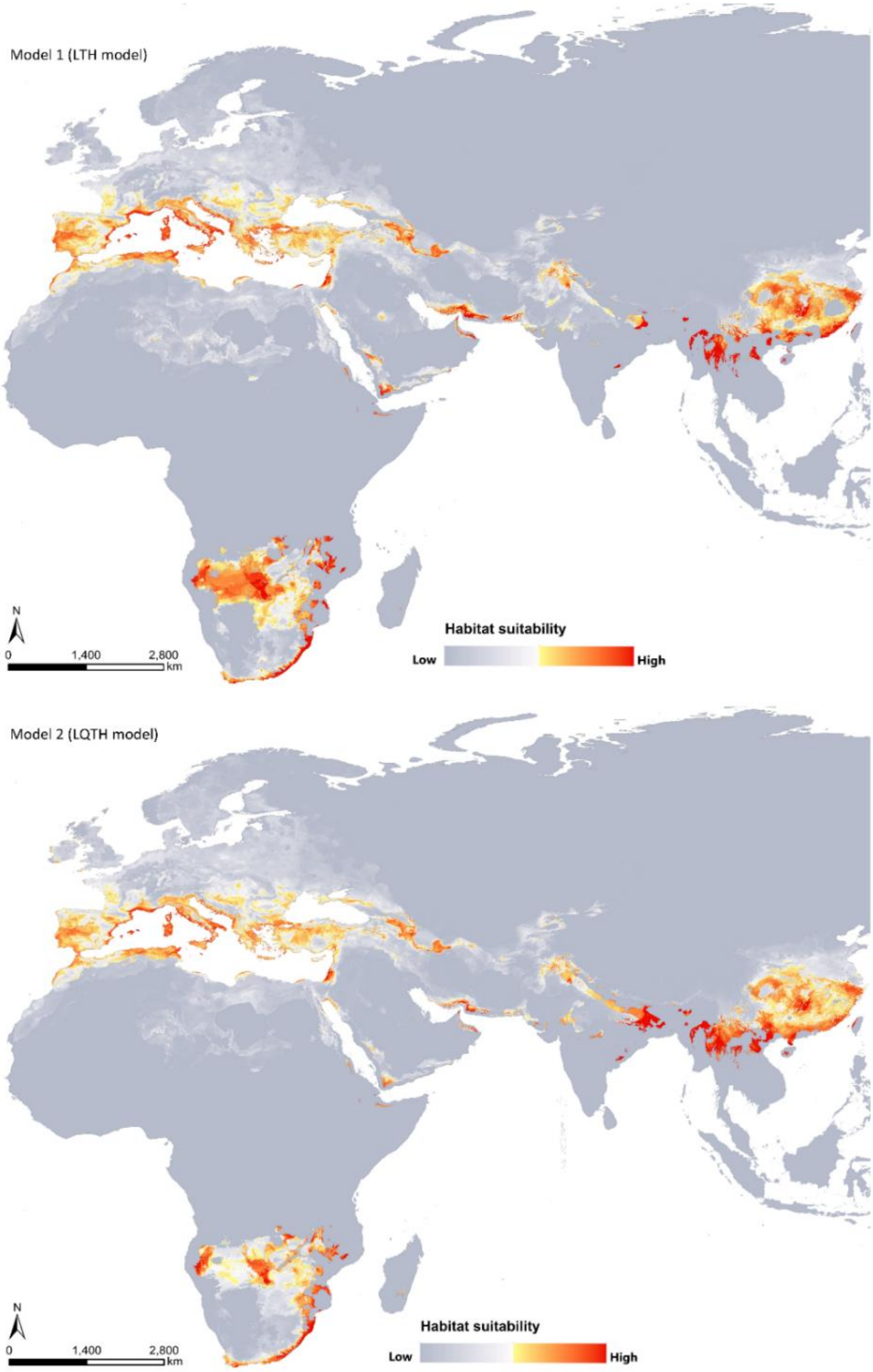

Figure 16. Predicted suitability maps for *Hyalomma marginatum* under current climatic conditions based on ENM results. Red areas indicate the highest habitat suitability.

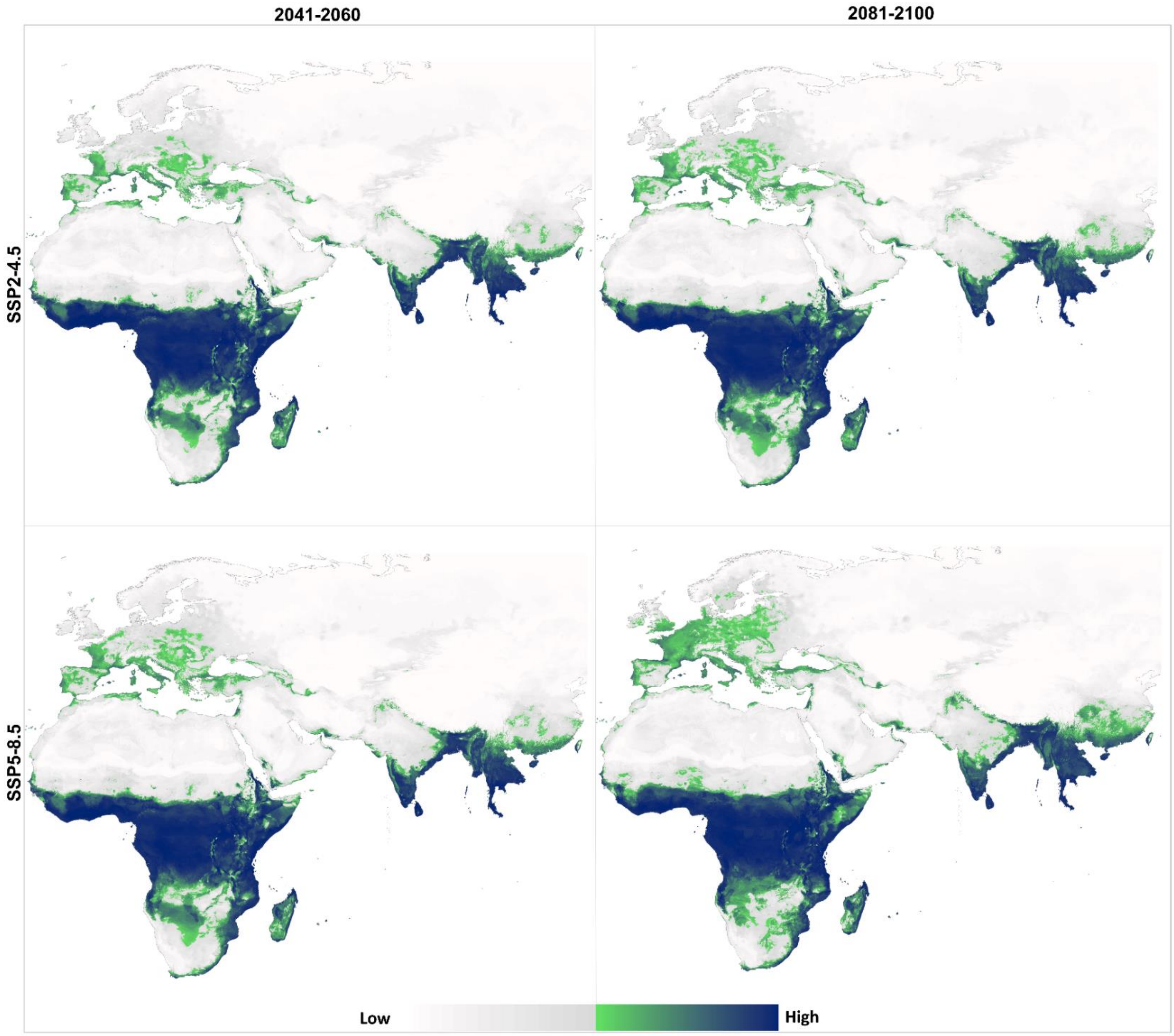

Figure 17. Projected habitat suitability of *Hyalomma marginatum* under future climate scenarios. The maps display predicted distribution changes for the periods 2041–2060 (left) and 2081–2100 (right) under ssp245 (top) and ssp585 (bottom) climate scenarios. Suitability ranges from low (white/gray) to high (green/blue).

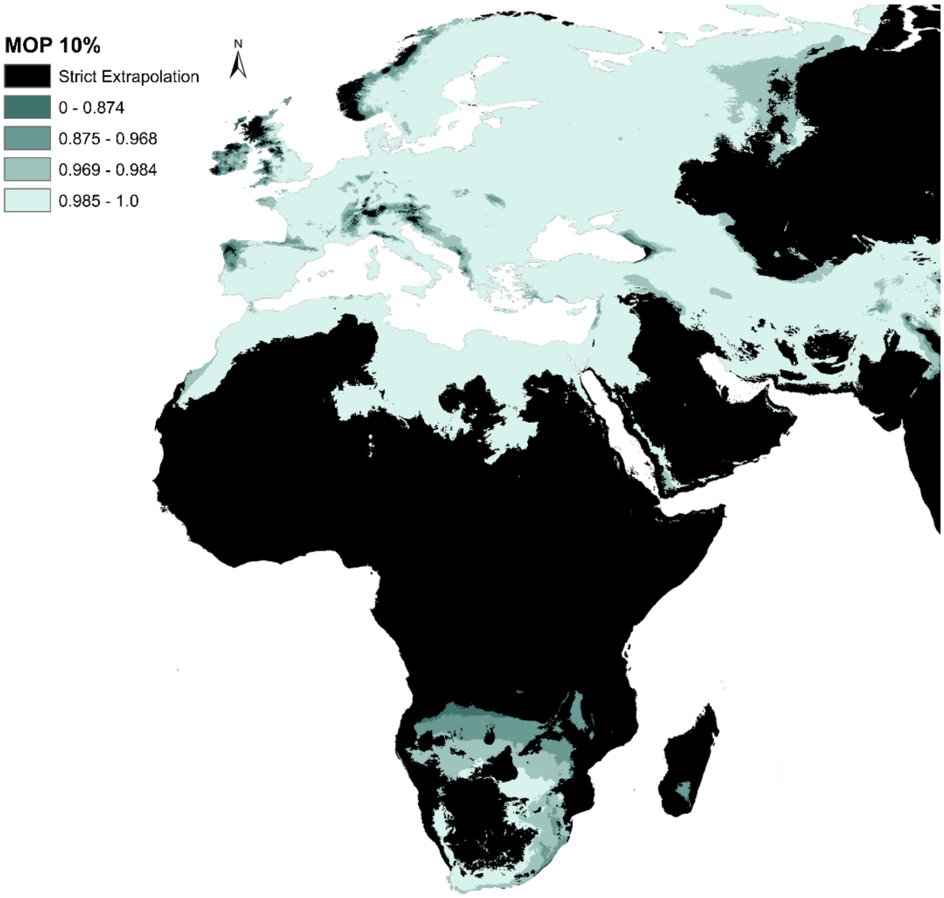

Figure 18. Extrapolation risk analysis for *Hyalomma marginatum* with MOP10% by using future scenarios. The map illustrates extrapolation risk based on the results from ssp245 for the 2061–2100 period. Dark areas represent regions with strict extrapolation, where environmental conditions fall outside the range of the calibration data, indicating high uncertainty in model predictions. Lighter shades indicate increasing environmental similarity to the calibration region, suggesting more reliable predictions.

20. Discussion

Our study presents an ENM analysis of *H. marginatum*, a principal vector of CCHFV, under current and projected climatic conditions. The results indicate that under present climatic conditions, *H. marginatum* exhibits high suitability across the Mediterranean Basin, North Africa, and western and central Asia, consistent with its known distribution. Future projections reveal a notable northward expansion, particularly in Europe, with increasing suitability in central and northern regions. These findings align with previous ENM studies on *H. marginatum* (Hekimoglu et al., 2023; Celina et al., 2023; Estrada-Peña et al., 2012), reinforcing concerns about the species' climate-driven range shifts and the implications for *Hyalomma*-borne disease transmission.

Several recent ENM studies have assessed the potential distribution of *H. marginatum* under both present and future climatic conditions. While our findings largely align with these projections, our study integrates a more extensive occurrence dataset and a refined set of bioclimatic predictors, enhancing the robustness of our results. A study by Hekimoglu et al. (2023) projected northward expansion into central and eastern Europe under ssp370 and ssp585 scenarios. Similarly, Celina et al. (2023) predicted increasing habitat suitability across southern, western, and central Europe, extending into southern Scandinavia. Our study aligns with these projections, particularly under ssp585, where rising temperatures facilitate tick survival in previously unsuitable regions. However, our more extensive dataset and different bioclimatic variable selection provide a comprehensive assessment of habitat shifts.

Temperature and precipitation remain the dominant factors influencing *H. marginatum* distribution. Our models identified maximum temperature of the warmest month (bio5), annual precipitation (bio12), and temperature seasonality (bio4) as the most influential variables, consistent with previous research emphasizing the species' dependence on warm, semi-arid conditions (Estrada-Peña et al., 2012; Requena-García et al., 2017).

The northern limit of *H. marginatum* is primarily determined by temperature, while the southern limit is constrained by precipitation and humidity (Gray et al., 2009). This explains the projected northward expansion into France, Germany, Poland, and Scandinavia, where warming temperatures improve habitat suitability. Conversely, southern Spain, Greece, and parts of Turkey may experience localized declines due to extreme heat stress.

Our future projections indicate substantial northward expansion of *H. marginatum* in Europe, with increasing suitability in Western and Central Europe, and the Balkans by 2041–2060. By 2081–2100, particularly under ssp585, high suitability expands into Northern Europe, reaching southern Scandinavia. These findings align with prior studies predicting tick range shifts due to warming winters and rising global temperatures (Williams et al., 2015; Fernández-Ruiz & Estrada-Peña, 2021). This expansion raises significant public health concerns, as *H. marginatum* is the primary vector of CCHFV. Increased tick populations in previously unaffected regions may elevate disease risk, necessitating enhanced surveillance and control measures in emerging hotspots. Veterinary and public health agencies should prioritize early detection and risk assessment in newly suitable areas.

Outside Europe, our models indicate stable habitat suitability in North Africa, the Middle East, and Central Asia, confirming their role as key regions for the long-term persistence of *H. marginatum*. In sub-Saharan Africa, suitability is expected to shift southward, with potential new habitats emerging in the region. However, South Africa remains largely unsuitable, as *Hyalomma rufipes*—another major CCHFV vector—dominates the region due to its greater ecological adaptability to arid and semi-arid environments (Apanaskevich & Horak, 2008).

One notable aspect of the future projections is the apparent increase in habitat suitability for *H. marginatum* in rainforest regions. As *H. marginatum* is primarily adapted to semi-arid and Mediterranean climates, its survival in dense, humid rainforests remains unlikely. The models used in this study rely solely on bioclimatic variables, particularly temperature and precipitation, without considering land cover, vegetation structure, or host availability—key factors for tick establishment. While climate change may create new marginally suitable microhabitats, dense vegetation, high humidity, and the absence of typical hosts make long-term tick survival in rainforests unlikely. This highlights a key limitation of climate-only ecological niche models, which can overpredict suitability in regions where additional ecological constraints are not considered. Furthermore, much of Africa, particularly rainforest regions, remains under-sampled, with sporadic studies and limited routine tick surveillance compared to Europe. As a result, the absence of records may reflect lack of sampling rather than true species absence. This lack of data, combined with climate-driven modeling, may contribute to projections of suitability in areas where the ecological requirements for *H. marginatum* are not fully met.

While climate is a primary driver of *H. marginatum* distribution, host availability is a crucial ecological factor influencing its establishment, persistence, and dispersal. *Hyalomma marginatum* follows a two-host life cycle, with immature stages feeding predominantly on small mammals and ground-dwelling birds, while adults parasitize larger vertebrates, including livestock (cattle, sheep, and horses), wild ungulates, and, occasionally, humans (Apanaskevich & Horak, 2008; Valcárcel et al., 2020). Given this broad host spectrum, the tick can exploit multiple ecological niches, increasing its potential for range expansion. Host movement patterns significantly impact the long-distance dispersal of *H. marginatum*. Migratory birds act as natural dispersal agents for nymphal stages, allowing the species to colonize new areas (Hasle, 2013; Hoogstraal et al., 1961). Similarly, livestock trade and transhumance facilitate the introduction of adult ticks into non-endemic regions, a factor implicated in recent reports of *H. marginatum* presence in central and northern Europe (Bah et al., 2022). However, the absence of suitable vertebrate hosts in newly climatically suitable regions could hinder the successful colonization and long-term persistence of tick populations.

Incorporating host distribution data into ENMs remains challenging due to the generalist nature of *H. marginatum* and the difficulty in obtaining reliable occurrence data for multiple host species. While certain vertebrates may serve as preferred hosts in specific regions, host preferences and availability are highly variable across different geographic areas, making their integration into models complex and uncertain (Valcárcel et al., 2020; Bonnet et al., 2022). Despite these limitations, future studies should explore ways to integrate host presence and abundance data to refine predictions of tick establishment and persistence. Additionally, land use changes and habitat fragmentation may further influence tick dispersal dynamics. The conversion of natural landscapes into agricultural and urbanized environments can create ecological barriers, limiting tick expansion, whereas semi-natural pastures and fragmented woodlands may increase contact between ticks and their hosts, facilitating their persistence in new areas (Suzán et al., 2012; Perez et al., 2016). A multifactorial approach, incorporating climatic, ecological, and anthropogenic variables, would provide a more comprehensive understanding of *H. marginatum*'s future distribution and its implications for *Hyalomma*-borne disease transmission.

21. Conclusion

Our study provides a comprehensive assessment of *H. marginatum*'s current and future distribution, predicting a clear northward expansion into Europe. These findings highlight the need for proactive surveillance, vector control, and public health preparedness to address the growing risk of *Hyalomma*-borne diseases in a changing climate. Given the ongoing environmental changes, integrating real-time surveillance data with ecological models will be crucial for tracking the species' expansion. Strengthening cross-border collaborations and developing early warning systems will further aid in mitigating the spread of *H. marginatum* and reducing the public health burden associated with its expansion.

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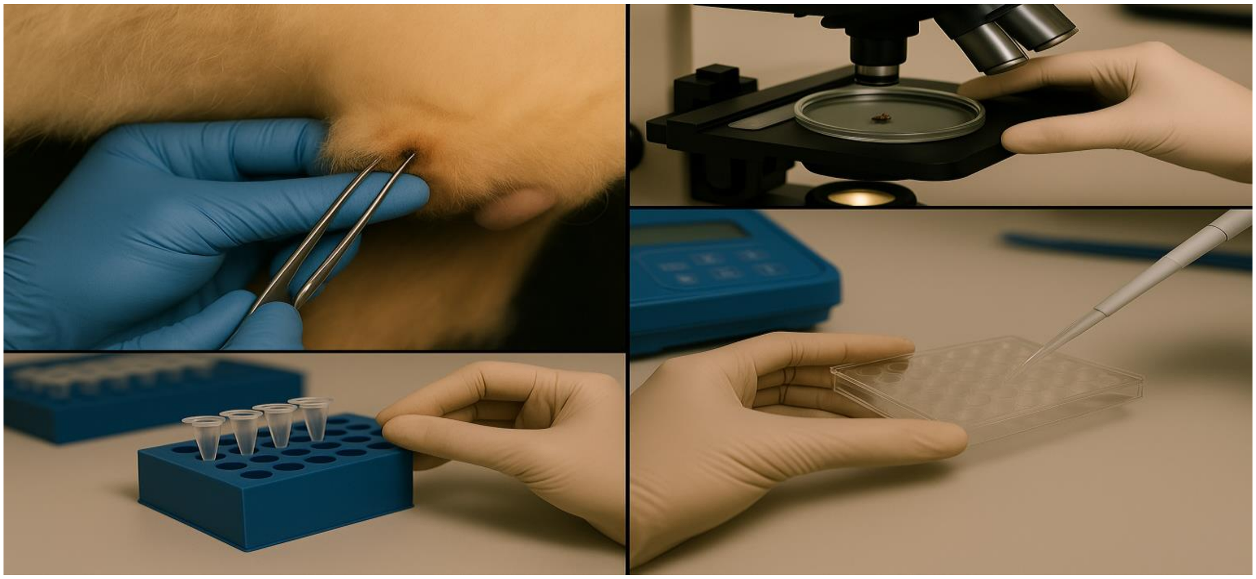
CHAPTER 4:

This chapter summarizes the ongoing research project.

Molecular and Serological Surveillance of Crimean-Congo Haemorrhagic Fever Virus in Ticks and Cattle in Kosovo: A 2022 – 2024 Study



Molecular and Serological Surveillance of Crimean-Congo Haemorrhagic Fever Virus in Ticks and Cattle in Kosovo: A 2022–2024 Study

Abstract

Crimean–Congo Haemorrhagic Fever (CCHF) is a severe viral disease transmitted by ticks, presenting a significant health threat to humans. The Balkan region is considered to be highly endemic area for the disease. Kosovo, particularly areas such as Skënderaj, Klinë, Malishevë, Rahovec, and Suharekë, has been identified as a prominent high-endemic zone for CCHF, significantly impacting the disease's dissemination. This study aimed to evaluate the prevalence of the CCHF virus (CCHFV) among ticks collected from livestock in Kosovo and to understand the transmission dynamics of the virus within tick populations. The samples were collected in June and July 2022 from cattle in different locations in Kosovo. Tick samples were identified and pooled by species and sex for CCHFV detection via RT-PCR. A total of 1470 ticks belonging to 8 species and 4 genera were collected. The species *Hyalomma marginatum* was the most abundant (60.3%), followed by *Rhipicephalus bursa* (20.3%), *Rhipicephalus annulatus* (16.6%), *Haemaphysalis punctata* (1.1%), *Hyalomma anatolicum* (0.9%), *Rhipicephalus sanguineus* (0.7%), *Hyalomma rufipes* (0.1%), and *Ixodes ricinus* (0.1%). CCHFV was detected in 41/381 of the tested pools. The infected tick species were identified as *H. marginatum*, *R. bursa*, and *R. annulatus*. In addition to tick testing, 100 serum samples from cattle were screened for CCHFV-specific antibodies, with 23% testing positive—indicating past or ongoing circulation of the virus among livestock in the region. This study confirmed the active circulation of CCHFV in ticks in Kosovo and highlights their role in the maintenance of CCHFV. The detection of CCHFV in prevalent tick species underscores the importance of adopting a comprehensive "One Health" approach that integrates animal and tick surveillance. It is imperative to take effective measures to control tick infestation in livestock to prevent future CCHFV infections in humans.

Keywords: Crimean-Congo Haemorrhagic Fever Virus, *Hyalomma*, *Rhipicephalus*, livestock, One Health.

23. Introduction

Crimean-Congo Haemorrhagic Fever (CCHF) is a zoonotic viral disease caused by the Crimean-Congo Haemorrhagic Fever Virus (CCHFV), a member of the genus *Orthonairovirus* within the family *Bunyaviridae*. As one of the most geographically widespread tick-borne viruses, CCHFV is present across more than 30 countries in Asia, Africa, Europe, and the Middle East (Hoogstraal, 1979). Its transmission involves a natural cycle between ticks, particularly those belonging to the *Hyalomma* genus, and vertebrate hosts, primarily livestock (Ergönül, 2006). Human infections occur through tick bites or contact with infected animal tissues, often leading to severe disease characterized by haemorrhagic manifestations and high fatality rates. Globally, an estimated 10,000 to 15,000 cases of CCHF are reported each year, though accurate numbers remain uncertain as up to 88% of cases may be asymptomatic, go undetected, or occur in regions with inadequate disease surveillance or laboratory testing infrastructure (WHO; Al-Abri et al., 2017; Hawman & Feldmann, 2018).

In Europe, CCHF is sporadic or endemic in southeastern countries, including Kosovo, Albania, Bulgaria, Greece, and Turkey. Kosovo, in particular, is a CCHF hotspot, with the virus being reported in 50% of its territory (Jameson et al., 2002; Ahmeti et al., 2019). The central and southwestern regions, including Malishevë, Rahovec, Suharekë, and Klinë, are hyper-endemic areas (Sherifi et al. 2018). These regions, characterized by low-altitude farmland and bush vegetation, provide suitable habitats for *H. marginatum*, the primary tick vector of CCHFV (Sherifi et al., 2018). CCHF cases in Kosovo predominantly occur between May and July, coinciding with the peak activity of *H. marginatum* ticks (Sherifi et al. 2014; Sherifi et al. 2018).

The earliest documented case of CCHF in Kosovo dates back to 1954 (Vesenjak-Hirjan et al. 1991). Since 1989, outbreaks have been reported approximately every 4–5 years. Since the mid-1990s human CCHF cases have been reported in Kosovo annually with an average mortality rate of 25.5% (Fajs et al., 2014a; Fajs et al., 2014b; Jameson et al., 2002; Sherifi et al., 2014). More recent data from 2013 to 2016 showed that 32 patients were treated for CCHF in Prishtina, with a case fatality rate of 34%, significantly higher than in neighboring countries like Turkey (11%) (Ahmeti et al. 2019).

Kosovo and the surrounding regions are notable for the genetic diversity of CCHFV. Two primary strains circulate in the area: Europe 1 (Clade V) and Europe 2 (Clade VI). The Europe 1 strain, predominantly transmitted by *Hyalomma marginatum*, is associated with severe outbreaks and high pathogenicity in humans (Sherifi et al., 2014). In contrast, the Europe 2 strain, linked to *Rhipicephalus bursa*, typically causes subclinical or mild cases of the disease (Papa et al. 2014). Historical evidence suggests that the Europe 1 strain may have been introduced to Kosovo from Turkey during the 1970s, with the region's high genetic diversity potentially playing a role in the virus's pathogenicity (Emmerich et al., 2018).

The taxonomy of the *Hyalomma* genus and its role in CCHF transmission in Kosovo remain complex and understudied. Historical records from 1967 indicated that *H. marginatum* accounted for a significant proportion of the tick population on domestic animals in Kosovo (Heneberg et al., 1967). However, earlier studies often used outdated or inaccurate species nomenclature, complicating comparisons with current tick population dynamics (Oswald, 1939). Recent research has reported CCHFV prevalence rates as high as 15% in *H. marginatum* ticks collected after outbreaks, emphasizing the critical role of these ticks in virus transmission (Duh et al., 2006).

Despite being one of the most affected regions in Europe, limited data are available on tick populations and CCHFV prevalence in Kosovo. This study aims to address this gap by analyzing tick populations collected from one of the hyper-endemic regions of Kosovo and assessing the presence of CCHFV.

24. Materials & Methods

24.1. Study Area and Sample Collection

This study was conducted in the Malishevë region of southern Kosovo, a hyper-endemic area for CCHFV. Tick samples were collected from cattle during June–July 2022 and June–July 2024 across 22 villages in 2022 and 12 villages in 2024, at altitudes ranging from 500 to 930 meters. Ticks were sampled from specific anatomical regions of the cattle, including the perineum, scrotum, udder, inner ear, and lower neck, using paired forceps under strict personal protective measures. The ticks were immediately placed in 2 mL cryovials and stored at −20°C for further analysis. For this study, only ticks from 2022 and cattle sera from 2024 were analyzed, while tick samples collected in 2024 remain under investigation as part of the ongoing project.

Tick species and gender were identified morphologically under a stereomicroscope using the classification key by Estrada-Peña et al. (2004). Ticks were grouped into pools for molecular analyses, categorized by species, gender, feeding status, and collection site, ensuring no intermixing between categories. The prepared pools were stored at −80°C to preserve RNA integrity.

In 2024, veterinary professionals collected 100 blood serum samples from farm-raised cattle. These serum samples were obtained from the same individual cattle from which tick specimens were collected during the 2024 fieldwork. Blood was drawn into sterile EDTA tubes, centrifuged, and the serum was isolated for subsequent serological analysis.

