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Dispersal of ticks and their microorganisms by African-Western Palaearctic migratory birds

TOVE HOFFMAN

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Abstract
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In Europe, tick-borne diseases are the most widespread and common vector-borne diseases and their geographical distribution is increasing. The dispersal of ticks depends on the movements of their vertebrate hosts. Avian hosts are more likely to be involved in long-distance range expansion of ticks due to their migration pattern. Billions of birds in the African-Palaearctic migration system migrate biannually between breeding grounds in the Palaearctic and wintering grounds in Africa and thereby create natural links between Africa, Europe, and Asia. In this thesis the dispersal of ticks and their microorganisms by northbound migratory birds utilizing flyways in the African-Western Palaearctic region has been investigated and the association between bird ecology and tick taxon addressed. The results suggest that long-distance migratory birds with wintering regions in Africa are involved in northward dispersal of the tick species *Hyalomma rufipes*, a known vector or Crimean-Congo hemorrhagic fever virus, and that birds with an open or wetland habitat have more *H. rufipes* in comparison to birds with a winter habitat comprising forest and shrubs. The results also suggest a role for birds in the ecology of Alkhurma hemorrhagic fever virus, a hemorrhagic flavivirus, and a potential mechanism for dispersal of the virus to new regions, including Europe and Asia Minor. The results did not provide evidence for immature ticks of the *Hyalomma marginatum* complex and birds having a major role in the ecology and northward dispersal of tick-borne *Anaplasma phagocytophilum*, a zoonotic bacterium causing febrile illness in humans and domestic animals. However, the results give support to the idea of a divergent enzootic cycle of *A. phagocytophilum* involving birds as hosts. Finally, the results of this thesis suggest that *H. rufipes* do not serve as vectors or contribute to the transmission of the tularemia-causing bacterium *Francisella tularensis* and that migratory birds do not contribute to northward dispersal of *F. tularensis*-infected ticks. However, the results suggest that migratory birds contribute to northward dispersal of *H. rufipes* carrying both *Francisella* and spotted fever group *Rickettsia* species, including *Francisella-like* endosymbionts and *Rickettsia aeschlimannii*. In conclusion, this thesis helps to clarify the knowledge about the dispersal of ticks and the microorganisms they carry by northbound migrating birds in the African-Western Palaearctic region. Furthermore, it highlights the need of establishing surveillance programs for monitoring the risk of introduction and establishment of important exotic tick species, such as *H. rufipes*, and tick-borne pathogens in the Western Palaearctic.

Keywords: African-Western Palaearctic, migratory birds, Ixodidae, *Hyalomma rufipes*, *Ixodes*, Alkhurma hemorrhagic fever virus, *Anaplasma phagocytophilum*, *Francisella*, *Midichloria*, *Rickettsia aeschlimannii*, PCR, microfluidic qPCR, metagenomics

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Till min son Leon

Inget i livet är viktigare än du.
Jag älskar dig till månen och tillbaka igen - oändligt många gånger.

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I **Hoffman, T**., Carra, L.G., Öhagen, P., Fransson, T., Barboutis, C., Figuerola, J., Kiat, Y., Onrubia, A., Jaenson, T.G.T., Nilsson, K., Lundkvist, Å., and Olsen, B. *Association between guilds of birds in the African-Western Palaearctic region and the tick species Hyalomma rufipes, one of the main vectors of Crimean-Congo hemorrhagic fever virus.* One Health, 2021. 13:100349.

II **Hoffman, T**., Lindeborg, M., Barboutis, C., Erciyas-Yavuz, K., Evander, M, Fransson, T., Figuerola, J., Jaenson, T.G.T, Kiat, Y., Lindgren, P-E, Lundkvist, Å., Mohamed, N., Moutailler, S., Nyström, F., Olsen, B., and Salaneck, E. *Alkhurma hemorrhagic fever virus RNA in Hyalomma rufipes ticks infesting migratory birds, Europe and Asia Minor.* Emerg Infect Dis, 2018. 24(5):879-882.

III **Hoffman, T**., Wilhelmsson, P., Barboutis, C., Fransson, T, Jaenson, T.G.T., Lindgren, P-E., Von Loewenich, F.D., Lundkvist, Å., Olsen, B. and Salaneck, E. *A divergent Anaplasma phagocytophilum variant in an Ixodes tick from a migratory bird; Mediterranean basin.* Infect Ecol Epidemiol, 2020. 10(1):1729653.

IV **Hoffman, T**., Sjödin, A., Öhrman, C., Karlsson, L., McDonough R.F., Sahl, J.W., Wilhelmsson, P., Pettersson, J.H.-O., Barboutis, C., Figuerola, J., Onrubia, A., Kiat, Y., Piacentini, D., Jaenson, T.G.T., Lindgren, P-E., Moutailler, S., Fransson, T., Forsman, M., Nilsson, K., Lundkvist, Å., and Olsen, B. *Co-occurrence of Francisella and spotted fever group Rickettsia in avian-associated Hyalomma rufipes.* Manuscript.

Additional Publications

Publications not included in the thesis:

Hoffman, T., Kolstad, L., Lindahl, J.F., Albinsson, B., Bergqvist, A., Rönnberg, B., and Lundkvist, Å. *Diagnostic potential of a Luminex-based Coronavirus Disease 2019 Suspension Immunoassay (COVID-19 SIA) for the detection of antibodies against SARS-CoV-2.* Viruses, 2021. 13(6):993.

Hoffman, T., Kolstad, L., Rönnberg, B., and Lundkvist, Å. *Evaluation of production lots of a rapid point-of-care lateral flow serological test intended for identification of IgM and IgG against the N-terminal part of the spike protein (S1) of SARS-CoV-2.* Viruses, 2021. 13(6):1043.

Krambrich, J., Akaberi, A., Ling, J., **Hoffman, T.**, Svensson, L., Hagbom, M., and Lundkvist, Å. *SARS-CoV-2 in hospital indoor environments is predominantly non-infectious.* Virol J, 2021. 18(1):109.

Nissen, K., Hagbom, M., Krambrich, J., Akaberi, D., **Hoffman, T.**, Sharma, S., Ling, J., Svensson, L., Bondesson, K., and Salaneck, E. *Presymptomatic viral shedding and infective ability of SARS-CoV-2; a case report*. Heliyon, 2021. 7(7):e06328.

Nissen, K., Krambrich, J., Akaberi, D., **Hoffman, T.**, Ling, J., Lundkvist, Å., Svensson, L., and Salaneck, E. *Long-distance airborne dispersal of SARS-CoV-2 in COVID-19 wards*. Sci Rep, 2020.10:19589.

Lindahl, J.F., **Hoffman, T.**, Esmaeilzadeh, M., Olsen, B., Winter, R., Amer, S., Molnár, C., Svalberg, A., and Lundkvist, Å. *High seroprevalence of SARS-CoV-2 in elderly care employees in Sweden.* Infect Ecol Epidemiol, 2020. 10(1).

Langenwalder, D.B., Schmidt, S., Silaghi, C., Skuballa, J., Pantchev, N., Matei, J.A., Mihalca, A.D., Gilli, U., Zajkowska, J., Ganter, M., **Hoffman, T.**, Salaneck, E., Petrovec, M., and von Loewenich, F.D. *The absence of the drhm gene is not a marker for human-pathogenicity in European Anaplasma phagocytophilum strains.* Parasit Vectors, 2020. 13:238.

Lindahl-Rajala, E., **Hoffman, T.**, Fretin, E., Godfroid, J., Sattorov, N., Boqvist, S., Lundkvist, Å., and Magnusson, U. *Detection and characterization of Brucella spp. in bovine milk in small-scale urban and peri-urban farming in Tadjikistan*. PLoS Negl Trop Dis, 2017. 11(3): e0005367.

Hoffman, T., Rock, K., Mugizi, DR., Muradrasoli, S., Lindahl-Rajala, E., Erume, J., Magnusson, U., Lundkvist, Å., and Boqvist, S. *Molecular detection and characterization of Brucella species in raw informally marketed milk from Uganda.* Infect Ecol Epidemiol, 2016. 6:10.3402/iee.v6.32442.

Contents

Abbreviations

12S rDNA	Mitochondrial gene encoding the 12S subunit of the ribosome
16S rDNA	Mitochondrial gene encoding the 16S subunit of the ribosome
17 kDa	17 kilo Dalton surface antigen
AHF	Alkhurma hemorrhagic fever
AHFV	Alkhurma hemorrhagic fever virus
ankA	Gene encoding a cytoplasmic protein antigen
ANSES	Agence Nationale de Sécurité Sanitaire de l'Alimentation
APMS	African-Palaearctic migration system
AWP	African-Western Palaearctic
AWPR	African-Western Palaearctic region
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Data System
bp	Base pair
CCHFV	Crimean-Congo hemorrhagic fever virus
CDC	Centers for Disease Control and Prevention
cDNA	Complementary DNA
CLE	*Coxiella*-like endosymbiont
COI	Cytochrome c oxidase subunit 1
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
FAO	Food and Agriculture Organization of the United Nations
FOHM	Folkhälsomyndigheten/Public Health Agency of Sweden
FOI	Totalförsvarets forskningsinstitut/Swedish Defense Research Agency
fopA	Gene encoding the outer membrane protein A
FV	Flavivirus
GA	Granulocytic anaplasmosis
GLM	Generalized linear model
gltA	Citrate synthase A gene
groEL	Gene encoding a heat shock protein
GTDB	Genome Taxonomy Database
HGA	Human granulocytic anaplasmosis

IFC	Integrated fluidic circuit
IgG	Immunoglobulin G
ITS2	Internal transcribed spacer 2
KFDV	Kyasanur Forest disease virus
LB	Lyme borreliosis
lpnA	Gene encoding the lipoprotein A
MAG	Metagenome-assembled genomes
MEGA	Molecular Evolution Genetics Analysis
ML	Maximum likelihood
NAU	Northern Arizona University
NCBI	National Center for Biotechnology Information
NCR	Non-coding region
NGI	National Genomics Infrastructure
NJ	Neighbor-joining
NS	Non-structural
OIE	World Organization for Animal Health
ORF	Open reading frame
PAM	Percent accepted mutation
PCR	Polymerase chain reaction
preM	Pre-membrane
qPCR	Real-time polymerase chain reaction
rDNA	Ribosomal DNA
RNA	Ribonucleic acid
RT	Reverse transcriptase
SFG	Spotted fever group
SFGR	Spotted fever group *Rickettsia*
s.l.	Sensu lato/complex
sp.	Species, singular
spp.	Species, plural
sucC	Gene encoding the succinyl-CoA ligase [ADP-forming] subunit beta
TBD	Tick-borne disease
TBE	Tick-borne encephalitis
TBEV	Tick-borne encephalitis virus
TBF	Tick-borne fever
TBFV	Tick-borne flavivirus
TBP	Tick-borne pathogen
UTR	Untranslated region
WHO	World Health Organization
WNV	West Nile virus
WP	Western Palaearctic

Glossary

African-Palaearctic – geographical region comprising Africa, Europe, parts of the Arabic Peninsula, and Asia north of the Himalayas
Afrotropical – zoogeographical region comprising sub-Saharan Africa, Madagascar, and part of the Arabian Peninsula
Amplifying host – species that increases pathogen transmission
Anthropogenic – caused by humans or human activity
Arabian Peninsula – geographical region comprising Saudi Arabia, Yemen, Oman, Kuwait, United Arab Emirates, Qatar, and Bahrain
Arthropod – member of the phylum Arthropoda, which includes spiders, insects, mites, etc.
Asia Minor – the westernmost part of the Asia continent comprising Turkey
Bacteremia – presence of bacteria in blood
Biome – areas with similar pattern of vegetation, fauna, and climate
Bootstrapping – re-sampling method used to estimate the confidence in an inferred tree topology
Bovine - cattle
Bridge vector – can acquire a pathogen from an infective enzootic host and transmit it to a secondary host or a human
Canine – dog
Caprine – goat
Clade – group including a common ancestor and all the descendants
Co-feeding transmission – transmission of pathogens between ticks feeding in close proximity
Dead-end host – species not involved in pathogen transmission due to not having high enough level of infection to infect a vector
Diapause – temporary pause in development
Dispersal – movement to a location where establishment may occur
Ecchymosis (bruising) – bleeding into tissues due to blood vessel rupture
Ecology – study of relationships among organisms and their environment
Embryogenesis – development and growth of an embryo
Emerging zoonosis – zoonosis that is newly recognized/evolved or previously has occurred but now presents an increasing incidence or geographical, host, or vector range
Encephalitis – inflammation of the brain
Endemic – regularly found in and restricted to a certain region
Endogenous – coming from within an organism

Endosymbiont – microorganism living inside a host as part of a symbiotic relationship
Engorged – blood-filled
Enzootic – constantly present in an animal population in a certain area
Epidemiology – study of the distribution (e.g., frequency) and the determinants (e.g., risk factors) of a disease in a defined population
Equine – horse
Feline – cat
Guild – group of species with shared characteristics
Hematophagous – feeding on blood
Hemorrhagic – escape of blood from damaged blood vessels
Hemorrhagic fever – infection associated with fever and bleeding
Horizontal transmission – infected vector transmits the pathogen to a host and reverse
Host – organism supporting replication of an infectious agent
Indian subcontinent – geographical region comprising Bangladesh, India, Maldives, Nepal, Pakistan, and Sri Lanka
Insectivores – mammalian order comprising shrews, moles, and hedgehogs
Intercontinental – between continents
Jaundice – yellow discoloration of skin, tissues, and body fluids caused by increase of bile pigments in the blood
Lagomorphs - mammalian order comprising hares, rabbits, and pikas
Malaise – feeling of discomfort
Mediterranean basin – geographical region comprising coastal areas around the Mediterranean Sea
Metagenomics – direct genetic analysis of genomes within an environmental sample
Molt – transition to the next life stage
Morphology – study of size, shape, and structure
Myalgia – muscular pain
Ornithology – study of birds
Ovine - sheep
Palaearctic – geographical region comprising Africa north of the Sahara, Europe, the temperate parts of the Arabian Peninsula, and Asia north of the Himalayas
Passerine – songbirds belonging to the order Passeriformes
Phylogenetic inference – to infer evolutionary relationships
Phylogeny (evolutionary tree) – inferred evolutionary relationship of organisms from observed data
Primordium (pl. primordia) – group of cells that represent the earliest stage in organ or tissue development
Reservoir competence – ability of an infected host to make an infectious agent available to a vector

Reservoir host – species that maintain pathogen transmission at a low but steady rate
Rhabdomyolysis – injury of the skeletal muscle resulting in release of intracellular contents into the circulation
Sahel – one of Africa´s eight biomes situated between the arid Sahara to the north and the moist savannas to the south
Sub-Saharan Africa – region of Africa south of the Sahara
Subspecies – subdivision of a species
Tabanid – biting fly
Taxon (pl. taxa) – biological classification unit
Transovarial transmission – passage of pathogen from mother to egg (offspring)
Transstadial transmission – passage of pathogen between developmental stages of the vector
Ungulate – group of hoofed mammals
Variant – group of organisms with altered forms of DNA
Vector – invertebrate that act as a transmitter of an infectious agent between vertebrate hosts
Vector competence – ability of a vector organism to acquire, maintain, and transmit an infectious agent
Viremia – presence of virus in blood
Western Palaearctic – geographic region comprising Europe, North Africa, and parts of the Arabian Peninsula and Asia
Zoonosis – infectious disease transmitted between vertebrate animals and humans

Introduction

Zoonotic diseases

Infectious diseases are a burden for both human and animal health globally. Among emerging infectious diseases, approximately 75% are zoonotic (transmitted between vertebrate animals and humans) and all originate in wildlife [1]. The World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the World Organization for Animal Health (OIE) have defined an emerging zoonosis as "*a zoonosis that is newly recognized or newly evolved, or that has occurred previously but shows an increase in incidence or expansion in geographical, host or vector range*" [1]. Zoonotic infectious diseases have multiple routes of transmission and a zoonotic pathogen can be transmitted to humans directly from a vertebrate host, indirectly via contaminated food or water, or via a vector. The rapid expansion of the human population has led to an urbanization and the emergence of many zoonotic diseases can be linked to anthropogenic factors, such as animal husbandry, habitat change, global travel, and trade. These factors have increased the interface between wildlife, domestic animals, and humans and provided more opportunities for spill-over events.

Vector-borne infections

Many human and animal pathogens are vector-borne and transmitted e.g., by infected hematophagous arthropods, such as mosquitoes or ticks. These include emerging human pathogens such as Crimean-Congo hemorrhagic fever virus (CCHFV) in Turkey [2], West Nile virus (WNV) in Europe and North America [3-6], Zika virus in South America [7], and *Rickettsia* species (spp.) worldwide [8]. Gathering information about the ecological details of transmission cycles, dispersal mechanisms, and pathogen-vector-host evolution is critical for better understanding of the underlying mechanisms of the emergence of vector-borne zoonotic pathogens. Movement of vectors, migration routes of reservoir hosts, adaptation of vector species to novel regions, and climate change are examples of ecological risk factors for the geographical spread of vector-borne zoonoses. Vector-borne zoonotic

infections can only be controlled when understanding the relationship between the pathogen, the vector(s), and the vertebrate host(s).

Bird biology and ecology

Migration and stopover ecology

Migration is an inherited trait and the timing of migration of birds is influenced by internal and external stimuli, such as endogenous rhythms within the bird (e.g., expressed in fat deposition and restlessness) and seasonal changes (e.g., photoperiod, temperature, food supply, and weather conditions). Migration occurs in most bird species that live in seasonal environments since regular biannual movements are needed in order to find favorable food and weather conditions. Many bird species therefore migrate seasonally along established migration flyways, mainly along north-south routes. The three major flyways are the American flyway, the African-Eurasian flyway, and the East Asian-Australasian flyway, which are further divided into eight flyways (Fig. 1) [9]. It should be noted that the major flyways are simplifications. Short-distance migrants can perform their migration route in one or a few single flights. Long-distance migrants, whose migration routes take several months and often include crossing ecological barriers, may break their journeys for hours up to weeks at a time at stopover sites along the migration route to rest and replenish fuel reserves. Where, when, and for how long they stay at a stopover site will affect their migration success and subsequently the following reproductive success. At some stopover sites large numbers of individuals gather at the same time. Arrival of many long-distance bird species at breeding and wintering areas usually occurs around the same time each year. The migratory behaviour of birds enables natural links between continents and thereby connects different biomes, and facilitates transfer of infesting ectoparasites, such as ticks, and avian- and vector-borne pathogens over ecological barriers - such as mountains, desserts, and oceans - and between distant geographical sites.[10, 11]

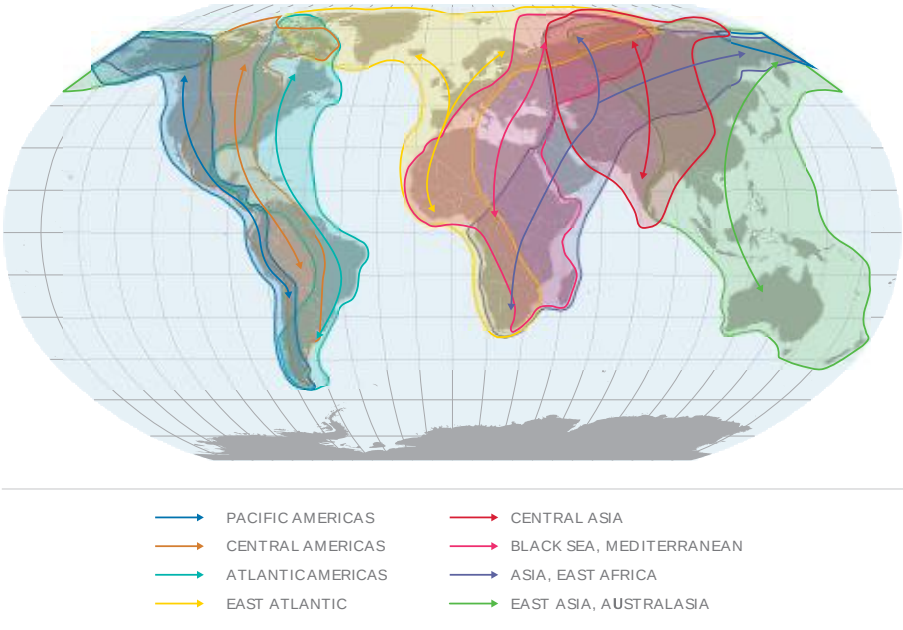

Figure 1. Map of the eight generalized flyways of migratory birds. Modified figure from [9], with permission by Birdlife International.

The African-Palaearctic migration system

Billions of birds in the African-Palaearctic migration system (APMS) migrate biannually between breeding grounds in the Palaearctic and wintering grounds in Africa [12], utilizing the East Atlantic, the Mediterranean/Black Sea, and the Asia/East Africa flyways to pass the Sahara desert or the Arabic peninsula (Fig. 1). These three migration routes constitute one of the world´s largest bird migration systems in which passerines are the most common [10, 12]. On the African continent, the Sahel region is an important transitional zone as well as a wintering area for migratory birds in terms of vegetation and food [13]. It stretches from east to west and is situated between the arid Sahara and the humid savannas. During the northern winter until the departure of Palaearctic birds in the spring, areas just south of the Sahara including the Sahel region are very dry due to the seasonal movement of the low air pressure belt at the Equator [14]. Consequently, many Palaearctic birds spend their non-breeding winter season in African regions with low rainfall.

Birds as hosts

Birds may be part of enzootic transmission cycles and serve as reservoirs and amplifying hosts for pathogens. For example, *Borrelia garinii* [15], Sindbis

virus [16], WNV [17], and avian influenza viruses [18] are known to have birds as reservoir hosts and to be carried by birds during migration.

Tick taxonomy, biology, and ecology

Tick taxonomy

Ticks belong to the phylum Arthropoda, the class Arachnida, the order Parasitiformes, and the suborder Ixodida [19]. The suborder Ixodida contains obligate hematophagous ectoparasites and is comprised of three families: Ixodidae (hard ticks), Argasidae (soft ticks), and Nuttalliellidae (one species) [19]. The Ixodidae family includes 742 species [20] while the Argasidae family includes about 190 species [21]. The family Ixodidae is divided into two groups: Prostriata and Metastriata. The Prostriata contains the genus *Ixodes* and 255 species [20]. The Metastriata comprises 486 species in 15 genera; e.g., *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, and *Rhipicephalus* [20].

Life cycle of Ixodidae ticks

The life cycle of Ixodidae ticks includes the embryonated egg and the three active stages; the larva, the nymph, and the adult. The life cycle is completed when the female tick has laid her eggs in the environment. A new cycle begins with embryogenesis of the eggs, followed by hatching of larvae, which disperse into the vegetation or nest environment to seek hosts, or enter diapause for over-wintering. Ticks require a blood meal from a host to be able to molt into the next stage of life and the female tick requires a blood meal to lay her eggs. The number of blood meals and hosts required for completion of the life cycle depend on the tick species. Some species require one host while others require two or three hosts. Most ticks have a three-host life cycle, which means that they seek a host, feed, and drop off to molt into a new stage in the environment. Thus, the active stages feed on separate hosts and are found free-living between feeding periods. In contrast, ticks with a two-host life cycle molt from larva to nymph on the host. The unfed nymph then re-attaches for a second blood meal on the same host before dropping off. In species of the subgenus *Boophilus* in the genus *Rhipicephalus* all stages feed on the same host individual. These ticks have a one-host life cycle. Engorgement is completed within several days, depending on tick species and its host.[19] The host seeking activity of ticks is driven by both abiotic (e.g., temperature and humidity (the amount of water vapor present in the air)) and biotic (e.g., vegetation structure and host abundance) factors. The majority of ticks die from desiccation or starvation.

Feeding preferences

Most ticks prefer to feed on certain groups of animals, with some being host specific (specialists) (e.g., *Ixodes trianguliceps*; swe: gnagarfästing), while others have a wide host range (generalists) (e.g., *I. ricinus*). Furthermore, different life stages of ticks have different host preferences. Larvae and nymphs often infest smaller mammals and birds while adults prefer larger mammals.[19]

Dispersal mechanisms

In general ticks have little mobility, and thus their dispersal depends on the movements of hosts such as by wild mammals, livestock, and migratory birds (Fig. 2). For example, animal movement and trade likely were involved in the recent introduction of the tick species *Rhipicephalus microplus*, an important disease vector of livestock, in West Africa [22]. A wide range of tick species, most commonly of the tick genera *Ixodes* and *Hyalomma* [23], can parasitize wild birds, especially ground-feeding and ground-breeding species [24]. Previous studies have shown that migratory birds can transport ticks carrying human pathogens over long distances [25, 26]. Tick species that spend long periods on their host are more likely to be transported over long distances, and migratory birds that pass through a wide range of areas are more likely to encounter a greater range of ticks and tick-borne microorganisms.

Figure 2. Golden oriole (*Oriolus oriolus*; swe: sommargylling) with infesting tick under the beak. Photo by Thord Fransson.

Vector competence

Vector competence is the ability of a vector organism to acquire, maintain, and transmit an infectious agent. Ticks are highly effective as vectors since they:

- inhabit every continent
- interact with different vertebrate hosts during their life cycle
- take several blood meals during their life cycle
- have a relatively long duration of blood-feeding
- remain infectious for long periods of time (including over winter)
- efficiently transmit pathogens both in immature and adult stages
- have relatively long lifespans (life cycle often measured in years)
- and have different modes of transmission: horizontal (passage of pathogen to host during feeding), transstadial (passage of pathogen during the molt to the next life stage), transovarial (passage of pathogen from the female tick to her eggs), and during co-feeding (passage of pathogen from infected tick to non-infected tick while feeding in close proximity on a non-infected host) [27, 28]. Besides being vectors for bacteria, viruses and parasites, ticks can also act as reservoir hosts - a phenomenon made possible by transovarial transmission of pathogens and recorded for *Rickettsia* bacteria [29], TBEV [28], and CCHFV [30]. In nature, tick-borne pathogens (TBPs) are maintained in enzootic cycles involving enzootic tick vectors and wild animals. A bridge (or link) vector can acquire the pathogen from an infective enzootic host and subsequently transmit the pathogen to a secondary host or a human.

Tick-borne diseases

Ticks are the most common vectors for human disease after mosquitoes, and ticks transmit a greater variety of pathogenic agents than any other arthropod-vector group [31-33]. See table 1 for an overview of a selection of TBPs and tick-borne diseases (TBDs). TBDs are the most widespread and common vector-borne diseases in Europe and their geographical distribution has increased since the 1980´s [34, 35], highlighting the need for increased surveillance of tick populations and TBP in the region. In Europe, Crimean-Congo hemorrhagic fever [2], Lyme borreliosis (LB) [36], and tick-borne encephalitis (TBE) [37] generate most concern in humans. In livestock, TBDs such as anaplasmosis, babesiosis, and theileriosis are global problems but most important in tropical and sub-tropical regions, leading to reduction in milk and animal production and resulting in economic losses [38, 39].

Hyalomma marginatum species complex

The genus *Hyalomma*, a.k.a. bont-legged ticks, contains 27 species [40] and includes species of both medical and veterinary importance [39, 41]. They are

medium to large-sized ticks with distinct brown and pale bands on the legs (Fig. 3) [19]. Their eyes and long legs enable them to actively seek their hosts in the adult stage [32]. From a taxonomic perspective, the *Hyalomma marginatum* species complex (*H. marginatum* sensu lato (s.l.)) is one of the most difficult groups. The complex is currently believed to comprise four species: *H. marginatum*, *H. rufipes*, *H. turanicum*, and *H. isaaci* [42], of which the latter is present on the Indian subcontinent. Identification of immature stages to species level is difficult and not advised [43]. *H. marginatum* and *H. rufipes* are important vectors and have a two-host life cycle, in which birds, lagomorphs, and insectivores most commonly act as hosts of the immature stages whereas wild and domestic ungulates act as hosts for the adult stage [42, 44]. Larvae molt on the host to become nymphs and remain on the same host up to 26 days [45-47], a period long enough to enable trans-continental (Africa to Europe or Asia) spread by trans-Saharan migrating birds. To date, permanent *Hyalomma* populations have not been recognized in Central and Northern Europe.

Figure 3. Adult *Hyalomma* tick. Photo by Tove Hoffman.

H. marginatum Koch, 1844, a.k.a. Mediterranean *Hyalomma* (swe: flyttfågelfästing), is present in southern Europe (native in many Mediterranean countries), northern Africa, and parts of Asia [42, 44, 48, 49]. Records of the species further south in Africa and north in Europe are likely due to import of immature stages by migratory birds [42, 50]. However, the distribution of the species may expand in the Mediterranean region into Central Europe [51]. *H. marginatum* infests humans and is the main vector of CCHFV in Europe [52]. *Rickettsia aeschlimannii* [53, 54] and *Anaplasma phagocytophilum* [55] have also been associated with the species.

Table 1. Causative agents, geographical distribution, vectors, and animal reservoirs of selected tick-borne diseases.

Group	Genus	Species	Disease or syndrome*(observed in)	Distribution	Vector(s)	Animal reservoir(s)	References
Bacteria	*Anaplasma*	*A. phagocytophilum*	Human granulocytic anaplasmosis (Human, cattle, goat, sheep, horse, dog cat)	E, NA	*Ixodes pacificus* *Ixodes persulcatus* *Ixodes ricinus* *Ixodes scapularis*	R, Ru	[56-59]
	Borrelia	*B. afzelii*	Lyme borreliosis (Human, cattle, dog, horse)	As, E	*Ixodes persulcatus* *Ixodes ricinus*	R	[60, 61]
		B. burgdorferi sensu stricto	Lyme borreliosis (Human, cattle, dog, horse)	As, E, NA	*Ixodes pacificus* *Ixodes persulcatus* *Ixodes ricinus* *Ixodes scapularis*	R	[57, 60, 61]
		B. garinii	Lyme borreliosis (Human, cattle, dog, horse)	As, E	*Ixodes persulcatus* *Ixodes ricinus*	B, R	[60, 61]
		Borrelia spp. (e.g., *B. duttonii*, *B. hermsii*, *B. parkeri*)	Tick-borne relapsing fever (Human)	A, As, E, NA	*Ornithodoros* spp.	H, R	[59, 62, 63]
		B. miyamotoi	Hard tick relapsing fever (Human)	As, E, NA	*Ixodes pacificus* *Ixodes scapularis* *Ixodes ricinus*	B, R	[63]
	Coxiella	*C. burnetii*	Q fever (Human, goat, sheep etc.)	A, As, Au, E, NA	Multiple species of different genera	Ru	[57, 59, 64]
	Ehrlichia	*E. chaffeensis*	Human monocytic ehrlichiosis (Human, dog, goat)	NA	*Amblyomma americanum*	Ru	[59, 65]
	Francisella	*F. tularensis*	Tularemia (Human, hare, rodent, sheep, goat etc.)	As, E, NA	Multiple species of different genera	U	[57, 59, 66, 67]
	Neoehrlichia	*Candidatus* Neoehrlichia mikurensis	Neoehrlichiosis (Human, dog)	As, E	*Ixodes ricinus*	R	[57, 68, 69]
	Rickettsia (SFG)	*R. aeschlimannii*	Unnamed (Human)	A	*Hyalomma marginatum* *Hyalomma rufipes*	U	[8]
		R. africae	African tick bite fever, LAR* (Human)	A, WI	*Amblyomma hebrum* *Amblyomma variegatum*	U	[8, 59]
		R. conorii	Mediterranean spotted fever (Human)	A, As, E	*Rhipicephalus sanguineus*	U	[8, 57]
		R. helvetica	Unnamed (Human)	As, E	*Ixodes ricinus*	U	[8, 57]
		R. massiliae	Unnamed (Human)	A, Am, As, E	*Rhipicephalus sanguineus*	U	[8]

Group	Genus	Species	Disease or syndrome*(observed in)	Distribution	Vector(s)	Animal reservoir(s)	References
		R. parkeri	Maculatum infection (Human)	Am	*Amblyomma maculatum*	U	[8]
		R. rickettsii	Rocky Mountain spotted fever (Human)	Am	*Amblyomma cajennense* *Dermacentor andersoni* *Dermacentor variabilis* *Rhipicephalus sanguineus*	U	[8, 59, 70]
		R. siberica	Siberian tick typhus, LAR* (Human)	A, As, E	*Dermacentor marginatus* *Hyalomma asiaticum*	U	[8]
		R. slovaca	Tibola/Debonel* (Human)	As, E	*Dermacentor marginatus*	U	[8]
Parasites	*Babesia*	*B. divergens*	Babesiosis (Human, cattle)	E	*Ixodes ricinus*	R, Ru	[71]
		B. microti	Babesiosis (Human)	E, NA	*Ixodes scapularis*	R	[71]
Viruses	Flaviviruses	Alkhurma hemorrhagic fever virus	Alkhumra hemorrhagic fever (Human)	AP	*Ornithodoros savignyi* *Hyalomma dromedarii*	U	[72, 73]
		Kyasanur Forest disease virus	Kyasanur forest disease (Human, NHP)	IS	*Haemaphysalis spinigera*	U	[32, 59, 74]
		Louping ill virus	Louping ill (Human, sheep)	WE	*Ixodes ricinus*	Ru	[57, 75, 76]
		Omsk hemorrhagic fever virus	Omsk hemorrhagic fever (Human)	As	*Dermacentor marginatus* *Dermacentor reticulatus* *Ixodes persulcatus*	U	[57, 59, 75]
		Tick-borne encephalitis virus	Tick-borne encephalitis (Human, dog)	As, E	*Ixodes persulcatus* *Ixodes ricinus*	R	[57, 59, 75]
	Bunyaviruses	Crimean-Congo hemorrhagic fever virus	Crimean-Congo hemorrhagic fever (Human)	A, As, EE	*Hyalomma marginatum* *Hyalomma rufipes*	U	[52, 57, 59, 75, 77]
		Severe fever with thrombocytopenia syndrome virus	Severe fever with thrombocytopenia syndrome (Human)	EAs, NA	*Haemaphysalis longicornis*	U	[78]

Host: B, birds; H, humans; NHP, non-human primates; R, rodents; Ru, ruminants; U, unknown.

Distribution: A, Africa; Am, the Americas; AP, Arabian Peninsula; As; Asia; Au, Australia; E, Europe; EAs, East Asia; EE, Eastern Europe; IS, Indian subcontinent; NA, North America; WE, Western Europe; WI, West Indies**.**

Abbreviation: LAR, lymphangitis-associated rickettsiosis; SFG, spotted fever group; spp., species; Tibola, tick-borne lymphadenopathy; Debonel, Dermacentor-borne necrosis erythema lymphadenopathy.

H. rufipes Koch, 1844, a.k.a. coarse bont-legged *Hyalomma* or hairy *Hyalomma*, is a native Afrotropical species that is distributed in the drier parts of Africa, including sub-Saharan Africa, and along the Red Sea cost on the Arabian Peninsula [42, 44, 79]. Records of this species from Europe and many regions of North Africa, with the exception of Egypt, and Asia are probably consequences of dispersal of immatures by birds migrating from Africa [42, 50, 80, 81]. *H. rufipes* frequently infest humans and are known to play a role in transmitting pathogens [82, 83], being a known vector for CCHFV in Africa [77]. Furthermore, DNA of *Borrelia burgdorferi* s.l., *Coxiella burnetii*, *Ehrlichia* spp., and *R. aeschlimannii* has been detected in nymphs collected from birds in Italy [84].

Ixodes ricinus species complex

The *I. ricinus* species complex, also referred to as the *I. persulcatus* complex, comprises closely related *Ixodes* species [85], which have a wide host range and an almost worldwide distribution, including the Neotropics (South America), Nearctic (North America), and the Palaearctic (Europe and Asia) [40]. Members of the complex, such as *I. ricinus*, *I. persulcatus*, and *I. scapularis* [86], have a three-host life cycle and are known vectors of several zoonotic pathogens, such as the agents causing TBE, human granulocytic anaplasmosis (HGA), and LB.

Tick-borne microorganisms

Arboviruses

The vast majority of emerging and re-emerging viral infectious diseases responsible for severe illness are caused by viruses with RNA genomes. Among these RNA viruses, arthropod-borne (arbo-) viruses, including alpha-, bunya- and flaviviruses, are important as they can cause fatal disease in domestic animals and humans. The life cycle of arboviruses is complex as it involves both arthropod vectors and vertebrate hosts.

Flaviviruses

Flaviviruses (FVs) are enveloped RNA viruses within the family *Flaviviridae*. The genomic RNA is single-stranded, linear, positive-sensed, and encodes a polyprotein. The genome is approximately 11 kilo bases in length and contains a single long open reading frame (ORF) flanked by 5´- and 3´- terminal non-coding/untranslated regions (NCR/UTRs), which form secondary structures needed for replication and translation. The genome encodes three structural (C, capsid; preM/M, membrane; E, envelope) and seven non-structural proteins (Fig. 4).[87]

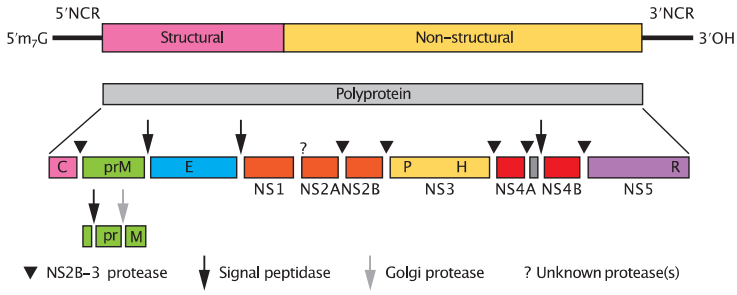

Figure 4. Genome organization and processing of the polyprotein of Flaviviruses. C, capsid; E, envelope; M, membrane; NCR, non-coding region; NS, non-structural. Figure by Simmonds et al. 2017 [88].

FVs have a broad geographical distribution, including Africa, the Americas, Asia, and Europe [89]. At present, the genus comprises 53 species [90] and most members infect both vertebrate and invertebrate species [91]. The genus is divided into three subgroups: i) the tick-borne FV group, ii) the mosquito-borne FV group, and iii) the no-known vector FV group (Fig. 5) [91, 92]. The tick-borne FVs (TBFVs) are further divided into three subgroups: i) the mammalian TBFV (M-TBFV) group, ii) the seabird TBFV group, and iii) the probably tick-borne group. The M-TBFV group comprises six human and animal pathogens: Alkhurma hemorrhagic fever virus (AHFV), Kyasanur Forest disease virus (KFDV), Langat virus, Louping-ill virus, Omsk hemorrhagic fever virus, Powassan virus, and TBEV [92].

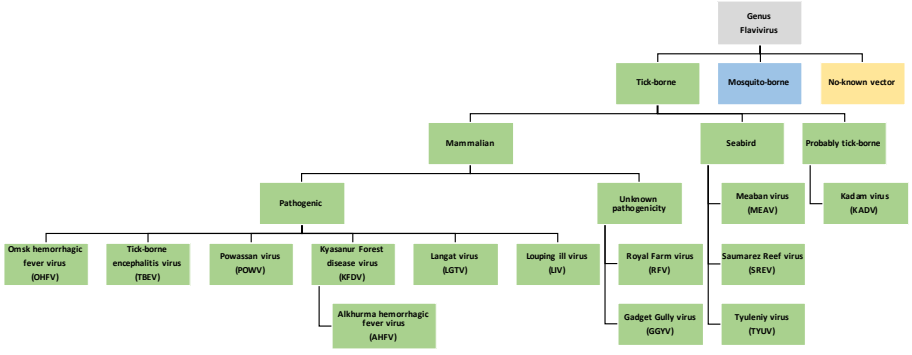

Figure 5. Division of the flavivirus genus into three major ecological groups of viruses: tick-borne flaviviruses (green), mosquito-borne flaviviruses (blue), and no-known vector flaviviruses (yellow). The group of tick-borne flaviviruses is further divided into the mammalian group, the seabird group, and the probably tick-borne group. The mammalian tick-borne flavivirus group comprises pathogenic tick-borne flaviviruses.

Alkhurma hemorrhagic fever virus

AHFV was identified in 1995 in Saudi Arabia [93]. Currently, Alkhurma hemorrhagic fever (AHF) is endemic in several provinces of Saudi Arabia and the case frequency has increased since 1995, with 620 cases of confirmed AHFV infection reported from 1995 to 2017 [94]. There are also reports from Africa, with four tourist-related cases detected near the Egypt-Sudan border in 2010 and 2013 [95-97] and a possible seropositive case as well as cattle-associated *Amblyomma lapidus* ticks testing positive for AHFV RNA detected in Djibouti, situated at the horn of Africa, in 2010 and 2011 [98, 99]. The clinical manifestation of AHF resembles that of other viral hemorrhagic fevers with initial malaise, high fever, headache, arthralgia, anorexia, and vomiting followed by encephalitis, jaundice, and ecchymosis (hemorrhagic manifestation). Rhabdomyolysis has also been reported [96]. The case fatality rate is reported to be 1-25%, with higher rates reported among a low number of hospitalized patients during an early outbreak [100] and later studies extending the disease spectrum by including also asymptomatic infections [101, 102]. Currently, there is no specific treatment or vaccine. Surveillance data is limited. The serological status of military staff stationed in different regions of Saudi Arabia was investigated in 2010 and a seroprevalence (AHFV-IgG) of 1.3% was found among the soldiers (n=1,024) [103].

AHFV is closely related to KFDV, a virus endemic in the south-western part of India and associated with *Haemaphysalis* ticks [104, 105]. Ticks, rodents, birds, and monkeys are believed to play a role in the enzootic cycle of KFDV [74, 105]. Analysis of full-length genomes has revealed an overall nucleotide sequence diversity of 8.4% between the two viruses [106]. An African origin has been suggested for AHFV and KFDV (Fig. 6) [107] and that, subsequently, AHFV spread to Saudi Arabia and KFDV to India.

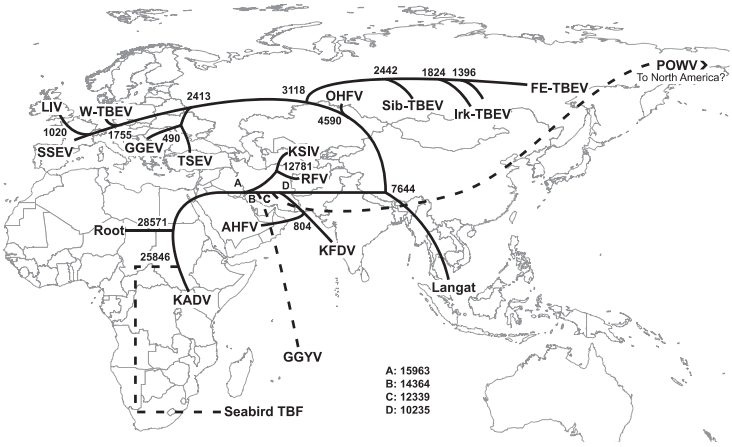

Figure 6. Model for dispersal of tick-borne flaviviruses. Figure by Heinze et al. 2012 [107], with permission from American Society for Microbiology.

The ecology of AHFV is largely unclear. The virus is believed to be zoonotic and the tick species *Ornithodoros savignyi* and *Hyalomma dromedarii* have been proposed as vectors, due to isolation of the virus from the two tick species [73] as well as molecular identification of the complete ORF of AHFV in *H. dromedarii* [72], which indicate replication of the virus within the two tick species. Additionally, goats, sheep, and camels have been suggested to be involved in the life cycle of AHFV due to their association with the mentioned tick species and their association with AHFV transmission to humans via slaughter and direct contact [72, 73, 100, 108]. Human-to-human transmission has not been reported.

Intracellular tick-borne bacteria

Ticks harbor pathogenic and symbiotic bacteria that require a host cell to replicate, including species of the genera *Anaplasma, Coxiella, Francisella, Midichloria, Rickettsia,* and *Spiroplasma* [109, 110].

Anaplasma phagocytophilum

A. phagocytophilum (Order: *Rickettsiales*; Family: *Anaplasmataceae*) is a zoonotic intracellular bacterium that replicates within neutrophils and has a wide geographical distribution, including Europe, the Americas, Africa, and Asia [58, 111]. The ecology of *A. phagocytophilum* is complex and involves different vectors and mammalian hosts. Hard ticks of the *I. ricinus* complex are the primary enzootic vectors and bridge vectors of the bacterium to humans: *I. ricinus* (Common tick, swe: vanlig fästing) in Western Eurasia, *I. persulcatus* (Taiga tick, swe: tajgafästing) in Eastern Eurasia, and *I. scapularis* (Deer tick) and *I. pacificus* (Western black-legged tick) in North America [58]. Other tick species may play a role in maintaining enzootic cycles in regions where the main vector is absent [112].

Clinical symptoms are observed in humans and domestic animals, including equines [113], canines [114], felines [115], bovines, ovines, and caprines [116]. In domestic ruminants, the disease is called tick-borne fever (TBF) and is characterized by high fever, anorexia, abortion, and drop in milk yield [58], resulting in economic losses for livestock owners in Europe. TBF has not been diagnosed in North America [58]. In equines, canines, and felines the disease is called granulocytic anaplasmosis (GA) and is characterized by fever and anorexia [58]. The clinical manifestation of human GA (HGA) ranges from asymptomatic to severe illness with symptoms such as fever, headache, myalgia, and malaise [117]. HGA was first described in the US in 1994 [118] but since then human cases have been described also in Europe and Asia. In Europe, the reported clinical cases are few and there are no reports of fatal outcome according to the European Centre for Disease Prevention and Control (ECDC) [119]. In contrast, clinical cases are more severe and frequent in

North America, with more than 5,000 cases reported to the Centers for Disease Control and Prevention (CDC) in 2019 [120] and reports indicating a fatality rate of less than 1% [121]. Underreporting of GA is likely in both humans and animals due to mild or asymptomatic infections. Currently, there is no vaccine and human infections are treated with antibiotics.

A. phagocytophilum is transmitted transstadially but is not proven to be transmitted transovarially within *Ixodes* species [122], indicating that a vertebrate reservoir host is needed to maintain the enzootic cycle. In North America, the white-footed mouse (*Peromyscus leucopus*) is considered to be the main reservoir host of human variants of *A. phagocytophilum* while the white-tailed deer (*Odocoileus virginianus*) is considered to be the major reservoir for the non-human variants of *A. phagocytophilum* [56, 58]. Knowledge of animal reservoirs of *A. phagocytophilum* in Europe is limited. *A. phagocytophilum* DNA has been detected in wildlife such as roe deer (*Caperolus caperolus*), red deer (*Cervus elaphus*), hedgehogs (*Erinaceus europaeus*), and small mammals [123-127], but their reservoir competence remains to be elucidated. *A. phagocytophilum* DNA has been detected in *I. ricinus* ticks and wild or domesticated animals in most European countries [56, 111], indicating that the bacterium and the infection is widespread in Europe. Birds have been proposed to have a role as reservoir hosts [128], but their role has been suggested to be limited [129]. Migratory birds may have a role in the dispersal of ticks infected by the bacterium [130-132]. Humans are dead-end hosts and are not involved in the transmission cycle of *A. phagocytophilum*.

A. phagocytophilum has evolved into different strains and genetic variants that display varying pathogenicity and preference of host and/or vector. The genetic diversity of *A. phagocytophilum* has been demonstrated by phylogenetical analyses of several genes, including *groEL* [133] and *ankA* [134-136]. The *ankA* gene encodes a cytoplasmic protein antigen with ankyrin repeats [137], might be involved in host-specific adaptation [136], and is considered to be a suitable marker for distinguishing variants by host [136, 138], allowing potential detection of zoonotic and distinct enzootic cycles.