24.2. Viral RNA Extraction from Tick Samples

Tick pools were homogenized in a Tissue Lyser LT (Qiagen, Hilden, Germany) using 7 mm stainless steel beads at 6000 rpm for 10 minutes. The homogenate was centrifuged at 14,000 × g for 5 minutes, and the supernatant was stored at −80°C. Total RNA was extracted using the TRIzol RNA isolation method following the manufacturer's protocol. Briefly, tick samples were immersed in 1 mL TRIzol reagent and incubated before adding 200 µL chloroform. The mixture was shaken for 15 seconds, centrifuged at 20,000 × g for 15 minutes at 4°C, and the RNA-containing upper aqueous phase was transferred to a new microcentrifuge tube. RNA was precipitated by adding isopropanol at a ratio of 0.5 mL per 1 mL of TRIzol, incubated at room temperature for 10 minutes, and centrifuged at 12,000 × g for 10 minutes at 4°C. The RNA pellet was washed with 1 mL of 75% ethanol and centrifuged at 7,500 × g for 5 minutes at 4°C. The pellet was air-dried for 5 minutes, resuspended in 20 µL of nuclease-free, DEPC-treated water, and incubated at 55°C for 10 minutes to ensure complete dissolution.

24.3. qRT-PCR Detection of CCHFV RNA

Quantitative reverse transcription PCR (qRT-PCR) was used to detect CCHFV RNA in tick samples. RNA was extracted as described previously and subjected to amplification using the Luna Universal Probe One-Step RT–qPCR Kit (New England Biolabs, Ipswich, MA). The reactions were prepared with 5 µL of RNA template, 10 µL of 2x Luna Universal Probe One-Step reaction mix, 1 µL of Luna WarmStart RT enzyme mix, 1.5 µL of each primer-probe mixture, and 2.5 µL of RNase-free water, resulting in a total reaction volume of 20 µL.

Modified primers and a probe specific to CCHFV were used for detection, as described by Kurtesh et al. (2014). The primers included CCHF S1-flap (5′-AATAAATCATAATCTCAAAGAAACACGTGCC-3′) and CCHF S122-flap (5′-AATAAATCATAACCTTTTTGAACTCTTCAAACC-3′), and the probe was labeled with FAM-BHQ1 (CCHF SP-ACTCAAGGKAACACTGTGGGCGTAAG).

qRT-PCR was performed on a CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Reverse transcription was conducted at 55°C for 10 minutes, followed by an initial denaturation step at 95°C for 1 minute. Amplification involved 50 cycles of denaturation at 95°C for 10 seconds and annealing/extension at 60°C for 30 seconds. A plate read was included at the end of each extension step.

24.4. Serological Detection of CCHFV in Cattle Sera

CCHFV-specific IgG antibodies were detected using the ID Screen® CCHF Double Antigen Multi-species ELISA kit (IDVet, Grabels, France), a validated diagnostic tool for multi-species serological analysis. The assay was conducted per the manufacturer's protocol. Serum samples were diluted and incubated at 25°C for 45 minutes, followed by conjugate and substrate reactions at 25°C for 30 and 15 minutes, respectively. The optical densities (ODs) were measured at a wavelength of 450 nm. The sample positivity percentage (S/P%) was calculated using the formula: S/P% = (ODS/ODPC) × 100. Samples with S/P% >30% were classified as positive, while those ≤30% were considered negative.

25.Results

A total of 1,470 ticks were collected from cattle during the study period, representing eight species across four genera (***Figure 19***). The predominant species identified was *H. marginatum*, which constituted 60.3% of the total collection, followed by *Rhipicephalus bursa* (20.3%) and *R. annulatus* (16.6%). The remaining species included *Haemaphysalis punctata* (1.1%), *H. anatolicum* (0.9%), *R. sanguineus* (0.7%), *H. rufipes* (0.1%), and *Ixodes ricinus* (0.1%) (***Figure***

20). These results highlight the predominance of *H. marginatum* within the region's tick population, consistent with its well-documented role as a primary vector of the virus.

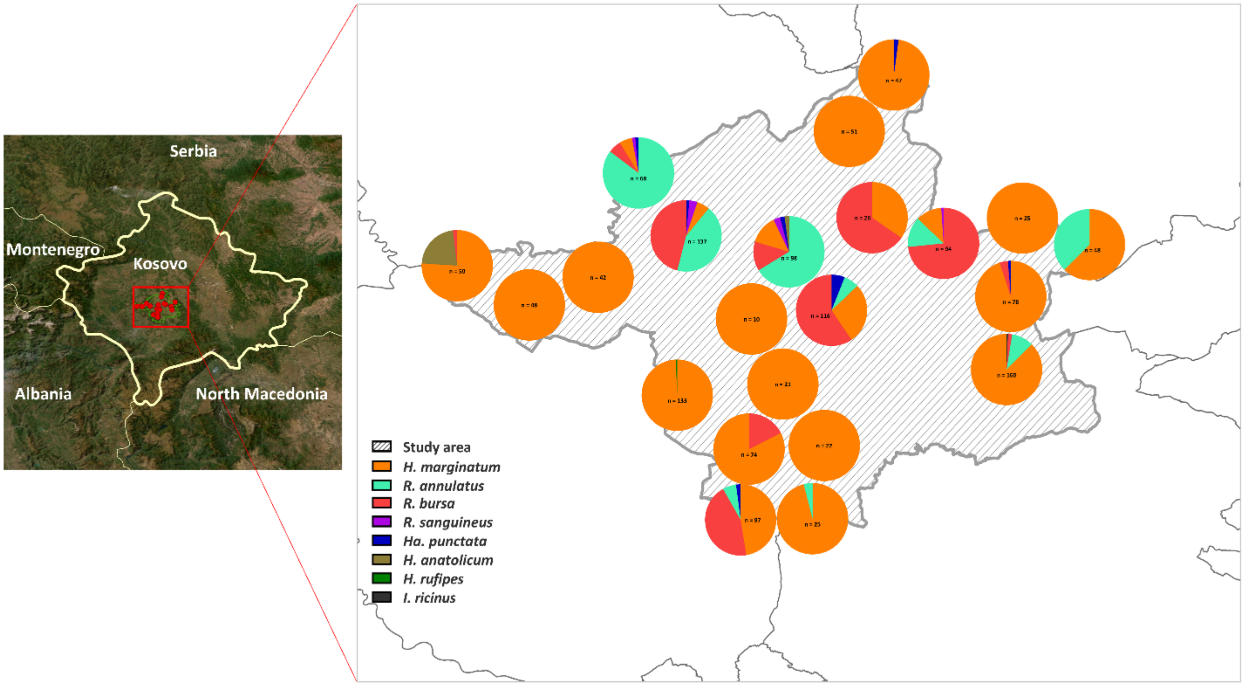


Figure 19. Geographic distribution and species composition of ticks collected in Kosovo.

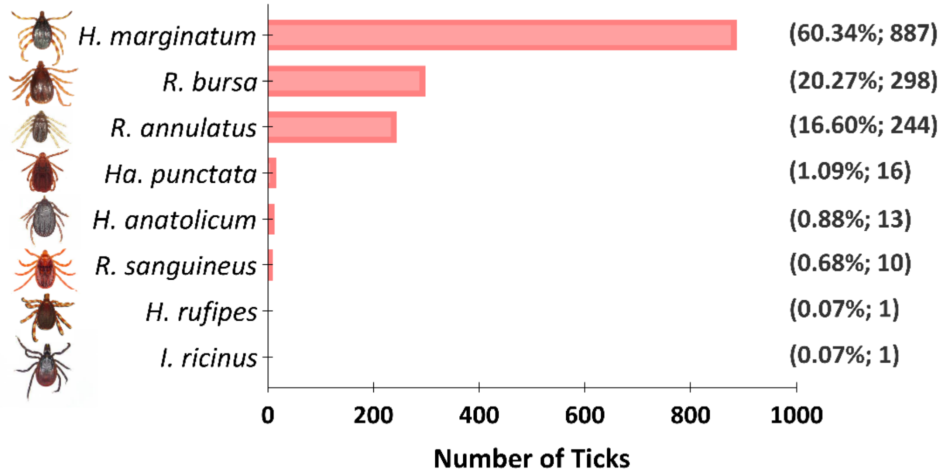


Figure 20. Distribution of tick species collected from cattle in Kosovo during 2022. The bar graph represents the total number and percentage of each tick species identified.

Regarding life stages and feeding statuses, *H. marginatum* populations were predominantly males (67.4%) and fed females (17.6%). Similarly, *R. bursa* and *R. annulatus* showed a high proportion of fed females (52.7% and 56.6%, respectively) and males (37.9% and 24.1%) (***Figure 21***).

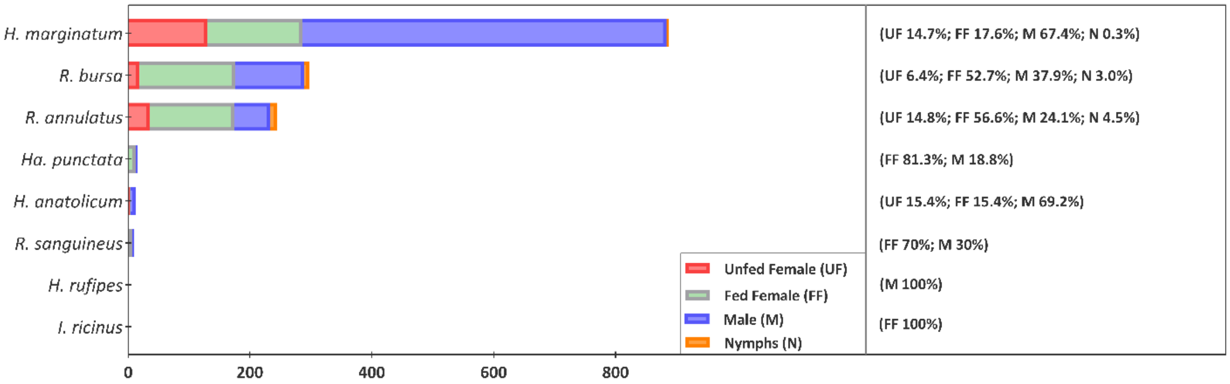


Figure 21. Distribution of life stages and feeding statuses among tick species collected from cattle in Kosovo.

Haemaphysalis punctata consisted mostly of fed females (81.3%), while *H. anatolicum* was largely composed of males (69.2%). In contrast, *R. sanguineus* was primarily made up of unfed females (70%). Rare species, such as *H. rufipes* and *Ixodes ricinus*, were represented by single individuals.

Molecular analyses were conducted on 381 tick pools, encompassing the 1,470 collected ticks. Of these, 340 pools (representing 1,293 ticks) tested negative for CCHFV. However, 41 pools, comprising 177 individual ticks, tested positive, resulting in a pool positivity rate of 10.8%. The cycle threshold (Ct) values for CCHFV-positive pools varied significantly, ranging from 10 to 40.

CCHFV-positive pools were associated with three tick species: *H. marginatum*, *R. bursa*, and *R. annulatus*. As the most frequently encountered species, *H. marginatum* was responsible for the majority of positive pools, further confirming its critical role as a primary vector in the transmission of CCHFV. The detection of CCHFV in *R. bursa* and *R. annulatus* also highlights their potential contributions to the transmission dynamics of the virus in the region, particularly as secondary vectors.

In addition to tick analyses, 100 serum samples from farm-raised cattle collected in 2024 were screened for CCHFV-specific antibodies. Of these, 23 samples tested positive, indicating active or past exposure to the virus in nearly a quarter of the sampled population.

This study is part of an ongoing investigation. Sequencing of tick samples collected in 2022 is underway at Aristotle University of Thessaloniki, Greece, and results are expected to provide insights into the genetic diversity of CCHFV in the region. Analysis of tick samples collected in 2024 has not yet commenced but is planned for later this year. Once these tick samples are analyzed, it will be possible to investigate any relationship between CCHFV-positive ticks and their associated cattle hosts, potentially linking positive tick pools to specific seropositive cattle. This additional layer of analysis will contribute to our understanding of the transmission dynamics of CCHFV in endemic regions like Kosovo. Findings from these ongoing and future analyses will be disseminated as separate studies.

26. Discussion

This study confirms the continued circulation of CCHFV in Kosovo, particularly within historically endemic regions such as Malishevë. The detection of CCHFV RNA in 41 pools of ticks collected from cattle in 2024 reflects active viral presence in the environment. As expected, *H. marginatum*, the primary vector of CCHFV, accounted for the majority of positive pools, reinforcing its central role in transmission. However, the detection of viral RNA in *R. bursa* and *R. annulatus* also suggests these species may act as secondary vectors in the region, highlighting the need to further explore the contributions of these tick species to the local and regional epidemiology of CCHFV.

Serological analysis of cattle from the same farms where ticks were collected revealed that 23% of animals had antibodies against CCHFV, indicating past or ongoing exposure. These results highlight the role of livestock not only as hosts for tick populations but also as important sentinels for monitoring viral circulation. This underscores the importance of integrating livestock surveillance into a broader "One Health" framework for early detection and control of CCHFV risks.

Between 2013 and 2016, a total of 32 patients were treated for CCHF in Kosovo, 11 of whom died, corresponding to a case-fatality rate of 34% (Ahmeti et al., 2019). This period represented one of the most active phases of CCHF in recent Kosovar history. Surveillance data from the Ministry of Health of Kosovo, supported by credible national news sources, indicate that the incidence of CCHF in Kosovo has remained relatively low in recent years. In 2016, all 46 suspected cases tested negative, and between 2014 and 2018 only eight laboratory-confirmed cases were recorded. A case in 2018 marked the first confirmed infection outside the traditional hotspot of Malishevë, in Gjilan, but the patient survived. No CCHF-related deaths occurred after 2013 until July 2020, when a fatal case was reported in Malishevë. Importantly, from 2021 to 2023, no confirmed human infections were reported despite ongoing tick exposures in endemic municipalities. A retrospective case report later described a 2013 infection in a pregnant woman which resulted in neonatal death (Ajazaj-Berisha et al., 2025). These observations suggest that although the virus remains enzootic in ticks and livestock, the intensified and widespread use of acaricides in recent years to control tick infestations in livestock may have been a decisive factor in reducing the number of human CCHF cases in recent years

Kosovo’s ecological and agricultural profile continues to provide suitable conditions for tick populations and CCHFV transmission. The Mediterranean-continental climate, fragmented farmland, bush vegetation, and semi-wild grazing practices create ideal habitats for *H. marginatum* and other vector species (Sherifi et al., 2014). Historical hotspots such as Malishevë, Rahovec, Suharekë, and Klinë have repeatedly experienced outbreaks with significant fatality rates, especially among farmers and agricultural workers (Ahmeti et al., 1996; Sherifi et al., 2018). Molecular studies show that Kosovo hosts two distinct viral lineages: the highly pathogenic Europe 1 (Clade V), primarily transmitted by *H. marginatum*, and the low-pathogenic Europe 2 (Clade VI), associated with *R. bursa* (Sherifi et al., 2014; Papa et al., 2014). This genetic diversity underlines the country’s critical role in the wider regional epidemiology of CCHFV.

The regional situation in the Western Balkans mirrors these patterns of low but persistent activity. In Albania, most cases occur in the northeastern districts bordering Kosovo, reflecting a persistent local tick-borne reservoir. For example, in March 2017 two confirmed CCHF cases were reported in Kukës district (Ndreu et al., 2018). No large outbreaks have been recorded in recent years; cases usually present as isolated or familial clusters. North Macedonia, after decades without reported cases, experienced a sudden re-emergence in 2023 when three infections were confirmed, including one fatality and one nosocomial case in a healthcare worker. Genetic sequencing demonstrated close similarity between the strain and those circulating in Kosovo, confirming transboundary viral movement (Jakimovski et al., 2025). By contrast, Serbia and Montenegro have not reported cases in recent years, although the presence of competent tick vectors and serological evidence of CCHFV in animals suggest underlying endemicity (EFSA, 2010). The absence of reported cases in these countries highlights the variable visibility of CCHF across the region, which may reflect differences in surveillance intensity rather than true absence.

The present study reinforces the importance of sustained surveillance in both ticks and livestock. The pending sequencing of tick samples collected in 2022 and 2024 will provide further insight into CCHFV strain diversity and the relationship between infected ticks and their animal hosts. These data will be essential for refining risk assessments and implementing targeted control measures, such as acaricide treatment and public education efforts. Given the sporadic but persistent nature of CCHFV in the region, continuous monitoring is critical to prevent potential re-emergence in the human population.

27. Conclusion

This study highlights the intricate relationship between ticks, cattle, and CCHFV in Kosovo, emphasizing the need for sustained vector surveillance and integrated control strategies. The findings add to the evidence of CCHFV's endemic presence in the Balkans and lay the groundwork for creating targeted strategies to reduce the disease's impact in the region. Continued research and collaboration across disciplines and borders are essential to address the persistent challenges posed by CCHFV in endemic areas.

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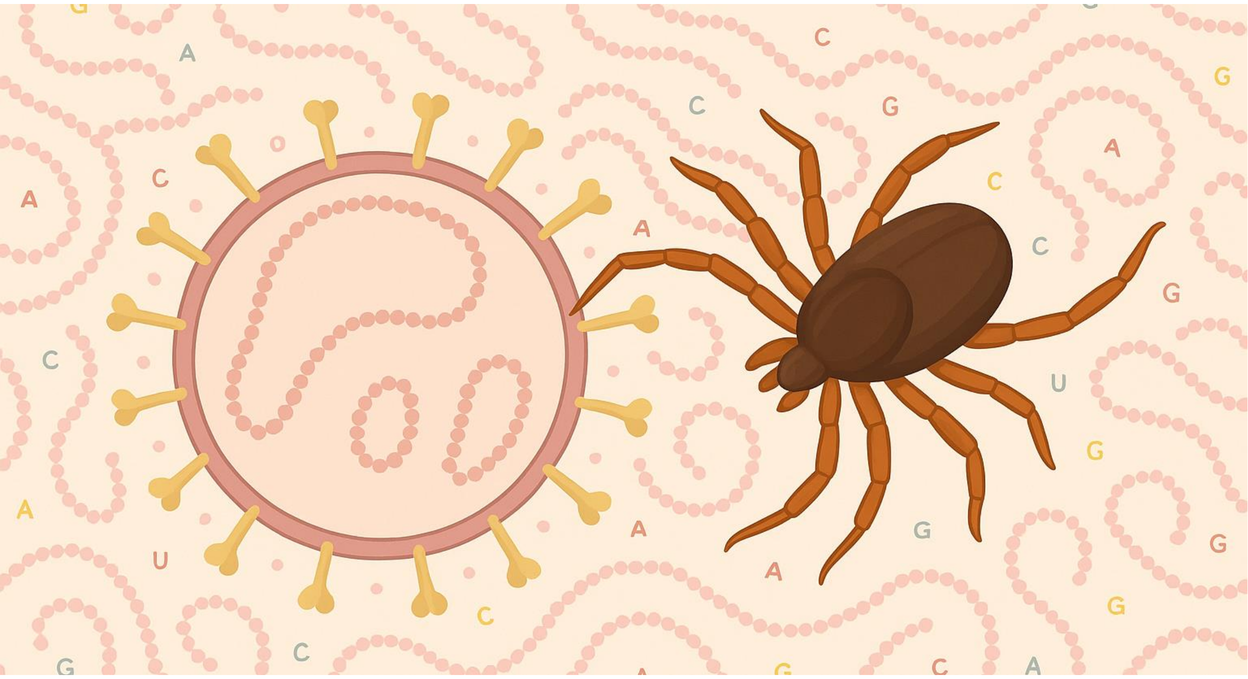
CHAPTER 5:

This chapter is a literal copy of the published article.

Genetic Background of Adaptation of Crimean-Congo Haemorrhagic Fever Virus to the Different Tick Hosts



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Authors' contribution: the first author, **Celina SS** participated in conceptualization, resources, methodology, data analysis, validation, visualization, writing - original draft, writing - review & editing. All authors provided comments, revisions, and final edits to the manuscript prior to publication.

Research Article

Genetic Background of Adaptation of Crimean-Congo Haemorrhagic Fever Virus to the Different Tick Hosts

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Abstract

Crimean-Congo Haemorrhagic Fever orthonairovirus (CCHFV) is a negative-sense, single-stranded RNA virus with a segmented genome and the causative agent of a severe Crimean-Congo Haemorrhagic Fever (CCHF) disease. The virus is transmitted mainly by tick species in *Hyalomma* genus but other ticks such as representatives of genera *Dermacentor* and *Rhipicephalus* may also be involved in virus life cycle.

To improve our understanding of CCHFV adaptation to its tick vectors, we compared nucleotide composition and codon usage patterns among the all CCHFV strains i) which sequences and other metadata as locality of collection and date of isolation are available in GenBank and ii) which were isolated from in-field collected tick vectors. These criteria fulfilled 70 sequences (24 coding for S, 23 for M, and 23 for L segment) of virus isolates originating from different representatives of *Hyalomma* and *Rhipicephalus* genera.

Phylogenetic analyses confirmed that *Hyalomma*- and *Rhipicephalus*-originating CCHFV isolates belong to phylogenetically distinct CCHFV clades. Analyses of nucleotide composition among the *Hyalomma*- and *Rhipicephalus*-originating CCHFV isolates also showed significant differences, mainly in nucleotides located at the 3rd codon positions indicating changes in codon usage among these lineages. Analyses of codon adaptation index (CAI), effective number of codons (ENC), and other codon usage statistics revealed significant differences between *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains. Despite both sets of strains displayed a higher adaptation to use codons that are preferred by *Hyalomma* ticks than *Rhipicephalus* ticks, there were distinct codon usage preferences observed between the two tick species. These findings suggest that over the course of its long coevolution with tick vectors, CCHFV has optimized its codon usage to efficiently utilize translational resources of *Hyalomma* species.

Keywords: Crimean-Congo Haemorrhagic Fever orthonairovirus, codon usage, coevolution, host adaptation, *Hyalomma*, *Rhipicephalus*.

28. Introduction

The Crimean-Congo Haemorrhagic Fever orthonairovirus (CCHFV), recently renamed *Orthonairovirus haemorrhagiae* by the International Committee on Taxonomy of Viruses (ICTV) Nairoviridae Study Group, is a member of the genus *Orthonairovirus*, family *Nairoviridae*, and the etiologic agent of Crimean-Congo Haemorrhagic Fever (CCHF) disease (Postler & Kuhn, 2021). It is a lipid-enveloped, single-stranded, negative-sense RNA virus with a segmented genome. Each of the three CCHFV genomic segments has a different function: the S (small) segment encodes nucleocapsid protein (N), the M (medium) segment encodes glycoproteins (Gn and Gc), and the L (large) segment encodes RNA-dependent RNA polymerase (RdRp; Bente et al., 2013).

CCHF is the most widely distributed tick-borne viral disease in humans, being endemic in many geographic regions across Africa, Asia, Eastern and Southern Europe, and the Middle East (Bente et al., 2013). In Africa and Eurasia, CCHFV is among the deadliest human pathogens (Shayan et al., 2015), and outbreaks of CCHF pose a significant threat due to its epidemic potential, high case fatality rates ranging from 5% to 80% (Mertens et al., 2013), potential for nosocomial outbreaks, and challenges in treatment and prevention (WHO, 2013). Therefore, due to its high potential for human-to-human transmission, the high risk of laboratory-acquired infections, and the lack of a specific vaccine, CCHFV is classified as a biosafety level 4 (BSL-4) agent (Ergönül, 2006).

The virus is transmitted through the bite of its main vector, ticks in the genus *Hyalomma* (Ixodidae). *Hyalomma marginatum* Koch, 1844 is the major vector of CCHFV in the Old World (Bente et al., 2013). Additionally, various other species within the *Hyalomma* genus have also been reported to carry CCHFV, such as *H. excavatum* Koch, 1844, *H. lusitanicum* Koch, 1844, *H. rufipes* Koch, 1844, and *H. truncatum* Koch, 1844 (Gonzalez et al., 1991; Madbouly et al., 1970; Estrada-Peña et al., 2012; Nasirian, 2022), playing significant roles as vectors in the Middle East, the Iberian Peninsula, and Africa, respectively (Orkun et al., 2017). However, the scope of potential vectors expands beyond *Hyalomma* species, with over 30 different tick species implicated in CCHFV transmission (Orkun et al., 2017).

Ticks from other genera of Ixodidae, including *Rhipicephalus* and *Dermacentor*, are also capable of transmitting CCHFV. *Rhipicephalus* ticks, such as *R. bursa* Koch, 1844, and *R. turanicus* Pomerantsev, 1936, have been identified as carriers of CCHFV in regions spanning Albania, Türkiye, Greece, and Russia (Papa et al., 2017; Tsapko et al., 2022; Yesilbag et al., 2013). Similarly, in the genus *Dermacentor*, *D. marginatus* Sulzer, 1776 has tested positive for CCHFV in Türkiye, Russia, and Spain (Papa et al., 2017; Tsapko et al., 2022; Sherifi et al., 2018; Moraga-Fernández et al., 2021; Fakoorziba et al., 2015), highlighting its potential as a vector for the virus. Despite the detection of CCHFV within *D. marginatus* eggs and its confirmed competency as a tick vector for the virus in laboratory studies, questions remain regarding their natural vectorial capacity within the enzootic cycle of the virus (Orkun et al., 2017; Sherifi et al., 2014).

Ticks transmit CCHFV to various mammals by taking blood meals for their maturation and egg production. Nevertheless, humans can acquire CCHFV infection not only from tick bites but also from direct contact with the blood or tissues of infected animals or human patients. Other possible routes of transmission for CCHFV include transmission from an infected mother to offspring, sexual contact, and blood transfusion (Pshenichnaya et al., 2017).

Due to its complex ecology, CCHFV is characterized by a great genetic diversity with complex evolutionary patterns (Anagnostou & Papa, 2009). CCHFV can be phylogenetically divided into eight distinguishable clades (Africa 1–3, Europe 1–3, and Asia 1 and 2). These clades differ not only by their geographic distribution and primary sequence but also by other features such as pathogenic potential. On the other hand, segment reassortment between the clades and long-range migration events of individual CCHFV lineages have been observed, demonstrating the dynamic nature of CCHFV evolution (Chinikar et al., 2010; Hewson et al., 2004).

In the genomes of each species, there is a distinct preference for specific synonymous codons, which encode the same amino acids, leading to unequal frequencies of codon usage within their respective genes (Plotkin & Kudla, 2011; Plotkin et al., 2006). This concept, referring to the differential usage of synonymous codons, is known as codon usage bias. Codon usage bias has been studied in a wide range of organisms, from prokaryotes to eukaryotes and viruses. However, how such biases arise is a much-debated area of molecular evolution. Different factors have been suggested to be related to codon usage bias, including gene expression level, guanine-cytosine content, guanine-cytosine skew, amino acid conservation, protein hydropathy, and transcriptional selection (Chen & Chen, 2014; Ermolaeva, 2001; Knight et al., 2001; Quax et al., 2015).

The codon usage bias in RNA viruses is mainly affected by compositional constraints under mutational pressure and natural selection (Shields et al., 1988; Stenico et al., 1994). Many studies on codon usage bias in various viruses have shown that the main driver shaping codon usage patterns is mutational pressure rather than natural selection (Jenkins & Holmes, 2003; Sharp et al., 2010; Wong et al., 2010). However, for many DNA and RNA viruses, mutational pressure is not the only factor influencing codon usage patterns (Butt et al., 2014; Rahman et al., 2018). Compared with prokaryotic and eukaryotic genomes, the viral genome has certain features, such as dependence on its hosts for replication, protein synthesis, and transmission of proteins. The interaction between virus and host is considered to affect the survival, adaptation, and evolution of the virus, as well as its ability to evade the host's immune system. In many viruses, including CCHFV, an optimal codon usage pattern has been shown to be an important factor in better adaptation of these viruses to their hosts (dos Passos Cunha et al., 2018; Singh et al., 2016). Moreover, major ecological shifts in the evolution of viruses have been linked to variations in their codon usage (Li et al., 2022). On the other hand, codon usage pattern deoptimization leads to decreased fitness in many viruses (de Fabritus et al., 2015; Manokaran et al., 2019; Nougairede et al., 2013).

Previously, an analysis describing the adaptation of CCHFV codon usage patterns to its vertebrate hosts was performed (Rahman et al., 2018), but tick species (except for *Hyalomma* ticks) were not included in the study, despite the fact that arthropod vectors have at least the same effect on arbovirus evolution as their vertebrate hosts (Sexton & Ebel, 2019).

Therefore, we performed a comprehensive analysis of codon usage patterns of three genomic segments (S, M, and L) of CCHFV isolates from *Hyalomma* and *Rhipicephalus* (no sequences of CCHFV strains isolated from other tick species are available) using available sequence data. While *Hyalomma* species are the primary virus reservoirs and vectors for CCHFV, there is experimental evidence suggesting the potential involvement of ticks from the *Rhipicephalus* genus in its transmission; however, concrete evidence supporting their significant role in viral maintenance and natural transmission remains inconclusive (Sherifi et al., 2014). Recent studies have detected CCHFV antigen within a *Rhipicephalus* species, along with viral RNA in different *Rhipicephalus* species across Albania, Kosovo, Greece, Türkiye, and Iran (Papa et al., 2017; Tsapko et al., 2022; Yesilbag et al., 2013; Fakoorziba et al., 2015; Sherifi et al., 2018). Notably, this highlights the exclusive presence of CCHFV strains belonging to Europe 2 (clade VI) within *R. bursa* species, indicating their potential role as vectors of the virus (Papa et al., 2017). Conversely, *R. sanguineus* sensu lato ticks are commonly linked with the Europe 1 clade. Further laboratory investigations are essential to establish the vector competence of *Rhipicephalus* species (Papa et al., 2017). Moreover, a recent comprehensive review categorizes *R. bursa* among confirmed vectors for CCHFV (Orkun et al., 2017). This classification is based on documented infection rates, records, and observations spanning over 30 distinct tick species (Orkun et al., 2017). Given this context, our study aimed to investigate the codon usage patterns of CCHFV in relation to both *Hyalomma* and *Rhipicephalus* tick species, providing a deeper understanding of its adaptation to various vector species.

29. Materials and Methods

29.1. Data Collection

In this study, complete or nearly complete genome sequences of CCHFV strains isolated from tick vectors were analyzed, while partial sequences, that could lead to biased results in terms of codon usage, were excluded. We compared nucleotide composition and codon usage patterns among the all CCHFV strains i) which sequences and other metadata as locality of collection and date of isolation are available in GenBank and ii) which were isolated from in-field collected tick vectors. These criteria fulfilled 70 sequences (24 coding for S, 23 for M, and 23 for L segment) of CCHFV isolates originating from different representatives of *Hyalomma* and *Rhipicephalus* genera, and were retrieved from the National Center for Biotechnology Information (NCBI) GenBank database (https://ncbi.nlm.nih.gov/nuccore/) on December 2019, together with information about isolation date, collection locality, and vector species. Sequence details were compiled in **Supplementary Table S1.**

29.2. Phylogenetic Analysis

Sequences were divided into groups according to CCHFV segments and aligned using MAFFT (v7.427). Maximum clade credibility (MCC) trees were constructed using Bayesian evolutionary analysis by sampling trees (BEAST, v1.10.4) (Drummond & Rambaut, 2007; Suchard et al., 2018) using HKY as the nucleotide substitution model with gamma distributed rate heterogeneity and a relaxed molecular clock. The Markov chain Monte Carlo (MCMC) algorithm was executed for 100-million generations, with the initial 10% of the chain discarded as burn-in using the TreeAnnotator package integrated within the BEAST software (Drummond & Rambaut, 2007). The resulting MCC tree files were subsequently imported into the Figtree tool (v1.4.4; http://tree.bio.ed.ac.uk/software/figtree/) for tree visualization and to estimate the time to the most recent common ancestor (tMRCA). Clades with Bayesian posterior probabilities exceeding 0.5 were displayed in the trees. A total of 70 sequences were used in this analysis.

29.3. Nucleotide Composition Analysis

Nucleotide compositional properties of the CCHFV coding sequences were calculated using CAIcal server (http://genomes.urv.es/CAIcal/) (Puigbò, Bravo, & Garcia-Vallve, 2008). The overall frequency of occurrence of nucleotides (A%, C%, U% and G%), frequency of each nucleotide at the third site of synonymous codons (A3%, C3%, U3% and G3%), frequencies of occurrence of nucleotides GC at the first (GC1), second (GC2) and third synonymous codon positions (GC3), the mean frequencies of nucleotide GC at the first and the second position (GC12), overall GC and AU contents, and AU and GC contents at the third codon positions (AU3, GC3) were calculated. AUG and UGG that are only the codons for Met and Trp (no synonymous codons) along with the termination codons (UAG, UAA, and UGA) which do not encode any amino acids were excluded from the analyses since they were not expected to show any codon usage bias.

29.4. Analysis of the Effective Number of Codons (ENC)

An effective number of codons (ENC) analysis was used to assess codon usage bias in CCHFV segments isolated from *Hyalomma* and *Rhipicephalus* ticks, calculated with CodonW software (v1.4.4) (http://sourceforge.net/projects/codonw). ENC values typically range from 20 to 61, with lower values signifying extreme codon usage bias and higher values indicating the opposite.

To explore whether the codon usage of given strains is solely due to mutational pressure or selection pressure, an ENC-plot was produced. To determine the correlation between the expected ENC and the GC3s values, the expected ENC values were calculated for different GC3s using the method of Singh et al. (2016) (Singh et al., 2016):

$ENC\mathrm{exp} = 2 + s + \frac{29}{(s^2+(1-s)^2)}$ where “s” indicates GC contents at the 3rd synonymous codon positions (GC3s%). When data points align with or near the standard curve, it suggests predominantly mutational pressure, while points falling below indicate codon usage subject to natural selection.

29.5. Relative Synonymous Codon Usage (RSCU) Analysis

RSCU analysis was performed to compare the codon usage preferences of three different segments of CCHFV being isolated either from *Hyalomma* or *Rhipicephalus*. RSCU was calculated using CAIcal server. RSCU values greater than 1 show positive codon usage bias and are described as “abundant” codons. The values less than 1 show negative codon usage bias and are described as “less-abundant” codons. A RSCU value of 1 indicates no bias in codon usage.

Hyalomma- and *Rhipicephalus*-isolated CCHFV coding sequences were compared with codon usage values of their natural vectors obtained from Codon and Codon Pair Usage Tables (CoCoPUTs) (https://hive.biochemistry.gwu.edu/review/codon2) (Alexaki et al., 2019), accessed in June 2020, and analyzed using CAIcal server.

29.6. Codon Adaptation Index (CAI) Analysis

CAI was performed to gain insight into the codon preferences of CCHFV in relation to its tick hosts. The CAI values vary from 0 to 1, and higher values indicate higher levels of codon usage bias towards the codons used in highly expressed genes (Butt et al., 2014). The most frequent codons signify the highest relative adaptation to the host, and sequences having higher CAI are known to be favoured over sequences having lower CAI. CAI analysis was performed using CAIcal server (Puigbò, Bravo, & Garcia-Vallve, 2008) for *Hyalomma* (*H. anatolicum, H. asiaticum, H. dromedarii, H. excavatum, H. lusitanicum, H. marginatum, H. rufipes* and *H. truncatum*) and *Rhipicephalus* (*R. bursa* and *R. sanguineus*) species. The reference data sets showing codon frequencies for these tick species were obtained from the CoCoPUTs database (Alexaki et al., 2019).

In addition, the expected CAI values (eCAI) at the 95% confidence interval were calculated in order to ascertain whether statistically significant differences in CAI values result from codon preferences and to provide statistical support to CAI analyses, a Kolmogorov-Smirnov test for the eCAI was also applied (Puigbò, Bravo, & Garcia-Vallvé, 2008).

29.7. Correspondence Analysis (COA)

COA analysis was performed to detect the variation of codon usage data (Singh et al., 2016). The COA was performed based on RSCU values for CCHFV strains (S, M, and L segments) isolated from *Hyalomma* and *Rhipicephalus*, and the distribution of the strains in the plane of the first two principal axes of COA was determined. CodonW (v1.4.4) software was used in order to examine the codon usage indices.

29.8. Selection Analysis

The dN/dS ratio (ω) is used to compare the non-synonymous substitution rate per non-synonymous site (dN) with the synonymous substitution rate per synonymous site (dS). This ratio estimates the selective pressures acting on a coding sequence by identifying the fraction of codons that are evolving under purifying/negative selection ($\omega < 1$), nearly neutral evolution ($\omega = 1$), or positive/diversifying selection ($\omega > 1$). To estimate the selection pressure on the S, M, and L segments of CCHFV isolated from *Hyalomma* and *Rhipicephalus* ticks, the Hypothesis Testing Using Phylogenies software v2.2.4 (HyPhy) was used (Pond et al., 2005). Pervasive site-specific selection pressure analysis was performed in this study, utilizing Fast Unconstrained Bayesian AppRoximation (FUBAR) method accessible at the Datamonkey webserver (Murrell et al., 2013) (https://www.datamonkey.org/). The dN/dS estimates for selection inference were analyzed using the posterior probability of ≥ 0.9.

29.9. Statistical Analysis

A statistically significant difference between all nucleotide compositional properties of *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains (A, C, U, G, A3, U3, G3, C3, AU, GC, GC1, GC2, AU3, GC3, GC12), and among ENC and CAI values was addressed by applying the t-test and Wilcoxon & Mann-Whitney test with Bonferroni correction ($p < 0.05$) in GraphPad Prism 9.

30. Results

30.1. *Hyalomma* and *Rhipicephalus* originating CCHFV isolates are phylogenetically isolated

The phylogenetic trees of S and L segments showed the genomic sequences were clearly assembled into clusters in relation to their tick hosts (***Figure 22***). The results revealed that CCHFV strains isolated from *Rhipicephalus* were genetically distant from *Hyalomma*-isolated strains. This highlighted the evidence of strong selection pressure on host adaptation. In contrast, the phylogenetic tree of M segment did not exhibit a clear separation of *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains. Notably, CCHFV strains isolated from *R. bursa* in Greece (Europe 2) formed a distinct cluster from *Hyalomma* strains, while strains from *R. bursa* in Türkiye (Europe 1) clustered together with *Hyalomma*-isolated strains. Previous studies showed that codon usage patterns in viral genomes can be influenced by geographic origins (Chen et al., 2014; Rahman et al., 2018; Singh et al., 2016; Wang et al., 2023; Yao et al., 2020). Therefore, the clustering of M segment strains may reflect both host adaptation and geographic distribution.

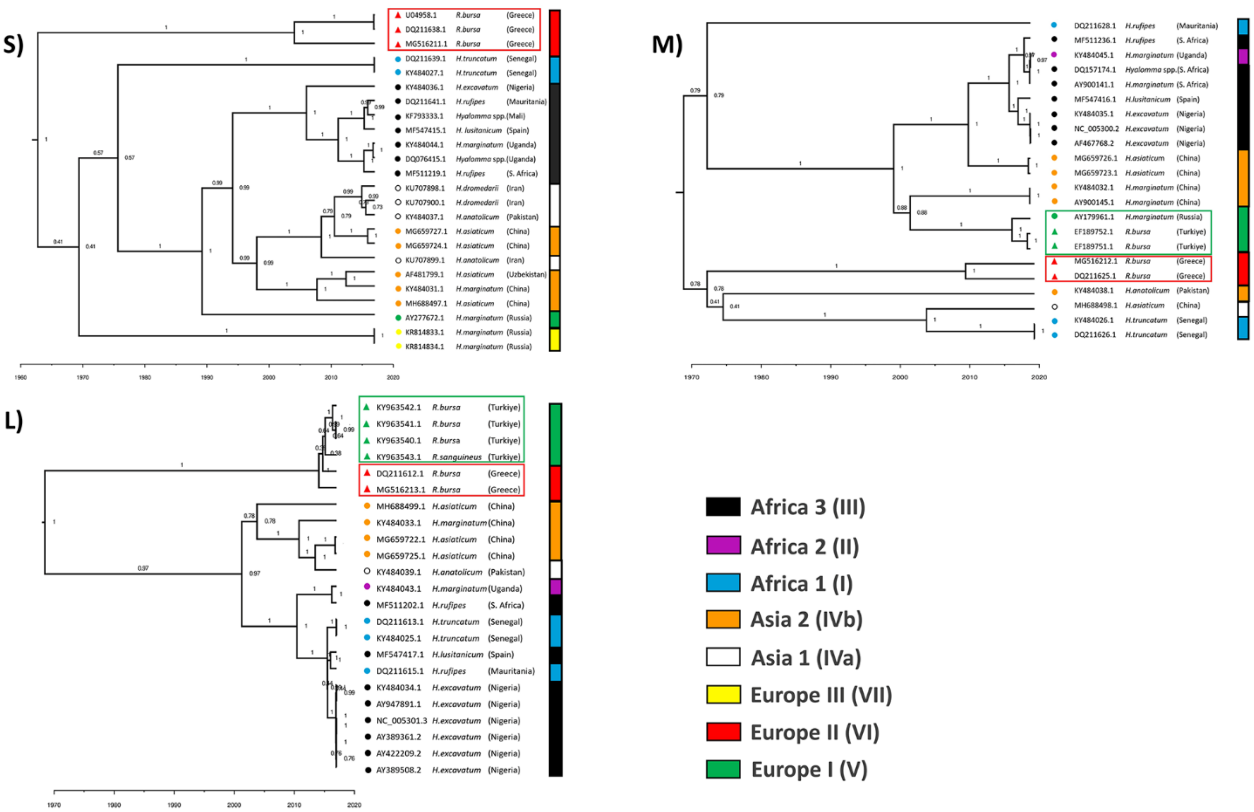

Figure 22. The maximum clade credibility tree for 70 CCHFV strains isolated from *Hyalomma* and *Rhipicephalus* species. The tree node shows the tMRCA in years. *Hyalomma*- and *Rhipicephalus*-isolated strains are distinguished in branches of different shapes (circle for *Hyalomma*-isolated strains and triangle for *Rhipicephalus*-isolated strains). Phylogenetic clades of strains are represented in the legend with different colors.

30.2. Nucleotide composition of CCHFV isolates from *Hyalomma* and *Rhipicephalus* ticks is different

Nucleotide composition strongly affects codon usage patterns. Important are especially nucleotides at the third position of codons (A3, U3, G3, C3) (dos Passos Cunha et al., 2018; Di Paola et al., 2018; Tao et al., 2009). There were statistically significant differences between *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains in the frequency of A3 and G3 in all segments (***Figure 23***; ***Supplementary Table S3***).

Further, C3 was significantly different between *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains in S and L (but not M) segments (***Figure 23; Supplementary Table S3***). No statistically significant difference was observed for U3 in any genomic segment of CCHFV strains isolated from *Hyalomma* and *Rhipicephalus*.

Moreover, GC content at all positions of codons (GC1, GC2, and GC3) and GC content at the first plus the second positions of codons (GC12) also play an important role in influencing overall codon usage preferences (Jenkins & Holmes, 2003; Nasrullah et al., 2015; Wong et al., 2010). In S and L segments, GC content significantly differed between *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains on almost all codon positions (***Figure 23; Supplementary Table S3***). In M segment, GC content significantly differs between *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains only at GC3 and GC12 positions. Nevertheless, results of these analyses showed substantial differences in frequencies of occurrence of nucleotides between *Hyalomma*- and *Rhipicephalus*-isolated CCHFV variants ($p < 0.05$).

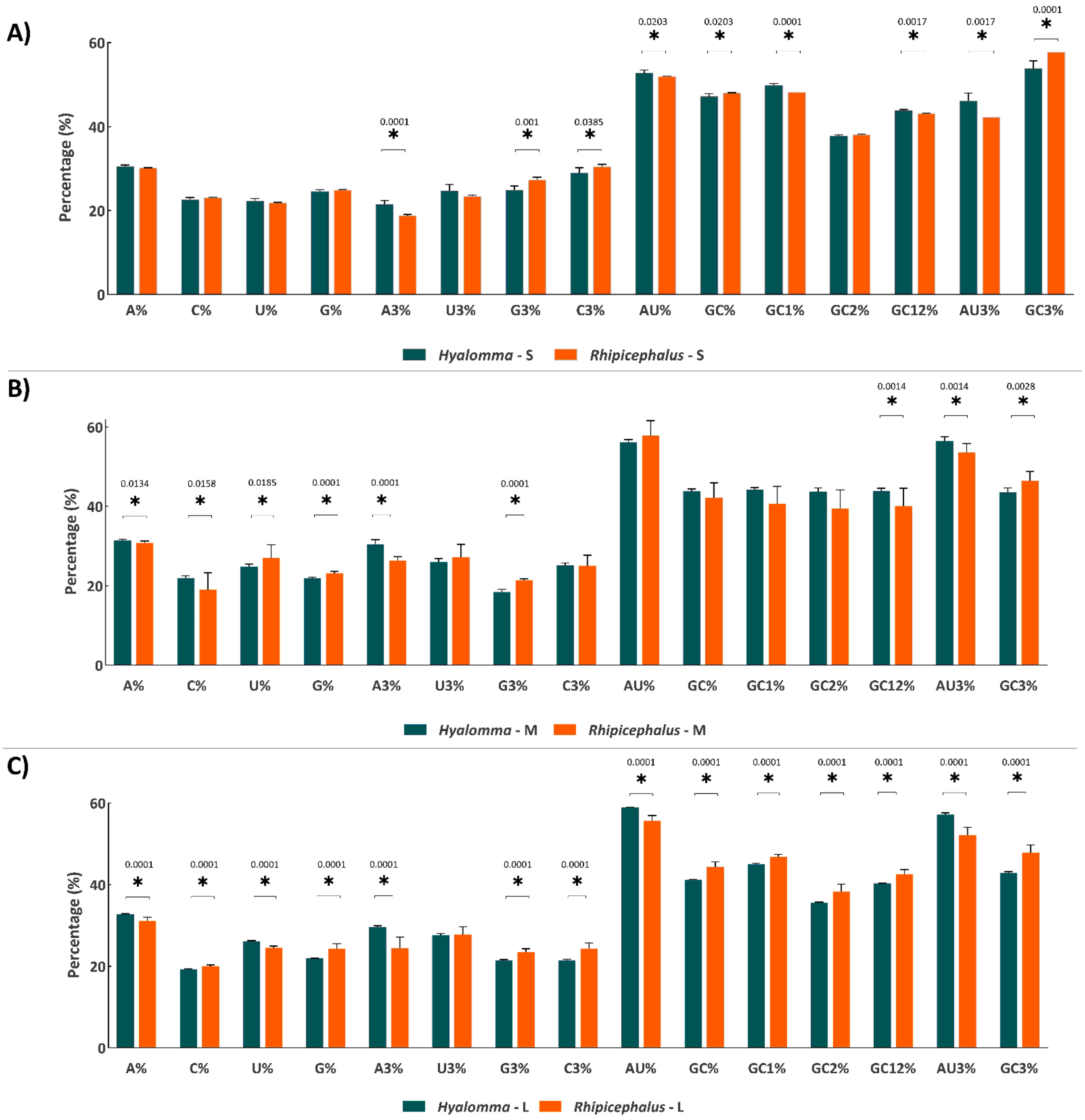

Figure 23. Nucleotide content distribution and composition in *Hyalomma*- and *Rhipicephalus*-isolated S (A), M (B), and L (C) segments, respectively. Standard deviation is marked in the plot by the error bars. Asterisk (*) shows a significant difference between variables ($p < 0.05$).

30.3. *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains preferentially use different codons

At S segments, G/C-ended codons were preferred over A/U-ended codons in CCHFV strains isolated from both *Hyalomma* and *Rhipicephalus* tick vectors. But, *Rhipicephalus*-isolated CCHFV strains had a strong preference to the A-ended codons whereas *Hyalomma*-isloated ones had a higher frequency in U-ended codons (***Figure 24***, ***Table** 7*).

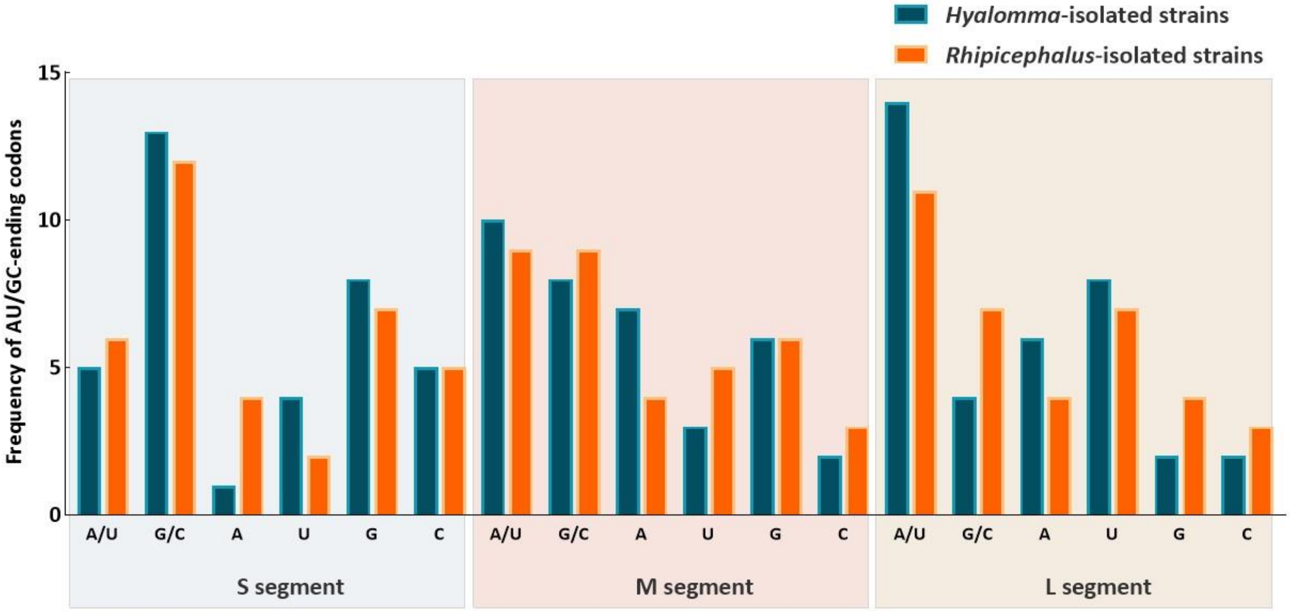

Figure 24. Preference for A/U- and G/C-ending codons, as well as A-, U-, G-, and C-ending codons separately, among *Hyalomma*- and *Rhipicephalus*- isolated S, M and L segments.

Table 7. The relative synonymous codon usage frequency (RSCU) of *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains. AA represents amino acid; grey colour represents the most optimal codons favoured by CCHFV isolated from two different hosts (the highest RSCU value for each particular amino acid); the bold represents over-represented codons (RSCU ≥ 1.6), the underline represents under-represented codons (RSCU ≤ 0.6) codons. Green color indicates the highest difference between RSCU values of *Hyalomma*- and *Rhipicephalus*-isolated CCHFV for each segment, while the red color show low difference.

AA	**CODONS**	**CCHFV - S SEGMENT**			**CCHFV - M SEGMENT**			**CCHFV - L SEGMENT**		
		Hyalomma	***Rhipicephalus***	**Difference**	***Hyalomma***	***Rhipicephalus***	**Difference**	***Hyalomma***	***Rhipicephalus***	**Difference**
Phe	UUU	0.96	0.85	0.11	1.09	1.33	0.24	1.14	1.16	0.02
	UUC	1.04	1.15	0.11	0.91	0.68	0.23	0.86	0.84	0.02
Leu	UUA	0.13	0	0.13	0.98	0.83	0.15	0.83	0.28	0.55
	UUG	0.56	0.84	0.28	1.17	1.19	0.02	1.32	**1.76**	0.44
	CUU	**2.13**	**1.80**	0.33	1.08	1.49	0.41	1.10	1.15	0.05
	CUC	1.43	1.57	0.14	0.67	0.62	0.05	0.92	1.22	0.3
	CUA	0.44	0.33	0.11	1.05	0.81	0.24	0.93	0.71	0.22
	CUG	1.30	1.46	0.16	1.06	1.08	0.02	0.91	0.89	0.02
Ile	AUU	1.25	1.20	0.05	1.13	1.41	0.28	1.20	**1.64**	0.44
	AUC	0.99	1.23	0.24	0.84	0.62	0.22	0.68	0.60	0.08
	AUA	0.76	0.57	0.19	1.03	0.98	0.05	1.12	0.76	0.36
Val	GUU	1.03	0.89	0.14	1.28	1.26	0.02	1.28	1.34	0.06
	GUC	1.23	1.18	0.05	0.96	1.18	0.22	0.86	0.80	0.06
	GUA	0.27	0.49	0.22	0.72	0.29	0.43	0.62	0.58	0.04
	GUG	1.47	1.43	0.04	1.04	1.27	0.22	1.23	1.28	0.05
Ser	UCU	1.46	1.49	0.03	0.83	0.47	0.36	1.18	0.88	0.3
	UCC	1.08	1.05	0.03	0.58	0.48	0.1	0.61	0.67	0.06
	UCA	0.97	0.72	0.25	**1.74**	1.05	0.69	1.15	0.50	0.65
	UCG	0.26	0.22	0.04	0.18	0.16	0.02	0.20	0.08	0.12
	AGU	1.02	0.72	0.3	1.21	**1.61**	0.4	1.40	**1.74**	0.34
	AGC	1.21	**1.82**	0.61	1.46	**2.23**	0.77	1.47	**2.13**	0.66
Pro	CCU	1.17	1.19	0.02	1.25	1.43	0.18	1.42	0.57	0.85
	CCC	0.49	0.77	0.28	0.83	0.41	0.42	0.76	0.90	0.14
	CCA	**1.85**	**1.68**	0.17	**1.71**	**1.93**	0.22	1.52	**2.44**	0.92
	CCG	0.49	0.35	0.14	0.22	0.24	0.02	0.29	0.09	0.2
Thr	ACU	0.98	1.16	0.18	1.0	0.66	0.34	1.21	0.86	0.35
	ACC	1.58	1.16	0.42	0.91	0.96	0.05	1.14	**1.88**	0.74
	ACA	1.31	1.30	0.01	**1.78**	**2.00**	0.22	1.44	0.98	0.46

	ACG	0.13	0.37	0.24	0.30	0.39	0.09	0.21	0.29	0.08
Ala	GCU	1.02	0.85	0.17	0.97	0.58	0.39	1.10	0.92	0.18
	GCC	1.46	1.39	0.07	1.13	**1.92**	0.79	0.62	0.46	0.16
	GCA	1.36	**1.63**	0.27	**1.78**	1.44	0.34	**2.02**	**2.19**	0.17
	GCG	0.15	0.13	0.02	0.12	0.06	0.06	0.26	0.42	0.16
Tyr	UAU	0.62	0.72	0.1	0.83	0.87	0.04	0.99	0.59	0.4
	UAC	1.38	1.28	0.1	1.17	1.13	0.04	1.01	1.41	0.4
His	CAU	0.71	0.56	0.15	0.93	0.53	0.4	1.23	**1.64**	0.41
	CAC	1.29	1.44	0.15	1.07	1.47	0.4	0.77	0.36	0.41
Glu	CAA	0.70	0.36	0.34	0.86	0.60	0.26	0.95	0.55	0.4
	CAG	1.30	**1.64**	0.34	1.14	1.40	0.26	1.05	1.45	0.4
Asn	AAU	0.64	0.70	0.06	0.97	1.12	0.15	1.01	1.11	0.1
	AAC	1.36	1.30	0.06	1.03	0.88	0.15	0.99	0.90	0.09
Lys	AAA	0.85	0.65	0.2	1.12	1.02	0.1	1.13	1.25	0.12
	AAG	1.15	1.35	0.2	0.88	0.99	0.11	0.87	0.75	0.12
Asp	GAU	0.93	0.67	0.26	0.97	1.12	0.15	1.13	1.47	0.34
	GAC	1.07	1.33	0.26	1.03	0.88	0.15	0.87	0.54	0.33
Glu	GAA	0.84	0.59	0.25	1.24	0.99	0.25	1.27	1.55	0.28
	GAG	1.16	1.41	0.25	0.76	1.01	0.25	0.73	0.45	0.28
Cys	UGU	1.25	1.33	0.08	0.98	0.84	0.14	1.07	0.93	0.14
	UGC	0.75	0.67	0.08	1.02	1.16	0.14	0.93	1.08	0.15
Arg	CGU	1.34	0.47	0.87	0.13	0.06	0.07	0.19	0.09	0.1
	CGC	0.23	0.59	0.36	0.16	0.04	0.12	0.29	0.53	0.24
	CGA	0.16	0.35	0.19	0.14	0.04	0.1	0.33	0.11	0.22
	CGG	0.37	0.12	0.25	0.14	0.06	0.08	0.21	0.03	0.18
	AGA	**1.71**	**1.76**	0.05	**3.49**	**3.89**	0.4	**2.66**	**2.04**	0.62
	AGG	**2.20**	**2.70**	0.5	**1.93**	**1.90**	0.03	**2.32**	**3.21**	0.89
Gly	GGU	0.77	1.14	0.37	1.00	0.73	0.27	1.26	1.38	0.12
	GGC	1.26	1.03	0.23	1.23	1.55	0.32	0.91	1.16	0.25
	GGA	1.20	1.22	0.02	1.06	1.10	0.04	1.14	0.80	0.34
	GGG	0.77	0.61	0.16	0.72	0.62	0.1	0.69	0.68	0.01

At M segments, A/U-ended codons were highly preferred in CCHFV strains isolated from both *Hyalomma* and *Rhipicephalus* tick vectors. M segments of *Hyalomma*-isolated CCHFV strains showed a higher preference to the A-ended codons, while M segments of CCHFV strains isolated from *Rhipicephalus* had a preference to highly use U-ended codons. Regarding the G/C-ended codons, M segments of CCHFV strains isolated from both tick vectors used different codons, but both preferred to use rather C-ended than G-ended codons (***Figure 43***, ***Table 7***).