Spotted fever group *Rickettsia*

Rickettsia are small obligate intracellular bacteria. The genus *Rickettsia* (Order: *Rickettsiales*; Family: *Rickettsiaceae*) is divided into four groups: the ancestral group (comprising *Rickettsia canadensis* and *R. bellii*), the transitional group (comprising *R. akari*, *R. felis*, and *R. australis*), the typhus group (comprising *R. prowazekii* and *R. typhi*), and the spotted fever group (SFG) that includes species such as: *R. aeschlimannii*, *R. africae*, *R. conorii*, *R. heilongjiangensis*, *R. helvetica*, *R. honei*, *R. japonica*, *R. massiliae*, *R. monacensis*, *R. montanensis*, *R. parkeri*, *R. peacockii*, *R. philipii*, *R. raoultii*,

R. rickettsii, R. sibirica, R. slovaca, and *R. tamurae* [8, 139]. All groups except the ancestral group contains pathogens known to cause human disease. The species *R. aeschlimannii, R. conorii, R. helvetica, R. massiliae, R. monacensis, R. slovaca*, and *R. sibirica* are considered to be emerging human pathogens in Europe [8, 140]. The clinical picture of SFG rickettsioses includes symptoms such as high fever, headache, and rash. An eschar at the inoculation site and neurological manifestation may also develop. The fatality rate of SFG rickettsioses ranges from 0% to 7% [140, 141]. Currently, there is no vaccine and human infections are treated with antibiotics.

SFG *Rickettsia* (SFGR) are transmitted horizontally, transstadially, and transovarially within ticks. Ticks therefore may serve both as vectors and reservoirs for SFGR. SFGR are transmitted to humans mainly by hard ticks of different genera, for example *Hyalomma, Ixodes,* and *Rhipicephalus* [8]. The role of vertebrates as reservoir hosts in maintaining zoonotic foci is still unclear. Humans are not involved in the transmission cycle of SFGR and are therefore considered to be accidental or dead-end hosts. SFGR have a worldwide distribution [8, 142], and their geographical distribution mimics the distribution of their vectors. See table 1 for additional information on geographic distribution and vectors of selected SFGR species. Migrating birds have been shown to be bacteremic with SFGR and to carry ticks infected by SFGR [143-145], suggesting a role of birds in the ecology and dispersal of SFGR.

Francisella

The genus *Francisella* (Order: *Thiotrichales*; Family: *Francisellaceae*) consists of closely related pathogenic and non-pathogenic species with an intracellular life style. Many of the non-pathogenic species are considered to be endosymbionts [146]. The species *Francisella tularensi*s is a facultative intracellular zoonotic bacterium that can multiply within macrophages [147]. It has a broad host range including mammals, birds, and arthropods [148], and is primarily present in the Northern hemisphere. The importance of vertebrate hosts as reservoirs for *F. tularensis* is poorly known. Humans are not involved in the life cycle and are considered dead-end hosts. Transmission to humans has been reported by direct contact with infected animals, arthropods bites (ticks, tabanids, and mosquitoes), contaminated water or food, or inhalation of contaminated particles [148]. Four subspecies of *F. tularensis* have been proposed: *F. tularensis tularensis* (Type A) that is endemic in North America, *F. t. holarctica* (Type B) that is found throughout the Northern hemisphere, *F. t. mediasiatica* that primarily is found in Central Asia, and *F. t. novicida* that is present in North America [149]. Of these, the subspecies *tularensis* and *holarctica* are of clinical importance in humans and responsible for tularemia (a.k.a rabbit fever) [146]. Tularemia can occur in several forms depending on the route of entry. The most common form of the disease is ulceroglandular

tularemia, which presents as a skin ulcer at the site of infection, swelling of regional lymph nodes, aches, and fever [150]. Human infections are treated with antibiotics. *F. tularensis* has a low infectious dose (as few as 10 organisms) [151], can become aerosolized (airborne) and cause a multisystemic disease with a fatality rate of up to 30% [152], and is therefore considered a potential agent of biological warfare [153]. Both subspecies *holarctica* and *tularensi*s are highly infectious for humans, stressing the need for both sensitive and specific diagnostic and surveillance methods [146]. Ticks of the genera *Amblyomma* and *Dermacentor* are the main human tick vectors for *F. tularensis* [148] while ticks of the genera *Ixodes* and *Haemaphysalis* are believed to be important enzootic vectors [148]. The genus *Francisella* was currently divided into four major clades and *F. tularensis* is located in Clade 1 [146]. Presence of *Francisella* spp. closely related to *F. tularensis* in tick species poses a challenge for molecular identification of *F. tularensis*.

Endosymbionts of ticks

Tick endosymbionts, mostly from the genera *Coxiella, Francisella* and *Rickettsia,* have a close evolutionary relationship with pathogens. Endosymbiotic bacteria present within tick cells may be necessary for host survival by providing nutrients that are missing in the blood meal, such as B-vitamins, and thereby improve the fitness of the tick [154-157]. Endosymbionts may alter transmission of TBPs, as illustrated by the reduced prevalence of the pathogenic *Rickettsia rickettsii* in the presence of the endosymbiotic *Rickettsia peacockii* in the tick species *Dermacentor andersoni* [157]. It has been speculated that presence of *Coxiella*-like endosymbionts (CLEs) in the salivary glands of *Amblyomma* ticks could influence maintenance or transmission of the pathogen *Ehrlichia chaffeensis* [158].

Francisella-like endosymbionts

Francisella-like endosymbionts (FLEs) (Order: *Thiotrichales*; Family: *Francisellaceae*) are intracellular bacteria that are capable of infecting the ovaries of female ticks, a feature that enables transovarial transmission [159, 160] and ensures continuation of symbiotic relationships. The genomes of FLEs contain many pseudogenes and inactivated versions of virulence genes of *F. tularensis,* suggesting that FLEs have evolved from a pathogenic ancestor [154, 156]. As the species *F. tularensis,* FLEs are found in Clade 1 of *Francisella* [146]. FLEs have a broad geographical distribution and they have been found in both hard and soft ticks, including the species *Amblyomma maculatum, D. andersoni, Dermacentor reticulatus, Dermacentor variabilis,* and *Ornithodoros moubata* [154, 161-171] - possibly due to spread of a pathogenic ancestor among different tick taxa during tick feeding on shared infectious hosts or through co-feeding [170]. In contrast to CLEs that are

considered to be obligate symbionts in most tick species [155, 172], FLEs have been suggested to be an alternative obligate symbiont in some tick species [154, 173]. An absolute reliance on a symbiont can become detrimental to a tick. The tick can escape the dependency by replacing the old symbiont with a new bacterium obtained from the environment [174]. It has been suggested that FLEs may have replaced CLEs in several tick lineages, including *A. maculatum* and *O. moubata* [154, 160, 173]. The knowledge about FLEs is limited since they are difficult to culture and few genomes have been assembled and characterized.

Midichloria

Candidatus Midichloriaceae is a novel family within the order *Rickettsiales* comprising intracellular bacteria associated with ticks [175]. The species *Midichloria mitochondrii* has an intramitochondrial lifestyle and is considered to be an endosymbiont of the tick species *I. ricinus* [176]. It resides primarily in the ovaries or ovarian primordia of ticks [177], a feature that enables transovarial transmission. The effect *M. mitochondrii* has on ticks and its potential to infect mammalian hosts are largely unknown. *M. mitochondrii* DNA has been detected in the salivary glands of ticks [178], DNA of bacteria related to *M. mitochondrii* has been detected in different mammalian species, including horses, sheep, and dogs [179], and anti-*Midichloria* antibodies have been detected in blood samples from humans and canines bitten by ticks [179, 180], which could indicate that *Midichloria* bacteria are transmitted horizontally between ticks and vertebrate hosts. Bacteria related to *M. mitochondrii* have been detected in other tick species of several tick genera, including *Ixodes*, *Rhipicephalus*, *Amblyomma*, and *Hyalomma* [181, 182].

Co-infections

Ticks may be co-infected by multiple pathogens [109, 183] and thereby potentially transmit several pathogens and cause co-infections in hosts, including humans [184, 185]. Multiple infections may show variable clinical symptoms and complicate the diagnosis of TBDs [186].

The Mediterranean basin tick project

Understanding of ecological systems requires large investigations. The Mediterranean basin tick project - in which the dispersal of avian-associated ticks and their microorganisms between the African, European, and Asian continent has been investigated - was initiated in 2009 and 2010. Early results indicated that northbound spring migratory birds utilizing the central Black Sea/Mediterranean flyway in the African-Western Palaearctic (AWP) region carried infected ticks from Africa to Europe and could thereby provide a

partial explanation for the increased geographical range of several TBPs in parts of Europe [53, 187]. The study therefore continued in 2014 and 2015 when additional study sites were included in an attempt to cover the three main flyways in the APMS. Since the beginning, the project has had an interdisciplinary approach in which experts within the fields of microbiology, medical entomology, medicine, virology, bioinformatics, statistics, and ornithology have collaborated.

Aims

General aim

The main aim of this thesis was to increase the knowledge about the dispersal of ticks and their microorganisms, including TBPs, in the AWP region (AWPR) by northbound spring migrating birds.

Specific aims

Study I

The ecology of the vertebrate host may contribute to the prevalence of tick infestation and the expansion of the geographical range of ticks. In Study I, we aimed to investigate which tick taxa northward migrating birds are infested by and transported along AWP flyways and whether bird ecology is associated with tick taxon.

Study II

In the light of an increasing case frequency in Saudi Arabia and the reported presence of AHF and AHFV on the African continent, the aim of Study II was to investigate presence of AHFV RNA in ticks infesting birds migrating from Africa to Europe and Asia during springtime.

Study III

Migratory birds have been found to be involved in the dispersal of ticks carrying *A. phagocytophilum*. The aim of Study III was to investigate the role of migratory birds and their infesting ticks in northward dispersal of *A. phagocytophilum* in the AWPR.

Study IV

The genus *Francisella* occurs in different tick taxa and consists of closely related pathogenic and non-pathogenic species. The aims of Study IV were to

investigate dispersal of *Francisella* as well as co-occurrence of *Francisella* and SFGR in ticks infesting northbound migratory birds in the AWPR.

Materials and Methods

Collection sites

Northbound spring migrating birds were trapped at bird observatories in the Mediterranean basin area, including several sites in the provinces of Huelva and Sevilla in Spain and on the Canary Islands (37°30′N, 5°30′W; 37°33′N, 6°55′W; 28°9′N, 15°25′W), Capri in Italy (40°33′N, 14° 15′E), Antikythira and Crete (Anapodaris river) in Greece (35°51′N, 23°18′E; 34°59′N, 25°17′E), the Kizilirmak Delta in Turkey (41°38′N, 36°05′E), and Jerusalem and its vicinity in Israel (31°47´N, 35°13´E) (Fig. 7). In 2009 and 2010, birds were trapped on Antikythira and Capri, two islands centrally located in the Mediterranean Sea and situated in the Mediterranean/Black Sea flyway. In 2014 and 2015 an effort was made to cover all three main flyways in the APMS, by including collection sites in the East Atlantic flyway (Spanish sites: Huelva, Sevilla, and the Canary Islands) and the Asia/East Africa flyway (Israelian site: Jerusalem; Turkish site: Kizilirmak Delta). The Kizilirmak Delta is a large wetland situated along the Turkish Black Sea coast. In contrast to the other collection sites, the Turkish site is not in direct connection with the Sahara passage. An additional site in the Mediterranean/Black Sea flyway was also included (Greece: Crete).

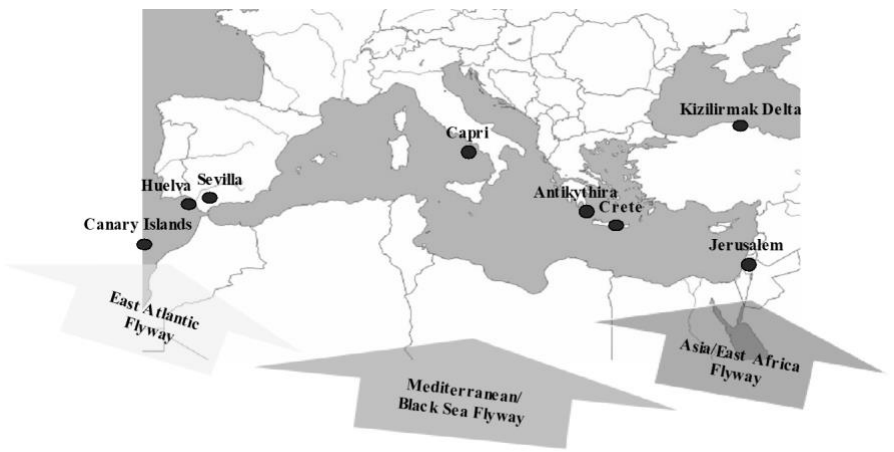

Figure 7. Map of the Mediterranean basin showing trapping and collection sites of the project and the three major flyways (simplified) of migratory birds in the Africa-Western Palaearctic migration system.

Maps

Maps of bird migration patterns and approximate distribution of *H. marginatum* s.l. ticks were based on information from the Handbook of birds of Europe, the Middle East and North Africa [188-190], the European Center for Disease Prevention and Control [48, 191], Walker et al. [44], and Estrada-Peña et al. [43].

Ethical consideration

Ticks were collected from birds in connection with ordinary scientific bird ringing activities at bird observatories by trained ringers with required permits and licenses:

Spain: 660117 and 180007 issued by the Ministerio de Agricultura, Pesca y Alimentación, and 66042 issued by Consejeria de Agricultura, Ganadería, Pesca y Desarrollo Sostenible.

Italy: 59019 issued by L´Instituto Superiore per la Protezione e Ricerca dell'Ambientale (ISPRA).

Greece: ΑΔΑ:ΒΛ9Σ0-Γ3Α, ΑΔΑ:Β4ΩΖ0-Ν6Χ, ΑΔΑ:ΩΗΛΔ465ΦΘΗ-31Γ, and ΑΔΑ: ΩΧΒΠ465ΦΘΗ-ΒΧΥ issued by the Hellenic Ministry of Environment and Energy.

Israel: A258 issued by the Israel Nature and Parks Authority.

Study material

Bird trapping (Studies I-IV)

In 2009, 2010, 2014, and 2015 migratory birds were trapped at bird observatories in the Mediterranean basin area. Trapping, using mist nets (Fig. 8), took place from March to October. At the bird observatories, birds are regularly ringed, measured, and weighed by ornithologists/ringers during the ringing season in order to study the migratory behaviour of birds (Fig. 9). Birds were carefully removed from the nets (Fig. 8) and for each individual bird the species, ring number, trapping date, and the number of ticks were documented. The birds were released after the examination (Fig. 10). The Turkish material was excluded in studies I and IV since it included a large number of bird individuals that were either resident or had not migrated over Sahara. Furthermore, the Spanish province Huelva and the Canary Islands

were excluded from studies I and IV since the birds had been trapped between July and October and thereby could represent southward migrating birds. Mist netting may have resulted in sampling biases and an over- or under-representation of bird species.

Figure 8. Trapping of birds at the bird observatory on Capri, Italy, using mist nests. Photos by Tove Hoffman.

Figure 9. Ringing activity at the bird observatory on Capri, Italy. Photo by Tove Hoffman.

Figure 10. Release of ringed bird. Photo by Tove Hoffman.

Tick collection (Studies I-IV)

Before the release, each bird was visually inspected for ticks on the head, throat, and chest by blowing apart the feathers (Fig. 11). Removal of ticks was performed using forceps or a tick remover (Fig. 11). During the field collection, ticks were stored individually in tubes containing RNA*later*™ (Invitrogen, Thermo Fisher Scientific, MA, USA), an RNA-stabilizing solution, at refrigerator temperature or -20°C. After the ringing season, the ticks were stored at -80°C. Ornithologists/ringers collected the ticks while conducting the annual trapping and ringing of birds. Visual inspection for ticks by ringers may have resulted in sampling biases and an over- or under-representation of tick taxa and life stage.

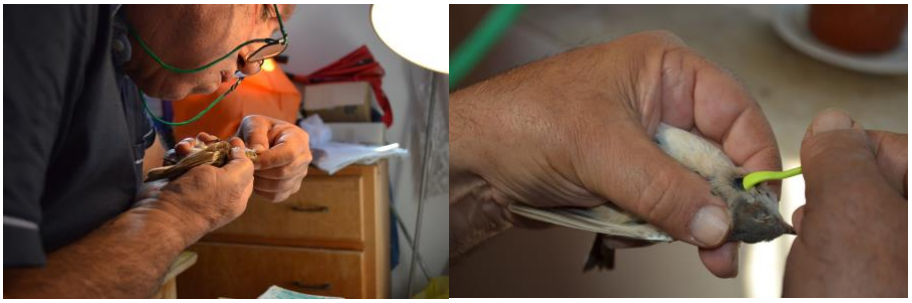

Figure 11. Collection of ticks from a white throat (*Sylvia communis*; swe: törnsångare) trapped at the bird observatory on Capri, Italy. Photos by Tove Hoffman.

Methods

Tick homogenization and extraction of nucleic acids (Studies I-IV)

For a detailed description of the homogenization and extraction of total nucleic acids for ticks collected in 2009 and 2010 (Studies II and III), see [192] and Study III. Ticks collected 2014 and 2015 (Studies I, II, and IV) were individually surface-sterilized using absolute ethanol (Sigma-Aldrich, Merck, Darmstadt, Germany) and rinsed twice in sterile H_2O before the extraction of nucleic acids. Mechanical homogenization of individual ticks and chemical inactivation of pathogens were conducted in the biosafety level 3 facility at the Public Health Agency of Sweden (FOHM) in Solna, due to the earlier detection of CCHFV RNA in ticks collected from a migratory bird in 2009 [187]. In brief, ticks were homogenized at 20 Hz for 5 minutes (min) using a TissueLyser II (Qiagen, Hilden, Germany), a 5 mm stainless-steel bead (Qiagen, Hilden, Germany), and 200 µL TRIzol™ (Invitrogen, Thermo Fisher Scientific, MA, USA). Additional 800 µL of TRIzol™ was added to the homogenate. Homogenates were centrifuged at 14,000 x g for 30 seconds (s) and the supernatant was used for isolation of nucleic acids. RNA was extracted using a manual phase separation technique with 0.2 volumes of chloroform (Sigma-Aldrich, Merck, Darmstadt, Germany). The solution was mixed by inversion, incubated for 5 min at room temperature, and centrifuged at 12,000 x g for 15 min at 4°C. The RNA from the aqueous phase was purified using the ZR-96 RNA Clean and Concentrator™ plate by Zymo Research (CA, USA), with the modification of eluting the RNA in 30 µL sterile H_2O. Extracted RNA was stored at -80°C. DNA was extracted from the organic phase using 0.75 mL of a back-extraction buffer containing guanidine thiocyanate, Trizma® base, sodium citrate (Sigma-Aldrich, Merck, Darmstadt, Germany), and sterile H_2O. The solution was mixed by inversion and centrifuged at 12,000 x g for 30 min at room temperature. The extracted DNA was concentrated and purified using the Nucleospin™ gDNA Clean up kit (Macherey Nagel, PA, USA), with minor modification (See Study I for details). Extracted DNA was stored at -20°C.

cDNA synthesis (Studies II and III)

For a detailed description of the synthesis of complementary (c) DNA for ticks collected in 2009 and 2010 (Studies II and III), see [192] and Study III. For ticks collected in 2014 and 2015 (Study II), the cDNA was synthesized using 1 µL of RNA and the qScript™ cDNA SuperMix by Quantabio (MA, USA) according to Gondard et al. [193]. The cDNA was stored at -20°C.

Tick genus/species determination (Studies I-IV)

In 2009 and 2010 (Studies II and III), ticks were photographed in the field with a USB-microscope [53, 192]. The life stage and sex of adult specimens were recorded and the degree of blood engorgement was estimated. The photographs were used by an entomologist to morphologically determine the genus of the ticks. Species determination was performed molecularly for ten randomly selected specimens, specimens lacking photographs, and specimens testing positive for a pathogen, using cDNA and targeting the mitochondrial 12S ribosomal DNA (rDNA) gene (320 base pairs (bp)) [53, 194] and the 16S rDNA gene (460 bp) [195]. Furthermore, the assay by Sormunen et al. [196] with primers targeting the second internal transcribed spacer (ITS2) gene of *Ixodes* spp. (95 bp) and two species-specific probes, allowing discrimination between *I. ricinus* and *I. persulcatus*, was used in Study III. For details, see Study III.

Due to difficulties in morphological species determination of immature *H. marginatum* s.l. ticks [42], ticks collected in 2014 and 2015 (Studies I, II, and IV) were determined to genus or species level by sequencing PCR-amplified fragments of the 12S and 16S rDNA genes [194, 195]. For details, see Study I and III. In Study IV, the tick species characterized by metagenomic analysis was confirmed by assembled mitochondrial genomes and animal identification via the cytochrome c oxidase subunit 1 (COI) gene in the Barcode of Life Data System (BOLD) [197].

Screening and confirmation analyses (Studies II-IV)

Microfluidic qPCR (Studies II and IV)

DNA and RNA of ticks collected in 2014 and 2015 were subjected to high-throughput screening for multiple tick-borne and tick-associated pathogens by microfluidic real-time (q) PCR at the Agence Nationale de Sécurité Sanitaire de l'Alimentation (ANSES) (Paris, France), using the BioMark™ qPCR system (Fluidigm, CA, USA) and primers and TaqMan probes targeting bacteria (25 species of 8 different genera: *Anaplasma*, *Bartonella*, *Borrelia*, *Coxiella*, *Ehrlichia*, *Francisella*, *Neoehrlichia*, and *Rickettsia*; one group (SFGR); one genus (*Borrelia* spp.)) and parasites (12 species of two genera: *Theileria* and *Babesia*) [198], as well as viruses (21 species of 7 different families: *Asfarviridae*, *Orthomyxoviridae*, *Reoviridae*, *Nairoviridae*, *Phenuiviridae*, *Peribunyaviridae*, and *Flaviviridae*) [193]. In brief, the cDNA was pre-amplified using the PerfeCTa® Preamp Supermix (Quantabio, MA, USA) and the assay primers. A 48.48 dynamic array chip (Fluidigm, CA, USA) was then primed in an Integrated Fluidic Circuit (IFC) Controller HX instrument (Fluidigm, CA, USA), followed by microfluidic assembly of 48 PCR mixes and 48 tick extracts on the chip using the IFC Controller HX, in

which PCR mixes and samples were dispensed into individual wells, and finally amplification and detection of microorganisms in the BioMark system [198].

Alkhurma hemorrhagic fever virus (Study II)

cDNA of samples collected in 2009 and 2010 were screened for AHFV by qPCR using primers and probe targeting the 5′UTR of AHFV (127 bp) [199] and the SuperScript™ III Platinum™ One-Step qRT-PCR kit by Invitrogen (Thermo Fisher Scientific, MA, USA). In brief, the master mix of 25 µL contained 1X Reaction mix, 400 nM of each primer, 200 nM of the probe, 0.25 µL of SuperScript III RT/Platinum Taq mix, 2 µL template, and sterile H_2O. The thermal profile comprised an initial denaturation step at 95°C for 2 min and 45 cycles of 95°C for 15 s and 57°C for 45 s. Positive results were analyzed twice.

cDNA of samples collected in 2014 and 2015 were screened for KFDV, including AHFV, at ANSES by microfluidic qPCR (BioMark™ Dynamic Arrays, Fluidigm, CA, USA), using a set of primers and probe that amplify a part of the preM region of KFDV and AHFV (72 bp) [193]. See Michelet et al. and Gondard et al. for details [198]. Positive results were analyzed twice. A subsequent confirmation qPCR was performed, using pre-amplified cDNA, the same KFDV/AHFV primers and probes, and the PerfeCTa® FastMix® II kit by Quantabio (MA, USA). The cDNA was pre-amplified using the PerfeCTa® PreAmp SuperMix (Quantabio, MA, USA) and primers targeting the preM region [193]. In brief, the master mix of 20 µL contained 1X Reaction mix, 200 nM of each primer, 200 nM of the probe, 2 µL pre-amplified and diluted (1:5) cDNA, and sterile H_2O. The thermal profile was as follows: an initial denaturation step at 95°C for 5 min and 45 cycles of 95°C for 15 s and 60°C for 60 s. A cycle threshold (Ct) value of 40 was selected as the cut-off in the confirmation qPCR.

***Anaplasma phagocytophilum* (Study III)**

cDNA of samples collected in 2010 were molecularly screened for *A. phagocytophilum* by collaborators at Linköping University, targeting a region of the *gltA* gene (64 bp) that encodes the enzyme citrate synthase [200]. A PCR targeting the 16S rDNA gene (507 bp) [201] was used to confirm the presence of *A. phagocytophilum*. For variant determination, a segment of the *ankA* gene (576 bp) was amplified by a collaborator at the Johannes Gutenberg University Mainz in Germany as previously described [135]. For details, see Study III.

***Francisella* (Study IV)**

Ticks collected in 2014 and 2015 were screened for *Francisella* species, including *F. tularensis*, at ANSES by microfluidic qPCR (BioMark™

Dynamic Arrays, Fluidigm, CA, USA), according to Michelet et al. [198]. To confirm the presence of *F. tularensis*, subsequent genus- and species-specific qPCRs were performed at the Swedish Defense Research Agency (FOI), using primers and probes targeting the genes encoding the outer membrane protein A (*fopA*; genus-specific) (91 bp), the succinyl-CoA ligase [ADP-forming] subunit beta (*sucC*; genus-specific) (125 bp), and the lipoprotein A (*lpnA*; *F. tularensis*-specific) (76 bp, 108 bp) [198, 202, 203]. In the confirmation step, DNA pre-amplified using the RepliG midi kit by Qiagen (Hilden, Germany), allowing unbiased amplification of whole genomes due to multiple displacement amplification (MDA), was used. For details, see Study IV.

Spotted fever group *Rickettsia* (Study IV)

Ticks collected in 2014 and 2015 were screened also for the presence of SFGR spp. by microfluidic qPCR, using primers and probes targeting the *gltA* gene (145 bp) [198]. Confirmational analyses were performed on 38 ticks with Ct values$_{gltA}$ ranging from 5.6 to 29.6, using primers targeting the 17kD antigen gene (434 bp) [204]. For details, see Study IV.

Cloning (Study II)

Amplicons in Study II were purified using the DNA Clean & Concentrator™-5 kit (Zymo Research, CA, USA) and TA-cloned using the TOPO TA cloning kit (Invitrogen, Thermo Fisher Scientific, MA, USA), kanamycin, and TOP10 cells prior sequencing. Transformants were screened for the insert using M13 primers and the PCR SuperMix High Fidelity kit (Invitrogen, Thermo Fisher Scientific, MA, USA). The M13 primers were used also as sequencing primers.

Sequencing (Studies I-IV)

Sanger sequencing (Studies I-IV)

PCR products were enzyme-treated with the illustra™ ExoProStar™ 1-step kit (Cytiva, MA, USA) to remove unincorporated primers and nucleotides before Sanger sequencing at Macrogen (Amsterdam, The Netherlands).

Metagenomic sequencing (Study IV)

Tick extracts testing positive for *F. tularensis* by microfluidic qPCR were subjected to further characterization by collaborators at FOI and Northern Arizona University (NAU), using two next generation sequencing technologies. The Illumina technology, in which short reads are generated, was used for metagenomic sequencing, i.e., characterization of the tick extracts, in a NovaSeq 6000 instrument (Illumina, CA, USA) at the SNP&SEQ Technology Platform at NGI Uppsala (Sweden). Illumina

sequencing of enriched samples was performed at NAU using a MiSeq sequencing instrument (Illumina, CA, USA). The technique is compatible with the captured-based enrichment method used in Study IV. The Nanopore technology, in which long sequence reads are generated, was used for sequencing the tick mitochondrion on the ONT MinION devise (Oxford Nanopore Technologies, Oxford, UK) at FOI (Umeå, Sweden).

Target enrichment (Study IV)

Due to few reads of *Francisella*, capture-based enrichment of the *Francisella* DNA present in the tick extracts was performed by collaborators at NAU, using short fragments (oligonucleotides) known as RNA baits that are complementary to the genome of interest. DNA extracts were uniquely indexed in approximately 300 bp libraries and subjected to RNA baits (Agilent Technologies Inc., CA, USA) designed against a *Francisella* pan-genome defined by 499 genome sequences [146]. Reads less than 120 bp were filtered out and regions with 80% homology with non-*Francisella* bacteria and rRNA genes were excluded. A 2x bait tiling frequency was included for capture optimization. Additionally, replicate copies of probes comprising of extremely high or low GC content (50%> GC <22%) were manufactured. The tick libraries were subjected to two rounds of target enrichment to maximize the purification of the *Francisella* sequences from background DNA.

Taxonomic classification of sequence reads (Study IV)

Samples from metagenomic sequencing were characterized at FOI using Kraken2 [205], a taxonomic classification system, and a custom-made database created with FlexTaxD [206] and bacteria based on the taxonomy from the Genome Taxonomy Database (GTDB) and viruses and eukaryotes from the National Center for Biotechnology Information (NCBI). Results were adjusted for different confidence scores using StringMeUp (https://github.com/danisven/StringMeUp).

Genome assembly and characterization (Study IV)

Illumina sequence reads from enriched samples were analysed at FOI using a bioinformatic pipeline controlled by the workflow manager Snakemake [207] and composed of the short read aligner tool BBMap (https://sourceforge.net/projects/bbmap/), the genome assembler tool SPAdes [208], the genome assembly improvement and variant detection tool Pilon [209], the assembly statistics tool assembly-stats (https://github.com/sanger-pathogens/assembly-stats), the genome quality assessment tool checkM [210], and the genome completeness assessment tool BUSCO [211]. In short, pre-processing of the data was performed by mapping reads to the collection of

499 *Francisella* genomes [146], keeping only mapped reads, and performing a digital normalization step. Remaining reads were *de novo* assembled and assemblies were post-processed by filtering away contigs less than 500 bp and subsequently performing a Basic Local Alignment Search Tool (BLAST)-based filtering in which contigs matching BLAST-results containing the string "*rancisella" were kept. Two rounds of polishing finalized the assembled sequences. Summary statistics were calculated and the quality of genome assemblies was evaluated. The same workflow was used for sequence reads from non-enriched samples, with the modification of keeping contigs matching "Midichloria" and "Rickettsia". Nanopore sequence reads were assembled using a long-read assembly algorithm (Flye) [212], polished using the nanopore sequencing correction tool Medaka (ONT, UK), and BLAST-based filtered where only contigs matching tick mitochondrial genomes were kept.

Phylogenetic analyses (Studies I-IV)

Tick speciation (Studies I-IV)

Obtained sequences were trimmed and assembled in the CLC Main Workbench version 7 (Qiagen, Aarhus, Denmark), and aligned using the MAFFT (Multiple Alignment using Fast Fourier Transform) algorithm (https://www.ebi.ac.uk/Tools/msa/mafft/) [213] and default settings. Partial 12S and 16S rDNA alignments were edited manually and compared to reference sequences retrieved from the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/genbank/), using standard nucleotide BLAST (BLASTN). In Study I, sequences from morphologically determined specimens of *H. marginatum*, *H. rufipes*, and *H. lusitanicum* were included as reference sequences. Phylogenies were built in the Molecular Evolution Genetics Analysis (MEGA) software (version 7) [214], using the maximum likelihood (ML) and neighbor-joining (NJ) algorithms. Model testing in MEGA7 was used for selection of substitution models combined with the models of gamma distribution (G) and invariable sites (I) in the ML analyses. For the NJ analyses, the maximum composite likelihood model (default choice) was used. The complete deletion option was chosen for gaps. In Study I, ticks were grouped according to their position in the ML phylogenies. The bootstrap analysis was conducted with 1,000 replicates.

Alkhurma hemorrhagic fever virus (Study II)

Sequences were assembled in the CLC Main Workbench 7 (Qiagen, Aarhus, Denmark) and aligned in MEGA7, using the Clustal W algorithm [215]. Reference sequences were retrieved from GenBank, using BLASTN.

Anaplasma phagocytophilum (Study III)

The phylogenetic inference of *ankA* sequences was performed at the Johannes Gutenberg University Mainz, using the MEGA X software (version 10.0.5) [216] and 398 previously published *ankA* sequences [135, 217]. *AnkA* sequences were codon-aligned by Clustal W [215], applying the PAM (percent accepted mutation) (Dayhoff) matrix [218]. Tree construction was achieved by ML and NJ algorithms, the latter with the complete deletion option. Bootstrap analysis was conducted with 1,000 replicates.

Metagenome-assembled genomes (Study IV)

Phylogenetic inference of metagenome-assembled genomes (MAGs) of *Francisella*, *Rickettsia*, *Midichloria,* and tick mitochondrion were performed at FOI using a bioinformatic pipeline. In brief, sequences of *Francisella*, *Rickettsia*, *Midichloria,* and tick mitochondria were downloaded from NCBI, using the NCBI-genome-download tool (https://github.com/kblin/ncbi-genome-download). Genome assemblies and public genomes were pairwise aligned to reference genomes (*F. t. tularensis* strain (str.) SCHUS4, *R. rickettsii* str. Iowa, *M. mitochondrii* str. IricVA, and *Hyalomma asiaticum* str. WY042-2 for *Francisella*, *Rickettsia*, *Midichloria*, and tick mitochondrial sequences, respectively), using the workflow manager Snakemake [207] and the multiple genome alignment tool progressiveMauve [219]. Alignments were set to the reference coordinates and merged into a multi-FASTA file using the genotype classifier tool CanSNPer [220]. Phylogenetic inference was conducted in IQ-TREE [221], selecting the best fit substitution models according to the Bayesian information criterion (BIC) with ModelFinder setting (-m TEST). Support values were generated by recalculating the trees with the selected models and bootstrap –b 100. The *Francisella* phylogeny was rooted in Clade 2 [146], the *Midichloria* and *Rickettsia* phylogenies in *Orientia tsutsugamushi* [222], and the tick mitochondria phylogeny in *Rhipicephalus decoloratus* [223].

Rickettsia (Study IV)

Rickettsia 17kD sequences were assembled in the CLC Main workbench 7 (Qiagen, Aarhus, Denmark) and compared to sequences deposited in GenBank, using BLASTN.

Average amino acid similarity and average nucleotide identity (Studies III and IV)

In Study III, net average amino acid similarities and net average nucleotide identities (ANI) between previously described *ankA* gene clusters [135] were calculated using the MEGA X software [216]. In Study IV, the ANI was calculated for all genomes pairwise within each dataset using the pyANI tool with the ANIb (BLASTN+) method setting [224].

Classification of bird species (Study I)

Tick-infested bird species were classified by an ornithologist at the Natural Museum of History in Sweden, according to the following variables: migration distance (resident/short; medium; long), wintering (non-breeding) region (South Europe; South Europe and North-West Africa; South Europe and Africa North of the Equator; Africa North of the Equator and South of the Sahel; Africa South of the Equator), foraging behaviour (ground; shrubs and trees; aerial), and winter habitat (open habitat; forest and shrubs; wetland) [188-190, 225-227]. The ecological characteristics of birds - such as migration distance, mating behaviour, favored habitats, foraging behaviour, and usage of resources - allow allocation of bird species into guilds, i.e., groups of species with shared characteristics [228, 229]. In Study I, bird species were further classified into guilds that were created by using different combinations of the bird characteristic variables (Table 2).

Statistics (Study I)

Data were analyzed by a statistician using descriptive measures, graphs, Chi2 tests, a generalized linear model (GLM), and the Tukey-Kramer adjustment for multiple comparisons test. The Chi2 test was applied to investigate if avian guilds differed in the degree of tick infestation and whether there was an association between avian guild and tick taxon. The GLM was applied to determine whether there was an association between bird characteristics and tick taxon. Adjustment for multiplicity at the study level was not performed, i.e., all tests were at 5%-level and there was no adjustment to control for Type-I error (false positives). However, all tests comparing more than two groups were adjusted for multiplicity issues. The Tukey-Kramer adjustment for multiple comparisons test was performed at test level and used for comparing bird characteristic variables. All results should therefore be viewed as exploratory. GLM was preferred over non-parametric test since GLM is better suited for adjusting for several covariates. For some of the GLM tests, a Wilcoxon test was used as a sensitivity analysis. A p value of <0.05 was considered statistically significant. The statistical analyses were conducted in SAS (Statistical Analysis Software) version 9.4 (SAS Institute Inc., NC, USA**).**

Table 2. Guilds of the tick-infested bird species [230]. The guilds were created based on information in the Handbook of the birds of Europe, the Middle East and North Africa [188-190, 225-227]. Table by Hoffman et al. 2021 [230] and licensed under CC BY-4.0 (https://creativecommons.org/licenses/by/4.0/).

	Bird characteristics				
Guild	**Migration distance**	**Wintering region**	**Foraging behaviour**	**Winter habitat**	**Bird species (Common name)**
A	Long	Africa North of the Equator and South of the Sahel	Shrubs and trees	Wetland	*Acrocephalus schoenobaenus* (Sedge warbler) *Acrocephalus scirpaceus* (Eurasian reed warbler)
B	Resident/short	South Europe	Ground	Wetland	*Charadrius alexandrinus* (Kentish plover)
C	Long	Africa North of the Equator and South of the Sahel	Ground	Open habitat	*Anthus trivialis* (Tree pipit) *Falco naumanni* (Lesser kestrel) *Monticola saxatilis* (Rufous-tailed rock thrush) *Oenanthe oenanthe* (Wheatear) *Saxicola rubetra* (Whinchat) *Upupa epops* (Eurasian hoopoe)
D	Long	Africa South of the Equator	Ground	Open habitat	*Motacilla flava* (Yellow wagtail)
E	Long	Africa South of the Equator	Arial	Forest and shrubs	*Caprimulgus europaeus* (European nightjar) *Muscicapa striata* (Spotted flycatcher)
F	Long	Africa North of the Equator and South of the Sahel	Shrubs and trees	Forest and shrubs	*Ficedula hypoleuca* (European pied flycatcher)
G	Long	South Europe and Africa North of the Equator	Shrubs and trees	Forest and shrubs	*Sylvia atricapilla* (Blackcap)
H	Medium	South Europe and Africa North of the Equator	Shrubs and trees	Forest and shrubs	*Phylloscopus collybita* (Chiffchaff)
I	Long	Africa South of the Equator	Shrubs and trees	Forest and shrubs	*Ficedula albicollis* (Collared flycatcher) *Hippolais icterina* (Icterine warbler) *Oriolus oriolus* (Eurasian golden oriole) *Phylloscopus sibilatrix* (Wood warbler) *Sylvia borin* (Garden warbler)
J	Long	Africa North of the Equator and South of the Sahel	Ground	Forest and shrubs	*Lanius senator* (Woodchat shrike) *Luscinia megarhynchos* (Nightingale) *Phoenicurus phoenicurus* (Common redstart) *Streptopelia turtur* (Turtle dove) *Sylvia communis* (Whitethroat)
K	Medium	South Europe and North-West Africa	Ground	Forest and shrubs	*Erithacus rubecul*a (European robin) *Turdus philomelos* (Song thrush)

Results and Discussion

Association between avian ecology and tick taxon (Study I)

The ecology of the vertebrate host contributes to the prevalence of tick infestation and the geographical range expansion of ticks. Avian hosts are more likely to be involved in long-distance range expansion of ticks compared to terrestrial hosts due to their migration patterns. In Study I, we addressed the association between bird ecology and tick taxon by investigating which tick taxa northbound migratory birds disperse in the AWP migration system, by classifying bird species into guilds and using molecular and statistical methods.

During the spring migration seasons of 2014 and 2015, 10,209 birds of 27 different species were trapped, ringed, and inspected for ticks at bird observatories in Spain, Italy, Greece, and Israel (Fig. 12). A total of 575 ticks were found attached to 244 (2.4%) birds, a number that may be an underestimation due to varying success of ringers to examine the birds for ticks. Most (98.0%; 239/244) of the tick-infested birds were long-distance intercontinental migrants that winter in sub-Saharan Africa. When trapped at the stopover sites most of them had likely been engaged in a flight over the Sahara. Of the collected ticks, 88.5% were determined to genus or species level and grouped (n = 11) according to their positioning in 12S rDNA phylogenies. A majority of the collected ticks was found to be *H. rufipes* (77.7%) and most likely were of Afrotropical origin. Association between bird ecology and tick taxon was investigated by classifying the tick-infested bird species according to the following variables: migration distance, wintering region, foraging behaviour, and winter habitat. As only one individual represented some of the tick-infested bird species, bird species were further classified into guilds (n = 11), by using different combinations of the bird characteristic variables. We found that guilds differed in their degree of tick infestation ($p = 0.00014$), that there was an association between avian guilds and tick taxon ($p < 0.0001$), and that guilds containing only long-distance migrants with wintering regions in Africa were associated with *H. rufipes* ($p < 0.0001$), a tick species with a preference for a dry open country habitat [79, 231] and a known vector of CCHFV and SFGR [232]. We also found an association between the winter habitat of the avian host and *H. rufipes* ($p =$

0.0032), and that birds occupying an open winter habitat had more *H. rufipes* (p = 0.014) as compared to birds wintering in a habitat comprising forest and shrubs (p = 0.82). It was also demonstrated that birds wintering in wetlands had more *H. rufipes* (p = 0.046) than birds in a forest and shrub habitat (p = 0.82), which may be related to foraging in *H. rufipes* habitats and/or being less selective of habitat during the migration.

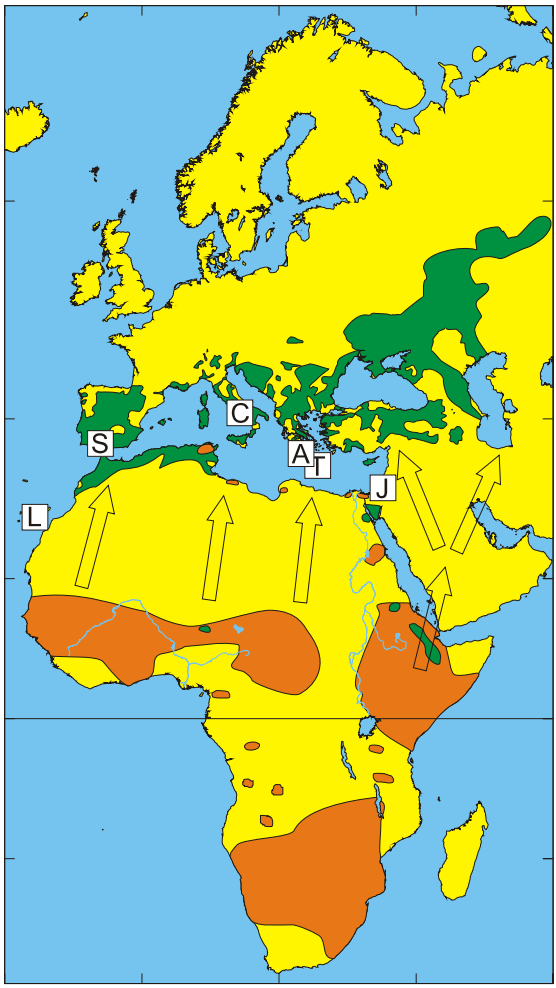

Figure 12. Map of the African-Western Palaearctic region, showing sites for trapping of birds and collection of infesting ticks as white squares: L) Canary Islands, Spain; S) continental Spain; C) Capri, Italy; A) Antikythira, Greece; T) Crete, Greece; and J) Jerusalem, Israel. Known distribution areas of *Hyalomma marginatum* are indicated by green and for *Hyalomma rufipes* by orange, based on tick maps from the European Centre for Disease Prevention and Control (ECDC) and Walker et al. [48, 232]. Arrows represent simplified northward migratory routes of birds. The black line indicates the Equator. Figure by Hoffman et al. 2021 [230] and licensed under CC BY-4.0 (https://creativecommons.org/licenses/by/4.0/).

Considering that more than a billion birds may cross the Mediterranean Sea during spring migration [12], millions of infesting ticks are likely to be transported from Africa to Europe and Asia each year. Adult *H. rufipes* ticks have been detected outside of their endemic areas, with reports from Central and Northern Europe [50, 233-236]. The presence of adult specimens outside endemic areas is likely a result of avian-associated introduction of immatures and prevailing weather conditions that allowed progression of the life cycle. The findings in Study I suggest that there is an association between bird ecology and tick taxon and that long-distance migrants are involved in the transportation of immature *H. rufipes* to countries at higher latitudes. With a warmer climate and available hosts for adults, the likelihood of completion of the life cycle and establishment of permanent *Hyalomma* populations in the central and northern regions of Europe will increase.

Alkhurma hemorrhagic fever virus-like RNA in avian-associated *Hyalomma rufipes* (Study II)

An African origin has been suggested for AHFV [106] and ticks have been proposed as vectors [72, 73]. In the light of this and recent reports of the presence of AHF and AHFV on the African continent [95-99], the potential role of AWP birds in the dispersal of AHFV to novel regions was addressed in Study II, by molecular investigation of the presence of AHFV RNA in ticks infesting birds migrating from Africa to Europe and Asia during the spring migration of 2009, 2010, 2014, and 2015.

A total of 36,893 birds (2009-2010: n = 14,824; 2014-2015: n = 22,069) were trapped at bird observatories in Spain, Italy, Greece, Turkey and Israel, and 1,771 infesting ticks (2009-2010: n = 747; 2014-2015: n = 1,024) were collected. RNA similar to AHFV and KFDV was detected by qPCR in six fully engorged *H. rufipes* (four nymphs, one larva, and one adult) collected from four long-distance migratory bird species with wintering areas in sub-Saharan Africa and breeding areas in Europe (Fig. 13). The nymphs were collected from an eastern woodchat shrike (*Lanius senator niloticus*; swe: rödhuvad törnskata) and two sedge warblers (*Acrocephalus schoenobaenus*; swe: sävsångare) while the larva was collected from a western yellow wagtail (*Motacilla flava*; swe: gulärla), all trapped on the Greek island Antikythira in 2010. One of the sedge warblers carried two of the AHFV/KFDV RNA containing nymphs. The fully engorged adult tick was collected from a common redstart (*Phoenicurus phoenicurus*; swe: rödstjärt) trapped in the Kizilirmak Delta in Turkey in 2014.

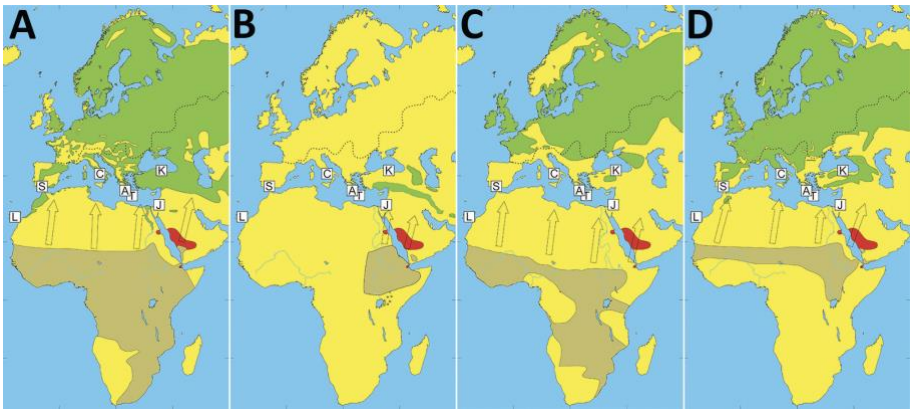

Figure 13. Maps of the African-Western Palaearctic region, showing wintering areas of birds infested by *Hyalomma* ticks testing positive for Alkhurma hemorrhagic fever virus (AHFV) RNA in light brown and breeding areas in green. Simplified springtime bird migratory routes are presented as arrows. The infested bird species were: **A**) western yellow wagtail (*Motacilla flava*), **B**) eastern woodchat shrike (*Lanius senator niloticus*), **C**) sedge warbler (*Acrocephalus schoenobaenus*), and **D**) common redstart (*Phoenicurus phoenicurus*). The sites for trapping of birds and collection of infesting ticks are labelled: L) Canary Islands, Spain; S) continental Spain; C) Capri, Italy; A) Antikythira, Greece; T) Crete, Greece; K) Kizilirmak Delta, Turkey, and J) Jerusalem, Israel. Red areas indicate where AHFV has been detected. The approximate northern geographic boundary of *Hyalomma marginatum* sensu lato ticks is indicated by a dashed line [191, 232]. The maps were created by information from the Handbook of birds of Europe, the Middle East and North Africa [189, 237, 238]. Figure by Hoffman et al. 2018 [239].