At L segments, CCHFV strains isolated from both tick vectors also had a high preference for A/U-ended codons. *Hyalomma*-isolated strains prefer A-ending codons, while *Rhipicephalus*-isolated strains prefer U-ending codons. However, L segments of *Rhipicephalus*-isolated CCHFV strains had higher frequencies in C-ended codons over G-ended codons (***Figure 24**, **Table** 7*).

Codon over- and underrepresentation analysis emphasized that RSCU values of the majority codons ranged from 0.6 to 1.6. Interestingly, we found that the nucleotide frequencies at the end of most over-represented codons (RSCU > 1.6) differ between *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains (***Figure 25***). Pro, Ala, and Arg are over-represented in all *Hyalomma*- and *Rhipicephalus* isolated CCHFV genomic segments. However, *Hyalomma*-isolated strains have a strong preference on GCA for Ala, while *Rhipicephalus* ones prefer to use GCC. Moreover, *Hyalomma*- and *Rhipicephalus* isolated L segments differ on codon usage preferences for Arg, where AGA is over-represented in *Hyalomma*-isolated strains, and *Rhipicephalus* ones show a higher tendency to use AGG codon (***Figure 25***).

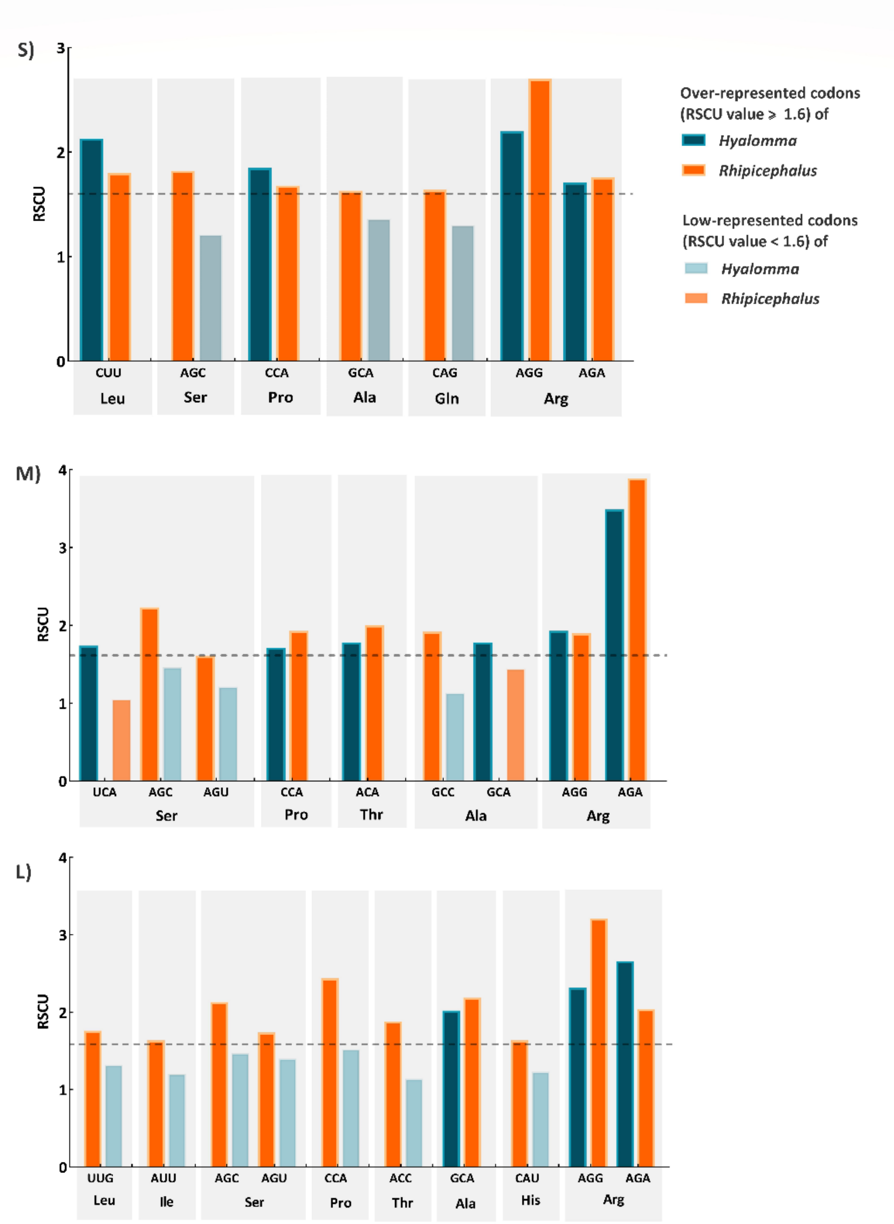

Figure 25. Over-represented (RSCU ≥ 1.6) and low-represented codons (RSCU < 1.6) between *Hyalomma*- and *Rhipicephalus*- isolated CCHFV strains for S, M and L segments.

Analysis of ENC values alone showed significant differences between the *Hyalomma*- and *Rhipicephalus*- isolated CCHFV strains for M and L but not for S segments ($p < 0.05$) (***Figure 26A***). While the S segment exhibited relatively similar ENC values for both *Hyalomma* (53.33 ± 1.33) and *Rhipicephalus* (52.85 ± 0.41), the M segment had higher ENC values for *Hyalomma*-isolated strains (50.89 ± 0.45) compared to *Rhipicephalus*-isolated strains (49.50 ± 1.91), suggesting a difference in codon usage bias among CCHFV isolates from two tick hosts. Further, the L segment showed the greatest difference in ENC values, with *Hyalomma*-isolated strains (51.92 ± 0.32) having markedly higher values than *Rhipicephalus*-isolated strains (47.64 ± 2.74), indicating a significant variation in codon usage bias of the L segment of CCHFV strains isolated from both hosts. When ENC was plotted as a function of GC3s (GC content at the third synonymous codon position), we could see a weak but significant codon usage bias for both *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains (***Figure 26B***). Further, it was apparent that both *Hyalomma*- and *Rhipicephalus*- isolated CCHFV strains form separated clusters. The results of COA revealed that CCHFV strains isolated from *Hyalomma* and *Rhipicephalus* ticks are separated into distinct clusters based on codons used by these strains (***Figure 26C***). This separation cannot be explained based on affiliation to the CCHFV phylogenetic clades. It clearly shows that *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains have considerable variation in codon usage patterns.

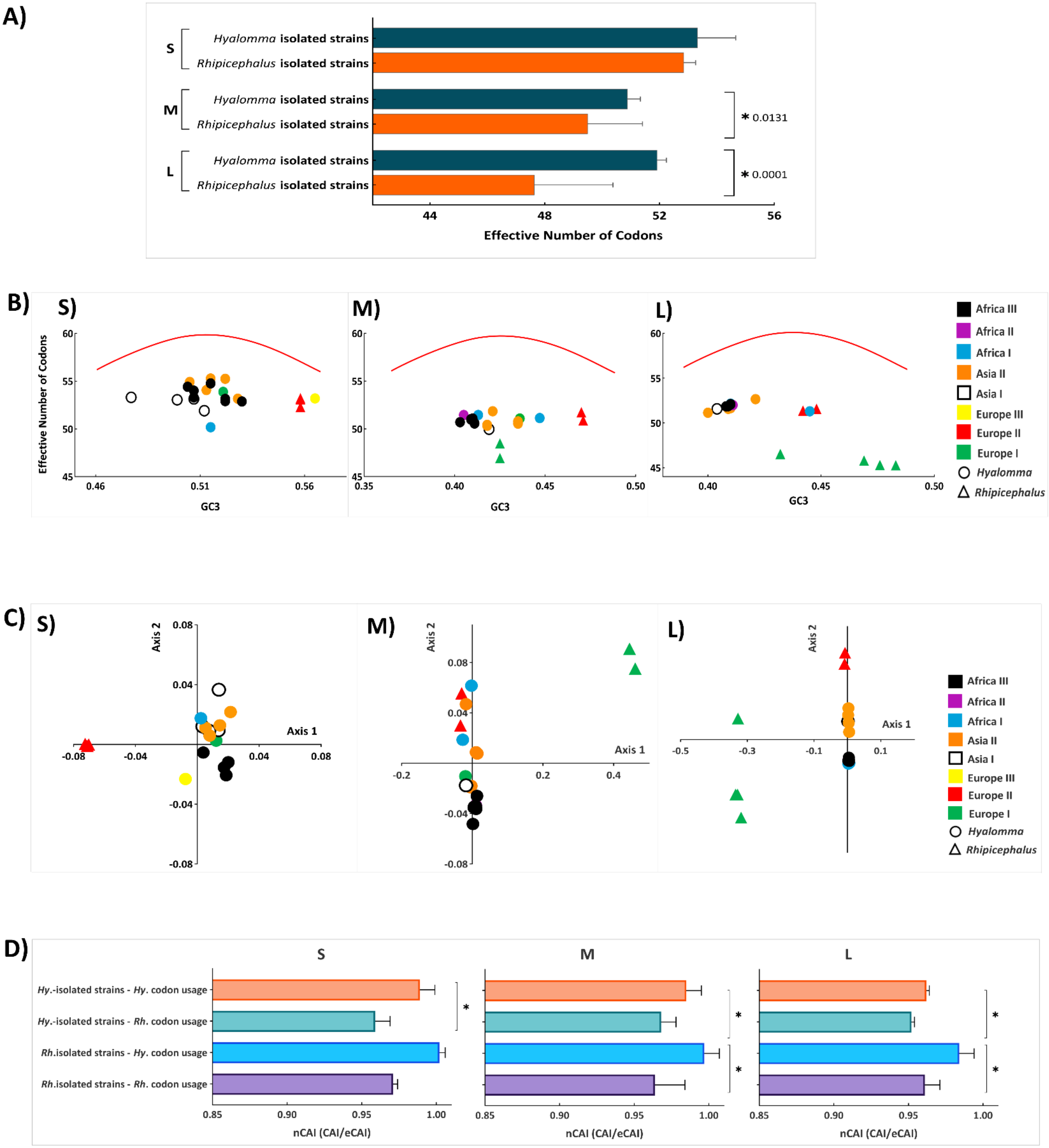
A)
S
Hyalomma isolated strains
Rhipicephalus isolated strains
M
Hyalomma isolated strains
Rhipicephalus isolated strains
L
Hyalomma isolated strains
Rhipicephalus isolated strains
* 0.0131
* 0.0001
44
48
52
56
Effective Number of Codons
B)
S)
M)
L)
Effective Number of Codons
60
55
50
45
0.46
0.51
0.56
GC3
0.35
0.40
0.45
0.50
GC3
0.40
0.45
0.50
GC3
Africa III
Africa II
Africa I
Asia II
Asia I
Europe III
Europe II
Europe I
Hyalomma
Rhipicephalus
C)
S)
Axis 2
Axis 1
0.08
0.04
-0.04
-0.08
-0.08
-0.04
0.04
0.08
M)
Axis 2
Axis 1
0.08
0.04
-0.04
-0.08
-0.2
0.2
0.4
L)
Axis 2
Axis 1
-0.5
-0.3
-0.1
0.1
Africa III
Africa II
Africa I
Asia II
Asia I
Europe III
Europe II
Europe I
Hyalomma
Rhipicephalus
D)
S
M
L
Hy.-isolated strains - Hy. codon usage
Hy.-isolated strains - Rh. codon usage
Rh.isolated strains - Hy. codon usage
Rh.isolated strains - Rh. codon usage
*
0.85
0.90
0.95
1.00
nCAI (CAI/eCAI)

Figure 26. Effective number of codons (ENC), correspondence analysis (COA) and codon adaptation index (CAI) analyses of *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains. A) Comparison of the values for ENC between *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains for S, M, and L segments. Standard deviation is marked in the plot by the error bars. **B)** ENC-GC3 plots of *Hyalomma*- and *Rhipicephalus*-isolated CCHFV genomes. ENC values (Y-axis) was plotted against the GC content at the third synonymous codon positions (GC3s values, X-axis). The curve (red line) indicates the expected codon usage if GC compositional constraints alone account for the codon usage bias. *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains are marked by different symbols (circle for *Hyalomma*-isolated strains and triangle for *Rhipicephalus*-isolated strains). Different colors mark for different CCHFV phylogenetic clades. **C)** COA values are based on the RSCU values of *Hyalomma*- and *Rhipicephalus*-isolated S, M, and L segments, respectively. **D)** nCAI values of *Hyalomma*-isolated strains to *Hyalomma* and *Rhipicephalus* codon usage, and *Rhipicephalus*-isolated strains to *Hyalomma* and *Rhipicephalus* codon usage in relation to S, M, and L segments, respectively.

30.4. Both *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains show higher codon adaptation index values for *Hyalomma* tick species

All genomic segments of both *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains show significantly higher codon adaptation index values for *Hyalomma* than for *Rhipicephalus* tick species ($p < 0.05$). Interesting is the fact that M and L segments of *Rhipicephalus*-isolated CCHFV strains show significantly higher CAI values to *Hyalomma* ticks than the M and L segments of *Hyalomma*-isolated CCHFV strains ($p = 0.0001$) (***Figure 26D***).

30.5. The pattern of selection in *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains varies

We utilized the FUBAR method, a site-specific pervasive selection approach, to assess the selective pressure on 70 CCHFV sequences isolated from both *Hyalomma* and *Rhipicephalus* ticks. The estimated dN/dS ratios were as follows: 0.04 and 0.02 for S segment, 0.05 and 0.17 for M segment, and 0.04 and 0.07 for L segment isolated from *Hyalomma* and *Rhipicephalus*, respectively. The results further revealed that the *Hyalomma*- and *Rhipicephalus*-isolated S segments had 380 and 103 sites identified under negative/purifying selection, respectively. For the *Hyalomma*- and *Rhipicephalus*-isolated M segments, 1696 and 397 sites were identified under negative/purifying selection, respectively. However, no sites were identified under positive/diversifying selection in both segments isolated from two ticks. Lastly, on the *Hyalomma*- and *Rhipicephalus*-isolated L segments, 2858 and 334 sites were identified under negative/purifying selection, respectively. Additionally, 1 site was identified under positive/diversifying selection for *Rhipicephalus*-isolated L segments.

Overall, these findings suggest that there are variations in the selective pressures acting on CCHFV isolates of different tick hosts, which may be influenced by the segment-specific codon usage biases and the tick species from which they were isolated.

31. Discussion

The differences in genome composition and codon usage patterns between *Hyalomma*- and *Rhipicephalus*-isolated CCHFV variants can influence viral fitness, evolution, and the ability to replicate within different tick species as well as mammalian hosts.

Phylogenies of S and L genomic segments of *Hyalomma*- and *Rhipicephalus*-isolated CCHFV variants revealed a clear phylogenetic separation of *Rhipicephalus*-isolated CCHFV strains from *Hyalomma* ones. These findings diverge from previous phylogenetic analyses, which primarily emphasized the influence of geographical spots on codon usage patterns (Rahman et al., 2018). Instead, our study highlights evidence of strong selection pressure on host adaptation, which is in agreement with CAI analysis. Our findings suggest that, beyond geographical factors, vector host species may significantly impact the codon usage patterns of the virus. Interestingly, the results of the phylogenetic analysis showed a phylogenetic separation among Europe 2 and Europe 1 clades in terms of the ticks in which the strains are vectored. Europe 1 circulates in various geographic regions, including southern Russia, Türkiye, the Balkan Peninsula, and Iran. The strains belonging to Europe 1 (clade V) are known to be highly pathogenic in humans, and *Hyalomma* species were observed to harbor particularly this clade. Likewise, the infection caused by strains belonging to Europe 2 (clade VI) has mild or non-pathogenic effects on humans, and this clade is exclusively found in ticks of the *Rhipicephalus* genus, in particular *R. bursa* (Papa et al., 2017; Volynkina et al., 2022).

In contrast to the clear separation observed in the phylogenetic trees of the S and L segments based on hosts, the M segment tree did not reveal a distinct clustering of *Hyalomma*- and

Rhipicephalus-isolated CCHFV strains. Previous studies showed that geographic origins might influence the codon usage patterns of the viral genome (Chen et al., 2014; Singh et al., 2016; Wang et al., 2016, 2023), and our findings suggest that a combination of both host adaptation and geographical origin may have contributed to the observed patterns in codon usage bias of *Hyalomma*- and *Rhipicephalus*-isolated CCHFV M segments. Additionally, potential variations in two different ticks hosting the same genetic structure of the virus should be explored, utilizing artificial feeding experiments to facilitate such investigations, thereby offering valuable suggestions for further studies in this field.

Previously, it has been shown that codon usage bias, or the preference for one type of codon over another, can be greatly influenced by the overall nucleotide composition in the genome (Jenkins & Holmes, 2003; Nasrullah et al., 2015). The nucleotide composition analysis revealed substantial differences in the frequencies of occurrence of nucleotides between *Hyalomma*- and *Rhipicephalus*-isolated CCHFV variants. Despite the differences, it is apparent that the M and L segments of *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains are AU-rich, and A/U-ended codons appear to be preferred, indicating that the usage of optional codons might be influenced by compositional constraints resulting in the presence of mutational pressure. This is consistent with a previous report indicating a substantial portion of mammalian-host isolated CCHFV strains are enriched with AU (Rahman et al., 2018). Further, this result is similar to other RNA viruses such as West Nile virus (Moratorio et al., 2013), dengue virus (Lara-Ramírez et al., 2014), Marburg virus (Nasrullah et al., 2015), Ebola virus (Luo et al., 2020), and bluetongue virus (Yao et al., 2020), where A/U-ended codons appear to be preferred. However, S segments isolated from both tick species are more GC-rich and preferentially use G/C-ended codons. The biological importance of this condition is uncertain; therefore, it is important to investigate the factors influencing different nucleotide frequencies of CCHFV segments (van Hemert et al., 2016).

Previous studies on codon usage bias have suggested that the composition of amino acids is a key factor in determining the nucleotide contents at the first and second codon positions of viral genomes, while the variation in proteins is influenced by functional selection. However, at the third codon position of a viral gene, a large proportion of the possible alterations (69%) result in synonymous or silent mutations that are not constrained by the functional selection of amino acids (van Hemert et al., 2016). Based on RSCU values, we explored the different codon usage preferences of *Hyalomma*- and *Rhipicephalus*-isolated CCHFV sequences. In a previous study, it was observed that *Hyalomma*-isolated CCHFV strains exhibited a preference for C-ended codons (Rahman et al., 2018). However, our study revealed that only CCHFV S segments isolated from *Hyalomma* and *Rhipicephalus* ticks demonstrated a preference for C-ended codons, while M and L segments showed contrary preferences toward A- and U-ended codons. Remarkably, *Hyalomma*-isolated M segments have a higher tendency to use A-ended codons, while strains isolated from *Rhipicephalus* have a strong preference for U-ended codons.

For the L segment, although the two hosts have similar preferences for A-ended codons over U, they exhibit different codon preferences for the same amino acids, such as Arg, Cys, and Thr. Furthermore, a previous study noted that CCHFV strains isolated from *Hyalomma* ticks exhibited a preference for CGC codons for Arg, while AGA and AGG were less favored (Rahman et al., 2018). Conversely, our analysis revealed an over-representation of AGA and AGG codons across S, M, and L segments of CCHFV isolates from *Hyalomma* and *Rhipicephalus* ticks. Regarding the codon preference for Ala, the previous study reported a strong preference for GCC in *Hyalomma*-isolated strains (Rahman et al., 2018). However, our findings showed that GCA was the preferred codon for Ala in *Hyalomma*-isolated S, M, and L segments. Additionally, our study revealed a strong preference for CCA codons for Pro in both *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains. This contrasts with the findings of the previous study (Rahman et al., 2018), which indicated a preference for CCC codons in *Hyalomma*-isolated CCHFV strains.

All genomic segments of *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains have remarkably different codon usage patterns. The codon usage bias of CCHFV isolated from the two tick genera was found to be low, with ENC values higher than 35. Similar low codon usage bias has also been reported among several other RNA viruses, for instance, Zika virus (ENC: 53.32; Wang et al., 2016), Ebola virus (ENC: 57.23; Cristina et al., 2015), chikungunya virus (ENC: 55.56; Butt et al., 2014), classical swine fever virus (ENC: 51.7; Tao et al., 2009), foot-and-mouth virus (ENC: 51.53; Zhong et al., 2007), hepatitis C virus (ENC: 52.62; Hu et al., 2011), Venezuelan equine encephalitis virus (ENC: 56.51; Wang et al., 2023), and West Nile virus (ENC: 53.81; Moratorio et al., 2013). It has been indicated that the low codon usage bias of the virus is beneficial for efficient replication in its host cells and reduces competition between the virus and its hosts for protein synthesis. Similarly, all segments of tick-isolated CCHFV strains show rather high ENC values, which indicate low codon usage bias. This suggests that the evolution of low codon bias within CCHFV coding sequences has allowed it to successfully maintain its survival cycle within its tick vectors as well as mammalian hosts.

The influence of a tick vector on CCHFV codon usage patterns was also visible from CAI analysis. Comparative analysis of CAI values revealed that both *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains display higher adaptation to use the codons that are preferred by *Hyalomma* tick species. These results suggest that, over the course of its long coevolution with tick vectors, CCHFV has optimized its codon usage patterns to utilize the translational resources of *Hyalomma* species more efficiently than those of *Rhipicephalus* ticks, which are vectors used only by specific CCHFV strains. The higher genetic adaptation of CCHFV strains isolated from the tick species of two genera, favoring the use of codons preferred by *Hyalomma* ticks, can be attributed to the role of *Hyalomma* species as the primary vectors of CCHFV. Contrarily, the lower adaptation of *Hyalomma*- and *Rhipicephalus*-isolated CCHFV segments to *Rhipicephalus* codon usage highlights that *Rhipicephalus* ticks are rather occasional vectors or evolutionary new vectors used by CCHFV in areas where *Hyalomma* ticks are either absent or at least rare.

The selection analysis of *Hyalomma*- and *Rhipicephalus*-isolated S, M, and L segments suggests that CCHFV isolates from these hosts are subject to strong purifying (negative) selection, as has been previously observed for other RNA viruses such as Zika virus, West Nile virus, dengue virus, yellow fever virus, and tick-borne encephalitis virus, which exhibit low dN/dS ratios ranging from 0.019 to 0.066 (Lin et al., 2019).

Overall, the results of this study show the strong differences in codon usage patterns between the CCHFV strains isolated from different tick species, which may mirror the differences in evolutionary processes shaping these virus strains. However, our study has identified a few limitations that should be considered when interpreting our findings. Firstly, the sample size of 70 sequences (24 for the S segment, 23 for the M segment, and 23 for the L segment) of CCHFV isolates from different representatives of the *Hyalomma* and *Rhipicephalus* genera may not be sufficient to provide robust conclusions. Additionally, there are limitations in the number of full CCHFV genome sequences isolated from *Rhipicephalus* and the lack of CCHFV sequences from *Dermacentor* species, despite experimental evidence suggesting their potential involvement in virus transmission and the frequent detection of viral RNA in these species (Orkun et al., 2017; Papa et al., 2017; Sherifi et al., 2018, 2014). Thus, generating full genome sequences of CCHFV strains isolated from *Dermacentor* ticks could provide valuable insights into the virus's adaptation to additional tick species. These limitations highlight the importance of generating full genome sequences of CCHFV strains from their tick hosts to achieve a more thorough understanding of virus-vector coevolution.

Despite a few limitations, this research not only provided knowledge about the variation in CCHFV codon usage patterns in relation to their two vectors but also contributed to analyzing the factors that influence the adaptation of the virus to its vector species. *In silico* studies are highly important in the case of CCHFV, as it is regarded as a BSL-4 pathogen, and therefore, studies on the virus are very limited.

32. Conclusion

Our study suggests that analysis of codon usage bias of *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains can provide an alternative strategy to understand the evolution and genetic background of adaptation of CCHFV to its vector species. Our findings indicate that CCHFV strains isolated from *Hyalomma* and *Rhipicephalus* have significant differences in codon usage variations and patterns. Furthermore, our study highlighted that *Hyalomma*- and *Rhipicephalus*-isolated CCHFV strains have a higher tendency to use the codons that are preferred by species of *Hyalomma* genus. The results of this study indicate the strong effect of evolutionary processes on codon usage patterns and highlight the evidence of strong selection pressure on host adaptation while codon usage bias patterns in *Hyalomma*- and *Rhipicephalus*-isolated CCHFV M segments may result from a combination of host adaptation and geographical origin. The data analyzed in this study contribute to our understanding of the virus's evolution and genetic adaptation to its vector species.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CHAPTER 6:

This chapter is a literal copy of the published article.

Crimean-Congo Haemorrhagic Fever Virus in Ticks, Domestic, and Wild Animals

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Review Article

Crimean-Congo Haemorrhagic Fever Virus in Ticks, Domestic, and Wild Animals

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Abstract

Crimean-Congo Haemorrhagic Fever Virus (CCHFV) poses a significant public health threat due to its potential for causing severe disease in humans and its wide geographic distribution. The virus, primarily transmitted by *Hyalomma* ticks, is prevalent across Africa, Asia, Europe, and the Middle East. Understanding the virus's spread among tick populations is crucial for assessing its transmission dynamics. Vertebrates play a key role in CCHF epidemiology by supporting tick populations and acting as virus carriers during viremia. Livestock, such as cattle, sheep, and goats, amplify the virus and increase tick numbers, posing zoonotic risks. Wildlife, while asymptomatic, can serve as reservoirs. Birds generally do not show signs of the virus but can introduce infected ticks to new regions. This review compiles information on CCHFV's tick vectors and vertebrate hosts, emphasizing their roles in the virus's transmission dynamics. Understanding these dynamics is essential for developing effective control and prevention strategies.

Keywords: Crimean-Congo Haemorrhagic Fever Virus, ticks, livestock, wildlife, zoonotic disease.