Since *H. rufipes* is a two-host tick species that molt from larva to nymph on the same host, the engorged larva and nymphs had probably fed only on the bird that they were attached to and could have acquired the virus horizontally from its avian host, transovarially from its mother, or during co-feeding [19, 240]. Co-feeding is less likely in this case since the two nymphs attached to the same host were not feeding close to each other. Furthermore, transovarial transmission is considered to be a rare event in nature [19]. In contrast to the immature stages, the adult tick could have acquired the virus also from a previous host.

Comparative sequence analysis revealed two identical 5′ UTR sequences of 72 bp with a high identity to reference sequences of AHFV and KFDV, except for a region of seven consecutive mismatching nucleotides and a deletion of 14 nucleotides (Fig. 14). A difference of one and three or four nucleotides was observed in the novel preM sequences of 31 bp as compared with available AHFV and KFDV sequences, respectively (Fig. 14). Acquisition of further sequence information was not possible. We suggest that the sequences represent AHFV since it is ecologically and geographically more likely as AHFV is endemic on the Arabic Peninsula while KFDV is endemic in India

[104], even though differentiation between AHFV and KFDV was impossible due to the short sequences.

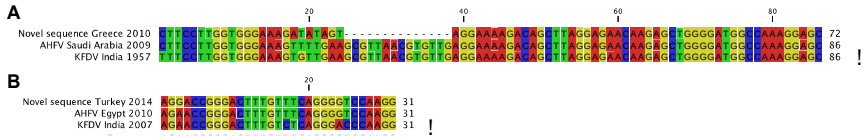

Figure 14. Partial nucleotide alignments of the 5´ untranslated region (**A**) and the premembrane region (**B**) with reference sequences of Alkhurma hemorrhagic fever virus (AHFV) and Kyasanur Forest disease virus (KFDV) obtained from GenBank and study sequences obtained from *Hyalomma rufipes* ticks collected from birds trapped on the Greek island Antikythira in 2010 (**A**) and in the Kizilirmak Delta in Turkey in 2014 (**B**). Figure by Hoffman et al. 2018 [239].

Information on possible vectors and vertebrate hosts is of importance for understanding the ecology and geographical distribution of a zoonotic vector-borne pathogen. Regarding the ecology of AHFV much is still unclear and remains to be investigated. The findings in Study I suggest a role for birds in the ecology of AHFV and a potential mechanism for the dispersal of the virus to new regions, including Europe and Asia Minor.

Divergent variant of *Anaplasma phagocytophilum* in an avian-associated *Ixodes* species tick (Study III)

A. phagocytophilum is an emerging zoonotic bacterium that causes febrile illness in humans and other mammals, mainly domesticated animals. The bacterium has a wide geographical distribution [58, 111] and a complex ecology involving different vectors, mammalian hosts, and genetic variants that display different host and vector tropism. In Study III, we addressed the role of northbound migratory birds in the dispersal of tick-borne *A. phagocytophilum* in the AWPR, by molecular investigation of the presence of *A. phagocytophilum* DNA in ticks infesting birds migrating from Africa to Europe during the 2010 spring migration season.

A total of 7,354 birds were trapped on the islands Antikythira (Greece) and Capri (Italy) and 2.8% were infested by ticks. *A. phagocytophilum* DNA was detected by PCR in one (0.28%; 1/358) of the collected and analyzed ticks, an *Ixodes* sp. tick of unknown life stage collected from a woodchat shrike (*Lanius senator senator*; swe: rödhuvad törnskata) trapped on the Greek island Antikythira, situated north-west of Crete. The woodchat shrike is a long-distance migrating passerine that winters in sub-Saharan Africa, breeds in many areas around the Mediterranean Sea and in the Middle East [238], and uses Antikythira only as a stopover site [241]. Phylogenetic inference of the *Ixodes* sp. tick revealed that the tick grouped with tick species of the *I. ricinus* complex and formed a clade with a bootstrap support of 68% with an *I. gibbosus* 16S rDNA sequence. A 5%-divergence between the study sequence and that of *I. gibbosus* was observed. The interspecific cut-off divergence levels in mitochondrial ribosomal genes are still to be determined for different tick genera [145]. Whether the observed divergence between the study 16S rDNA sequence and that of *I. gibbosus* reflects different species, subspecies, or geographic origins of the specimens [195] remains to be investigated. Furthermore, whether the tick species is involved in the transmission of *A. phagocytophilum* in the ecosystems of the southern parts of the Mediterranean basin and Africa and whether it transmits pathogens to human and animals remains to be studied. For this, its presence in the region, vector competence, and efficiency of transstadial transmission of *A. phagocytophilum* should be investigated.

In *ankA* and *groEL* analyses, variants of *A. phagocytophilum* found in rodents and shrews and in birds and bird-derived ticks have been found to differ [133, 135, 138, 242], possibly indicating divergent enzootic cycles. We found that the *ankA* sequence of the *Ixodes* sp. tick exhibited highest similarity to Cluster 5 (net ANI: 82%) with sequences from rodents and shrews and Cluster 4 (net ANI: 80%) with sequences from ruminants (Fig. 15), and that it formed an outgroup to Cluster 4, leading to the suggestion that the *A. phagocytophilum*

variant represents a reservoir-driven enzootic cycle with birds as hosts, an enzootic cycle resulted by geographic isolation, or an influx from another area by avian migration.

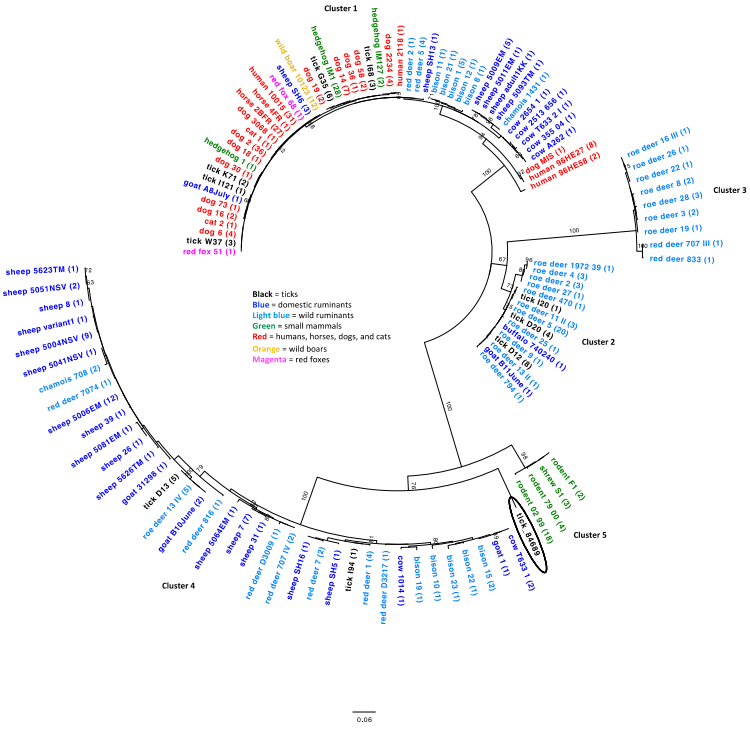

Figure 15. Phylogenetic inference of *Anaplasma phagocytophilum*, using partial *ankA* sequences. Sequence names are colored by host group. The study *ankA* sequence is circled. The tree construction was achieved in MEGA X, using the maximum likelihood algorithm. Bootstrap values <60% are not presented. The scale bar indicates the number of substitutions per site. Figure by Hoffman et al. 2020 [243].

Most (92.5%) of the collected ticks belonged to the *H. marginatum* complex and most likely represented the species *H. rufipes* and *H marginatum*. Furthermore, most (87%) of the *Hyalomma* specimens were of immature life stages. *A. phagocytophilum* DNA has previously been detected in adult *H. marginatum* ticks collected from animals in France, Israel, and Africa [55, 244, 245], but was not detected in any of the *H. marginatum* s.l. ticks in Study III. Thus, the findings of Study III did not provide evidence for immature *H. marginatum* s.l. ticks and birds having a major role in the ecology and northward dispersal of tick-borne *A. phagocytophilum* in the AWPR. However, the result of Study III gives support the theory of distinct enzootic cycles of *A. phagocytophilum* involving birds and bird-associated ticks [133, 242, 246].

Co-occurrence of *Francisella* and spotted fever group *Rickettsia* in avian-associated *Hyalomma rufipes* (Study IV)

Ticks are known to harbor both pathogenic and symbiotic bacteria and may contain multiple bacterial species. The bacterial genus *Francisella* is present in different tick taxa and consists of closely related non-pathogenic and pathogenic species, such as FLEs and the zoonotic species *F. tularensis*, respectively. The bacterial genus *Rickettsia* comprises the SFG, whose members are transmitted to humans by different tick genera and are considered to be emerging human pathogens in Europe. In Study IV, we addressed the role of migratory birds in the dispersal of ticks carrying *Francisella* spp. in the AWPR, by molecularly investigating and characterizing *Francisella* spp. in ticks infesting birds migrating from Africa to Europe and Asia during the spring migration of 2014 and 2015. We also investigated co-occurrence of *Francisella* and SFGR.

The birds were trapped at stopover sites in Spain, Italy, Greece, and Israel (Fig. 12). A total of 575 ticks were collected from mainly long-distance migrants and *H. rufipes* was found to be the most common tick species at 77.7%. Based on previous findings, the majority of the ticks were assumed to be immatures. We screened the ticks for the presence of *Francisella* spp. by microfluidic qPCR and the results indicated a high prevalence of *Francisella* in *H. rufipes* (*fopA*+: 76.7%; 343/447) and the presence of *F. tularensis* DNA in two *H. rufipes* specimens (*fopA*+*lpnA*+: 0.3%; 2/575). The putatively *lpnA* positive ticks were subjected to subsequent confirmational qPCR assays (genus- and species-specific), genome enrichment, and metagenomics characterization using Illumina technology, in which the presence of *F. tularensis* could not be confirmed. Analysis of the primer and probe regions of the study FLE genomes revealed several mismatches, indicating that the used qPCR assays were suboptimal. Phylogenetic inference of the two metagenome-assembled *Francisella* genomes detected in the *H. rufipes* specimens revealed highest resemblance to the FLE group that belongs to Clade 1 of *Francisella*, which includes the FLE species found in the soft tick *O. moubata* (FLE-Om) and the hard tick *A. maculatum* (FLE-Am). The FLEs of *H. rufipes* (FLE-Hr) had the highest identity to FLE-Om (ANIb: 96.7 - 97.0%), indicating that they belong to the same species [247]. Phylogenetic inference and calculations of ANI of the two metagenomic-assembled tick mitochondrial genomes revealed high resemblance to *H. rufipes* (ANIb: 98.0 - 98.1), confirming the initial speciation in which the 12S rDNA gene was used as genetic marker.

We found that presence of *Francisella* did not appear to have prevented occurrence by additional intracellular bacteria, as 47.1% (271/575; *fopA*+*gltA*+) of the investigated ticks and 50.6% (226/447; *fopA*+*gltA*+) of the *H. rufipes* ticks seemed to carry both *Francisella* and SFGR spp. A similar observation has previously been reported [170]. Phylogenetic inference and calculations of the ANI of the two metagenome-assembled *Rickettsia* genomes detected in *H. rufipes* revealed a high resemblance to *R. aeschlimannii* (ANIb: 98.8 - 99.9%), a bacterium previously detected at similar prevalences in *H. marginatum* s.l. ticks infesting northward migratory birds [53, 248] and reported to cause infection in humans [249, 250]. *Midichloria* was also detected in the two *H. rufipes* specimens. Phylogenetic inference and ANI calculations of the two metagenome-assembled *Midichloria* genomes revealed resemblance to *M. mitochondrii* (ANIb: 91.5 - 92.3%), an intracellular bacterium whose influence on ticks and potential to cause infection in mammals are not well defined. DNA of bacteria related to *M. mitochondrii* has previously been detected in *Hyalomma* ticks [181, 182]. Furthermore, avian-associated *H. marginatum* s.l. ticks and their trans-Saharan migratory avian hosts, trapped in Italy during spring migration, have been found to carry *Midichloria* sp. DNA [251].

The findings of Study IV suggest that migratory birds contribute to the geographical spread of ticks, including *H. rufipes*, containing *Francisella* and SFGR spp. in the AWPR. Furthermore, the findings illustrate the complexity of designing primers for bacteria with unknown diversity, and suggest that immature *H. rufipes* do not serve as vectors or contribute to the transmission of *F. tularensis* and that migratory birds do not contribute to the northward dispersal of *F. tularensis*-infected ticks in the AWPR.

Conclusion

The mechanisms that drive the emergence of zoonotic infectious diseases are complex and vector-borne infections are a growing concern globally. The life cycle of vector-borne pathogens involves the pathogen, the vector, and the vertebrate host. Identifying the species that act as vectors, vertebrate hosts, and vehicles of vector-borne zoonotic pathogens are imperative for elucidating the transmission cycles, dispersal mechanisms, and establishment of vector-borne pathogens in nature. Introduction of pathogens and vector species, abundance of vectors and hosts, and adaptation of pathogens to new vectors and vertebrate hosts are determinants in the dispersal and establishment of vector-borne infections. An important geographical determinant of ticks is the climate. With global warming, the geographical distribution and abundance of ticks could increase in the Western Palaearctic (WP). The influx of millions of African ticks and their microorganisms into the WP via avian transport might therefore pose a greater risk for the public and animal health in the future. An integrated approach in surveillance of avian-imported tick species and TBPs and the establishment of exotic tick species in the WP should therefore be established.

In this thesis we have investigated the dispersal of ticks and their microorganisms in the AWPR by northbound spring migratory birds. In Study I, we showed that long-distance migratory birds are involved in the dispersal of immature *H. rufipes* ticks in the AWPR and that there is an association between the ecology of the avian host and the infesting tick taxon in the investigated system, i.e., bird species that share the same habitat as *H. rufipes* during the non-breeding season or spend time in the same habitat as *H. rufipes* during the migration are more likely to become infested by *H. rufipes*. With a warmer climate and availability of hosts for adults, the likelihood of completion of the life cycle and establishment of permanent *Hyalomma* populations in central and even northern regions of Europe will increase. The results of Study I therefore highlight the need of establishing surveillance programs for monitoring the risk of introduction and establishment of *H. rufipes* in the WP. Our study suggests that migratory bird species wintering in African open habitats and wetlands are good candidates for monitoring the introduction.

In Study II, we reported to our knowledge the first molecular evidence of RNA similar to AHFV in *H. rufipes* ticks infesting northbound migrating birds trapped at two stopover sites in the Mediterranean basin. Our study shows that AHFV may be dispersed over large geographical areas via ticks on trans-Saharan migratory birds, which motivates further investigations of AHFV ecology, including the role of long-distance passerine birds as reservoirs or vehicles of potentially infected ticks. Furthermore, our findings together with clinical and serological cases and AHFV RNA positive ticks in Africa, suggest a wide geographic distribution of the virus in Africa and in novel regions, which motivate increased surveillance for this pathogenic emerging zoonotic virus outside of endemic areas.

In Study III, we reported the detection of *A. phagocytophilum* DNA in an *Ixodes* sp. tick found feeding on a trans-Saharan migratory bird trapped at a stopover site in the Mediterranean Sea. Sequences of the detected *Ixodes* sp. tick and *A. phagocytophilum* variant differed from published sequences, which raised questions as to whether these are both novel variants and whether this could reflect a divergent enzootic cycle of *A. phagocytophilum* with birds as hosts, geographic isolation, or influx from another area by avian migration. Further investigations of both *A. phagocytophilum* and *Ixodes* ticks in the study area and surrounding regions, including the African continent, are needed to address these questions. The findings of Study III did not provide evidence for immature *H. marginatum* s.l. ticks and birds having a major role in the ecology and northward dispersal of tick-borne *A. phagocytophilum* in the AWPR.

Detection of genetic material of pathogens in ticks collected from a host could reflect infection in the host via the tick´s blood meal or carriership of the pathogen by the tick. However, detection of gene segments of pathogens does not imply presence of viable microorganisms or vector competence. Thus, our findings are insufficient to establish the role of *H. rufipes*, the *Ixodes* sp., and migratory birds, including their competence as vectors and hosts, in the life cycle of AHFV and *A. phagocytophilum*. However, our data demonstrate potential involvement of migrating birds in the dispersal of mentioned pathogens in the Mediterranean basin and between Africa, Europe, and Asia. Our data also demonstrate that genetic variants can be detected by screening avian-associated ticks and that the pathogen-tick-avian system thereby could possibly aid in identifying enzootic cycles.

In Study IV, we showed that northbound migratory birds contribute to the geographical spread of *H. rufipes* carrying both *Francisella* and SFGR spp. in the AWPR. The phylogenetic inference and calculations of ANI of the FLE, *Rickettsia*, and *Midichloria* MAGs detected in the hard tick *H. rufipe*s revealed resemblance to an FLE detected in the soft tick *O. moubata*, *R.*

aeschlimannii, and *M. mitochondrii*, respectively. The influence of FLEs on *H. rufipes* ticks and their interaction with pathogenic and non-pathogenic symbiotic bacteria, such as *R. aeschlimannii* and *Midichloria*, remains to be investigated. The results of Study IV also suggest that immature *H. rufipes* do not serve as vectors or contribute to the transmission of the tularemia-causing agent *F. tularensis* and that migratory birds do not contribute to a northward dispersal of *F. tularensis*-infected ticks in the AWPR. Furthermore, the results illustrate the complexity of designing primers for bacteria with unknown diversity and indicate that the genetic 12S rDNA marker is suitable for speciation of *H. rufipes*.

Since vector-borne zoonotic infection systems can only be understood by elucidating the details of the systems, future research on tick-borne zoonotic infection systems should have an integrated approach and focus on the systems´ biological, ecological, clinical, and epidemiological features. Furthermore, identification of involved vectors, vertebrate hosts, and transmission mechanisms as well as increased awareness and vector control are imperative for the prevention of infectious diseases where there is an underreporting of clinical cases and absence of treatment and/or vaccines, as is the case for AHF and HGA. Hopefully, the knowledge gained from this thesis will be useful for both the scientific community and the public by adding to the understanding of tick-borne infections.

Future considerations

Continued and targeted sampling

In this thesis, we studied the dispersal of ticks and their microorganisms by northbound migratory birds, mainly trans-Saharan migrants, in the western part of the APMS. Birds that have a more eastern migration route were therefore not included. Future research should therefore focus on the Asia/East Africa flyway that connects the African and Asian continents to get a broader understanding of the dispersal role of spring migratory birds in the APMS. For this, Mongolia would be an ideal study site since migratory birds also enter Mongolia via three major flyways: the Asia/East Africa flyway, the Central Asia flyway, and the East Asia/Australasia flyway (Fig. 1). Studies on KFDV, TBEV, and severe fever with thrombocytopenia syndrome virus would then be of interest. A collaboration with ornithologist in Mongolia has been established and collections of ticks from spring migrating birds have been performed.

It is important to perform research on and surveillance of ticks and tick-borne microorganisms, including different genotypes circulating in nature, as they may turn out to be of public health interest. By performing yearly and structured collections of ticks from migrating birds, livestock, and the vegetation at the stopover sites and in non-endemic areas of *H. rufipes*, in areas where adult *H. rufipes* ticks have been reported, the findings of this thesis could be further investigated and surveillance of establishment of exotic tick vectors such as *H. rufipes* in the WP could be performed. Targeted sampling has been initiated on Antikythira for identification of possible divergent enzootic cycle of *A. phagocytophilum*, by collecting ticks also from the vegetation.

Egypt and Saudi Arabia import livestock, such as cattle, sheep, and camels from East African countries (e.g., South Sudan, Ethiopia, Djibouti, and Somalia) to meet an increasing demand for animal food sources and transport. This trade of livestock creates biological links between the African continent and the Arabian Peninsula. By performing targeted sampling of potential animal hosts at camel markets and livestock farms located at or nearby Egyptian seaports, analyzing collected ticks using next generation sequencing,

and performing serological investigations the ecology and the concept of an African evolutionary origin of AHFV could be further investigated.

Vector competence

Future studies should focus on the vector competence of *H. rufipes* and the investigated *Ixodes* sp. concerning AHFV and *A. phagocytophilum*, respectively, as detection of genetic material of microorganisms in an engorged tick does not imply presence of viable microorganisms and could reflect infection in the host. Future studies should perform detection, quantification, and viability testing of the microorganisms in the primary organs and barriers for pathogen acquisition (the midgut) and transmission (the salivary glands) in ticks, since it could aid in the determination whether acquisition of microorganisms was from the host or the tick as well as provide some insight into the vector competence [252]. Droplet digital PCR (ddPCR) (Bio-Rad, CA, US) should be evaluated for detection and absolute quantification of microorganisms in the different tick tissues [253].

Virus isolation

Analyses of the RNA integrity in a small set of investigated avian-associated ticks revealed RNA degradation and indicated that the RNA*later*™ did not penetrate the tick enough to preserve the RNA. Furthermore, RNA*later*™ may prevent isolation and propagation of enveloped RNA viruses. For future phenotypic studies and whole genome sequencing, which would result in an increased amount of information and lead to deepened knowledge about the investigated viral agent, ticks should therefore be stored alive or directly in -80°C after the collection.

Reservoir competence

In order to define the role of migratory birds in the life cycle of AHFV and *A. phagocytophilum*, investigations on reservoir competence (i.e., the ability of an infected host to make an infectious agent available to a vector) including initial studies identifying infection in birds through serological evidence, level and duration of viremia and bacteriemia, and isolation of the infectious agent are needed. For this sampling of host blood will be necessary. If birds are proven to be competent hosts of AHFV and *A. phagocytophilum*, they could play an important role in their dispersal as well as in the establishment of novel enzootic foci.

Svensk sammanfattning

Fåglar som uppehåller sig i områden med säsongsvarierande klimat och tillgång på föda förflyttar sig, säsongsmigrerar, långa sträckor för att maximera sina chanser för överlevnad. Det afrikanska-palaearktiska migrationssystemet som i huvudsak består av tre migrationsvägar - en västlig, en central och en östlig - är det största migrationssystemet i världen, i vilken biljoner fåglar förflyttar sig två gånger per år mellan häckningsområden i norr och övervintringsområden i syd. Förflyttningen skapar naturliga länkar mellan geografiska områden, knyter samman olika biomer, och möjliggör spridning av blodsugande fästingar och de mikrorganismer som fästingarna bär på. Fästingar är effektiva spridare av bakterier, virus och parasiter. De kan även vara bärare av endosymbiontiska bakterier som kan vara nödvändiga för fästingens överlevnad. Fästingburna sjukdomar är de mest spridda och vanligaste vektorburna sjukdomarna i Europa och deras geografiska utbredning ökar.

I denna avhandling har vi studerat vilka fästingar och fästingburna mikroorganismer som nordmigrerande fåglar transporterar i den afrikanska-västpalaearktiska regionen (AVPR). Nordmigrerande fåglar fångades in med slöjnät på olika fågelstationer i medelhavsregionen samt vid Svarta havet under vårmigrationen 2009, 2010, 2014 och 2015. Efter fångst utförde licensierade ringmärkare ringmärkning av fåglarna samt insamling av parasiterande fästingar. Därefter återbördades fåglarna till frihet. Fästingarna analyserades för olika mikroorganismer i Sverige, Frankrike och Tyskland med hjälp av multiplexad mikrofluidik-realtids-PCR, realtids-PCR, PCR samt Sanger- och helgenomssekvensering.

Avhandling består av fyra studier och syftet med första studien var att undersöka vilka taxa av fästingar som nordmigrerande fåglar sprider i den AVPR och om fågelns ekologi är associerad med fästing-taxon. Fästingar insamlade 2014 och 2015 bestämdes till genus eller art molekylärt med genmarkören 12S rDNA och delades in i olika grupper med hjälp av fylogenetisk analys. Fågelarter klassificerades utifrån variablerna: migrationsdistans, övervintringsområde, födosöksbeteende och vinterhabitat. Då vissa arter endast var representerade av enstaka fågelindivider så skapades grupper (gillen) för fågelarter med delade karaktärer. Därefter analyserade association mellan fågelgille och fästingbärarskap. Majoriteten av fåglarna

med fästing var långdistansflyttare med övervintringsområden i subsahariska Afrika och den vanligaste fästingarten var *Hyalomma rufipes*. *H. rufipes* förekommer bland annat i torra områden i Afrika och är en känd vektor av Krim-Kongoviruset som orsakar hemorragisk feber med hög dödlighet och som är endemisk i Afrika, sydöstra Europa, mellanöstern och Asien. Vi fann att fågelgillen skiljde sig åt i grad av fästingbärarskap, att det fanns en association mellan fågelgillen och fästing-taxon samt att fågelgillen med endast långdistansflyttare var associerade med *H. rufipes*. Vi fann även en association mellan vinterhabitat och *H. rufipes* samt att fåglar som uppehöll sig i ett öppet habitat och våtmarker under vintersäsong hade fler *H. rufipes* än fåglar med ett vinterhabitat bestående av skog och buskar. Att fåglar som uppehöll sig i våtmarker under vintersäsongen hade fler *H. rufipes* beror troligtvis på att de födosökte i samma habitat som *H. rufipes* och/eller vistades i samma habitat som *H. rufipes* under migrationen. Fynd av vuxna *H. rufipes*-fästingar har rapporterats från centrala och norra Europa där *H. rufipes* normalt inte förekommer. Vår studie visar på att de kan ha transporterats dit av långmigrerande fåglar.

Alkhurmaviruset orsakar hemorragisk feber i Saudi Arabien på den arabiska halvön. Lite är känt om viruset men ett afrikanskt ursprung har föreslagits och det finns indikationer på att fästingar kan vara vektorer. Kliniska fynd och molekylär detektion av viruset i fästingar plockade från boskap har gjorts på senare tid på den afrikanska kontinenten. Syftet med den andra studien var att undersöka om fåglar kan vara involverade i den geografiska spridningen av Alkhurmaviruset. Fästingar insamlade 2009, 2010, 2014 och 2015 analyserades för viruset arvsmassa molekylär och vi rapporterade så vitt vi vet det första fyndet av RNA likt Alkhurmaviruset och dess nära släkting Kyasanurviruset, som förekommer i Indien, i sex *H. rufipes* plockade från långdistansflyttare fåglar med övervintringsområden i subsahariska Afrika. De yngre livsstadierna, fyra nymfer och en larv, var insamlade på den grekiska ön Antikythira 2010 medan den vuxna fästingen samlades in i Turkiet 2014. En av fåglarna bar på två positiva nymfer. I och med att fåglars roll i viruset ekologi är okänd kan fästingarna ha plockat upp viruset från sin värd, fått det från sin moder eller genom att suga blod nära en infekterad fästing på en icke-viremisk värd. De två senare är mindre troliga på grund av att överföring från fästingmoder till avkomma troligtvis förekommer sällan i naturen samt att fästingarna på samma fågel inte satt nära varandra. Den vuxna fästingen kan ha fått i sig viruset även från en tidigare värd. Vi kunde inte fastslå om de korta sekvenserna representerade Alkhurma- eller Kyasanurviruset på grund av för lite genetisk information men vi föreslår att sekvenserna representerar Alkhurmaviruset då det är ekologiskt och geografiskt mest troligt. Vår studie visar på att fåglar kan ha en roll i Alkhurmavirusets ekologi och spridning till nya geografiska områden, så som Europa och Mindre Asien.

Anaplasma phagocytophilium är en zoonotisk intracellulär bakterie som orsakar febril sjukdom i människor och domesticerade djur så som får, hundar, katter och hästar. Bakterien har en världsomspännande utbredning och fästingar i *Ixodes ricnus*-komplexet verkar både som enzootiska och zoonotiska vektorer. Det är troligt att ett reservoarvärddjur behövs i livscykeln då det inte finns tillräckligt med vetenskapliga bevis för att *A. phagocytophilium* sprids från fästingmoder till avkomma. Olika genetiska varianter av *A. phagocytophilium* förekommer och de har varierande grad av patogenicitet och preferens för vektorer och värddjur. Lite är känt om vilka djur som utgör värddjur för bakterien i Europa. Fåglar har föreslagits ha en begränsad reservoarroll. Syftet med tredje studien var att studera migrerande fåglars roll i den nordliga spridningen av fästingar som bär på *A. phagocytophilium* i den AVPR. Fågelassocierade fästingar insamlade 2010 på den italienska ön Capri och den grekiska ön Antikythira analyserades för *A. phagocytophilum* och DNA från bakterien detekterades i en av fästingarna. Artbestämning av fästingen var ej möjlig men fylogenetisk inferens visade på tillhörighet till *I. ricinus*-komplexet. *Ixodes*-fästingen plockades från en rödhuvad törnskata fångad på Antikythira. Den rödhuvade törnskatan är en transsaharaflyttare med häckningsområden i Europa. Fylogenetisk inferens av *ankA*-genen visade att den detekterade *A. phagocytophilum*-sekvensen skiljde sig från andra publika sekvenser och att den hade högst likhet med sekvenser från gnagare och sorkar samt sekvenser från idisslare. Vi föreslår därför att den här varianten av *A. phagocytophilum* representerar en avgränsad enzootisk cykel med fåglar som värddjur eller att den är ett resultat av geografisk isolering. Den skulle även kunna vara ett resultat av introduktion från annat geografiskt område via fågelmigration. Vi kunde inte finna bevis för att yngre livsstadier av fästingkomplexet *H. marginatum*, vanligast förekommande i vår studie, har en betydande roll i *A. phagocytophilum*s ekologi eller nordlig spridning i den AVPR. Dock ger studien ytterligare stöd till teorin om en avgränsad enzootisk cykel med fåglar som värddjur.

Genuset *Francisella* består av närbesläktade patogena arter, så som bakterien *F. tularensis* som kan vara fästingburen och orsaka harpest i människan, och icke-patogena arter som ofta klassas som endosymbionter. Syftet med den fjärde studien var att öka kunskapen om den geografiska spridningen av *Francisella* genom att molekylärt undersöka och helgenomskaraktärisera *Francisella*-bakterier i fästingar plockade från nordmigrerande fåglar i den AVPR under vårmigrationen 2014 och 2015, samt att undersöka samförekomst av *Francisella* och fläckfeberrickettsia då samförekomst av mikroorganismer är vanligt förekommande i fästingar. Vi fann en hög prevalence av *Francisella* i *H. rufipes* med hjälp av mikrofluidik-realtids-PCR samt en indikation på närvaro av *F. tularensis*-DNA i två *H. rufipes*-fästingar. Närvaro av *F. tularensis* gick inte att konfirmera varken med uppföljande realtids-PCR-analyser eller metagenomisk karaktärisering. Fylogenetisk

inferens av de två francisellagenomen från *H. rufipes* visade att den detekterade *Francisella*-bakterien var av samma art som en *Francisella*-lik endosymbiont (FLE) detekterad i den mjuka fästingarten *Ornithodoros moubata* (FLE-Om). Vi fann även att hälften av *H. rufipes*-fästingarna var bärare av genetiskt material från både *Francisella* och fläckfeberrickettsia som är en grupp av intracellulära fästingburna bakterier med stor geografisk utbredning och som orsakar febril sjukdom med utslag. Fylogenetisk inferens av de två rickettsiagenomen från *H. rufipes* visade på hög likhet med arten *Rickettsia aeschlimannii. R. aeschlimannii* kan orsaka infektion i människor och har tidigare påvisats med liknande prevalensnivåer i fästingar som tillhör fästingkomplexet *H. marginatum* och som plockats från nordmigrerande fåglar i studieområdet. Vi karaktäriserade även två midichloriagenom från *H. rufipes* och den fylogenetiska analysen visade på likhet med arten *M. mitochondrii* vars effekt på fästingar och möjlighet att infektera däggdjur är okänd. Vårmigrerande fåglar i studieområdet har tidigare rapporterats vara bärare av fästingar som testat positivt för *Midichloria*-DNA. Vår studie visar att migrerande fåglar kan bidra till geografisk spridning av fästingar som bär på *Francisella* och FLE:s i den AVPR. Studien visar även på att samförekomst av *Francisella* och fläckfeberrickettsia kan förekomma i *H. rufipes* samt att de yngre livsstadierna av *H. rufipes* och migrerande fåglar inte verkar bidra till geografisk spridning av *F. tularensis* i regionen.

Det är rimligt att anta att risken för spridning av fästingburna patogener samt etablering av exotiska fästingvektorer kan komma att öka med klimatförändringar. Övervakningssystem bör därför upprättas i den västpalaearktiska regionen för att kunna följa introduktion och etablering av exotiska fästingvektorer så som *H. rufipes*.

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References

1. WHO, FAO, and OIE, *Report of the WHO/FAO/OIE Joint Consultation on Emerging Zoonotic Diseases*. 2004, WHO, FAO, OIE: Geneva, Switzerland.
2. Maltezou, H.C., L. Andonova, R. Andraghetti, M. Bouloy, O. Ergonul, F. Jongejan, N. Kalvatchev, S. Nichol, M. Niedrig, A. Platonov, G. Thomson, K. Leitmeyer, and H. Zeller, *Crimean-Congo hemorrhagic fever in Europe: current situation calls for preparedness.* Euro Surveill, 2010. **15**(10): 19504.
3. Gubler, D.J., *The continuing spread of West Nile virus in the western hemisphere.* Clin Infect Dis, 2007. **45**(8): 1039-1046. DOI: 10.1086/521911.
4. Kramer, L.D., A.T. Ciota, and A.M. Kilpatrick, *Introduction, spread, and establishment of West Nile virus in the Americas.* J Med Entomol, 2019. **56**(6): 1448-1455. DOI: 10.1093/jme/tjz151.
5. Holt, E., *West Nile virus spreads in Europe.* Lancet Infect Dis, 2018. **18**(11): 1184. DOI: 10.1016/S1473-3099(18)30616-9.
6. Sambri, V., M. Capobianchi, R. Charrel, M. Fyodorova, P. Gaibani, E. Gould, M. Niedrig, A. Papa, A. Pierro, G. Rossini, S. Varani, C. Vocale, and M.P. Landini, *West Nile virus in Europe: emergence, epidemiology, diagnosis, treatment, and prevention.* Clin Microbiol Infect, 2013. **19**(8): 699-704. DOI: 10.1111/1469-0691.12211.
7. Gatherer, D. and A. Kohl, *Zika virus: a previously slow pandemic spreads rapidly through the Americas.* Journal of General Virology, 2016. **97**: 269-273.
8. Parola, P., C.D. Paddock, C. Socolovschi, M.B. Labruna, O. Mediannikov, T. Kernif, M.Y. Abdad, J. Stenos, I. Bitam, P.E. Fournier, and D. Raoult, *Update on tick-borne rickettsioses around the world: a geographic approach.* Clin Microbiol Rev, 2013. **26**(4): 657-702. DOI: 10.1128/CMR.00032-13.
9. BirdLife International, *State of the world's birds: taking the pulse of the planet*, T. Allinson, E. Vovk, I. Burfield, S. Butchart, A. Dale, and M. Heath, Editors. 2018: Cambridge, UK: BirdLife International.
10. Newton, I., *The migration ecology of birds*. 2008: Academic Press.
11. Moreau, R., *The Palaearctic-African bird migration systems*. 1972, London: Academic Press.
12. Hahn, S., S. Bauer, and F. Liechti, *The natural link between Europe and Africa - 2.1 billion birds on migration.* Oikos, 2009. **118**: 624-626.
13. Zwarts, L., R.G. Bijlsma, J. van der Kamp, and E. Wymenga, *Living on the edge: Wetlands and birds in a changing Sahel*. 2nd ed. 2010, Zeist, The Netherlands: KNNV Publishing
14. Zwarts, L., R.G. Bijlsma, J. van der Kamp, and E. Wymenga, *Living on the edge: Wetlands and birds in a changing Sahel*. 2009, Zeist, The Netherlands: KNNV Publishing.

15. Comstedt, P., T. Jakobsson, and S. Bergstrom, *Global ecology and epidemiology of Borrelia garinii spirochetes.* Infect Ecol Epidemiol, 2011. **1**: 1087-1095. DOI: 10.3402/iee.v1i0.9545.
16. Lundström, J., *Mosquito-borne viruses in Western Europe: a review.* J Vector Ecol, 1999. **24**: 1-39.
17. Campbell, G.L., A.A. Marfin, R.S. Lanciotti, and D.J. Gubler, *West Nile virus.* Lancet Infect Dis, 2002. **2**(9): 519-529. DOI: 10.1016/s1473-3099(02)00368-7.
18. Olsen, B., V.J. Munster, A. Wallensten, J. Waldenstrom, A.D. Osterhaus, and R.A. Fouchier, *Global patterns of influenza a virus in wild birds.* Science, 2006. **312**(5772): 384-388. DOI: 10.1126/science.1122438.
19. Sonenshine, D.E., *Biology of Ticks*. Vol. 1. 1991, New York, Oxford: Oxford University Press.
20. Guglielmone, A.A., T.N. Petney, and R.G. Robbins, *Ixodidae (Acari: Ixodoidea): descriptions and redescriptions of all known species from 1758 to December 31, 2019* 2020, Auckland, New Zealand Magnolia Press.
21. Estrada-Peña, A., A.J. Mangold, S. Nava, J.M. Venzal, M. Labruna, and A.A. Guglielmone, *A review of the systematics of the tick family Argasidae (Ixodida).* Acarologia, 2010. **50**(3): 317-333. DOI: https://doi.org/10.1051/acarologia/20101975.
22. Madder, M., E. Thys, L. Achi, A. Toure, and R. De Deken, *Rhipicephalus (Boophilus) microplus: a most successful invasive tick species in West-Africa.* Exp Appl Acarol, 2011. **53**(2): 139-145. DOI: 10.1007/s10493-010-9390-8.
23. Santos-Silva, M.M., R. Sousa, A.S. Santos, P. Melo, V. Encarnacao, and F. Bacellar, *Ticks parasitizing wild birds in Portugal: detection of Rickettsia aeschlimannii, R. helvetica and R. massiliae.* Exp Appl Acarol, 2006. **39**(3-4): 331-338. DOI: 10.1007/s10493-006-9008-3.
24. Hoogstraal, H., M. Kaiser, M. Traylor, S. Geber, and E. Guindy, *Ticks (Ixodoidea) on birds migrating from Africa to Europe and Asia.* Bull World Health Organ, 1961. **24**(2): 197-212.
25. Elfving, K., B. Olsen, S. Bergström, J. Waldenström, Å. Lundkvist, A. Sjöstedt, H. Mejlon, and K. Nilsson, *Dissemination of spotted fever Rickettsia agents in Europe by migrating birds.* PloS One, 2010. **5**(1): e8572.
26. Olsen, B., T.G. Jaenson, and S. Bergström, *Prevalence of Borrelia burgdorferi sensu lato-infected ticks on migrating birds.* Appl Environ Microbiol, 1995. **61**(8): 3082-3087.
27. Philip, C.B. and W. Burgdorfer, *Arthropod vectors as reservoirs of microbial disease agents.* Annu. Rev. Entomol, 1961. **6**: 391-412.
28. Nuttal, P. and M. Labuda, *Dynamics of infection in tick vectors and at the tick-host interface.* Adv. Virus Res, 2003. **60**: 233-272.
29. Parola, P., C.D. Paddock, and D. Raoult, *Tick-borne rickettsioses around the world: emerging diseases challenging old concepts.* Clin Microbiol Rev, 2005. **18**(4): 719-756. DOI: 10.1128/CMR.18.4.719-756.2005.
30. *Crimean-Congo hemorrhagic fever. A global perspective*, ed. O. Ergonul and C.A. White. 2007, The Netherlands: Springer.
31. Estrada-Peña, A. and F. Jongejan, *Ticks feeding on humans: a review of records on human-biting Ixodoidea with special reference to pathogen transmission.* Experimental and Applied Acarology, 1999. **23**: 685-715.
32. Jongejan, F. and G. Uilenberg, *The global importance of ticks.* Parasitology, 2004. **129** S3-14. DOI: 10.1017/S0031182004005967.

33. Parola, P. and D. Raoult, *Tick-borne bacterial diseases emerging in Europe.* Clin Microbiol Infect, 2001. **7**(2): 80-83.
34. Randolph, S.E., *To what extent has climate change contributed to the recent epidemiology of tick-borne diseases?* Veterinary Parasitology 2010. **167**: 92-94.
35. Amato-Gauci, A.J. and H. Zeller, *Tick-borne encephalitis joins the diseases under surveillance in the European Union.* Eurosurveillance, 2012. **17**(42): pii=20299.
36. Rizzoli, A., H. Hauffe, G. Carpi, H.G. Vourc, M. Neteler, and R. Rosa, *Lyme borreliosis in Europe.* Euro Surveill, 2011. **16**(27): 21794218.
37. Bogovic, P. and F. Strle, *Tick-borne encephalitis: A review of epidemiology, clinical characteristics, and management.* World J Clin Cases, 2015. **3**(5): 430-441. DOI: 10.12998/wjcc.v3.i5.430.
38. Bram, R. *Tick-borne livestock diseases and their vectors. The global problem*. 2014 [cited 2021 Nov 16]; Available from: http://www.fao.org/docrep/004/x6538e/x6538e02.htm.
39. Brown, C.G., *Control of tropical theileriosis (Theileria annulata infection) of cattle.* Parassitologia, 1990. **32**(1): 23-31.
40. Guglielmone, A.A., R.G. Robbins, D.A. Apanaskevich, T.N. Petney, A. Estrada-Peña, and I.G. Horak, *The hard ticks of the world (Acari: Ixodida: Ixodidae)*. 2014: Springer.
41. Bente, D.A., N.L. Forrester, D.M. Watts, A.J. McAuley, C.A. Whitehouse, and M. Bray, *Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity.* Antiviral Res, 2013. **100**(1): 159-189. DOI: 10.1016/j.antiviral.2013.07.006.
42. Apanaskevich, D. and I. Horak, *The genus Hyalomma Koch, 1844: V. Re-evaluation of the taxonomic rank of taxa comprising the H. (Euhyalomma) marginatum Koch complex of species (Acari: Ixodidae) with redescription of all parasitic stages and notes on biology.* International Journal of Acarology, 2008. **34**(1): 13-42.
43. *Ticks of Europe and North Africa. A guide to species identification.*, ed. A. Estrada-Peña, A. Mihalca, and T. Petney. 2017, Switzerland: Springer.
44. Walker, A., A. Bouattour, J.-L. Camicas, A. Estrada-Peña, I. Horak, A. Latif, R. Pegram, and P. Preston, *Ticks of domestic animals in Africa: A guide to identificaiton of species*. 2014: Edinburgh Scotland, UK.
45. Knight, M.M., R.A. Norval, and Y. Rechav, *The life cycle of the tick Hyalomma marginatum rufipes Koch (Acarina: Ixodidae) under laboratory conditions.* J Parasitol, 1978. **64**(1): 143-146.
46. Estrada-Peña, A., J. de la Fuente, T. Latapia, and C. Ortega, *The impact of climate trends on a tick affecting public health: A retrospective modeling approach for Hyalomma marginatum (Ixodidae).* PLoS One, 2015. **10**(5): e0125760. DOI: 10.1371/journal.pone.0125760.
47. Hoogstraal, H., *The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa* J Med Entomol, 1979. **15**(4): 307-417.
48. ECDC. *Hyalomma marginatum - current known distribution: March 2021*. 2021 [cited 2021 April 6]; Available from: https://www.ecdc.europa.eu/en/publications-data/hyalomma-marginatum-current-known-distribution-march-2021.

49. Santos-Silva, M. and Z. Vatansever, *Hyalomma marginatum Koch, 1844*, in *Ticks of Europe and North Africa. A guide to species identification*, A. Estrada-Peña, A.D. Mihalca, and T.N. Petney, Editors. 2017, Springer Nature: Switzerland. p. 349-354.
50. Chitimia-Dobler, L., S. Nava, M. Bestehorn, G. Dobler, and S. Wolfel, *First detection of Hyalomma rufipes in Germany.* Ticks Tick Borne Dis, 2016. **7**(6): 1135-1138. DOI: 10.1016/j.ttbdis.2016.08.008.
51. Estrada-Peña, A. and J.M. Venzal, *Climate niches of tick species in the Mediterranean region: modeling of occurrence data, distributional constraints, and impact of climate change.* J Med Entomol, 2007. **44**(6): 1130-1138.
52. Ergonul, O., *Crimean-Congo haemorrhagic fever.* Lancet Infect Dis, 2006. **6**(4): 203-214. DOI: 10.1016/S1473-3099(06)70435-2.
53. Wallménius, K., C. Barboutis, T. Fransson, T.G. Jaenson, P.E. Lindgren, F. Nyström, B. Olsen, E. Salaneck, and K. Nilsson, *Spotted fever Rickettsia species in Hyalomma and Ixodes ticks infesting migratory birds in the European Mediterranean area.* Parasit Vectors, 2014. **7**: 318. DOI: 10.1186/1756-3305-7-318.
54. Beati, L., M. Meskini, B. Thiers, and D. Raoult, *Rickettsia aeschlimannii sp. nov., a new spotted fever group rickettsia associated with Hyalomma marginatum ticks.* Int J Syst Bacteriol, 1997. **47**(2): 548-554. DOI: 10.1099/00207713-47-2-548.
55. Keysary, A., R.F. Massung, M. Inbar, A.D. Wallach, U. Shanas, K.Y. Mumcuoglu, and T. Waner, *Molecular evidence for Anaplasma phagocytophilum in Israel.* Emerg Infect Dis, 2007. **13**(9): 1411-1412. DOI: 10.3201/eid1309.070455.
56. Matei, I.A., A. Estrada-Peña, S.J. Cutler, M. Vayssier-Taussat, L. Varela-Castro, A. Potkonjak, H. Zeller, and A.D. Mihalca, *A review on the eco-epidemiology and clinical management of human granulocytic anaplasmosis and its agent in Europe.* Parasit Vectors, 2019. **12**(1): 599. DOI: 10.1186/s13071-019-3852-6.
57. Vayssier-Taussat, M., M. Kazimirova, Z. Hubalek, S. Hornok, R. Farkas, J.F. Cosson, S. Bonnet, G. Vourch, P. Gasqui, A.D. Mihalca, O. Plantard, C. Silaghi, S. Cutler, and A. Rizzoli, *Emerging horizons for tick-borne pathogens: from the 'one pathogen-one disease' vision to the pathobiome paradigm.* Future Microbiol. , 2015. **10**(12): 2033-2043. DOI: 10.2217/fmb.15.114.
58. Dugat, T., A.C. Lagree, R. Maillard, H.J. Boulouis, and N. Haddad, *Opening the black box of Anaplasma phagocytophilum diversity: current situation and future perspectives.* Front Cell Infect Microbiol, 2015. **5**: 61. DOI: 10.3389/fcimb.2015.00061.
59. Piesman, J. and L. Eisen, *Prevention of tick-borne diseases.* Annu Rev Entomol, 2008. **53**: 323-343. DOI: 10.1146/annurev.ento.53.103106.093429.
60. Steere, A.C., F. Strle, G.P. Wormser, L.T. Hu, J.A. Branda, J.W. Hovius, X. Li, and P.S. Mead, *Lyme borreliosis.* Nat Rev Dis Primers, 2016. **2**: 16090. DOI: 10.1038/nrdp.2016.90.
61. Chomel, B., *Lyme disease.* Rev Sci Tech, 2015. **34**(2): 569-576. DOI: 10.20506/rst.34.2.2380.
62. Talagrand-Reboul, E., P.H. Boyer, S. Bergstrom, L. Vial, and N. Boulanger, *Relapsing fevers: Neglected tick-borne diseases.* Front Cell Infect Microbiol, 2018. **8**: 98. DOI: 10.3389/fcimb.2018.00098.