34. Introduction

Crimean-Congo Haemorrhagic Fever Virus (CCHFV) is a lipid-enveloped, single-stranded RNA virus in the *Orthonairovirus* genus (*Nairoviridae* family). It causes Crimean-Congo Haemorrhagic Fever (CCHF) in humans, a severe disease with significant public health implications due to its widespread prevalence. CCHF is among the most widely distributed tick-borne viral diseases, endemic across Africa, Asia, Eastern and Southern Europe, and the Middle East, with case fatality rates ranging from 5 to 40% (Bente et al., 2013; Mertens et al., 2013; Shayan et al., 2015).

Hyalomma ticks, particularly *Hyalomma marginatum*, are the primary vectors for CCHFV. They feed on various domestic ruminants (e.g., sheep, goats, and cattle) and wild animals (e.g., hares, hedgehogs, certain rodents, and ostriches) (Hoogstraal, 1979). Ticks play a crucial role in spreading the virus to humans through bites or direct contact with infected animal tissues. Infected vertebrates, although asymptomatic, sustain tick populations and facilitate CCHFV transmission during viremia (Spengler et al., 2016).

Small mammals, such as hares and hedgehogs, amplify immature ticks, while larger domestic animals, including cattle, goats, and sheep, host adult ticks (***Figure 27***). Although CCHFV has a short viremia in small mammals, their role in CCHFV ecology is significant, as population surges, especially among hares, are linked to disease outbreaks (Hoch et al., 2016; Ergönül, 2006). Large domestic mammals inadvertently expose humans to CCHFV, especially during slaughter (Shahhosseini et al., 2018; Sargianou et al., 2013; Sharifi-Mood et al., 2014; Chinikar et al., 2010). Birds, except for ostriches, generally do not show viremia but may carry infected ticks to new regions (Ergönül, 2006).

Serological evidence confirms CCHFV exposure in various domestic and wild animals, with experimental infections validating their susceptibility (Spengler et al., 2016). Understanding the virus's persistence within tick populations, their role as vectors, and the factors influencing viral transmission is essential for effective control strategies. Examining CCHFV in livestock, which often serve as amplifying hosts, provides insights into the virus's impact on animal health and potential spillover to humans. Additionally, studying CCHFV within wildlife populations is essential for understanding its broader epidemiology.

This review compiles information on CCHFV tick vectors and vertebrate hosts, focusing on their roles in virus transmission and providing a comprehensive resource for understanding CCHFV in animals.

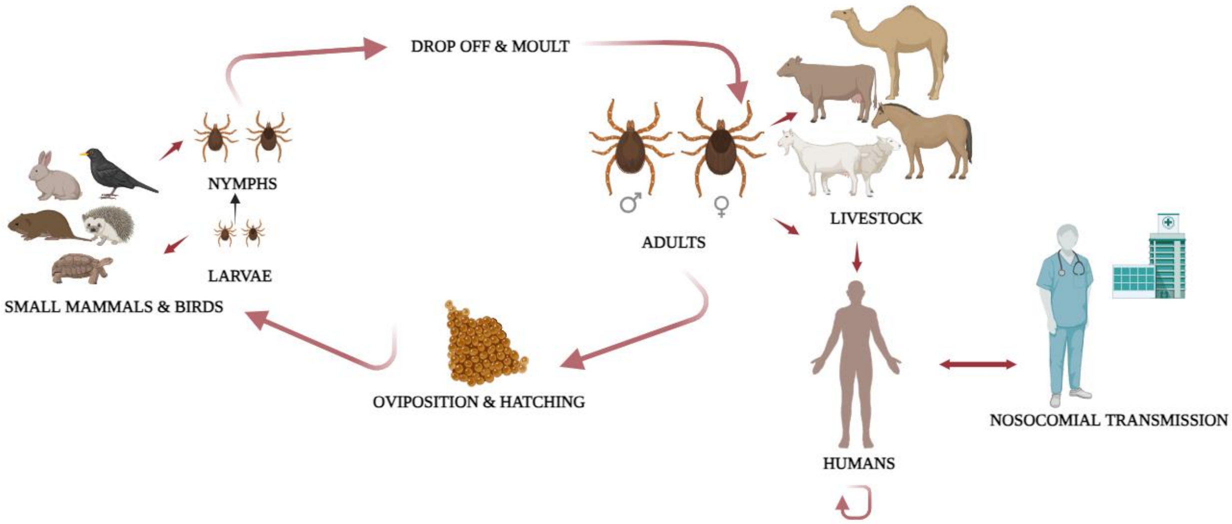

Figure 27. Life cycle of *Hyalomma marginatum* and transmission route of Crimean-Congo Haemorrhagic Fever Virus (CCHFV). *Hyalomma marginatum* is a two-host tick. Upon hatching, larvae seek small animal hosts, such as birds, lagomorphs or rodents, for their first blood meal. After engorgement, the larvae molt into nymphs while remaining on the same host. The nymphs then continue feeding on the same animal until they engorge and drop off to molt into adults. Adult ticks seek larger vertebrate hosts, such as livestock, for feeding and mating. Engorged females then detach to oviposit in the environment. CCHFV transmission occurs between ticks and vertebrate hosts and through co-feeding between ticks. Humans can become infected through tick bites, contact with infected animal fluids, or nosocomial transmission. Secondary human-to-human transmission occurs through direct exposure to the blood, bodily fluids, organs, or secretions of infected individuals. The original figure was created with BioRender (https://Biorender.com/).

35. CCHFV in Animals

35.1 CCHFV in Ticks

The first documented outbreak of CCHF was reported in the Crimean region of the former Soviet Union in 1944, where 200 military personnel suffered from an acute febrile illness with haemorrhagic symptoms, resulting in a 10% fatality rate (Bente et al., 2013). Investigating the situation, a team led by Mikhail Chumakov found that tick exposure caused these cases. Collecting over 3,000 blood-sucking arthropods, they observed an abundance of ticks, particularly the *H. marginatum* species, now recognized as the primary CCHFV vector (Ergönül, 2006). These infections were linked to abandoned cultivated lands during the German occupation, enabling tick host expansion. Subsequently, the virus was independently recognized as the cause of illness in the Congo in 1969, leading to the name Crimean-Congo Haemorrhagic Fever Virus (Ergönül, 2006). Since then, comprehensive studies have consistently reaffirmed ticks as the primary transmission source and reservoir for CCHFV in nature.

CCHFV infection persists throughout the tick life cycle without detrimental effects, allowing the virus to survive transstadially and vertically. Although the frequency of transstadial transmission, the percentage of infected eggs, and the number of generations that can sustain the virus are not well understood, ticks can survive for extended periods without feeding, which supports the overwintering of CCHFV, allowing them to act as reservoirs even when vertebrate hosts are absent (Gargili et al., 2017).

Ticks of the *Ixodidae* family, especially those of the genus *Hyalomma*, are considered both as reservoirs and vectors for CCHFV. *Hyalomma marginatum* has the most prominent role globally in the natural history of CCHF in the Old World. Dramatic increases in the circulation of CCHFV coincide with significant expansions in *H. marginatum* populations, driven by favorable weather conditions and human-induced ecological alterations (Gray et al., 2009; Celina et al., 2023).

Although the virus is transmitted mainly by tick species in the *Hyalomma* genus, CCHFV has been isolated from other ticks belonging to the genera *Amblyomma*, *Dermacentor*, *Haemaphysalis*, and *Rhipicephalus*. However, there is limited evidence indicating the active circulation of CCHFV among non-*Hyalomma* tick species in natural transmission cycles (Nasirian, 2022).

CCHFV has been reported in 39 tick species collected from a variety of hosts (Gargili et al., 2017; Nasirian, 2022). These include one species from *Amblyomma*, two species from *Dermacentor*, 15 species from *Hyalomma*, five species from *Haemaphysalis*, one species from *Ixodes*, 12 species from *Rhipicephalus*, and three species from the *Argasidae* family within the genera *Argas* and *Ornithodoros* (***Table 8; Figure 28***). This wide range of tick species highlights the potential role of numerous ticks in both spreading and maintaining the virus across various regions and host ecosystems.

Detection of CCHFV in ticks predominantly relies on reverse transcriptase-polymerase chain reaction (RT-PCR) due to its high specificity and sensitivity in amplifying viral RNA. Additionally, a few studies employed immunological methods, including enzyme-linked immunosorbent assay (ELISA) (e.g., studies by Gülce-İz et al., 2021; Hartlaub et al., 2020), immunofluorescence assay (IFA) (Hartlaub et al., 2021), and a combination of indirect hemagglutination fluorescence assay (IHFA) with RT-PCR (Suda et al., 2018).

Hyalomma marginatum is recognized as the primary vector in the Old World. Among the tick species found infected with CCHFV, 15 are confirmed vectors, while 16 are considered potential vectors (Nasirian, 2022). In addition to *H. marginatum*, confirmed vectors of CCHFV include *Amblyomma variegatum*, *H. aegyptium*, *H. anatolicum*, *H. asiaticum*, *H. asiaticum kozlovi*, *H. detritum*, *H. dromedarii*, *H. excavatum*, *H. impeltatum*, *H. rufipes*, *H. schulzei*, *H. truncatum*, *H. turanicum*, and *R. bursa*. This classification is based on documented infection rates, infection records, and observations across over 30 tick species. Potential vectors include *D. marginatus*, *D. nuttalli*, *Ha. concinna*, *Ha. inermis*, *Ha. parva*, *Ha. punctata*, *Ha. sulcata*, *I. ricinus*, *R. annulatus*, *R. appendiculatus*, *R. decoloratus*, *R. evertsi evertsi*, *R. geigyi*, *R. guilhoni*, *R. sanguineus*, and *R. turanicus* (Nasirian, 2022). *Hyalomma lusitanicum* has been repeatedly found carrying CCHFV RNA in Spain and the Iberian Peninsula. Although experimental vector competence studies remain limited, its consistent field detection suggests a potential role in transmission. Several recent reviews and surveillance reports also highlight *H. lusitanicum* as a species of concern, warranting its consideration among potential vectors of CCHFV.

Detecting a virus within an arthropod does not necessarily mean it is an active vector (Gargili et al., 2017). Studies on the vector competence of ticks for CCHFV reveal that ixodid (hard) ticks, particularly those in the *Hyalomma* genus, are highly susceptible to infection and can transmit the virus through biting. Conversely, argasid (soft) ticks are generally not efficient CCHFV vectors (Gargili et al., 2017). The evolutionary dynamics of CCHFV are closely constrained by the necessity to maintain high adaptability within both arthropod and vertebrate host environments. To validate a tick species as a vector, several steps are necessary: the ticks must feed on naturally infected hosts without artificial virus exposure, the virus must be detected in the ticks after molting, and the infected ticks must then feed on naïve hosts. The virus should then be found in these hosts and subsequently in the new generation of ticks from the initially infected adults. Strict adherence to these procedures is essential for accurately determining the vectorial abilities of specific tick species. However, these experiments are particularly challenging to perform because CCHFV is a biosafety level 4 (BSL-4) pathogen, requiring high-level containment facilities for safety.

Further studies are needed to evaluate the vector competence of various tick species for CCHFV transmission and to explore factors influencing the spread of the virus. Understanding both vector competence and vectorial capacity—the extent of transmission—is essential for predicting CCHFV's spread into new areas. Surveillance of the virus in ticks is an effective tool for monitoring the virus's introduction or circulation within vulnerable populations. This surveillance helps assess human exposure risk, identify high-risk areas, and establish early warning systems for potential outbreaks. This surveillance is essential not only for accumulating information about pathogen epidemiology but also for clarifying the role of CCHFV tick vectors in public and veterinary health (Nasirian, 2022).

Table 8. List of tick species infected by Crimean-Congo Haemorrhagic Fever Virus.

Order	Family	Scientific name	Country	References
Ixodida	Ixodidae	*Amblyomma variegatum*	Côte d'Ivoire, Ghana, Guinea, Senegal	(Adjogoua et al., 2021; Akuffo et al., 2016; Badji et al., 2023; Naidenova et al., 2020; Zeller et al., 1997a)
		Dermacentor marginatus	Greece, Iran, Russia, Spain, Türkiye	(Albayrak et al., 2010b; Cuadrado-Matías et al., 2024; de Mera et al., 2017; Orkun et al., 2017; Sánchez-Seco et al., 2021; Shafei et al., 2016; Tsapko et al., 2022; Yesilbag et al., 2013a)
		Dermacentor nuttalli	China	(Moming et al., 2018)
		Haemaphysalis concinna	Türkiye	(Tekin et al., 2012)
		Haemaphysalis inermis	Iran	(Telmadarraiy et al., 2015)
		Haemaphysalis parva	Greece, Türkiye	(de Mera et al., 2017; Hekimoglu et al., 2012)
		Haemaphysalis punctata	Iran, Russia	(Telmadarraiy et al., 2015; Tsapko et al., 2022)
		Haemaphysalis sulcata	Iran	(Biglari et al., 2016a)
		Hyalomma aegyptium	Algeria, Iran, Syria, Türkiye	(Kar et al., 2020; Kautman et al., 2016; Široký et al., 2014; Telmadarraiy et al., 2010)
		Hyalomma anatolicum	Armenia, India, Iran, Kazakhstan, Pakistan, Oman, Tajikistan, Türkiye, Turkmenistan, Uzbekistan	(Albayrak et al., 2010c; Aristova et al., 1973; Biglari et al., 2016a; Champour, Chinikar, Mohammadi, Razmi, Shah-Hosseini, et al., 2016; Chumakov et al., 1974a; Fakoorziba et al., 2012; Farhadpour et al., 2016a; Kasi, Arnim, et al., 2020; Kayedi et al., 2015a; Mohammadian, Chinikar, Telmadarraiy, Vatandoost, Oshaghi, Hanafi-Bojd, Sedaghat, Noroozi, Faghihi, Jalali, et al., 2016; Mourya et al., 2012a; Onishchenko et al., 2005; Pak, 1974; Petrova et al., 2013; Saghafipour et al., 2019a; Sedaghat et al., 2017; Shahid et al., 2021; Tahmasebi et al., 2010b; Tekin et al., 2012; Telmadarraiy et al., 2015; Williams et al., 2000a)
		Hyalomma asiaticum	China, Iran, Kazakhstan, Mongolia, Turkmenistan, Uzbekistan	(Bryant-Genevier et al., 2022; Fakoorziba et al., 2015; Kong et al., 2022; Li et al., 2020; Moming et al., 2018; S. E. Smirnova et al., 1978; Sultankulova et al., 2022; Telmadarraiy et al., 2015; Voorhees et al., 2018)
		Hyalomma detritum - (syn. *Hyalomma scupense*)	China, Iran, Pakistan, Russia, Türkiye	(Albayrak et al., 2010d; Kasi, Arnim, et al., 2020; Moming et al., 2018; Tahmasebi et al., 2010a; Tekin et al., 2012; Telmadarraiy et al., 2015; Tsapko et al., 2022)

Hyalomma dromedarii	China, Egypt, Iran, Mauritania, Pakistan, Saudi Arabia, Turkmenistan, United Arab Emirates	(Bendary et al., 2022; Camp et al., 2021; Champour, Chinikar, Mohammadi, Razmi, Shah-Hosseini, et al., 2016; Chisholm et al., 2012; Kasi, Arnim, et al., 2020; Kong et al., 2022; R. A. E. H. Mohamed et al., 2017; Schulz, Barry, Stoek, Pickin, et al., 2021; Sedaghat et al., 2017; Shahid et al., 2021; S. E. Smirnova et al., 1978; Telmadarraiy et al., 2015)
Hyalomma excavatum	Egypt, Ghana, Oman, Pakistan, Türkiye	(Akuffo et al., 2016; Chisholm et al., 2012; Kasi, Arnim, et al., 2020; Orkun et al., 2017; Williams et al., 2000a)
Hyalomma impeltatum	Mauritania, Senegal, Sudan, Tunisia	(Badji et al., 2023; Bouaicha et al., 2021a; Chitimia-Dobler et al., 2019; Nabeth et al., 2004)
Hyalomma impressum	Côte d'Ivoire, Pakistan	(Adjogoua et al., 2021; Shahid et al., 2021)
Hyalomma lusitanicum	Spain	(Sánchez-Seco et al., 2021)
Hyalomma nitidum	Central African Republic	(Burt et al., 2009)
Hyalomma marginatum	Albania, Bosnia, Bulgaria, Iran, Kosovo, Mauritania, Romania, Pakistan, Russia, Spain, Türkiye, United Arab Emirates, Zambia	(Fakoorziba et al., 2015; Gargili et al., 2011; Gergova et al., 2012a; Goletic et al., 2022; Kajihara et al., 2021; Kasi, Arnim, et al., 2020; Negredo et al., 2019; Panayotova et al., 2016; Papa et al., 2017; Saluzzo et al., 1985; Sedaghat et al., 2017; Shahid et al., 2021; Sherifi et al., 2014, 2018; Tekin et al., 2012; Telmadarraiy et al., 2010, 2015; Tsapko et al., 2022)
Hyalomma rufipes	Egypt, Ghana, Iran, Italy, Kenya, Mauritania, Nigeria, Pakistan, Senegal, South Africa	(Badji et al., 2023; Mancuso et al., 2019; Nabeth et al., 2004; Nasirian, 2022; Sang, 2011; Sene et al., 2024; Shahid et al., 2021; Zeller et al., 1997b)
Hyalomma schulzei	Iran, Saudi Arabia	(Mohamed et al., 2017; Telmadarraiy et al., 2010, 2015)
Hyalomma truncatum	Cameroon, Kenya, Senegal, Zambia	(Badji et al., 2023; Kajihara et al., 2021; Sang, 2011; Simo Tchetgna et al., 2023a)
Hyalomma turanicum	Türkiye	(Tekin et al., 2012)
Ixodes ricinus	Bulgaria, Kazakhstan, Kosovo, Poland, Russia, Spain, Türkiye	(Albayrak et al., 2010b, 2010a; Cuadrado-Matías et al., 2024; Gergova et al., 2012b; Jarosław et al., 2017; Sánchez-Seco et al., 2021; Sultankulova et al., 2022; Tsapko et al., 2022)
Rhipicephalus (Boophilus) annulatus	Guinea, Russia, Spain, Türkiye	(Gargili et al., 2011; Naidenova et al., 2020; Sánchez-Seco et al., 2021; Tsapko et al., 2022)
Rhipicephalus appendiculatus	Iran, Uganda	(Atim et al., 2023; Fakoorziba et al., 2015)
Rhipicephalus bursa	Albania, Greece, Iran, Kosovo, Russia, Spain, Türkiye	(Gargili et al., 2011; Hekimoglu et al., 2012; Tekin et al., 2012; Telmadarraiy et al., 2010; Tsapko et al., 2022)
Rhipicephalus (Boophilus) decoloratus	Guinea, Kenya, Senegal, Uganda	(Badji et al., 2023; Chiuya et al., 2021; Naidenova et al., 2020; Wampande et al., 2021)
Rhipicephalus e. evertsi	Senegal	(Badji et al., 2023; Mhamadi et al., 2022)
Rhipicephalus geigyi	Côte d'Ivoire, Guinea	(Adjogoua et al., 2021; Naidenova et al., 2020)
Rhipicephalus guilhoni	Senegal	(Mhamadi et al., 2022)
Rhipicephalus microplus	Côte d'Ivoire, Madagascar, Pakistan	(Adjogoua et al., 2021; Shahid et al., 2021)

		Rhipicephalus pumilio	Armenia	(Chumakov et al., 1974b)
		Rhipicephalus rossicus	Russia	(Tsapko et al., 2022)
		Rhipicephalus sanguineus	Albania, Bulgaria, Côte d'Ivoire, Egypt, France, Greece, Iran, Ghana, Guinea, Mauritania, Pakistan, Spain, Türkiye	(Adjogoua et al., 2021; Biglari et al., 2016b; Cuadrado-Matías et al., 2024; Darwish et al., 1978; Farhadpour et al., 2016b; Jafari et al., 2022; Kayedi et al., 2015b; Kiwan et al., 2024; Mohammadian, Chinikar, Telmadarraiy, Vatandoost, Oshaghi, Hanafi-Bojd, Sedaghat, Noroozi, Faghihi, & Jalali, 2016; Mohammadian, Chinikar, Telmadarraiy, Vatandoost, Oshaghi, Hanafi-Bojd, Sedaghat, Noroozi, Faghihi, Jalali, et al., 2016; Nabeth et al., 2004; Naidenova et al., 2020; Nasirian, 2022; Panayotova et al., 2016; Saghafipour et al., 2019b; Sedaghat et al., 2017; Tahmasebi et al., 2010a; Telmadarraiy et al., 2008, 2015)
		Rhipicephalus turanicus	Egypt, Iran, Kazakhstan, Russia, Türkiye	(Albayrak et al., 2010a, 2010d; Bryant-Genevier et al., 2022; Darwish et al., 1978; Fakoorziba et al., 2015; Orkun et al., 2017; Tahmasebi et al., 2010a; Tekin et al., 2012; Tsapko et al., 2022; Yesilbag et al., 2013b)
	Argasidae	*Argas persicus*	Uzbekistan	(Chumakov et al., 1974b)
		Argas reflexus	Iran	(Tahmasebi et al., 2010a)
		Ornithodoros lahorensis	Iran	(Telmadarraiy et al., 2010)

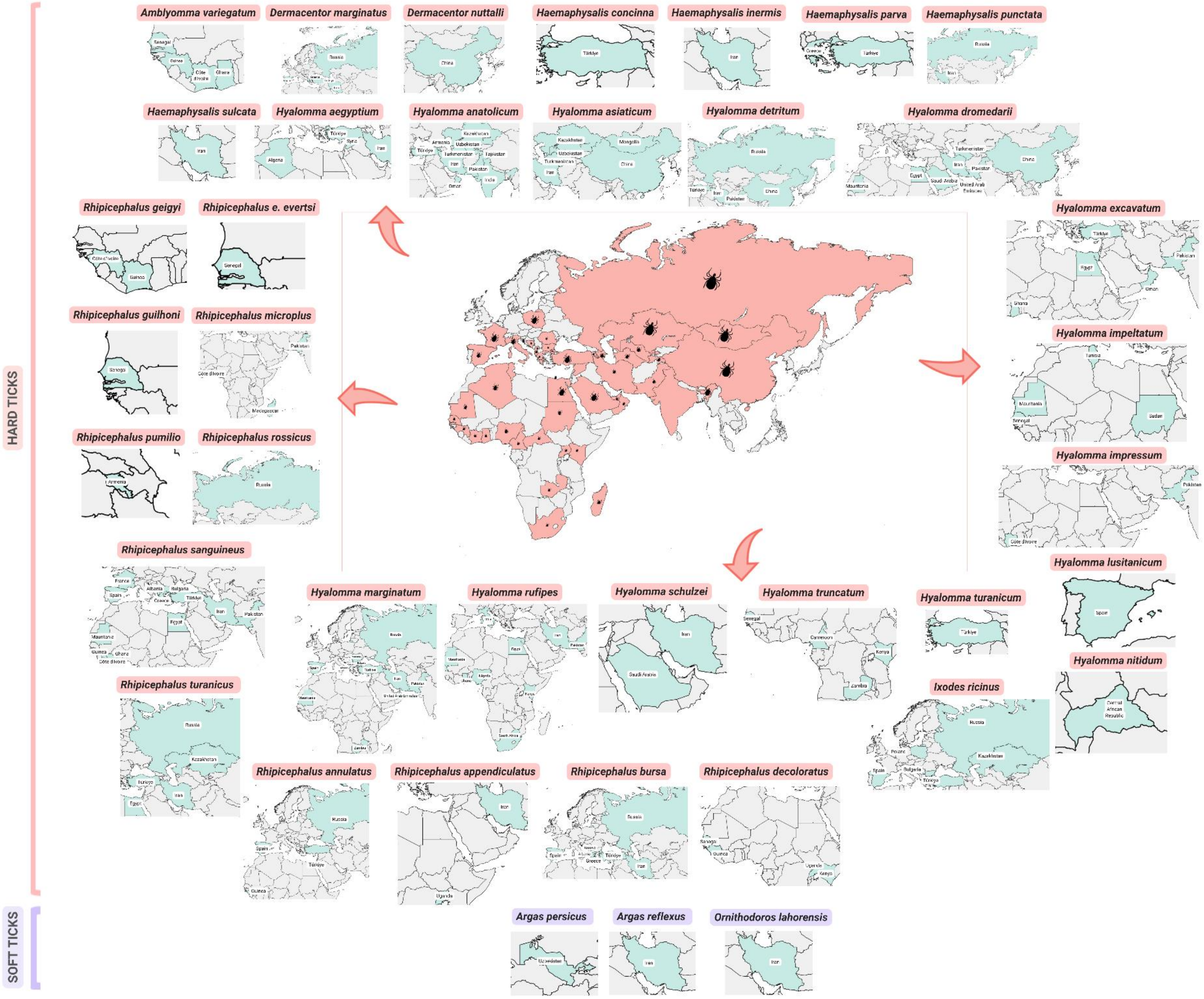

Figure 28. Geographic distribution of Crimean-Congo Haemorrhagic Fever Virus detection in ticks.

35.2. Serological Detection of CCHFV in Animals

Serological detection of CCHFV in animals provides crucial information about its ecological role. Because CCHFV often causes a short-lived viremia and can be asymptomatic, directly detecting the virus can be difficult. Thus, serological surveys are essential for monitoring the spread of CCHFV in animal populations and assessing spillover risk to humans.

Common serological methods include ELISA, IFA, and neutralization tests. These techniques help identify animals exposed to the virus, even when symptoms are absent or the infection is not active (Mazzola & Kelly-Cirino, 2019).

ELISA is the most frequently used method for detecting anti-CCHFV antibodies across various animal species. This technique typically targets the nucleocapsid protein (NP) of the virus (Gülce-İz et al., 2021). However, because the Hazara virus (HAZV) and CCHFV belong to the same serogroup, their NPs are genetically similar, leading to cross-reactivity in tests. Studies have shown that sera from animals vaccinated with HAZV can weakly cross-react with CCHFV in immunofluorescence and immunoblot assays, although commercial CCHFV ELISAs used in field studies generally do not show this cross-reactivity (Hartlaub et al., 2020). Similarly, Dugbe orthonairovirus (DUGV), while genetically and antigenically close to CCHFV, can produce false positives in certain CCHFV tests, particularly immunofluorescence assays (Hartlaub et al., 2021). Therefore, CCHFV prevalence might be overestimated in areas where HAZV and DUGV are present. ELISAs are considered to have the highest specificity, followed by micro-virus neutralization tests (mVNT), indirect immunofluorescence assays (iIFA), and plaque reduction neutralization tests (PRNT) (Hartlaub et al., 2021).

Virus neutralization assays, known for their high specificity, are rarely used for diagnosing CCHFV due to the requirement of high-containment laboratories (BSL-3/BSL-4) for handling live viruses. The level of containment depends on whether the area is endemic or non-endemic. Alternative methods, such as the pseudovirus neutralization test (pVNT), which uses pseudotyped Vesicular Stomatitis Virus, and the surrogate virus neutralization test (sVNT), can be performed in lower-containment BSL-2 laboratories, making them more accessible for diagnostic purposes (Wang et al., 2024; Suda et al., 2018).

35.3. CCHFV in Domestic Animals

CCHFV circulates silently in an enzootic tick-vertebrate-tick cycle, without manifesting disease in animals. In humans, however, it triggers severe illness. Seroepidemiological surveys have identified CCHFV antibodies in various domestic animals (Spengler et al., 2016; ***Table 9; Figure 29***). These surveys are crucial for identifying potential sources of CCHFV that might otherwise remain undetected. Since infected animals show no clinical symptoms, serological investigations are essential for assessing CCHFV exposure in animals and the associated risks for human exposure to infected ticks (Gray et al., 2009).

These surveys, especially in CCHFV-endemic regions, reveal high levels of antibodies in cattle, sheep, goats, horses, camels, and other domestic animals, indicating their significant role in the epidemiology of CCHF. These animals support tick reproduction and facilitate the movement of ticks across large areas, aiding the spread of the virus. Large mammals serve as hosts for the virus during viremia, acting as intermediaries and amplifiers between ticks. Various vertebrate hosts, particularly large ungulates, can transiently increase infection opportunities, enabling the virus to spread to other ticks feeding on these hosts. They can also contribute to CCHFV spread through co-feeding transmission, where ticks acquire the virus from infected ticks nearby, even if the host animal is not viremic (Gargili et al., 2017). The movement of livestock, which may harbor infected ticks, significantly influences the spread of the virus (Gargili et al., 2017). When livestock travel long distances, they can unknowingly transport infected ticks, as these ticks feed for an extended period. Unregulated trade movements of domestic animals could greatly elevate the risk of introducing infected ticks to new areas (Alam et al., 2013).

The prevalence of CCHFV antibodies among livestock varies based on factors like age and breed, highlighting different levels of susceptibility and exposure. Older animals typically have higher antibody levels due to repeated exposure, while younger animals, such as calves, are more likely to contract the infection while grazing, increasing their chances of encountering infected ticks (Barthel et al., 2014; Lotfollahzadeh et al., 2011; Ibrahim et al., 2015; Adam et al., 2013). Cross-bred cattle often show higher seropositivity compared to native breeds, possibly due to genetic or environmental factors (Adam et al., 2013). Longitudinal studies suggest that animals with existing antibodies and tick infestations may be at risk of reinfection (Zeller et al., 1997).

Antibodies against CCHFV can persist in naturally infected livestock for up to two months, emphasizing the need for effective surveillance and control strategies (Zeller et al., 1997).

The detection of CCHFV antibodies in domestic animals has been crucial in identifying the presence of the virus and localizing areas with higher risks of human infection. Livestock such as cattle, sheep, camels, and goats commonly become infected with CCHFV through tick bites, often experiencing asymptomatic transient viremia for 7–15 days (Spengler et al., 2016; Spengler et al., 2019). Other domestic species, including buffaloes, horses, donkeys, dogs, chickens, and ostriches, occasionally show CCHFV seropositivity, though less commonly than livestock.

Buffaloes play an important role in CCHFV epidemiology as definitive hosts for *Hyalomma* and *Rhipicephalus* ticks. In a study examining the sera of 880 buffaloes using ELISA, 145 were found to have been exposed to the virus (El-Alfy et al., 2023). Their resistance to tick bites, attributed to thicker hides and mud-wallowing habits, reduces the likelihood of tick-borne pathogen transmission (Aubry & Geale, 2011; Romero-Salas et al., 2016; Woolhouse et al., 2002). However, in densely populated regions like India, buffaloes may increase the risk of CCHFV transmission to humans (Mourya et al., 2012; Sarangi et al., 2023). In Africa, the coexistence of buffaloes and cattle within integrated wildlife-livestock ranching systems suggests a potential reservoir role for buffaloes in CCHFV transmission. A recent study in Kenya observed higher CCHFV prevalence in buffaloes compared to cattle, indicating that buffaloes could act as a reservoir, potentially transmitting the infection to cattle due to shared habitats and overlapping ranges (Obanda et al., 2021).

Horses are susceptible to CCHFV infection and can serve as hosts for infected adult ticks, thereby contributing to virus transmission. They can produce antibody levels similar to other animals, but their viremia is too low to infect new naive ticks and sustain transmission through blood feeding (Spengler et al., 2016). Seroprevalence studies have documented CCHFV prevalence in horses across various endemic regions, including Bulgaria (Hoogstraal, 1979; Vasilenko et al., 1971), India (Shanmugam et al., 1976), Iraq (Tantawi et al., 1981), Russia (Badalov et al., 1969; Berezin et al., 1969), Tajikistan (Smirnova et al., 1971), and Türkiye (Saltık, 2022). The role of horses in CCHFV transmission varies depending on environmental conditions, tick prevalence, and the density of horse populations in endemic regions. In regions invaded by *H. marginatum* ticks, such as the Czechia (Lesiczka et al., 2022) and France (Grech-Angelini et al., 2016), horses exhibit higher infestation rates compared to other ungulates, likely due to regular ectoparasite checks.

Donkeys play a crucial role in the spread of CCHFV as they frequently encounter ticks during rural activities. Along with mules, they have historically been vital in agriculture and transportation. The high seroprevalence of CCHFV in donkeys is influenced by factors such as climate, animal movement, living conditions, and cohabitation with other livestock, highlighting their role in sustaining the virus within communities. Although donkeys might not directly transmit the virus like viremic livestock, they significantly contribute to its persistence. Spengler et al. (2016) reported seroprevalence rates of 18.8% in Azerbaijan, 17.4% and 50% in Bulgaria, and 39.5% in Tajikistan. In Kenya, Omoga et al. (2023) found the highest seropositivity in donkeys at 31.4% compared to other livestock species. In Senegal, Mangombi et al. (2020) reported a seropositivity rate of 17.2% in donkeys. The highest recorded seroprevalence was in Türkiye, where Saltık (2022) reported a rate of 53.48% in donkeys.

Dogs can harbor CCHFV asymptomatically or with mild symptoms when exposed to infected ticks. Studies in Africa have shown varying seroprevalence rates among domestic dogs. Antibodies to CCHFV were found in 6% of dogs (n = 1978) in South Africa and Zimbabwe (Shepherd et al., 1987). In Senegal, Mangombi et al. (2020) found a seropositivity rate of 18.2% in dogs. In Uganda, Atim et al. (2022) reported a high seropositivity rate of 56.2% in dogs. While the role of dogs in CCHFV epidemiology is not as well understood as that of livestock, their close association with humans raises concerns about the potential introduction of infected ticks into human environments. Further research into companion animals and their interactions with vector species is essential to better understand their role in the ecology of CCHFV.

While various domestic mammals are susceptible to CCHFV infection, birds generally seem refractory. For example, Spengler et al. (2016) stated a seroprevalence of 0.2% in chickens and ducks in Kazakhstan. Interestingly, ostriches demonstrate the presence of both CCHFV antibodies and viremia, unlike most other bird species. Ostriches appear to be the only birds in which there is detectable circulation of the virus in blood comparable to mammals (Ergönül, 2006). Viremia in ostriches is short, lasting 1–4 days, while the virus persists in visceral organs such as the spleen, liver, and kidneys for up to 5 days (Swanepoel et al., 1998). Their role in transmitting the virus to humans is uncertain, but instances of notable viremia associated with CCHFV infection in humans have been noted (Swanepoel et al., 1998; Shepherd et al., 1987; Mostafavi et al., 2013; EFSA Panel on Animal Health and Welfare [AHAW], 2010).

Table 9. List of seropositive domestic animals infected by Crimean-Congo Haemorrhagic Fever Virus.

Order	Family	Common name	Scientific name	Country	References
Artiodactyla	Bovidae	Buffalo	*Bubalus* spp.	Egypt, India, Kenya, Pakistan	(Darwish et al., 1983; M. Mohamed et al., 2008; Mourya et al., 2012a; Obanda et al., 2021)
		Cattle	*Bos* spp.	Afghanistan, Albania, Armenia, Azerbaijan, Bosnia, Bulgaria, Cameroon, Egypt, Hungary, India, Iran, Iraq, Ireland, Kazakhstan, Kenya, Kosovo, Malawi, Mauritania, Niger, Nigeria, North Macedonia, Oman, Russia, Saudi Arabia, Senegal, Somalia, South Africa, Sudan, Tajikistan, Turkmenistan, Tanzania, Tunisia, Turkey, UAE, Uganda, Zambia, Zimbabwe	(Adam et al., 2013c, 2013a; Badalov, 1969a; Barthel et al., 2014a; Berezin et al., 1969a; Blanco-Penedo et al., 2021; Chinikar et al., 2012; Chumakov, 1972; Chumakov et al., 1970; Chunikhin, Chumakov, Butenko, et al., 1969; Darwish et al., 1983; Deézsi-Magyar et al., 2024; Dinçer et al., 2014; El Ghassem et al., 2023; Erasmus et al., 1987; Fajs et al., 2014; Gergova & Kamarinchev, 2013; Goletic et al., 2022; Hassanein, el-Azazy, et al., 1997; Hassanein, El-Azazy, et al., 1997; Horton et al., 2014; Horváth, 1975; Ibrahim et al., 2015a; Karinskaya et al., 1974; Khan et al., 1997a, 1997b; Kirya et al., 1972; Kuchin et al., 1974; Lotfollahzadeh et al., 2011; Lugaj, Koni, et al., 2014; Lugaj, Mertens, et al., 2014; Mariner et al., 1995; Matevosyan, n.d.; Mertens et al., 2015; M. Mohamed et al., 2008; Mohammad et al., 2016; Mostafavi, Haghdoost, et al., 2013; Mourya et al., 2012a, 2012b, 2015; Mustafa et al., 2011; Ngom et al., 2024; Obanda et al., 2021; Özüpak & Albayrak, 2020; Saidi et al., 1975; Sas et al., 2017; Schulz, Barry, Stoek, Ba, et al., 2021; Semashko, 1975; J. Shanmugam et al., 1976a; Simo Tchetgna et al., 2023b; Smienova, 1971a; Swanepoel et al., 1985; Tantawi et al., 1981; TEKELİOĞLU et al., 2021; Telmadarraiy et al., 2010; Umoh et al., 1983; Vasilenko et al., 1971a; Williams et al., 2000a; Yadav et al., 2014a; Zouaghi et al., 2021)
		Goats	*Capra* spp.	Afghanistan, Albania, Bulgaria, Cameroon, Egypt, India, Iran, Iraq, Kenya, Kosovo, Mauritania, Niger, Oman, Saudi Arabia, Senegal, Somalia, Sudan, Tunisia, Turkey, UAE, Uganda	(Barthel et al., 2014b; Blanco-Penedo et al., 2021; Chinikar et al., 2012; Chumakov, 1974; Dinçer et al., 2014; El Ghassem et al., 2023; Fajs et al., 2014; Hassanein, el-Azazy, et al., 1997; Hassanein, El-Azazy, et al., 1997; Khan et al., 1997b; Mariner et al., 1995; M. Mohamed et al., 2008; Mohammad et al.,

					2016; Mostafavi, Haghdoost, et al., 2013; Mourya et al., 2012c, 2014, 2015; Nabeth et al., 2004; Ngom et al., 2024; Özüpak & Albayrak, 2020; Papa et al., 2009; Saidi et al., 1975; Sene et al., 2024; D. Shanmugam, 1973; J. Shanmugam et al., 1976a; Simo Tchetgna et al., 2023b; Tantawi et al., 1981; TEKELİOĞLU et al., 2021; Telmadarraiy et al., 2010; Vasilenko et al., 1971a; Williams et al., 2000a; Yadav et al., 2014b; Zouaghi et al., 2021)
		Sheep	*Ovis* spp.	Afghanistan, Azerbaijan, Bulgaria, Cameroon, China, Egypt, Greece, Hungary, India, Iran, Iraq, Kazakhstan, Kenya, Kosovo, Mauritania, Oman, Pakistan, Romania, Russia, Saudi Arabia, Senegal, Tajikistan, Tunisia, Turkey, Turkmenistan, UAE	(Barthel et al., 2014a; Blanco-Penedo et al., 2021; Ceianu et al., 2012; Chumakov et al., 1970; Darwish et al., 1978; Deézsi-Magyar et al., 2024; El Ghassem et al., 2023; Fajs et al., 2014; Hassanein, El-Azazy, et al., 1997; Hoogstraal, 1979b; Kasi, Sas, et al., 2020; Khamassi Khbou et al., 2021; Khan et al., 1997b; Li et al., 2020; Mahzounieh et al., 2012; Mariner et al., 1995; M. Mohamed et al., 2008; Mohammad et al., 2016; Mourya et al., 2015; Mustafa et al., 2011; Ngom et al., 2024; Omoga et al., 2023; Özüpak & Albayrak, 2020; Papa et al., 2014; Schulz, Barry, Stoek, Ba, et al., 2021; Schuster et al., 2017; Sene et al., 2024; Simo Tchetgna et al., 2023b; Smienova, 1971a; Spengler, Bergeron, et al., 2016; Tantawi et al., 1981; Taraku et al., 2015; TEKELİOĞLU et al., 2021; Vasilenko et al., 1971a; Williams et al., 2000a; Zouaghi et al., 2021)
	Camelidae	Camels	*Camelus* spp.	China, Egypt, Iran, Iraq, Kenya, Mauritania, Niger, Oman, Pakistan, Russia, Sudan, Tunisia, UAE	(Bouaicha et al., 2021b; Camp et al., 2020; Champour, Chinikar, Mohammadi, Razmi, Mostafavi, et al., 2016; Champour et al., 2014; Chumakov, 1972; Darwish et al., 1978; El Ghassem et al., 2023; Khalafalla et al., 2021; Khan et al., 1997b; Li et al., 2020; Mariner et al., 1995; Mohammad et al., 2016; Morrill et al., 1990; Schulz, Barry, Stoek, Ba, et al., 2021; Suliman et al., 2017; Tantawi et al., 1981; Williams et al., 2000b)
Perissodactyla	Equidae	Donkey	*Equus africanus*	Azerbaijan, Bulgaria, Kenya, Senegal, Tajikistan, Turkey	(Barthel et al., 2014a; Chumakov et al., 1970; Mangombi et al., 2020; Saltık, 2022; Smienova, 1971a; Vasilenko et al., 1971a)
		Horses	*Equus caballus*	Bulgaria, India, Iraq, Russia, Senegal, Tajikistan, Turkey	(Badalov, 1969a; Berezin et al., 1969a; Mangombi et al., 2020; Saltık, 2022; J. Shanmugam et al., 1976a; Smienova, 1971a; Tantawi et al., 1981; Vasilenko et al., 1971a)
Carnivora	Canidae	Dogs	*Canis familiaris*	Senegal, South Africa, Uganda, Zimbabwe	(Atim et al., 2022; Mangombi et al., 2020; Shepherd, Swanepoel, Shepherd, et al., 1987)
Galliformes	Phasianidae	Chickens	*Gallus domesticus*	Kazakhstan	(Semashko, 1975)

Struthioniformes	Struthionidae	Ostriches	*Struthio* spp.	Iran, South Africa	(Mostafavi, Chinikar, et al., 2013; Shepherd, Swanepoel, Leman, et al., 1987)

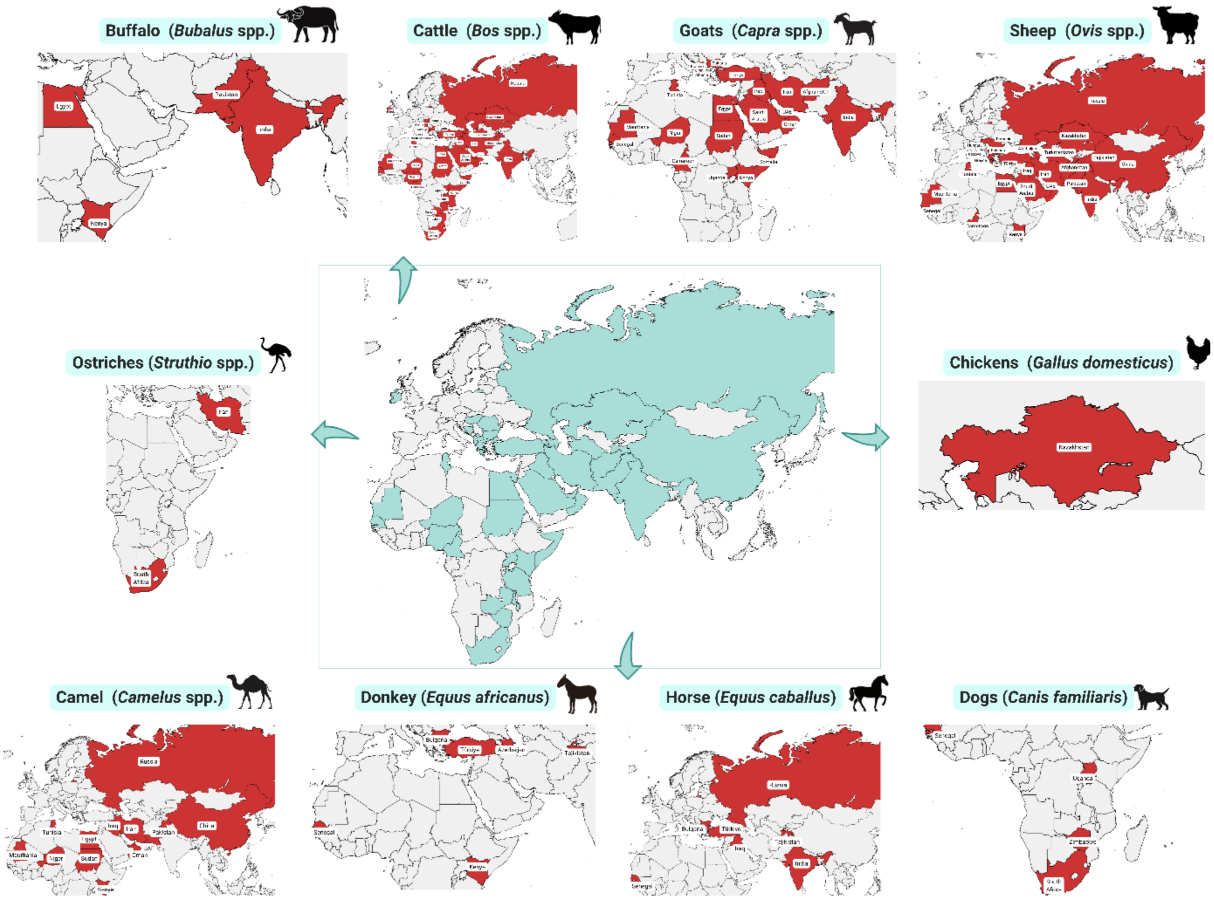

Figure 29. Geographic distribution of Crimean-Congo Haemorrhagic Fever Virus exposure detected in domestic animals.

35.4. CCHFV in Wild Animals

Numerous serological studies across a wide range of wild animals have highlighted the diverse responses observed in populations regarding CCHFV infections. These studies suggest their roles as amplifying hosts, facilitating virus transmission between infected and uninfected ticks during co-feeding or when feeding on a viremic animal.

A comprehensive review of nearly 7,000 samples from over 175 mammalian, avian, and reptilian species revealed varying levels of seroprevalence (Spengler et al., 2016; ***Table 10***; ***Figure 30***). Certain mammals, such as hares (3–22%), buffalo (10–75%), and rhinoceroses (40–68%), exhibited considerable seropositivity. Rodents and lagomorphs are crucial in CCHFV epidemiology (Hoogstraal, 1979; Ergönül, 2006; Bernard et al., 2022). Several rodent and lagomorph species, including the European hare (*Lepus europaeus*), scrub hare (*Lepus saxatilis*), Cape hare (*Lepus capensis*), bushveld gerbil (*Gerbilliscus leucogaster*), four-striped grass mouse (*Rhabdomys pumilio*), and multimammate mouse (*Mastomys* spp.), act as amplifying hosts, facilitating virus replication and transmission to ticks during their feeding (Ergönül, 2006). Infected rodents contribute significantly to the spread of CCHFV by transmitting the virus to ticks, thereby influencing its presence in the environment. Understanding the role of rodents in CCHFV transmission is important for developing effective surveillance and control strategies. Various rodent species such as the Cape porcupine (*Hystrix africaeaustralis*) (Shepherd et al., 1987), black rat (*Rattus rattus*) (Darwish et al., 1983), brown rat (*R. norvegicus*) (Darwish et al., 1983), bushveld gerbil (*G. leucogaster*) (Shepherd et al., 1987), four-striped grass mouse (*R. pumilio*) (Shepherd et al., 1987), Highveld gerbil (*Tatera brantsii*) (Shepherd et al., 1987), Indian desert jird (*Meriones hurrianae*) (Darwish et al., 1983), Indian gerbil (*T. indica*) (Darwish et al., 1983), multimammate mouse (*Mastomys* spp.) (Shepherd et al., 1987), Namaqua rock rat (*Aethomys namaquensis*) (Shepherd et al., 1987), Sundevall's jird (*M. crassus*) (Saidi et al., 1975), South African springhare (*Pedetes capensis*) (Shepherd et al., 1987), and Cape ground squirrel (*Xerus inauris*) (Shepherd et al., 1987) have displayed seropositivity to CCHFV in different regions, indicating their potential involvement in the virus's transmission cycle.

Additionally, other animals, including many large herbivorous mammals within the Artiodactyla and Perissodactyla orders, such as the African buffalo (*Syncerus caffer*), blesbok (*Damaliscus dorcas*) (Shepherd et al., 1987), common eland (*Taurotragus oryx*) (Shepherd et al., 1987), duiker (*Sylvicapra grimmia*) (Shepherd et al., 1987), gemsbok (*Oryx gazella*) (Shepherd et al., 1987), greater kudu (*Tragelaphus strepsiceros*) (Shepherd et al., 1987; Burt et al., 1993), impala (*Aepyceros melampus*) (Shepherd et al., 1987; Burt et al., 1993), mountain reedbuck (*Redunca fulvorufula*) (Shepherd et al., 1987), nyala (*Tragelaphus angasii*) (Shepherd et al., 1987; Burt et al., 1993), red hartebeest (*Alcelaphus buselaphus*) (Shepherd et al., 1987), sable antelope (*Hippotragus niger*), southern reedbuck (*Redunca arundinum*) (Shepherd et al., 1987), springbok (*Antidorcas marsupialis*) (Shepherd et al., 1987), waterbuck (*Kobus ellipsiprymnus*) (Shepherd et al., 1987), giraffe (*Giraffa camelopardalis*) (Shepherd et al., 1987), warthog (*Phacochoerus aethiopicus*) (Shepherd et al., 1987), white rhinoceros (*Ceratotherium simum*) (Shepherd et al., 1987; Burt et al., 1993), black rhinoceros (*Diceros bicornis*) (Shepherd et al., 1987; Burt et al., 1993), and Burchell's zebra (*Equus burchelli*) (Shepherd et al., 1987), as well as the African bush elephant (*Loxodonta africana*) (Shepherd et al., 1987; Burt et al., 1993) in South Africa and Zimbabwe, have demonstrated seropositivity to CCHFV.

Certain members of the Carnivora order also exhibited seropositivity in specific regions, including the African wild dog (*Lycaon pictus*) (Burt et al., 1993) in South Africa, red fox (*Vulpes vulpes*) in Russia and Turkmenistan (Hoogstraal, 1979), and Pallas's cat (*Otocolobus manul*) in Turkmenistan (Hoogstraal, 1979).

Bats, such as the common noctule (*Nyctalus noctula*) and large mouse-eared bat (*Myotis blythii omari*) in Iran (Saidi et al., 1975), also displayed seropositivity to CCHFV.

The potential involvement of birds in transmitting and maintaining CCHFV poses a significant concern in disease ecology. Migratory birds, traveling long distances through various habitats, carry a range of ectoparasites such as ticks, mites, fleas, and lice. Their movements, especially between Africa and Europe, coincide with environmental changes that may influence the spread of tick-borne diseases. Studies indicate that migratory birds can transport *H. marginatum* ticks from Africa to Europe, with certain Passerine bird species (e.g., *Acrocephalus arundinaceus*, *A. scirpaceus*, *A. palustris*, *A. schoenobaenus*, *Locustella luscinioides*, and *Luscinia megarhynchos*) facilitating the dispersion of infected ticks along their migratory routes (Bernard et al., 2022). Although avian species may generally be refractory to CCHFV infection (Spengler et al., 2016; Spengler et al., 2019; Shepherd et al., 1987; Capek et al., 2014), they can serve as blood sources for immature *H. marginatum* ticks, potentially contributing to disease spread. While most wild birds do not show evidence of CCHFV infection, exceptions like magpies (*Pica pica*), which have displayed CCHFV antibodies, suggest a more complex situation (Ergönül, 2006). Ostriches, however, show unique susceptibility to CCHFV, displaying both antibodies and viremia, unlike other birds (Ergönül, 2006). Further research is crucial to understand how different bird species contribute to CCHFV transmission.

Among reptiles, only one species—the Horsfield's tortoise (*Testudo horsfieldii*) in Tajikistan—has been reported as seropositive for CCHFV (Hoogstraal, 1979). Notably, the tick species *H. aegyptium*, which is closely associated with tortoises and often linked to CCHFV transmission (Kar et al., 2020; Široký et al., 2007), primarily infests hosts within the *Testudo* genus. This suggests a possible role of tortoises in virus transmission. However, the overall susceptibility of reptiles to CCHFV remains unclear, despite evidence pointing to potential transmission through tortoise-associated ticks.