63. Wagemakers, A., P.J. Staarink, H. Sprong, and J.W. Hovius, *Borrelia miyamotoi: a widespread tick-borne relapsing fever spirochete.* Trends Parasitol, 2015. **31**(6): 260-269. DOI: 10.1016/j.pt.2015.03.008.
64. Eldin, C., C. Melenotte, O. Mediannikov, E. Ghigo, M. Million, S. Edouard, J.L. Mege, M. Maurin, and D. Raoult, *From Q Fever to Coxiella burnetii Infection: a Paradigm Change.* Clin Microbiol Rev, 2017. **30**(1): 115-190. DOI: 10.1128/CMR.00045-16.
65. Paddock, C.D. and J.E. Childs, *Ehrlichia chaffeensis: a prototypical emerging pathogen.* Clin Microbiol Rev, 2003. **16**(1): 37-64. DOI: 10.1128/CMR.16.1.37-64.2003.
66. Pilo, P., *Phylogenetic lineages of Francisella tularensis in animals.* Front Cell Infect Microbiol, 2018. **8**: 258. DOI: 10.3389/fcimb.2018.00258.
67. Oyston, P.C., A. Sjostedt, and R.W. Titball, *Tularaemia: bioterrorism defence renews interest in Francisella tularensis.* Nat Rev Microbiol, 2004. **2**(12): 967-978. DOI: 10.1038/nrmicro1045.
68. Portillo, A., P. Santibanez, A.M. Palomar, S. Santibanez, and J.A. Oteo, *'Candidatus Neoehrlichia mikurensis' in Europe.* New Microbes New Infect, 2018. **22**: 30-36. DOI: 10.1016/j.nmni.2017.12.011.
69. Li, H., J.F. Jiang, W. Liu, Y.C. Zheng, Q.B. Huo, K. Tang, S.Y. Zuo, K. Liu, B.G. Jiang, H. Yang, and W.C. Cao, *Human infection with Candidatus Neoehrlichia mikurensis, China.* Emerg Infect Dis, 2012. **18**(10): 1636-1639. DOI: 10.3201/eid1810.120594.
70. Tomassone, L., A. Portillo, M. Novakova, R. de Sousa, and J.A. Oteo, *Neglected aspects of tick-borne rickettsioses.* Parasit Vectors, 2018. **11**(1): 263. DOI: 10.1186/s13071-018-2856-y.
71. Homer, M.J., I. Aguilar-Delfin, S.R. Telford, 3rd, P.J. Krause, and D.H. Persing, *Babesiosis.* Clin Microbiol Rev, 2000. **13**(3): 451-469. DOI: 10.1128/CMR.13.3.451.
72. Charrel, R.N., S. Fagbo, G. Moureau, M.H. Alqahtani, S. Temmam, and X. de Lamballerie, *Alkhurma hemorrhagic fever virus in Ornithodoros savignyi ticks.* Emerg Infect Dis, 2007. **13**(1): 153-155. DOI: 10.3201/eid1301.061094.
73. Mahdi, M., B.R. Erickson, J.A. Comer, S.T. Nichol, P.E. Rollin, M.A. AlMazroa, and Z.A. Memish, *Kyasanur Forest Disease virus Alkhurma subtype in ticks, Najran Province, Saudi Arabia.* Emerging infectious diseases, 2011. **17**(5): 945. DOI: 10.3201/eid1705.101824.
74. Holbrook, M.R., *Kyasanur forest disease.* Antiviral Res, 2012. **96**(3): 353-362. DOI: 10.1016/j.antiviral.2012.10.005.
75. Labuda, M. and P.A. Nuttall, *Tick-borne viruses.* Parasitology, 2004. **129 Suppl**: S221-245.
76. Gilbert, L., *Louping ill virus in the UK: a review of the hosts, transmission and ecological consequences of control.* Exp Appl Acarol, 2016. **68**(3): 363-374. DOI: 10.1007/s10493-015-9952-x.
77. Gargili, A., A. Estrada-Peña, J.R. Spengler, A. Lukashev, P.A. Nuttall, and D.A. Bente, *The role of ticks in the maintenance and transmission of Crimean-Congo hemorrhagic fever virus: A review of published field and laboratory studies.* Antiviral Res, 2017. **144**: 93-119. DOI: 10.1016/j.antiviral.2017.05.010.
78. Liu, Q., B. He, S.Y. Huang, F. Wei, and X.Q. Zhu, *Severe fever with thrombocytopenia syndrome, an emerging tick-borne zoonosis.* Lancet Infect Dis, 2014. **14**(8): 763-772. DOI: 10.1016/S1473-3099(14)70718-2.

79. Vatansever, Z., *Hyalomma rufipes Koch, 1844*, in *Ticks of Europe and North Africa. A guide to species identification.*, A. Estrada-Peña, A.D. Mihalca, and N. Petney, Editors. 2017, Springer Nature: Switzerland. p. 355-359.
80. Capek, M., I. Literak, E. Kocianova, O. Sychra, T. Najer, A. Trnka, and P. Kverek, *Ticks of the Hyalomma marginatum complex transported by migratory birds into Central Europe.* Ticks Tick Borne Dis, 2014. **5**(5): 489-493. DOI: 10.1016/j.ttbdis.2014.03.002.
81. Hasle, G., G. Bjune, E. Edvardsen, C. Jakobsen, B. Linnehol, J.E. Roer, R. Mehl, K.H. Roed, J. Pedersen, and H.P. Leinaas, *Transport of ticks by migratory passerine birds to Norway.* J Parasitol, 2009. **95**(6): 1342-1351. DOI: 10.1645/GE-2146.1.
82. Horak, I.G., L.J. Fourie, H. Heyne, J.B. Walker, and G.R. Needham, *Ixodid ticks feeding on humans in South Africa: with notes on preferred hosts, geographic distribution, seasonal occurrence and transmission of pathogens.* Exp Appl Acarol, 2002. **27**(1-2): 113-136.
83. Hoogstraal, H., *African Ixodidae. VoI. I. Ticks of the Sudan (with special reference to Equatoria province and with preliminary reviews of the genera Boophilus, Margaropus, and Hyalomma*. 1956, Dept. of the Navy, Bureau of Medicine and Surgery, Washington, D.C.
84. Toma, L., F. Mancini, M. Di Luca, J.G. Cecere, R. Bianchi, C. Khoury, E. Quarchioni, F. Manzia, G. Rezza, and A. Ciervo, *Detection of microbial agents in ticks collected from migratory birds in central Italy.* Vector Borne Zoonotic Dis, 2014. **14**(3): 199-205. DOI: 10.1089/vbz.2013.1458.
85. Keirans, J.E., G.R. Needham, and J.J.H. Oliver, *The Ixodes ricinus complex worldwide: diagnosis of the species in the complex, hosts and distribution. In Acarology IX: Symposia*, ed. G.R. Needham, R. Michell, D.J. Horn, and W.C. Welburn. Vol. 2. 1999, Columbus, Ohio: The Ohio Biological Survey.
86. Xu, G., Q.Q. Fang, J.E. Keirans, and L.A. Durden, *Molecular phylogenetic analyses indicate that the Ixodes ricinus complex is a paraphyletic group.* J. Parasitol, 2003. **89**(3): 452-457.
87. Lindenbach, B.D. and C.M. Rice, *Molecular biology of flaviviruses.* Adv Virus Res, 2003. **59**: 23-61.
88. Simmonds, P., P. Becher, J. Bukh, E.A. Gould, G. Meyers, T. Monath, S. Muerhoff, A. Pletnev, R. Rico-Hesse, D.B. Smith, J.T. Stapleton, and I.R. Consortium, *ICTV virus taxonomy profile: Flaviviridae.* Journal of General Virology, 2017. **98**: 2-3. DOI: 10.1099/jgv.0.000672.
89. Gould, E.A. and T. Solomon, *Pathogenic flaviviruses.* Lancet, 2008. **371**(9611): 500-509. DOI: 10.1016/S0140-6736(08)60238-X.
90. International Committee on Taxonomy of Viruses. *Genus: Flavivirus*. 2017 [cited 2018 Oct 10]; Available from: https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/flaviviridae/360/genus-flavivirus.
91. Grard, G., G. Moureau, R. Charrel, J.-J. Lemasson, J.-P. Gonzalez, P. Gallian, T. Gritsun, E. Holmes, E. Gould, and X. de Lamballerie, *Genetic characterization of tick-borne f laviviruses: New insights into evolution, pathogenetic determinants and taxonomy*. Virology, 2007. **361**: 80-92.
92. International Committee on Taxonomy of Viruses. *International Committee on Taxonomy of Viruses (ICTV), 9th report*. 2011 [cited 2018 September 2018]; Available from: https://talk.ictvonline.org/ictv-reports/ictv_9th_report/.
93. Al-Tawfiq, J. and Z. Memish, *Alkhurma hemorrhagic fever virus.* Microbes Infect, 2017. **19**(6): 305-310. DOI: 10.1016/j.micinf.2017.04.004.

94. Madani, T.A. and E.M.E. Abuelzein, *Alkhumra hemorrhagic fever virus infection.* Arch Virol, 2021. **166**(9): 2357-2367. DOI: 10.1007/s00705-021-05083-1.
95. Carletti, F., C. Castilletti, A. Di Caro, M.R. Capobianchi, C. Nisii, F. Suter, M. Rizzi, A. Tebaldi, A. Goglio, C. Passerini Tosi, and G. Ippolito, *Alkhurma hemorrhagic fever in travelers returning from Egypt, 2010.* Emerg Infect Dis, 2010. **16**(12): 1979-1982. DOI: 10.3201/eid1612.101092.
96. Ravanini, P., E. Hasu, E. Huhtamo, M.G. Crobu, V. Ilaria, D. Brustia, A.M. Salerno, and O. Vapalahti, *Rhabdomyolysis and severe muscular weakness in a traveler diagnosed with Alkhurma hemorrhagic fever virus infection.* J Clin Virol, 2011. **52**(3): 254-256. DOI: 10.1016/j.jcv.2011.08.001.
97. Musso, M., V. Galati, M.C. Stella, and A. Capone, *A case of Alkhumra virus infection.* J Clin Virol, 2015. **66**: 12-14. DOI: 10.1016/j.jcv.2015.02.019.
98. Horton, K.C., N.T. Fahmy, N. Watany, A. Zayed, A. Mohamed, A.A. Ahmed, P.E. Rollin, and E.L. Dueger, *Crimean Congo Hemorrhagic Fever Virus and Alkhurma (Alkhumra) Virus in Ticks in Djibouti.* Vector Borne Zoonotic Dis, 2016. DOI: 10.1089/vbz.2016.1951.
99. Andayi, F., R.N. Charrel, A. Kieffer, H. Richet, B. Pastorino, I. Leparc-Goffart, A.A. Ahmed, F. Carrat, A. Flahault, and X. de Lamballerie, *A sero-epidemiological study of arboviral fevers in Djibouti, Horn of Africa.* PLoS neglected tropical diseases, 2014. **8**(12). DOI: 10.1371/journal.pntd.0003299.
100. Madani, T.A., *Alkhumra virus infection, a new viral hemorrhagic fever in Saudi Arabia.* J Infect, 2005. **51**(2): 91-97. DOI: 10.1016/j.jinf.2004.11.012.
101. Memish, Z.A., S.F. Fagbo, A.O. Ali, R. AlHakeem, F.M. Elnagi, and E.A. Bamgboye, *Is the epidemiology of Alkhurma hemorrhagic fever changing?: A three-year overview in Saudi Arabia.* PLoS One, 2014. **9**(2): e85564. DOI: 10.1371/journal.pone.0085564.
102. Alzahrani, A.G., H.M. Al Shaiban, M.A. Al Mazroa, O. Al-Hayani, A. Macneil, P.E. Rollin, and Z.A. Memish, *Alkhurma hemorrhagic fever in humans, Najran, Saudi Arabia.* Emerg Infect Dis, 2010. **16**(12): 1882-1888. DOI: 10.3201/eid1612.100417.
103. Memish, Z.A., A. Albarrak, M.A. Almazroa, I. Al-Omar, R. Alhakeem, A. Assiri, S. Fagbo, A. MacNeil, P.E. Rollin, N. Abdullah, and G. Stephens, *Seroprevalence of Alkhurma and other hemorrhagic fever viruses, Saudi Arabia.* Emerg Infect Dis, 2011. **17**(12): 2316-2318. DOI: 10.3201/eid1712.110658.
104. Sadanandane, C., A. Elango, N. Marja, P.V. Sasidharan, K.H. Raju, and P. Jambulingam, *An outbreak of Kyasanur forest disease in the Wayanad and Malappuram districts of Kerala, India.* Ticks Tick Borne Dis, 2017. **8**(1): 25-30. DOI: 10.1016/j.ttbdis.2016.09.010.
105. Pattnaik, P., *Kyasanur forest disease: an epidemiological view in India.* Rev Med Virol, 2006. **16**(3): 151-165. DOI: 10.1002/rmv.495.
106. Dodd, K.A., B.H. Bird, M.L. Khristova, C.G. Albarino, S.A. Carroll, J.A. Comer, B.R. Erickson, P.E. Rollin, and S.T. Nichol, *Ancient ancestry of KFDV and AHFV revealed by complete genome analyses of viruses isolated from ticks and mammalian hosts.* PLoS Negl Trop Dis, 2011. **5**(10): e1352. DOI: 10.1371/journal.pntd.0001352.
107. Heinze, D.M., E.A. Gould, and N.L. Forrester, *Revisiting the clinal concept of evolution and dispersal for the tick-borne flaviviruses by using phylogenetic and biogeographic analyses.* J Virol, 2012. **86**(16): 8663-8671. DOI: 10.1128/JVI.01013-12.

108. Zaki, A.M., *Isolation of a flavivirus related to the tick-borne encephalitis complex from human cases in Saudi Arabia.* Trans R Soc Trop Med Hyg, 1997. **91**(2): 179-181.
109. Moutailler, S., C. Valiente Moro, E. Vaumourin, L. Michelet, F.H. Tran, E. Devillers, J.-F. Cosson, P. Gasqui, V.T. Van, P. Mavingui, G. Vourc`h, and M. Vayssier-Taussat, *Co-infection of ticks: the rule rather than the exception.* PLoS Neglected Tropical Diseases, 2016. **10**(3): e0004539. DOI: 10.1371/journal.pntd.0004539.
110. Zhang, Y.-K., *Maternally inherited symbiotic bacteria in ticks: Incidence and biological importance.* Systematic and Applied Acarology, 2019. **24**(1): 158-179. DOI: 10.11158/saa.24.1.12.
111. Stuen, S., E.G. Granquist, and C. Silaghi, *Anaplasma phagocytophilum - a widespread multi-host pathogen with highly adaptive strategies.* Front Cell Infect Microbiol, 2013. **3**. DOI: 10.3389/fcimb.2013.00031.
112. Bown, K.J., M. Begon, M. Bennett, Z. Woldehiwet, and N.H. Ogden, *Seasonal dynamics of Anaplasma phagocytophila in a rodent-tick (Ixodes trianguliceps) system, United Kingdom.* Emerg Infect Dis, 2003. **9**(1): 63-70. DOI: 10.3201/eid0901.020169.
113. Dziegiel, B., L. Adaszek, M. Kalinowski, and S. Winiarczyk, *Equine granulocytic anaplasmosis.* Res Vet Sci, 2013. **95**(2): 316-320. DOI: 10.1016/j.rvsc.2013.05.010.
114. Carrade, D.D., J.E. Foley, D.L. Borjesson, and J.E. Sykes, *Canine granulocytic anaplasmosis: a review.* Journal of Veterinary Internal Medicine, 2009. **23**(6): 1129-1141. DOI: 10.1111/j.1939-1676.2009.0384.x.
115. Savidge, C., P. Ewing, J. Andrews, D. Aucoin, M.R. Lappin, and S. Moroff, *Anaplasma phagocytophilum infection of domestic cats: 16 cases from the northeastern USA.* J Feline Med Surg, 2016. **18**(2): 85-91. DOI: 10.1177/1098612X15571148.
116. Woldehiwet, Z., *The natural history of Anaplasma phagocytophilum.* Vet Parasitol, 2010. **167**(2-4): 108-122. DOI: 10.1016/j.vetpar.2009.09.013.
117. Dumler, J.S., K.S. Choi, J.C. Garcia-Garcia, N.S. Barat, D.G. Scorpio, J.W. Garyu, D.J. Grab, and J.S. Bakken, *Human granulocytic anaplasmosis and Anaplasma phagocytophilum.* Emerg Infect Dis, 2005. **11**(12): 1828-1834. DOI: 10.3201/eid1112.050898.
118. Chen, S.M., J.S. Dumler, J.S. Bakken, and D.H. Walker, *Identification of a granulocytotropic Ehrlichia species as the etiologic agent of human disease.* J Clin Microbiol, 1994. **32**(3): 589-595. DOI: 10.1128/jcm.32.3.589-595.1994.
119. ECDC. *Factsheet on Human granulocytic anaplasmosis*. 2021 [cited 2021 Oct 13]; Available from: https://www.ecdc.europa.eu/en/all-topics-z/human-granulocytic-anaplasmosis/factsheet-human-granulocytic-anaplasmosis.
120. CDC. *Anaplasmosis. Epidemiology and Statistics*. 2021 [cited 2021 Oct 13]; Available from: https://www.cdc.gov/anaplasmosis/stats/index.html.
121. Dumler, J.S., *The biological basis of severe outcomes in Anaplasma phagocytophilum infection.* FEMS Immunol Med Microbiol, 2012. **64**(1): 13-20. DOI: 10.1111/j.1574-695X.2011.00909.x.
122. Ogden, N.H., K. Bown, B.K. Horrocks, Z. Woldehiwet, and M. Bennett, *Granulocytic Ehrlichia infection in ixodid ticks and mammals in woodlands and uplands of the U.K.* Med Vet Entomol, 1998. **12**(4): 423-429.

123. Alberdi, M.P., A.R. Walker, and K.A. Urquhart, *Field evidence that roe deer (Capreolus capreolus) are a natural host for Ehrlichia phagocytophila.* Epidemiol. Infect., 2000. **124**: 315–323.
124. Stuen, S., K.S. Pettersen, E.G. Granquist, K. Bergström, K.J. Bown, and R.J. Birtles, *Anaplasma phagocytophilum variants in sympatric red deer (Cervus elaphus) and sheep in southern Norway.* Ticks Tick Borne Dis, 2013. **4**(3): 197-201. DOI: 10.1016/j.ttbdis.2012.11.014.
125. Petrovec, M., W. Sixl, R. Schweiger, S. Mikulasek, L. Elke, G. Wust, E. Marth, K. Strasek, D. Stunzner, and T. Avsic-Zupanc, *Infections of wild animals with Anaplasma phagocytophila in Austria and the Czech Republic.* Ann N Y Acad Sci, 2003. **990**: 103-106. DOI: 10.1111/j.1749-6632.2003.tb07345.x.
126. Skuballa, J., T. Petney, M. Pfäffle, and H. Taraschewski, *Molecular detection of Anaplasma phagocytophilum in the European hedgehog (Erinaceus europaeus) and its ticks.* Vector Borne Zoonotic Dis, 2010. **10**(10): 1055-1057. DOI: 10.1089/vbz.2009.0150.
127. Liz, J.S., L. Anderes, J.W. Sumner, R.F. Massung, L. Gern, B. Rutti, and M. Brossard, *PCR detection of granulocytic ehrlichiae in Ixodes ricinus ticks and wild small mammals in western Switzerland.* J Clin Microbiol, 2000. **38**(3): 1002-1007.
128. Daniels, T.J., G.R. Battaly, D. Liveris, R.C. Falco, and I. Schwartz, *Avian reservoirs of the agent of human granulocytic ehrlichiosis?* Emerg Infect Dis, 2002. **8**(12): 1524-1525. DOI: 10.3201/eid0812.010527.
129. Skotarczak, B., A. Rymaszewska, B. Wodecka, M. Sawczuk, M. Adamska, and A. Maciejewska, *PCR detection of granulocytic Anaplasma and Babesia in Ixodes ricinus ticks and birds in west-central Poland.* Ann Agric Environ Med, 2006. **13**(1): 21-23.
130. De La Fuente, J., V. Naranjo, F. Ruiz-Fons, U. Hofle, I.G. Fernandez De Mera, D. Villanua, C. Almazan, A. Torina, S. Caracappa, K.M. Kocan, and C. Gortazar, *Potential vertebrate reservoir hosts and invertebrate vectors of Anaplasma marginale and A. phagocytophilum in central Spain.* Vector Borne Zoonotic Dis, 2005. **5**(4): 390-401. DOI: 10.1089/vbz.2005.5.390.
131. Bjoersdorff, A., S. Bergström, R.F. Massung, P.D. Haemig, and B. Olsen, *Ehrlichia-infected ticks on migrating birds.* Emerg Infect Dis, 2001. **7**(5): 877-879. DOI: 10.3201/eid0705.017517.
132. Alekseev, A.N., H.V. Dubinina, A.V. Semenov, and C.V. Bolshakov, *Evidence of ehrlichiosis agents found in ticks (Acari : Ixodidae) collected from migratory birds.* Journal of Medical Entomology, 2001. **38**(4): 471-474. DOI: Doi 10.1603/0022-2585-38.4.471.
133. Jahfari, S., E.C. Coipan, M. Fonville, A.D. van Leeuwen, P. Hengeveld, D. Heylen, P. Heyman, C. van Maanen, C.M. Butler, G. Foldvari, S. Szekeres, G. van Duijvendijk, W. Tack, J.M. Rijks, J. van der Giessen, W. Takken, S.E. van Wieren, K. Takumi, and H. Sprong, *Circulation of four Anaplasma phagocytophilum ecotypes in Europe.* Parasit Vectors, 2014. **7**: 365. DOI: 10.1186/1756-3305-7-365.
134. von Loewenich, F.D., B.U. Baumgarten, K. Schroppel, W. Geissdorfer, M. Rollinghoff, and C. Bogdan, *High diversity of ankA sequences of Anaplasma phagocytophilum among Ixodes ricinus ticks in Germany.* J Clin Microbiol, 2003. **41**(11): 5033-5040.