***Table 10*. List of seropositive wild animals infected by Crimean-Congo Haemorrhagic Fever Virus.**

Class	Order	Family	Common name	Scientific name	Country	References
Mammals	Artiodactyla	Bovidae	African buffalo	*Syncerus caffer*	Kenya	(Obanda et al., 2021)
			Blesbok	*Damaliscus dorcas*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
			Common eland	*Taurotragus oryx*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
			Duiker	*Sylvicapra grimmia*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
			Gemsbok	*Oryx gazella*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
			Greater kudu	*Tragelaphus strepsiceros*	South Africa, Zimbabwe	(Burt et al., 1993; Shepherd, Swanepoel, Shepherd, et al., 1987)
			Impala	*Aepyceros melampus*	South Africa, Zimbabwe	(Burt et al., 1993; Shepherd, Swanepoel, Shepherd, et al., 1987)
			Mountain reedbuck	*Redunca fulvorufula*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
			Nyala	*Tragelaphus angasii*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
			Red hartebeest	*Alcelaphus buselaphus*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
			Sable antelope	*Hippotragus niger*	South Africa	(Burt et al., 1993; Shepherd, Swanepoel, Shepherd, et al., 1987)
			Southern reedbuck	*Redunca arundinum*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
			Springbok	*Antidorcas marsupialis*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
			Waterbuck	*Kobus ellipsiprymnus*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
		Giraffidae	Giraffe	*Giraffa camelopardalis*	South Africa, Zimbabwe	(Burt et al., 1993; Shepherd, Swanepoel, Shepherd, et al., 1987)
		Suidae	Warthog	*Phacochoerus aethiopicus*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
	Perissodactyla	Rhinocerotidae	White rhinoceros	*Ceratotherium simum*	South Africa, Zimbabwe	(Burt et al., 1993; Shepherd, Swanepoel, Shepherd, et al., 1987)
			Black rhinoceros	*Diceros bicornis*	South Africa	(Burt et al., 1993; Shepherd, Swanepoel, Shepherd, et al., 1987)
		Equidae	Burchell's zebra	*Equus burchelli*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
	Proboscidea	Elephantidae	African bush elephant	*Loxodonta africana*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
	Carnivora	Canidae	African wild dog	*Lycaon pictus*	South Africa	(Burt et al., 1993)
			Red fox	*Vulpes vulpes*	Russia, Turkemenistan	(S. Smirnova et al., 1969; Vy. Zarubinsky et al., 1975)
		Felidae	Pallas's cat	*Otocolobus manul*	Turkmenistan	(S. Smirnova et al., 1969)
		Herpestidae	Meerkat	*Suricata suricatta*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
	Chiroptera	Vespertilionidae	Common noctule	*Nyctalus noctula*	Iran	(Saidi et al., 1975)
			Large mouse-eared bat	*Myotis blythii omari*	Iran	(Saidi et al., 1975)
	Lagomorpha	Leporidae	Cape hare	*Lepus capensis*	South Africa, Zimbabwe, Turkmenistan	(Hoogstraal, 1979b; Shepherd, Swanepoel, Shepherd, et al., 1987)
			Hare	*Lepus* spp.	South Africa, Zimbabwe, Bulgaria, Iran	(Arata, 1975; Hoogstraal, 1979b; Shepherd, Swanepoel, Shepherd, et al., 1987; Vasilenko et al., 1971a)
			European hare	*Lepus europaeus*	Russia, Hungary	(Hoogstraal, 1979b; Németh et al., 2013; Vy. Zarubinsky et al., 1975)
			Scrub hare	*Lepus saxatilis*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)

	Eulipotyphla	Erinaceidae	Long-eared hedgehog	*Hemiechinus auritus*	Turkmenistan	(Chunikhin, Chumakov, Smirnova, et al., 1969; Hoogstraal, 1979b)
	Rodentia	Cricetidae	Bank vole	*Myodes glareolus*	Hungary	(Földes et al., 2019)
		Hystricidae	Cape porcupine	*Hystrix africaeaustralis*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
		Muridae	Black rat	*Rattus rattus*	Pakistan	(Darwish et al., 1983)
		Muridae	Brown rat	*Rattus norvegicus*	Pakistan	(Darwish et al., 1983)
		Muridae	Bushveld gerbil	*Tatera leucogaster*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
		Muridae	Four-striped grass mouse	*Rhabdomys pumilio*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
		Muridae	Highveld gerbil	*Tatera brantsii*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
		Muridae	Indian desert jird	*Meriones hurrianae*	Pakistan	(Darwish et al., 1983)
		Muridae	Indian gerbil	*Tatera indica*	Pakistan	(Darwish et al., 1983)
		Muridae	Multimammate mouse	*Mastomys* spp. *(coucha, natalensis)*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
		Muridae	Namaqua rock rat	*Aethomys namaquensis*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
		Muridae	Striped field mouse	*Apodemus agrarius*	Hungary	(Földes et al., 2019)
		Muridae	Sundevall's jird	*Meriones crassus*	Iran	(Saidi et al., 1975)
		Muridae	Yellow-necked mouse	*Apodemus flavicollis*	Hungary	(Földes et al., 2019)
		Pedetidae	South African springhare	*Pedetes capensis*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
		Sciuridae	Cape ground squirrel	*Xerus inauris*	South Africa, Zimbabwe	(Shepherd, Swanepoel, Shepherd, et al., 1987)
Aves	Passeriformes	Corvidae	Eurasian magpie	*Pica pica*	Russia	(Vy. Zarubinsky et al., 1975)
Reptilia	Testudines	Testudinidae	Russian tortoise	*Testudo horsfieldii*	Tajikistan	(Sonenshine & Mather, 1994)

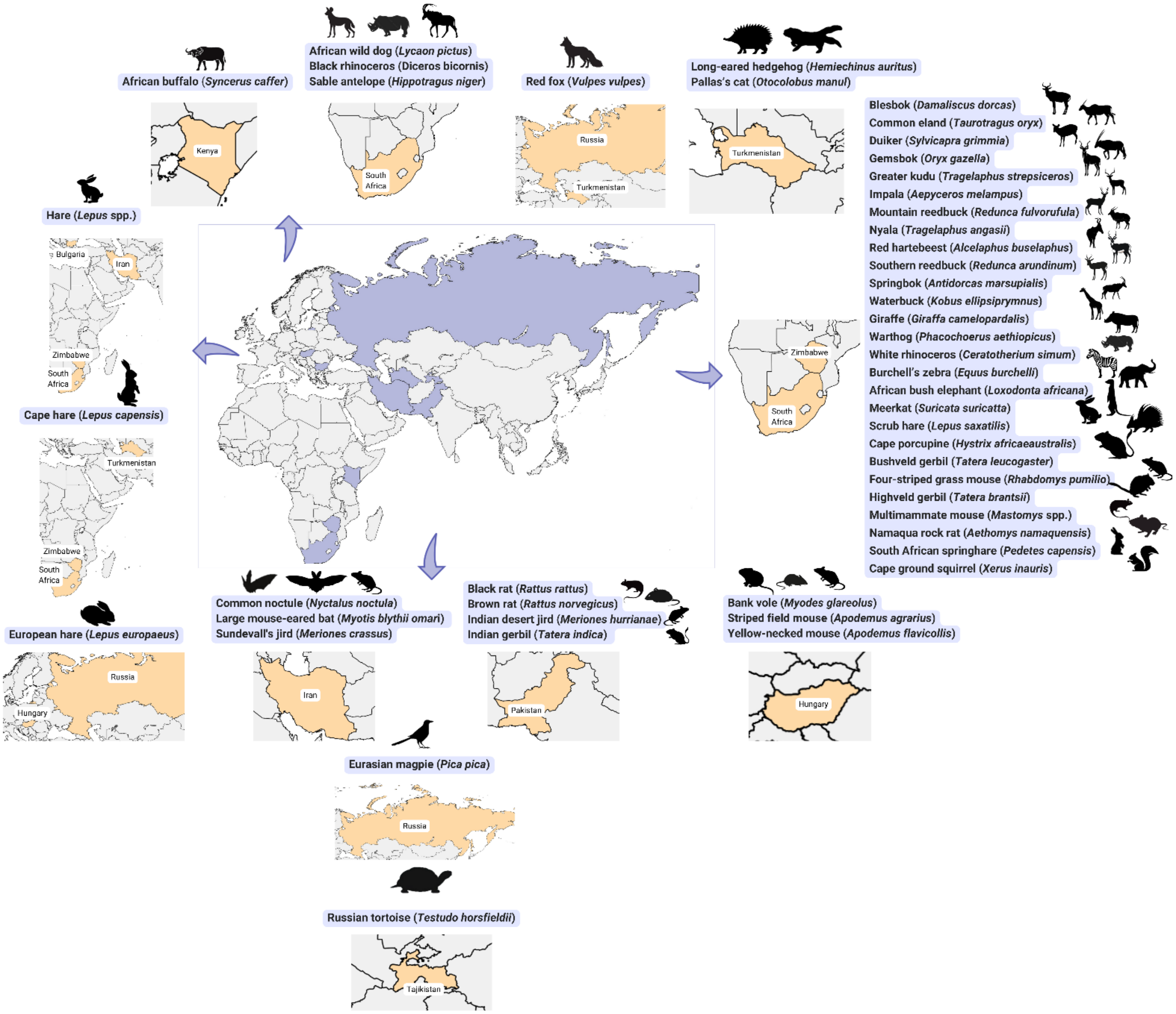

Figure 30. Geographic distribution of Crimean-Congo Haemorrhagic Fever Virus exposure detected in wild animals.

35.5. Molecular Detection of CCHFV in Animals

Despite evidence of seropositivity among domestic and wild animals, isolating CCHFV directly from these hosts has proven challenging, with documented instances remaining scarce (Spengler et al., 2016). Notable cases include a febrile cow in Kenya (Shanmugam et al., 1976), cattle and a goat from a Nigerian abattoir (Tantawi et al., 1981), a sentinel goat in Senegal (Hoogstraal, 1979; Shanmugam et al., 1976), European hares in Crimea (Chumakov, 1974), and a hedgehog in Nigeria (Tantawi et al., 1981). These sporadic cases highlight the challenges in identifying and isolating the virus due to the typically short viremic period in infected animals and the absence or mildness of clinical symptoms (Ergönül, 2006). Consequently, most successful isolations have come from ticks or human cases, where the virus is more prominent.

Molecular detection of CCHFV infection relies on both real-time and end-point PCR techniques (Muzammil et al., 2024). These methods amplify specific segments of the viral RNA, such as the S segment encoding the nucleoprotein, enabling precise detection and quantification of the virus. In resource-limited settings, loop-mediated isothermal amplification (RT-LAMP) offers a cost-effective alternative, amplifying viral RNA under isothermal conditions without the need for advanced equipment (Febrer-Sendra et al., 2023).

Enhanced molecular detection methods, longitudinal studies, and comprehensive monitoring programs are essential for fully understanding the role of various animal species in the ecology of CCHFV. This knowledge is critical for mitigating potential transmission risks to humans and preventing outbreaks of this serious zoonotic disease.

36. Experimental CCHFV Infections in Animals

Experimental studies investigating CCHFV infections across various animal species have provided valuable insights into susceptibility patterns, infection dynamics, and immune responses.

Small mammals, despite displaying short viremic periods of 2 to 15 days followed by antibody development, are not considered long-term reservoirs for CCHFV (Spengler et al., 2016). Nonetheless, population surges in species like hares have been linked to disease outbreaks, implying their ecological significance in CCHFV transmission (Ergönül, 2006; Spengler et al., 2016). Studies on small African wild mammals and laboratory animals showed diverse responses to CCHFV, with some species showing viremia and antibody responses, while others did not. South African hedgehogs, for instance, display resistance but develop neutralizing antibodies (Shepherd et al., 1989). Furthermore, the virus was recovered from the blood of experimentally infected long-eared hedgehogs (*Hemiechinus auritus*) (Hoogstraal, 1979), while European hedgehogs (*Erinaceus europaeus*) did not exhibit similar susceptibility (Blagoveshchenskaya et al., 1975). The varying outcomes among hedgehog species indicate that susceptibility to CCHFV and infection dynamics may vary even within closely related species.

Experimental studies have shown that various rodent and lagomorph species respond differently to CCHFV infection. European hares (*Lepus europaeus*), for example, showed varying viremic intervals (2, 4, 5, 9 dpi) and generated an antibody response by day 7, which was maintained throughout the study (Spengler et al., 2016). Similarly, scrub hares (*Lepus saxatilis*) and bushveld gerbils (*Gerbilliscus leucogaster*) exhibited viremia within the first week after infection, along with the production of antibodies (Shepherd et al., 1989). However, some species like the Cape ground squirrel (*Xerus inauris*) and the four-striped grass mouse (*Rhabdomys pumilio*) showed limited or no viremia and inconsistent antibody responses (Shepherd et al., 1989). On the other hand, the Southern multimammate mouse (*Mastomys coucha*), white-tailed rat (*Uromys caudimaculatus*), and red veld rat (*Aethomys chrysophilus*) demonstrated viremia (ranging from 1 to 6 dpi) and produced antibodies, indicating different responses to CCHFV among rodent species (Shepherd et al., 1989). Guinea pigs displayed low-level viremia accompanied by elevated temperatures. The onset of viremia correlated with the route of infection (Shepherd et al., 1989). The varied responses among small mammals highlight the complexity of CCHFV interactions, emphasizing the need for species-specific understanding in ecological dynamics. For a more comprehensive list, refer to the detailed experimental infection data of various small mammals infected with CCHFV, as thoroughly discussed in these studies (Ergönül, 2006; Spengler et al., 2016).

Experimental studies have investigated how CCHFV infects livestock, focusing on ruminants like sheep, cattle, horses, and donkeys. Similar to small mammals, these ruminants experienced a brief period of viremia and developed antibodies about a week after inoculation (Spengler et al., 2016). In sheep, maternal transfer of these antibodies was demonstrated, indicating a form of passive immunity (Gonzalez et al., 1998). Additionally, experiments on West African sheep highlighted diverse clinical manifestations following infection (Gonzalez et al., 1998). Some infected sheep developed moderate fever, hepatic dysfunction, and abnormal blood cell counts, including marked neutrophilia, that persisted for weeks. These observations highlight the potential impacts of CCHFV infection in livestock, particularly in sheep, affecting their health and possibly contributing to the virus's circulation in nature.

Calves have also been subjects of experimental infections, showing varying responses based on their age at the time of infection (Zarubinsky et al., 1976). When infected, 2-month-old calves displayed mild illness, with the virus detected in their blood. In contrast, 6-month-old calves did not show signs of viremia. However, only the younger calves, with detectable viremia, would be significant for the virus's circulation, despite both age groups exhibiting high levels of antibodies against CCHFV. Horses and donkeys showed different responses: donkeys exhibited low-level viremia (Rabinovich et al., 1972), while horses displayed minimal or no viremia but developed strong virus-neutralizing antibodies for up to three months (Milyutin, 1969). This highlights horses as valuable sources of serum for diagnostic and therapeutic purposes due to the stability of their virus-neutralizing antibodies.

It is important to note that these experimental studies were conducted in the 1970s. These studies revealed low viremia levels and asymptomatic cases in many animals, yet some could still transmit the virus to ticks during feeding. These results emphasize the need for updated research to better understand current CCHFV dynamics in livestock and improve prevention strategies. On the other hand, performing such research would be very complicated or even impossible nowadays as CCHFV is classified as a BSL-4 pathogen.

Efforts to establish animal models for CCHF have faced challenges, with limited success achieved so far. Newborn mice are the only animals besides humans that display symptoms of the disease, providing a basis for research (Ergönül, 2006). Additionally, genetically modified adult mice and hamsters, deficient in specific immune components, mimic human disease and exhibit uncontrolled viral replication, inflammatory immune reactions, liver pathology, and mortality (Zivcec et al., 2013; Ranadheera et al., 2020; Bereczky et al., 2010; Bente et al., 2010; Lindquist et al., 2018).

Non-human primate models, such as cynomolgus macaques, reflect varied disease outcomes similar to humans, aiding in preclinical assessments of therapeutics and vaccines (Haddock et al., 2018). In experimental infections with African green monkeys (*Chlorocebus sabaeus*), the majority of subjects exhibited either limited symptoms or remained asymptomatic, although one monkey developed fever post-infection, with some monkeys showing detectable antibodies against the virus (Butenko et al., 1970). In a separate study, Patas monkeys (*Erythrocebus patas*) and a Guinea baboon (*Papio papio*) displayed low-level viremia following inoculation, ultimately leading to the development of neutralizing antibodies in the baboon (Fagbami et al., 1975).

Studies investigating CCHFV infection in birds suggest that avian species, both wild and domestic, are generally refractory to the virus. Early experiments found that birds remained healthy after CCHFV inoculation, displaying no signs of viremia or detectable antibody responses (Berezin et al., 1971). However, several studies indicated that ground-feeding birds may contribute to the virus's ecological dynamics by facilitating viremic and non-viremic transmission or cofeeding (Ergönül, 2006; Shepherd et al., 1987; Zeller et al., 1994; Berezin et al., 1971).

Ostriches, however, appear to be significant hosts for CCHFV, showing detectable viremia and being epidemiologically linked to human infections (Zeller et al., 1994). In controlled experiments, infected ostriches developed viremia and subsequently produced antibodies against CCHFV (Erasmus et al., 1987). Other bird species, such as the red-billed hornbill (*Tockus erythrorhynchus*), demonstrated replication of CCHFV without viremia but were able to infect immature naïve ticks (Zeller et al., 1994). Similarly, helmeted guineafowl (*Numida meleagris*) exhibited low-level viremia followed by a transient antibody response starting 5–6 days post-infection (Shepherd et al., 1987). Additionally, birds like the glossy starling (*Lamprotornis* spp.) did not display viremia but generated an antibody response (Zeller et al., 1994). Further research is needed to clarify the role of birds in CCHFV transmission and its ecological implications.

37. Prevention and Control of CCHFV in Animals

Preventing and controlling the transmission of CCHFV in animals is crucial not only for animal health but also for preventing the virus from spreading to humans, where it poses a significant health risk. These measures aim to minimize the risk of virus transmission to humans and prevent CCHFV from reaching non-endemic regions.

The primary strategy to control CCHFV in animals involves managing tick populations, the main vectors for the virus. Using acaricides and other tick control methods is the most practical approach, although complete prevention of tick bites is unlikely (Kumar et al., 2020). Efforts often focus on periods surrounding slaughter, when exposure of slaughterhouse workers to CCHFV in animal blood or tissues is most likely. Additional practices to reduce tick exposure include environmental adjustments, treating animals with tick repellents, maintaining clean pastures, establishing quarantine measures for new animals, and improving animal housing (Kumar et al., 2020). Preventing or controlling CCHF infection in animals and ticks is complex due to the typically unnoticed tick-animal-tick-CCHFV life cycle and the often asymptomatic nature of the infection in most animals. The widespread presence of tick vectors further complicates control efforts, making acaricide-based tick control feasible only in well-managed livestock facilities.

Surveillance systems play a crucial role in early detection and response to CCHFV outbreaks in animals. Regular monitoring of animal populations in endemic areas for the presence of CCHFV antibodies or viral RNA could help identify potential reservoirs and understand disease dynamics. Timely detection enables prompt interventions to prevent further spread.

Finally, implementing biosecurity measures in farms, slaughterhouses, and veterinary facilities is essential to prevent CCHFV transmission between animals and humans, as these facilities have been identified as major risk areas for human infection (Shahhosseini et al., 2018; Akuffo et al., 2016; Msimang et al., 2021; Mostafavi et al., 2017).

Control strategies for CCHF infection in animals also extend to human protection. These strategies include avoiding tick bites through the use of repellents and employing adequate protection when handling or slaughtering animals (Hawman & Feldmann, 2023). Preventing the movement of naive animals into endemic areas is crucial, as it minimizes the risk of vertebrate amplification of the virus, reducing occupational risks for workers involved in animal processing. Educating livestock owners, veterinarians, and the general public about CCHFV transmission, symptoms in animals, and preventive measures is vital. Raising awareness about the disease’s impact, emphasizing the importance of early reporting of suspected cases, and promoting proper biosecurity measures are key components of effective disease control efforts.

38. Conclusion

CCHFV, a highly virulent virus transmitted by *Hyalomma* ticks, poses a significant global health threat by causing severe haemorrhagic fever in humans. Its widespread presence across Africa, Asia, and Europe highlights the urgent need to understand its behavior within tick vectors and animal hosts.

Both wild and domestic animals, acting as asymptomatic carriers, play critical roles in maintaining tick populations and transmitting the virus, thereby potentially spreading the disease. Further, small mammals like hares and hedgehogs support immature tick populations, while larger domestic animals such as cattle, goats, and sheep can inadvertently expose humans to CCHFV during handling and slaughter. The complex interplay between the virus, ticks, and vertebrate hosts presents significant challenges in controlling CCHFV transmission. Despite often lacking visible symptoms, animals play a crucial role in the maintenance and spread of the virus, highlighting the necessity for rigorous surveillance, serological screening, and a deeper understanding of their roles in CCHFV ecology. Experimental infections confirm that various animal species are susceptible to CCHFV, emphasizing the need for ongoing research and monitoring.

Control strategies mainly focus on managing tick populations through the use of acaricides and improving hygiene in animal habitats. However, the virus's elusive nature within animals and the difficulties in identifying infected hosts continue to pose significant challenges to disease control. Continued research and a deeper understanding of CCHFV in animal populations are essential for developing more effective control strategies, mitigating zoonotic risks, and protecting the health of both animals and humans.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CHAPTER 7

General Discussion

This thesis presents significant findings on the ecology, distribution, and genetic adaptation of *H. marginatum*, the principal vector of CCHFV, and the molecular surveillance of the virus in ticks and livestock. Research on the ecology and vector capacity of *H. marginatum* in Transalpine Europe remains a neglected topic within tick biology, especially compared to other tick species. Given the increasing reports of *H. marginatum* in regions previously deemed unsuitable, understanding the natural processes driving its distribution and its role as a vector is critical for public health and disease mitigation. This thesis addresses these knowledge gaps by providing an integrative approach to studying the molecular ecology of CCHFV and its primary vector across the Western Palaearctic, with a particular focus on Europe.

The first component of this thesis, presented in Chapter 2, is a systematic review of *H. marginatum* in Europe, examining its historical and current distribution patterns. The findings reveal an ongoing range expansion driven by climate change, migratory bird transport, and human activities. Temperature and humidity were identified as key environmental factors shaping *H. marginatum* habitats, supporting the observation that its populations are shifting towards temperate regions, including Central Europe. In many parts of Europe, the species has been increasingly recorded, with subadult stages frequently detected in new areas and adult stages reported sporadically. While *H. marginatum* has already established stable populations in regions such as southern France, its presence in Central and Northern Europe, as well as the UK, remains limited to sporadic records, often linked to bird migration. Although cooler temperatures and higher humidity continue to restrict its permanent establishment in certain areas, climate projections suggest that future warming may facilitate colonization. Despite these insights, the review highlights inconsistencies in data collection methods, host selection, and taxonomic classifications, which hinder direct comparisons across studies. These findings underscore the need for standardized surveillance strategies to improve monitoring of *H. marginatum* and its associated pathogens.

In Chapter 3, ENM was applied to predict the potential distribution of *H. marginatum* under current and future climatic scenarios. The results identified extensive suitable habitats across the Old World, including parts of Europe where the species is not yet permanently established. The results strongly indicate that *H. marginatum* will continue expanding northward into temperate regions, particularly across Central, Western, Eastern, and Northern Europe. Compared to previous studies, the model identified broader areas of medium to high suitability, with extensive expansion predicted in Central Europe and parts of Northern Europe. The northward shift is primarily influenced by rising temperatures, which enhance survival conditions in previously unsuitable regions. However, extreme heat stress in parts of Southern Europe may lead to localized declines in tick populations. Additionally, host availability remains a crucial factor in tick expansion, as migratory birds and livestock trade continue to facilitate dispersal. These findings highlight the need for proactive vector surveillance and control measures, particularly in regions where *H. marginatum* is projected to establish in the future.

Chapter 4 presents molecular and serological surveillance of CCHFV in Kosovo, confirming the active circulation of the virus. The identification of CCHFV in 41 tick pools, particularly from *H. marginatum*, reinforces the primary role of this species in the local transmission cycle. Additionally, the detection of CCHFV in *R. bursa* and *R. annulatus* suggests that these ticks may act as secondary vectors. The serological detection of CCHFV antibodies in 23% of cattle highlights livestock exposure to the virus, further emphasizing their role as sentinel species in disease surveillance. Phylogenetic studies from previous work have shown that two main strains circulate in the region: Europe 1 (Clade V), largely associated with *H. marginatum* and severe outbreaks, and Europe 2 (Clade VI), linked to *R. bursa* and usually associated with mild or subclinical cases especially in non-endemic regions. Continued surveillance, including genomic sequencing of tick samples, will be crucial for tracking viral evolution and transmission dynamics.

The fifth chapter of this thesis involves the genetic adaptation of CCHFV to *Hyalomma* and *Rhipicephalus* tick vectors. Molecular analyses reveal that CCHFV exhibits high genetic diversity, with frequent reassortment events among viral segments, which may enhance its ability to adapt to different hosts. Our phylogenetic analyses of the S and L genome segments show a clear distinction between strains carried by *Hyalomma* and *Rhipicephalus* ticks, suggesting that host-specific selection pressures play a crucial role in viral evolution. Interestingly, the phylogeny of the M segment does not show a distinct clustering pattern based on tick hosts, suggesting that geographic factors may have influence on the genetic structure of this segment. Further comparative analyses reveal significant nucleotide composition differences between strains isolated from the two tick genera, along with distinct codon usage patterns. The selection pressures exerted by different hosts appear to drive these differences, as *Hyalomma*- and *Rhipicephalus*-isolated strains exhibit significant variations in codon usage bias. CAI analysis indicates that CCHFV strains demonstrate a stronger adaptation to codons preferentially used by *Hyalomma* species. This aligns with the fact that *Hyalomma* ticks are the primary vectors of the virus, while *Rhipicephalus* ticks may serve as occasional or evolutionary new vectors, particularly in regions where *Hyalomma* is absent.

The final research component, covered in Chapter 6, extends the investigation of CCHFV beyond domestic livestock to wild animals. The detection of CCHFV in ticks infesting wildlife suggests a broader reservoir host range, raising concerns about zoonotic spillover events. Wild animals likely play a crucial role in maintaining viral circulation within natural ecosystems, necessitating a "One Health" approach to *Hyalomma*-borne disease management. The findings emphasize the necessity of cross-sectoral collaborations in monitoring CCHFV transmission in both domestic and wildlife hosts.

Overall, this thesis provides a comprehensive analysis of the ecology and epidemiology of *H. marginatum* and CCHFV, highlighting climate change, land-use alterations, migratory birds, and global animal movements as key drivers of their expansion. Given the zoonotic nature of CCHFV and its transmission cycle, a "One Health" approach is essential, integrating animal, human, and environmental health to address emerging risks.

Future research should focus on long-term surveillance, genetic characterization of CCHFV strains, and predictive modeling to assess the impact of environmental changes on *Hyalomma*-borne disease risks. Effective control strategies require a multidisciplinary approach, strengthening surveillance, improving tick control, and enhancing public health preparedness to mitigate the spread of *H. marginatum* and CCHFV.

CHAPTER 8

General Conclusion

Based on the findings from the six studies presented in this thesis, the following conclusions can be drawn:

Hyalomma marginatum Distribution in Europe (Chapter 2)

- *Hyalomma marginatum* has undergone a notable northward and westward expansion beyond its traditional Mediterranean range, with verified establishment or transient records in Central and Northern Europe. This expansion is facilitated by a combination of ecological, climatic, and anthropogenic drivers.
- Specifically, the tick thrives under warm, dry conditions, with optimal development occurring at temperatures between 20–35 °C and relative humidity levels ranging from 30% to 70%, provided microclimatic conditions (e.g., vegetation cover) offer adequate moisture retention. The lower thermal development threshold is approximately 14–16 °C, below which development slows significantly .
- Evidence suggests that climate warming, especially increasing minimum winter temperatures, has extended the tick's potential habitat into regions such as Germany, Czechia, Hungary, and parts of southern Scandinavia, where overwintering was previously unlikely. Moreover, the role of migratory birds in long-distance dispersal of immature stages is well-documented, with multiple records of *H. marginatum* larvae and nymphs retrieved from birds along European migratory flyways.
- The species' establishment potential is further supported by field reports of host-seeking adults and presence of all developmental stages in some parts of Central Europe. However, these detections remain sporadic, emphasizing the need for standardized, long-term surveillance systems.

Current and Future Distribution of *Hyalomma marginatum* (Chapter 3)

- Ecological niche modeling confirmed that *H. marginatum* occupies a broader climatically suitable habitat across Central, Western, Eastern, and Northern Europe than previously recognized, extending beyond its historical Mediterranean range.
- By 2100 (under RCP8.5), substantial habitat expansion is predicted into temperate regions, particularly affecting Germany (e.g., Bavaria, North Rhine–Westphalia), France (central and northern departments), Austria, Switzerland, Czechia (South Moravia), the UK (southern regions), and southern parts of Sweden and Norway.
- Densely populated metropolitan zones—such as Paris, Frankfurt, Vienna, London, and Stockholm—fall within future climatically suitable zones, indicating heightened potential for human exposure to *H. marginatum* and possible emergence of CCHFV in previously non-endemic areas.
- The tick's ecological niche is shifting due to both climatic (e.g., warmer winters, milder annual temperatures) and non-climatic factors (e.g., anthropogenic land use, host mobility), indicating adaptation to temperate continental climates.
- Localized habitat declines are projected for southern Spain, southern coastal Turkey, and parts of North Africa due to increasing aridity and extreme summer temperatures (exceeding 35°C), which may exceed the tick's physiological limits for survival and development.

CCHFV Circulation in Kosovo (Chapter 4)

- Molecular and serological surveillance confirms active circulation of CCHFV in Kosovo, particularly in hyper-endemic regions such as Malishevë.
- A total of 1,470 ticks representing eight species were collected from cattle in the hyper-endemic Malishevë region of southern Kosovo during 2022; *H. marginatum* was the dominant species (60.3%), followed by *R. bursa* (20.3%) and *R. annulatus* (16.6%).
- Molecular analysis via RT-qPCR detected CCHFV RNA in 41 out of 381 tick pools, yielding a pool positivity rate of 10.8%. Positive pools were identified in *H. marginatum*, *R. bursa*, and *R. annulatus*, confirming their role in virus maintenance.

- *Hyalomma marginatum*, as the most abundant and frequently infected species, remains the primary vector; however, detection in *R. bursa* and *R. annulatus* highlights their potential involvement as secondary vectors.
- Serological testing of 100 cattle sera from the same region in 2024 revealed 23% IgG positivity, suggesting substantial virus exposure and reinforcing the role of cattle as sentinel hosts.
- The term "southern regions" refers specifically to CCHF hyper-endemic zones in central and southwestern Kosovo, including Malishevë, Klinë, Rahovec, Suharekë, and Skënderaj, which have documented recurrent outbreaks, favorable tick habitats, and high livestock density.
- The findings support a “One Health” approach by integrating livestock serosurveillance and tick monitoring to inform risk assessment and intervention planning for human CCHF prevention.

Genetic Adaptation of CCHFV to Tick Vectors (Chapter 5)

- Phylogenetic analyses of the S and L genomic segments reveal clear separation between *Hyalomma*- and *Rhipicephalus*-vectored CCHFV strains, indicating strong host-specific adaptation.
- The M segment did not demonstrate distinct clustering by vector genus; instead, its codon usage patterns appear influenced by a combination of geographic origin and host-vector associations.
- Nucleotide composition analysis demonstrated significant differences in A3, G3, C3, and GC content at various codon positions, particularly in the S and L segments, between the two tick genera ($p < 0.05$).
- Codon usage bias analysis showed that the effective number of codons (ENC) differed significantly between *Hyalomma*- and *Rhipicephalus*-isolated strains for the M and L segments, with the L segment showing the greatest variation (51.92 ± 0.32 vs 47.64 ± 2.74, respectively).

- Codon usage patterns also diverged: *Hyalomma*-associated viruses preferred U- and A-ended codons, while *Rhipicephalus*-isolated strains favored A- and C-ended codons.
- Specific codon choices, such as GCA (Ala) and AGA (Arg) for *Hyalomma*-isolated strains and GCC (Ala) and AGG (Arg) for *Rhipicephalus*-isolated strains, further emphasized this divergence.
- Correspondence analysis (COA) confirmed that these patterns were not driven by phylogenetic clade affiliation but by tick genus.
- Moreover, codon adaptation index (CAI) values were significantly higher for *Hyalomma* than for *Rhipicephalus* across all segments, with the M and L segments of *Rhipicephalus*-isolated strains unexpectedly showing higher adaptation to *Hyalomma* than *Rhipicephalus* ($p = 0.0001$).
- These findings support that *Hyalomma* species are the main vector of CCHFV, with viral genomic segments showing stronger codon optimization and higher CAI values in *Hyalomma*-vectored strains. While *Rhipicephalus* species may serve as a secondary vector, especially in localized or *Hyalomma*-absent regions, its associated viral strains exhibit less optimized codon usage and reduced evolutionary adaptation.

The Role of Wild and Domestic Animals in CCHFV Transmission (Chapter 6)

- Both domestic and wild animals play a vital role in maintaining tick populations and facilitating the silent transmission of CCHFV, often without showing clinical symptoms.
- Serological studies confirm widespread exposure in livestock species such as cattle, sheep, goats, and camels across endemic regions, with exposure rates occasionally exceeding 70%, particularly in older or cross-bred animals.
- Experimental and field studies show that livestock, especially ruminants, serve as amplifying hosts, allowing virus acquisition by ticks during transient viremia and potentially contributing to co-feeding transmission.
- Wild mammals such as hares, hedgehogs, and various rodent species are ecologically important for immature tick stages and can act as local amplifiers, especially following sharp increases in their numbers.

- Donkeys, buffaloes, and dogs also show seropositivity in various regions, supporting their epidemiological relevance, especially in rural and pastoral settings.
- While most birds are resistant to infection, ostriches can develop viremia and have been linked to human infections. Migratory and ground-feeding birds may disperse infected ticks over long distances.
- Molecular detection of viral RNA in animals is rare due to the short duration of viremia, but successful isolations have occurred sporadically in cattle, goats, hares, and hedgehogs.
- Experimental infections confirm short-lived viremia and antibody responses in various domestic and wild species, underlining the need for species-specific surveillance.
- Preventing animal-associated transmission focuses on tick control, biosecurity in slaughterhouses and farms, and reducing unregulated animal movement, especially in and out of endemic zones.
- A "One Health" approach that integrates animal, human, and environmental surveillance is essential for monitoring virus circulation and minimizing spillover risks to humans.

Public Health and Control Strategies

- A "One Health" approach is crucial for CCHFV control, integrating environmental, veterinary, and public health sectors to address host-vector-pathogen dynamics effectively

Diagnostics

- Targeted surveillance should focus on high-risk regions identified through ecological niche models.
- Molecular detection (RT-PCR) from ticks and livestock blood samples enables early identification of circulating CCHFV, while serological assays (ELISA, IFA) help assess prior exposure.
- Human diagnostics should be prioritized for symptomatic individuals with occupational exposure, especially in endemic zones.
- Field-deployable diagnostics are critical in rural and low-resource settings where outbreaks often occur.

Vector Control Strategies

- Model-based hotspot mapping should guide targeted vector control, especially in hyperendemic zones like the Balkans and Anatolia.
- Anti-tick vaccines, such as those targeting *Hyalomma* spp. antigens (e.g., Bm86, Subolesin), have shown up to 65.4–80% efficacy in trials and should be integrated with acaricide rotation strategies to prevent resistance.
- Natural repellents and phytoacaricides offer environmentally safe alternatives and should be included in integrated tick management programs.

Livestock and Human Protection

- Livestock management improvements, such as rotational grazing and reducing tick-host interface in semi-natural grasslands, are necessary to disrupt the tick-host life cycle.
- Emphasis should be placed on PPE use among high-risk groups (farmers, veterinarians, abattoir workers), including gloves and facial protection, especially during tick removal or contact with blood/tissue.
- Education programs targeting occupational exposure and hygiene practices significantly reduce nosocomial transmission risk.

Medical Countermeasures

- Although there is no licensed vaccine for humans, promising candidates such as ChAdOx2-CCHF and MVA-CCHFV are in Phase I clinical trials. mRNA-based vaccines encoding CCHFV glycoproteins have demonstrated robust immunity in preclinical models.
- For animals, research into anti-CCHFV vaccines is ongoing, but the epidemiological impact of vaccinating livestock remains uncertain.
- Ribavirin continues to be used off-label as a post-exposure therapeutic, but its availability and efficacy remain variable.
- Advancing both therapeutic options and vaccine deployment—especially in high-risk rural settings—is essential.

Intersectoral Collaboration and Monitoring

- A global surveillance system to share real-time data on CCHFV cases and vector emergence is urgently needed.
- Local governments should develop risk maps, health education campaigns, and subsidized insurance coverage for vulnerable populations (e.g., shepherds, butchers) to encourage early treatment-seeking and reduce transmission chains.

CHAPTER 9

General References

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CHAPTER 10:

APPENDICES

Appendix 1: Supplementary material for Chapter 2

Appendix 2: Supplementary material for Chapter 5

Appendix 3: Supplementary material for Chapter 5

Appendix 1

Supplementary material for Chapter 2:
Hyalomma marginatum in Europe: The Past, Current Status and Future Challenges – A Systematic Review

Supplementary Table S1: **Detailed dataset presenting all records of *Hyalomma marginatum* distribution, associated hosts, and detected pathogens across Europe, compiled and analyzed for this systematic review.**

https://doi.org/10.6084/m9.figshare.28816430.v1.

Appendix 2

Supplementary material for Chapter 5: Genetic Background of Adaptation of Crimean-Congo Haemorrhagic Fever Virus to the Different Tick Hosts

Supplementary Table S2 - The strain name, accession number, origin, isolation host and collection date of polyprotein-coding region of each CCHFV isolates used in this study.

	SEQUENCES	**STRAIN**	**HOST**	**COUNTRY**	**COLLECTION DATE**
S segment	DQ076415.1	SPU128/81/7	*Hyalomma* spp.	Uganda	1981
	KF793333.1	Daral 2012	*Hyalomma* spp.	Mali	2012
	KU707899.1	CJH	*Hyalomma anatolicum*	Iran	2015
	KY484036.1	IbAr10200	*Hyalomma excavatum*	Nigeria	1996
	KY484037.1	JD-206	*Hyalomma anatolicum*	Pakistan	1965
	AF481799.1	Uzbek/TI10145	*Hyalomma asiaticum*	Uzbekistan	1985
	MG659724.1	WJQ16206	*Hyalomma asiaticum*	China	2017
	MG659727.1	FK16116	*Hyalomma asiaticum*	China	2017
	MH688497.1	YL16204	*Hyalomma asiaticum*	China	2016
	KU707898.1	CHH	*Hyalomma dromedarii*	Iran	2015
	KU707900.1	GRH	*Hyalomma dromedarii*	Iran	2015
	MF547415.1	Caceres 2014	*Hyalomma lusitanicum*	Spain	2014
	AY277672.1	ROS/TI28044	*Hyalomma marginatum*	Russia	2000
	KR814833.1	59-TK-2012	*Hyalomma marginatum*	Russia	2012
	KR814834.1	128-TK-2012	*Hyalomma marginatum*	Russia	2012
	KY484031.1	HY-13	*Hyalomma marginatum*	China	1968
	KY484044.1	SPU 128/81	*Hyalomma marginatum*	Uganda	1981
	DQ211641.1	ArD39554	*Hyalomma rufipes*	Mauritania	1984
	MF511219.1	SPUD8_81_7_813051_S	*Hyalomma rufipes*	South Africa	1981
	DQ211639.1	ArD8194	*Hyalomma truncatum*	Senegal	1969
	KY484027.1	DAK8194	*Hyalomma truncatum*	Senegal	1969
	DQ211638.1	AP92	*Rhipicephalus bursa*	Greece	1975
	MG516211.1	Pentalofos-Greece-2015	*Rhipicephalus bursa*	Greece	2015
	U04958.1	AP92	*Rhipicephalus bursa*	Greece	1994
M segment	DQ157174.1	SPU128/81/7	*Hyalomma* spp.	South Africa	1981
	KY484038.1	JD-206	*Hyalomma anatolicum*	Pakistan	1965
	MG659726.1	FK16116	*Hyalomma asiaticum*	China	2017
	MG659723.1	WJQ16206	*Hyalomma asiaticum*	China	2017
	MH688498.1	YL16204	*Hyalomma asiaticum*	China	2016
	NC_005300.2	IbAr10201	*Hyalomma excavatum*	Nigeria	1996
	KY484035.1	IbAr10200	*Hyalomma excavatum*	Nigeria	1996
	AF467768.2	IbAr10200	*Hyalomma excavatum*	Nigeria	2002
	MF547416.1	Caceres 2014	*Hyalomma lusitanicum*	Spain	2014
	KY484045.1	SPU 128/81	*Hyalomma marginatum*	Uganda	1981
	KY484032.1	HY-13	*Hyalomma marginatum*	China	1968
	AY900145.1	Hy13	*Hyalomma marginatum*	China	2005

	AY900141.1	SPU128/84	*Hyalomma marginatum*	South Africa	2005
	AY179961.1	VLG/TI29414	*Hyalomma marginatum*	Russia	2000
	DQ211628.1	ArD39554	*Hyalomma rufipes*	Mauritania	1984
	MF511236.1	SPUD8_81_7_813051_M	*Hyalomma rufipes*	South Africa	1981
	KF793334.1	Daral 2012	*Hyalomma spp.*	Mali	2012
	KY484026.1	DAK8194	*Hyalomma truncatum*	Senegal	1969
	DQ211626.1	ArD8194	*Hyalomma truncatum*	Senegal	1969
	MG516212.1	Pentalofos-Greece-2015	*Rhipicephalus bursa*	Greece	2015
	DQ211625.1	AP92	*Rhipicephalus bursa*	Greece	1975
	EF189752.1	Kelkit/Türkiye-RB2/2005	*Rhipicephalus bursa*	Türkiye	2005
	EF189751.1	Kelkit/Türkiye-RB1/2005	*Rhipicephalus bursa*	Türkiye	2005
L segment	KY484039.1	JD-206	*Hyalomma anatolicum*	Pakistan	1965
	MG659725.1	FK16116	*Hyalomma asiaticum*	China	2017
	MG659722.1	WJQ16206	*Hyalomma asiaticum*	China	2017
	MH688499.1	YL16204	*Hyalomma asiaticum*	China	2016
	KY484034.1	IbAr10200	*Hyalomma excavatum*	Nigeria	1996
	AY389508.2	IbAr10200	*Hyalomma excavatum*	Nigeria	1966
	NC_005301.3	IbAr10200	*Hyalomma excavatum*	Nigeria	1966
	AY422209.2	IbAr10200	*Hyalomma excavatum*	Nigeria	1966
	AY389361.2	IbAr10200	*Hyalomma excavatum*	Nigeria	1966
	AY947891.1	IbAr10200	*Hyalomma excavatum*	Nigeria	1966
	MF547417.1	Caceres 2014	*Hyalomma lusitanicum*	Spain	2014
	KY484043.1	SPU 128/81	*Hyalomma marginatum*	Uganda	1981
	KY484033.1	HY-13	*Hyalomma marginatum*	China	1968
	DQ211615.1	ArD39554	*Hyalomma rufipes*	Mauritania	1984
	MF511202.1	SPUD8_81_7_813051_L	*Hyalomma rufipes*	South Africa	1981
	KY484025.1	DAK8194	*Hyalomma truncatum*	Senegal	1969
	DQ211613.1	ArD8194	*Hyalomma truncatum*	Senegal	1969
	MG516213.1	Pentalofos-Greece-2015	*Rhipicephalus bursa*	Greece	2015
	DQ211612.1	AP92	*Rhipicephalus bursa*	Greece	1975
	KY963542.1	ET35	*Rhipicephalus bursa*	Türkiye	2016
	KY963541.1	ET37	*Rhipicephalus bursa*	Türkiye	2016
	KY963540.1	ET36	*Rhipicephalus bursa*	Türkiye	2016
	KY963543.1	KM6	*Rhipicephalus sanguineus*	Türkiye	2015

Appendix 3

Supplementary material for Chapter 5: Genetic Background of Adaptation of Crimean-Congo Haemorrhagic Fever Virus to the Different Tick Hosts

Supplementary Table S3 - Nucleotide composition analysis of S, M, and L segments of CCHFV isolated from *Hyalomma* and *Rhipicephalus* species (%). Significant values on the 95% confidence limit between strains isolated from two tick hosts are marked in bold (p<0.05).

SEQUENCES	**HOST**	**COUNTRY**		**A%**	**C%**	**U%**	**G%**	**A3%**	**U3%**	**G3%**	**C3%**	**AU%**	**GC%**	**GC1%**	**GC2%**	**GC12 %**	**AU3%**	**GC3%**
DQ076415.1	*Hyalomma* spp.	Uganda	***S segment***	30.23	22.29	22.36	25.12	20.5	25.26	26.29	27.95	52.59	47.41	50.1	37.89	44	45.76	54.24
KF793333.1	*Hyalomma* spp.	Mali		30.71	22.77	22.22	24.29	22.15	25.05	23.6	29.19	52.93	47.07	50.31	38.1	44.2	47.2	52.8
KU707899.1	*H.anatolicum*	Iran		30.99	21.6	23.4	24.02	22.57	27.54	23.4	26.5	54.38	45.62	49.28	37.68	43.48	50.1	49.9
KY484036.1	*H.excavatum*	Nigeria		30.5	22.43	22.57	24.5	21.33	25.88	24.43	28.36	53.07	46.93	49.9	38.1	44	47.2	52.8
KY484037.1	*H.anatolicum*	Pakistan		30.23	22.22	22.98	24.57	20.7	25.88	25.05	28.36	53.21	46.79	49.28	37.68	43.48	46.58	53.42
AF481799.1	*H.asiaticum*	Uzbekistan		30.78	22.57	22.02	24.64	22.36	23.4	24.84	29.4	52.8	47.2	49.69	37.68	43.69	45.76	54.24
MG659724.1	*H.asiaticum*	China		30.16	22.15	22.98	24.71	20.5	26.09	25.26	28.16	53.14	46.86	49.28	37.89	43.58	46.58	53.42
MG659727.1	*H.asiaticum*	China		30.02	22.5	22.64	24.84	20.08	25.05	25.67	29.19	52.66	47.34	49.28	37.89	43.58	45.13	54.87
MH688497.1	*H.asiaticum*	China		30.64	22.15	22.64	24.57	22.15	25.26	24.64	27.95	53.28	46.72	49.48	38.1	43.79	47.41	52.59
KU707898.1	*H.dromedarii*	Iran		30.43	22.22	22.91	24.43	21.33	25.88	24.43	28.36	53.35	46.65	49.28	37.89	43.58	47.2	52.8
KU707900.1	*H.dromedarii*	Iran		30.57	22.22	22.98	24.22	21.95	25.88	23.81	28.36	53.55	46.45	49.28	37.89	43.58	47.83	52.17
MF547415.1	*H. lusitanicum*	Spain		30.57	22.77	22.08	24.57	21.74	24.64	24.43	29.19	52.66	47.34	50.31	38.1	44.2	46.38	53.62
AY277672.1	*H.marginatum*	Russia		30.37	22.71	22.36	24.57	20.91	24.84	25.26	28.99	52.73	47.27	49.9	37.68	43.79	45.76	54.24
KR814833.1	*H.marginatum*	Russia		30.09	23.81	21.12	24.98	20.5	21.12	26.5	31.88	51.21	48.79	50.31	37.68	44	41.61	58.39
KR814834.1	*H.marginatum*	Russia		30.09	23.81	21.12	24.98	20.5	21.12	26.5	31.88	51.21	48.79	50.31	37.68	44	41.61	58.39
KY484031.1	*H.marginatum*	China		31.19	22.71	21.74	24.36	23.6	22.77	24.02	29.61	52.93	47.07	50.1	37.47	43.79	46.38	53.62
KY484044.1	*H.marginatum*	Uganda		30.23	22.29	22.29	25.19	20.5	25.26	26.29	27.95	52.52	47.48	50.1	38.1	44.1	45.76	54.24
DQ211641.1	*H.rufipes*	Mauritania		30.71	22.64	22.36	24.29	21.95	25.47	24.02	28.57	53.07	46.93	50.31	37.89	44.1	47.41	52.59
MF511219.1	*H.rufipes*	S. Africa		30.23	22.5	22.02	25.26	20.5	24.43	26.5	28.57	52.24	47.76	50.1	38.1	44.1	44.93	55.07
DQ211639.1	*H.truncatum*	Senegal		31.06	23.05	21.81	24.09	22.36	24.02	23.6	30.02	52.86	47.14	50.31	37.47	43.89	46.38	53.62
KY484027.1	*H.truncatum*	Senegal		31.06	23.05	21.81	24.09	22.36	24.02	23.6	30.02	52.86	47.14	50.31	37.47	43.89	46.38	53.62

Mean ± STD				30.52 ± 0.35	22.59 ± 0.51	22.30 ± 0.58	24.59 ± 0.36	**21.45 ± 0.93**	24.71 ± 1.53	**24.86 ± 1.05**	**28.98 ± 1.23**	**52.82 ± 0.68**	**47.18 ± 0.68**	**49.87 ± 0.43**	37.83 ± 0.22	**43.85 ± 0.23**	**46.16 ± 1.82**	**53.84 ± 1.82**
DQ211638.1	*R.bursa*	Greece		30.16	22.98	21.88	24.98	18.63	23.6	27.74	30.02	52.04	47.96	48.24	37.89	43.06	42.24	57.76
MG516211.1	*R.bursa*	Greece		29.95	23.26	21.95	24.84	19.25	22.98	26.5	31.26	51.9	48.1	48.24	38.3	43.27	42.24	57.76
U04958.1	*R.bursa*	Greece		30.23	22.98	21.81	24.98	18.63	23.6	27.74	30.02	52.04	47.96	48.24	37.89	43.06	42.24	57.76
Mean ± STD				30.11 ± 0.12	23.07 ± 0.13	21.88 ± 0.06	24.94 ± 0.07	**18.84 ± 0.29**	23.40 ± 0.29	**27.33 ± 0.58**	**30.43 ± 0.58**	**51.99 ± 0.07**	**48.01 ± 0.07**	**48.24 ± 0.00**	38.03 ± 0.19	**43.13 ± 0.10**	**42.24 ± 0.00**	**57.76 ± 0.00**

CHAPTER 11

Curriculum Vitae

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PERSONEL

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SKILLS

- Molecular Biology
- Molecular Virology
- Molecular Diagnostics
- Molecular Phylogenetics
- Medical Entomology
- Ticks
- Tick-Borne Pathogens
- Disease Mapping

EXPERIENCE

PhD Researcher/Research Assistant — Sep 2021 - ongoing

Faculty of Tropical AgriSciences, CZU, Prague, Czechia

Junior Researcher — Sep 2021- ongoing

Spatial Epidemiology Lab, Cairo, Egypt

- I am a part of Spatial Epidemiology Group of Ain Shams University and our work focuses on using a combination of field and lab experiments related to vector-borne diseases, GIS, remote sensing, and ecological modelling, trying to understand the major drivers of disease spread, and identifying the possible shifts at disease risk in response to global warming.

Junior Researcher — Sep 2021 - Sep 2022

Center for Infectious Animal Diseases (CINeZ), CZU, Prague, Czechia

- Project HERA: Enhancing Whole Genome Sequencing (WGS) and/or Reverse Transcription Polymerase Chain Reaction (RT-PCR) national infrastructures and capacities to respond to the COVID-19 pandemic in the European Union and European Economic Area.

Health & Science Editor — Nov 2021- ongoing

Prizma Medium, Kosovo

Visiting Researcher — 2022

The Pirbright Institute, England, UK

- Molecular virology, virus culture, virus isolation, whole genome sequencing, NGS, bioinformatics.

Intern — Sep 2013 - May 2016

Vector Ecology Research Unit, Ankara, Turkey

- Research activities: understanding the population structure, intra/inter-specific interactions of sand fly and mosquito species, understanding the effects of climatic changing on vector populations and vector-borne diseases, epidemiology of cutaneous/visceral leishmaniasis and malaria.

Intern — Jun 2015 - Aug 2015

INTERGEN Genetics and Rare Diseases Diagnosis Research Center, Ankara, Turkey

- Learning methods/skills: DNA & RNA isolation, PCR, qPCR, QF-PCR, Gel electrophoresis, Comparative genomic hybridization, MLPA, NGS, Misec-Illumina, Sanger Sequencing, Karyotype, Tissue culture, Fluorescence in situ hybridization, Chromosomal staining techniques.

Trainee — 2020

Molecular Ecology of Vectors & Pathogens Lab- Czech Academy of Sciences

- Molecular methods on detection of Lyme disease pathogen.

Volunteer — 2014

EU Project: EFSA-ECDC VectorNet Project

- This project supports the data collection on vectors and pathogens related to animal and human health. The project includes information on the distribution of ticks, mosquitoes, sand flies and biting midges.

INFORMATICS

MS Office	★★★★★
ArcGIS	★★★★★
SPSS	★★★★★
GraphPad Prism	★★★★★
Statistica	★★★★★
CyberTracker	★★★★★
Photoshop	★★★★
MATLAB	★★★
R	★★★
Python	★

LANGUAGES

Turkish (Native)	★★★★★
Albanian	★★★★★
English (TOEFL)	★★★★

REFERENCES

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EDUCATION

MSc Wildlife Management and Conservation 2019 - 2021

Czech University of Life Sciences, Prague, Czechia

- Master thesis: *Genetic Background of Adaptation of Crimean-Congo Haemorrhagic Fever Virus to the Different Tick Hosts.*
- Czech Government Scholarship (Full Scholarship International Student *2019-2021*).
- Merit Scholarship, MŠMT, Czech Republic (*2020*).
- Scholarship of Achievement, FTZ, CZU (*2020*).
- Final grade honors (*1.00/1.00*), Graduated Summa Cum Laude.

BSc Degree in Biology 2012 - 2016

Hacettepe University, Ankara, Turkey

- Turkey Government Scholarship (Full Scholarship International Student *2012-2016*).
- Award from Hacettepe University, Faculty of Science - 'The 2nd Best Student' among 1200 students/High Honor Student (*3.61/4.00*), Graduated Cum Laude.

High School 2009 - 2012

Gymnasium Gjon Buzuku, Prizren, Kosovo.

- Science & Mathematics (*5.00/5.00*).

TRAINING/WORKSHOP/CONFERENCE

9th European Congress of Virology April 2025

Dubrovnik, Croatia

- Poster Presentation: Genetic Background of Adaptation of Crimean-Congo Haemorrhagic Fever Virus to the Different Tick Hosts.

CzechoSlovak Virology Conference Feb 2024

Prague, Czechia

- Poster Presentation: Detection of Crimean-Congo hemorrhagic fever virus from livestock ticks in Kosovo in 2022.

WSV2023 One Health – One World – One Virology June 2023

Riga, Latvia

- Poster Presentation: Mapping the potential distribution of the principal vector of Crimean-Congo hemorrhagic fever virus *Hyalomma marginatum* in the Old World.

8th European Congress of Virology May 2023

Gdansk, Poland

- Poster Presentation: Re-Emergence of BTV-4 in Sheep Farms in Kosovo, 2020: A Retrospective Study.

10th Tick and Tick-Borne Pathogens Aug 2022

Murighiol, Romania

- Oral Presentation: Genetic Background of Adaptation of Crimean-Congo Haemorrhagic Fever Virus to the Different Tick Hosts.

International Virus Bioinformatics Meeting 2022 (ViBioM 2022) Mar 2022

Training on Advanced Molecular Analysis and Phylogenetics Oct 2018

Institut Pasteur

- Sequencing, Multiple Alignments, Phylogenetics for Veterinarian Virology and Medical Entomology, Phylodynamics, Evolutionary Models (Distance, Parcimony, ML), Bayesian Methods.

PUBLICATIONS

1. **Seyma S. Celina** & Jiří Černý. *Hyalomma marginatum* in Europe: The Past, Current Status and Future Challenges - A Systematic Review. *Transboundary and Emerging Diseases*, 2025. https://doi.org/10.1155/tbed/7771431.
2. **Seyma S. Celina**, Jignesh Italiya, Allan Obonyom Tekkara, Jiří Černý. Crimean-Congo haemorrhagic fever virus in ticks, domestic, and wild animals. *Frontiers in Veterinary Science*, 2025. http://dx.doi.org/10.3389/fvets.2024.1513123.
3. **Seyma S. Celina**, Jiří Černý. Genetic Background of Adaptation of Crimean-Congo Haemorrhagic Fever Virus to the Different Tick Hosts. *PLOS One*, 2024. http://dx.doi.org/10.1371/journal.pone.0302224.
4. Johana Alaverdyan, **Seyma S. Celina**, Miloslav Jirků, Marina Golovchenko, Jignesh Italiya, Libor Grubhoffer, Natalie Rudenko, Jiří Černý. A First Look at the Relationship Between Large Herbivore-Induced Landscape Modifications and *Ixodes ricinus* Tick Abundance in Rewilding Sites. *Vector-Borne and Zoonotic Diseases*, 2024. https://doi.org/10.1089/vbz.2023.0146.
5. **Seyma S. Celina**, Jiří Černý, Abdallah M. Samy. Mapping the potential distribution of the principal vector of Crimean-Congo hemorrhagic fever virus *Hyalomma marginatum* in the Old World. *PLOS Neglected Tropical Diseases*, 2023. https://doi.org/10.1371/journal.pntd.0010855.
6. Jignesh Italiya, Vojtěch Vacek, Petr Matějů, Christophe Dering, **Seyma S. Celina**, Arame Ndiaye, Jiří Černý. First Detection of SARS-CoV-2 in White Rhinoceros during a Small-Scale Coronavirus Surveillance in the Bandia Reserve, Senegal. *Animals*, 2023. https://doi.org/10.3390/ani13162593.
7. **Seyma S. Celina**, Simon King, Martin Ashby, Katie Harris, Noemi Polo, Mentor Alishani, Avni Robaj, Afrim Hamidi, Driton Sylejmani, Carrie Batten, Jiří Černý. Re-Emergence of BTV-4 in Sheep Farms in Kosovo, 2020: A Retrospective Study. *Transboundary and Emerging Diseases*, 2023. https://doi.org/10.1155/2023/3112126.
8. **Seyma S. Celina** & Jiří Cerný. *Coxiella burnetii* in ticks, livestock, pets and wildlife: A mini-review. *Frontiers in Veterinary Science*, 2022. https://doi.org/10.3389/fvets.2022.1068129.

MEMBERSHIP

- Czechoslovak Society for Microbiology: Prague, CZ
- World Society for Virology: Northampton, Massachusetts, US