135. Huhn, C., C. Winter, T. Wolfsperger, N. Wuppenhorst, K. Strasek Smrdel, J. Skuballa, M. Pfäffle, T. Petney, C. Silaghi, V. Dyachenko, N. Pantchev, R.K. Straubinger, D. Schaarschmidt-Kiener, M. Ganter, M.L. Aardema, and F.D. von Loewenich, *Analysis of the population structure of Anaplasma phagocytophilum using multilocus sequence typing.* PLoS One, 2014. **9**(4): e93725. DOI: 10.1371/journal.pone.0093725.
136. Scharf, W., S. Schauer, F. Freyburger, M. Petrovec, D. Schaarschmidt-Kiener, G. Liebisch, M. Runge, M. Ganter, A. Kehl, J.S. Dumler, A.L. Garcia-Perez, J. Jensen, V. Fingerle, M.L. Meli, A. Ensser, S. Stuen, and F.D. von Loewenich, *Distinct host species correlate with Anaplasma phagocytophilum ankA gene clusters.* J Clin Microbiol, 2011. **49**(3): 790-796. DOI: 10.1128/JCM.02051-10.
137. Caturegli, P., K.M. Asanovich, J.J. Walls, J.S. Bakken, J.E. Madigan, V.L. Popov, and J.S. Dumler, *ankA: an Ehrlichia phagocytophila group gene encoding a cytoplasmic protein antigen with ankyrin repeats.* Infect Immun, 2000. **68**(9): 5277-5283. DOI: 10.1128/IAI.68.9.5277-5283.2000.
138. Majazki, J., N. Wuppenhorst, K. Hartelt, R. Birtles, and F.D. von Loewenich, *Anaplasma phagocytophilum strains from voles and shrews exhibit specific ankA gene sequences.* BMC Vet Res, 2013. **9**: 235. DOI: 10.1186/1746-6148-9-235.
139. Gillespie, J.J., M.S. Beier, M.S. Rahman, N.C. Ammerman, J.M. Shallom, A. Purkayastha, B.S. Sobral, and A.F. Azad, *Plasmids and rickettsial evolution: insight from Rickettsia felis.* PLoS One, 2007. **2**(3): e266. DOI: 10.1371/journal.pone.0000266.
140. Brouqui, P., P. Parola, P.E. Fournier, and D. Raoult, *Spotted fever rickettsioses in southern and eastern Europe.* FEMS Immunol Med Microbiol, 2007. **49**(1): 2-12. DOI: 10.1111/j.1574-695X.2006.00138.x.
141. Regan, J.J., M.S. Traeger, D. Humpherys, D.L. Mahoney, M. Martinez, G.L. Emerson, D.M. Tack, A. Geissler, S. Yasmin, R. Lawson, V. Williams, C. Hamilton, C. Levy, K. Komatsu, D.A. Yost, and J.H. McQuiston, *Risk factors for fatal outcome from rocky mountain spotted Fever in a highly endemic area-Arizona, 2002-2011.* Clin Infect Dis, 2015. **60**(11): 1659-1666. DOI: 10.1093/cid/civ116.
142. Portillo, A., S. Santibanez, L. Garcia-Alvarez, A.M. Palomar, and J.A. Oteo, *Rickettsioses in Europe.* Microbes Infect, 2015. **17**(11-12): 834-838. DOI: 10.1016/j.micinf.2015.09.009.
143. Hornok, S., D. Kováts, T. Csörgő, M.L. Meli, E. Gönczi, Z. Hadnagy, N. Takács, R. Farkas, and R. Hofmann-Lehmann, *Birds as potential reservoirs of tick-borne pathogens: first evidence of bacteraemia with Rickettsia helvetica.* Parasit Vectors, 2014. **7**: 128.
144. Berthová, L., V. Slobodnik, R. Slobodnik, M. Oleksak, Z. Sekeyova, Z. Svitalkova, M. Kazimirova, and E. Spitalska, *The natural infection of birds and ticks feeding on birds with Rickettsia spp. and Coxiella burnetii in Slovakia.* Exp Appl Acarol, 2016. **68**(3): 299-314. DOI: 10.1007/s10493-015-9975-3.
145. Mukherjee, N., L. Beati, M. Sellers, L. Burton, S. Adamson, R.G. Robbins, F. Moore, and S. Karim, *Importation of exotic ticks and tick-borne spotted fever group rickettsiae into the United States by migrating songbirds.* Ticks Tick Borne Dis, 2014. **5**(2): 127-134. DOI: 10.1016/j.ttbdis.2013.09.009.

146. Öhrman, C., J.W. Sahl, A. Sjödin, I. Uneklint, R. Ballard, L. Karlsson, R.F. McDonough, D. Sundell, K. Soria, S. Backman, K. Chase, B. Brindefalk, S. Sozhamannan, A. Vallesi, E. Hagglund, J.G. Ramirez-Paredes, J. Thelaus, D. Colquhoun, K. Myrtennas, D. Birdsell, A. Johansson, D.M. Wagner, and M. Forsman, *Reorganized genomic taxonomy of Francisellaceae enables design of robust environmental PCR assays for detection of Francisella tularensis*. Microorganisms, 2021. **9**(1): 146. DOI: 10.3390/microorganisms9010146.
147. Fortier, A.H., S.J. Green, T. Polsinelli, T.R. Jones, R.M. Crawford, D.A. Leiby, K.L. Elkins, M.S. Meltzer, and C.A. Nacy, *Life and death of an intracellular pathogen: Francisella tularensis and the macrophage.* Immunol Ser, 1994. **60**: 349-361.
148. Friend, M., *Tularemia*. 2006, Reston, Va., U.S. Geological Survey, Circular 1297.
149. Timofeev, V., I. Bakhteeva, G. Titareva, P. Kopylov, D. Christiany, A. Mokrievich, I. Dyatlov, and G. Vergnaud, *Russian isolates enlarge the known geographic diversity of Francisella tularensis subsp. mediasiatica.* PLoS One, 2017. **12**(9): e0183714. DOI: 10.1371/journal.pone.0183714.
150. Ellis, J., P.C. Oyston, M. Green, and R.W. Titball, *Tularemia.* Clin Microbiol Rev, 2002. **15**(4): 631-646. DOI: 10.1128/CMR.15.4.631-646.2002.
151. Health Canada. *Pathogen Safety Data Sheets: Infectious Substances – Francisella tularensis*. 2011 2011-02-18 [cited 2021 Nov 1]; Available from: https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/francisella-tularensis-material-safety-data-sheets-msds.html.
152. Dienst, F.T., Jr., *Tularemia: a perusal of three hundred thirty-nine cases.* J La State Med Soc, 1963. **115**: 114-127.
153. Dennis, D.T., T.V. Inglesby, D.A. Henderson, J.G. Bartlett, M.S. Ascher, E. Eitzen, A.D. Fine, A.M. Friedlander, J. Hauer, M. Layton, S.R. Lillibridge, J.E. McDade, M.T. Osterholm, T. O'Toole, G. Parker, T.M. Perl, P.K. Russell, K. Tonat, B. for the Working Group on Civilian, B. Working Grp Civilian, and B. Working Group on Civilian, *Tularemia as a biological weapon: medical and public health management.* JAMA : the journal of the American Medical Association, 2001. **285**(21): 2763-2773. DOI: 10.1001/jama.285.21.2763.
154. Gerhart, J.G., A.S. Moses, and R. Raghavan, *A Francisella-like endosymbiont in the Gulf Coast tick evolved from a mammalian pathogen.* Sci Rep, 2016. **6**: 33670. DOI: 10.1038/srep33670.
155. Smith, T.A., T. Driscoll, J.J. Gillespie, and R. Raghavan, *A Coxiella-like endosymbiont is a potential vitamin source for the Lone Star tick.* Genome Biol. Evol., 2015. **7**(3): 831-838.
156. Gerhart, J.G., H. Auguste Dutcher, A.E. Brenner, A.S. Moses, L. Grubhoffer, and R. Raghavan, *Multiple acquisitions of pathogen-derived Francisella endosymbionts in soft ticks.* Genome Biol Evol, 2018. **10**(2): 607-615. DOI: 10.1093/gbe/evy021.
157. Ahantarig, A., W. Trinachartvanit, V. Baimai, and L. Grubhoffer, *Hard ticks and their bacterial endosymbionts (or would be pathogens).* Folia Microbiological, 2013. **58**: 419-428.

158. Klyachko, O., B. Stein, N. Grindle, K. Clay, and C. Fuqua, *Localization and visualization of a Coxiella-type symbiont within the lone star tick, Amblyomma americanum.* Appl Environ Microbiol., 2007. **73**(20): 6584-6594. DOI: 10.1128/AEM.00537-07.
159. Baldridge, G.D., G.A. Scoles, N.Y. Burkhardt, B. Schloeder, T.J. Kurtti, and U.G. Munderloh, *Transovarial transmission of Francisella-like endosymbionts and Anaplasma phagocytophilum variants in Dermacentor albipictus (Acari: Ixodidae).* J Med Entomol, 2009. **46**(3): 625-632.
160. Duron, O., O. Morel, V. Noêll, M. Buysse, F. Binetruy, R. Lancelot, E. Loire, C. Ménard, O. Bouchez, F. Vavre, and L. Vial, *Tick-bacteria mutualism depends on B vitamin synthesis pathways.* Current Biology, 2018. **28**: 1896-1902. DOI: https://doi.org/10.1016/j.cub.2018.04.038.
161. Sun, L.V., G.A. Scoles, D. Fish, and S.L. O'Neill, *Francisella-like endosymbionts of ticks.* J Invertebr Pathol, 2000. **76**(4): 301-303. DOI: 10.1006/jipa.2000.4983.
162. Kaufman, E.L., N.E. Stone, G.A. Scoles, C.M. Hepp, J.D. Busch, and D.M. Wagner, *Range-wide genetic analysis of Dermacentor variabilis and its Francisella-like endosymbionts demonstrates phylogeographic concordance between both taxa.* Parasit Vectors, 2018. **11**: 306. DOI: 10.1186/s13071-018-2886-5.
163. Michelet, L., S. Bonnet, N. Madani, and S. Moutailler, *Discriminating Francisella tularensis and Francisella-like endosymbionts in Dermacentor reticulatus ticks: evaluation of current molecular techniques.* Vet Microbiol, 2013. **163**: 399-403. DOI: 10.1016/j.vetmic.2013.01.014.
164. Noda, H., U.G. Munderloh, and T.J. Kurtti, *Endosymbionts of ticks and their relationship to Wolbachia spp. and tick-borne pathogens of humans and animals.* Applied and Environmental Microbiology, 1997. **63**(10): 3926-3932. DOI: 10.1128/AEM.63.10.3926-3932.1997.
165. Niebylski, M.L., M.G. Peacock, E.R. Fischer, S.F. Porcella, and T.G. Schwan, *Characterization of an endosymbiont infecting wood ticks, Dermacentor andersoni, as a member of the genus Francisella.* Applied and environmental microbiology, 1997. **63**(10): 3933-3940. DOI: 10.1128/AEM.63.10.3933-3940.1997.
166. Machado-Ferreira, E., J. Piesman, N.S. Zeidner, and A.G.S. Carlos, *Francisella-like endosymbiont DNA and Francisella tularensis virulence-related genes in Brazilian ticks (Acari: Ixodidae).* Journal of Medical Entomology, 2009. **46**(2): 369-374. DOI: 10.1603/033.046.0224.
167. Sumrandee, C., V. Baimai, W. Trinachartvanit, and A. Ahantarig, *Molecular detection of Rickettsia, Anaplasma, Coxiella and Francisella bacteria in ticks collected from Artiodactyla in Thailand.* Ticks and tick-borne diseases, 2016. **7**(5): 678-689. DOI: 10.1016/j.ttbdis.2016.02.015.
168. Szigeti, A., Z. Kreizinger, S. Hornok, G. Abichu, and M. Gyuranecz, *Detection of Francisella-like endosymbiont in Hyalomma rufipes from Ethiopia.* Ticks Tick Borne Dis, 2014. **5**(6): 818-820. DOI: 10.1016/j.ttbdis.2014.06.002.
169. Sréter-Lancz, Z., Z. Széll, T. Sréter, and K. Márialigeti, *Detection of a novel Francisella in Dermacentor reticulatus: a need for careful evaluation of PCR-based identification of Francisella tularensis in Eurasian ticks.* Vector borne and zoonotic diseases (Larchmont, N.Y.), 2009. **9**(1): 123-126. DOI: 10.1089/vbz.2008.0010.
170. Scoles, G.A., *Phylogenetic Analysis of the Francisella-like endosymbionts of Dermacentor ticks.* Journal of medical entomology, 2004. **41**(3): 277-286.

171. Ivanov, I.N., N. Mitkova, A.L. Reye, J.M. Hübschen, R.S. Vatcheva-Dobrevska, E.G. Dobreva, T.V. Kantardjiev, and C.P. Muller, *Detection of new Francisella-like tick endosymbionts in Hyalomma spp. and Rhipicephalus spp. (Acari: Ixodidae) from Bulgaria.* Applied and Environmental Microbiology, 2011. **77**(15): 5562-5565.
172. Gottlieb, Y., I. Lalzar, and L. Klasson, *Distinctive genome reduction rates revealed by genomic analyses of two Coxiella-like endosymbionts in ticks.* Genome Biol Evol, 2015. **7**(6): 1779-1796. DOI: 10.1093/gbe/evv108.
173. Duron, O., F. Binetruy, V. Noel, J. Cremaschi, K.D. McCoy, C. Arnathau, O. Plantard, J. Goolsby, A.A. Perez de Leon, D.J.A. Heylen, A.R. Van Oosten, Y. Gottlieb, G. Baneth, A.A. Guglielmone, A. Estrada-Peña, M.N. Opara, L. Zenner, F. Vavre, and C. Chevillon, *Evolutionary changes in symbiont community structure in ticks.* Mol Ecol, 2017. **26**(11): 2905-2921. DOI: 10.1111/mec.14094.
174. Bennett, G.M. and N.A. Moran, *Heritable symbiosis: The advantages and perils of an evolutionary rabbit hole.* Proc Natl Acad Sci U S A, 2015. **112**(33): 10169-10176. DOI: 10.1073/pnas.1421388112.
175. Montagna, M., D. Sassera, S. Epis, C. Bazzocchi, C. Vannini, N. Lo, L. Sacchi, T. Fukatsu, G. Petroni, and C. Bandi, *"Candidatus Midichloriaceae" fam. nov. (Rickettsiales), an ecologically widespread clade of intracellular alphaproteobacteria.* Appl Environ Microbiol, 2013. **79**(10): 3241-3248. DOI: 10.1128/AEM.03971-12.
176. Epis, S., M. Mandrioli, M. Genchi, M. Montagna, L. Sacchi, D. Pistone, and D. Sassera, *Localization of the bacterial symbiont Candidatus Midichloria mitochondrii within the hard tick Ixodes ricinus by whole-mount FISH staining.* Ticks Tick Borne Dis, 2013. **4**: 39-45. DOI: 10.1016/j.ttbdis.2012.06.005.
177. Sassera, D., T. Beninati, C. Bandi, E.A.P. Bouman, L. Sacchi, M. Fabbi, and N. Lo, *'Candidatus Midichloria mitochondrii', an endosymbiont of the tick Ixodes ricinus with a unique intramitochondrial lifestyle.* Int J Syst Evol Microbiol, 2006. **56**: 2535-2540. DOI: 10.1099/ijs.0.64386-0.
178. Di Venere, M., M. Fumagalli, A. Cafiso, L. De Marco, S. Epis, O. Plantard, A. Bardoni, R. Salvini, S. Viglio, C. Bazzocchi, P. Iadarola, and D. Sassera, *Ixodes ricinus and Its endosymbiont Midichloria mitochondrii: A comparative proteomic analysis of salivary glands and ovaries.* PLoS One, 2015. **10**(9): e0138842. DOI: 10.1371/journal.pone.0138842.
179. Bazzocchi, C., M. Mariconti, D. Sassera, L. Rinaldi, E. Martin, G. Cringoli, S. Urbanelli, C. Genchi, C. Bandi, and S. Epis, *Molecular and serological evidence for the circulation of the tick symbiont Midichloria (Rickettsiales: Midichloriaceae) in different mammalian species.* Parasit Vectors, 2013. **6**: 350. DOI: 10.1186/1756-3305-6-350.
180. Mariconti, M., S. Epis, P. Gaibani, C. Dalla Valle, D. Sassera, P. Tomao, M. Fabbi, F. Castelli, P. Marone, V. Sambri, C. Bazzocchi, and C. Bandi, *Humans parasitized by the hard tick Ixodes ricinus are seropositive to Midichloria mitochondrii: is Midichloria a novel pathogen, or just a marker of tick bite?* Pathog Glob Health, 2012. **106**(7): 391-396. DOI: 10.1179/2047773212Y.0000000050.
181. Epis, S., D. Sassera, T. Beninati, N. Lo, L. Beati, J. Piesman, L. Rinaldi, K.D. McCoy, A. Torina, L. Sacchi, E. Clementi, M. Genchi, S. Magnino, and C. Bandi, *Midichloria mitochondrii is widespread in hard ticks (Ixodidae) and resides in the mitochondria of phylogenetically diverse species.* Parasitology, 2008. **135**(4): 485-494. DOI: 10.1017/S0031182007004052.

182. Harrus, S., A. Perlman-Avrahami, K.Y. Mumcuoglu, D. Morick, O. Eyal, and G. Baneth, *Molecular detection of Ehrlichia canis, Anaplasma bovis, Anaplasma platys, Candidatus Midichloria mitochondrii and Babesia canis vogeli in ticks from Israel.* Clin Microbiol Infect, 2011. **17**(3): 459-463. DOI: 10.1111/j.1469-0691.2010.03316.x.
183. Milhanoa, N., I. Lopes de Carvalhoa, A.S. Alvesa, S. Arroubeb, J. Soaresc, P. Rodriguezc, M. Carolinod, M.S. Núncioa, J. Piesmane, and R. de Sousaa, *Coinfections of Rickettsia slovaca and Rickettsia helvetica with Borrelia lusitaniae ticks collected in a safari park, portugal.* Ticks and Tick-Borne Diseases, 2010. **1**(4): 172-177.
184. Tijsse-Klasen, E., H. Sprong, and N. Pandak, *Co-infection of Borrelia burgdorferi sensu lato and Rickettsia species in ticks and in an erythema migrans patient.* Parasit Vectors, 2013. **6**: 347. DOI: 10.1186/1756-3305-6-347.
185. Horowitz, H., M. Aguero-Rosenfeld, D. Holmgren, D. McKenna, I. Schwartz, M. Cox, and G. Wormser, *Lyme disease and human granulocytic anaplasmosis coinfection: impact of case definition on coinfection rates and illness severity.* Clin Infect Dis. , 2013. **56**(1): 93-99. DOI: 10.1093/cid/cis852.
186. Belongia, E.A., K.D. Reed, P.D. Mitchell, P.H. Chyou, N. Mueller-Rizner, M.F. Finkel, and M.E. Schriefer, *Clinical and epidemiological features of early Lyme disease and human granulocytic ehrlichiosis in Wisconsin.* Clin Infect Dis, 1999. **29**(6): 1472-1477. DOI: 10.1086/313532.
187. Lindeborg, M., C. Barboutis, C. Ehrenborg, T. Fransson, T.G. Jaenson, P.E. Lindgren, Å. Lundkvist, F. Nyström, E. Salaneck, J. Waldenström, and B. Olsen, *Migratory birds, ticks, and Crimean-Congo hemorrhagic fever virus.* Emerg Infect Dis, 2012. **18**(12): 2095-2097. DOI: 10.3201/eid1812.120718.
188. *Handbook of the birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic. Tyrant flycatchers to thrushes*, ed. S. Cramp. Vol. 5. 1988, Oxford: Oxford University Press.
189. *Handbook of the birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic. Warblers*, ed. S. Cramp. Vol. 6. 1992, Oxford: Oxford University Press.
190. *Handbook of the birds of Europe the Middle East and North Africa. The birds of the Western Palearctic. Flycatchers to shrikes*, ed. S. Cramp and C.M. Perrins. Vol. 7. 1993, Oxford: Oxford University Press.
191. ECDC. *Hyalomma marginatum - current known distribution: May 2018*. [cited 2018 Oct 8]; Available from: https://www.ecdc.europa.eu/en/publications-data/hyalomma-marginatum-current-known-distribution-may-2018.
192. Hagman, K., C. Barboutis, C. Ehrenborg, T. Fransson, T. Jaenson, P.-E. Lindgren, Å. Lundkvist, F. Nyström, J. Waldenström, and E. Salaneck, *On the potential roles of ticks and migrating birds in the ecology of West Nile virus.* Infection Ecology & Epidemiology, 2014. **4**(1): 20943.
193. Gondard, M., L. Michelet, A. Nisavanh, E. Devillers, S. Delannoy, P. Fach, A. Aspan, K. Ullman, J. Chirico, B. Hoffmann, F.J. van der Wal, A. de Koeijer, C. van Solt-Smits, S. Jahfari, H. Sprong, K.L. Mansfield, A.R. Fooks, K. Klitgaard, R. Bodker, and S. Moutailler, *Prevalence of tick-borne viruses in Ixodes ricinus assessed by high-throughput real-time PCR.* Pathog Dis, 2018. **76**(8): 30423120. DOI: 10.1093/femspd/fty083.

194. Beati, L. and J.E. Keirans, *Analysis of the systematic relationships among ticks of the genera Rhipicephalus and Boophilus (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters.* J Parasitol, 2001. **87**(1): 32-48. DOI: 10.1645/0022-3395(2001)087[0032:AOTSRA]2.0.CO;2.
195. Manogold, A., M. Bargues, and S. Mas-Coma, *Mitochondrial 16S rDNA sequences and phylogenetic relationships of species of Rhipicephalus and other tick genera among Metastriata (Acari: Ixodidae).* Parasitol Res. , 1998. **84**(6): 478-484.
196. Sormunen, J.J., R. Penttinen, T. Klemola, J. Hänninen, I. Vuorinen, M. Laaksonen, I.E. Sääksjärvi, K. Ruohomäki, and E.J. Vesterinen, *Tick-borne bacterial pathogens in southwestern Finland.* Parasit Vectors, 2016. **9**: 168. DOI: 10.1186/s13071-016-1449-x.
197. Ratnasingham, S. and P.D. Hebert, *bold: The Barcode of Life Data System (http://www.barcodinglife.org).* Mol Ecol Notes, 2007. **7**(3): 355-364. DOI: 10.1111/j.1471-8286.2007.01678.x.
198. Michelet, L., S. Delannoy, E. Devillers, G. Umhang, A. Aspan, M. Juremalm, J. Chirico, F.J. van der Wal, H. Sprong, T.P. Boye Pihl, K. Klitgaard, R. Bodker, P. Fach, and S. Moutailler, *High-throughput screening of tick-borne pathogens in Europe.* Front Cell Infect Microbiol, 2014. **4**: 103. DOI: 10.3389/fcimb.2014.00103.
199. Madani, T.A., M. Kao, E.I. Azhar, E.-T.M.E. Abuelzein, H.M.S. Al-Bar, H. Abu-Araki, and T.G. Ksiazek, *Successful propagation of Alkhumra (misnamed as Alkhurma) virus in C6/36 mosquito cells.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 2012. **106**(3): 180-185. DOI: 10.1016/j.trstmh.2011.11.003.
200. Henningsson, A.J., D. Hvidsten, B.E. Kristiansen, A. Matussek, S. Stuen, and A. Jenkins, *Detection of Anaplasma phagocytophilum in Ixodes ricinus ticks from Norway using a realtime PCR assay targeting the Anaplasma citrate synthase gene gltA.* BMC Microbiol, 2015. **15**: 153. DOI: 10.1186/s12866-015-0486-5.
201. Stuen, S., A.S. Oppegaard, K. Bergstrom, and T. Moum, *Anaplasma phagocytophilum infection in north Norway. The first laboratory confirmed case.* Acta Vet Scand, 2005. **46**(3): 167-171.
202. Cronhjort, S., P. Wilhelmsson, L. Karlsson, J. Thelaus, A. Sjödin, P. Forsberg, and P.-E. Lindgren, *The Tick-Borne Diseases STING study: Real-time PCR analysis of three emerging tick-borne pathogens in ticks that have bitten humans in different regions of Sweden and the Åland islands, Finland.* Infection Ecology & Epidemiology, 2019. **9**(1): 1683935. DOI: 10.1080/20008686.2019.1683935.
203. Thelaus, J., A. Andersson, P. Mathisen, A.L. Forslund, L. Noppa, and M. Forsman, *Influence of nutrient status and grazing pressure on the fate of Francisella tularensis in lake water.* FEMS Microbiol Ecol, 2009. **67**(1): 69-80. DOI: 10.1111/j.1574-6941.2008.00612.x.
204. Carl, M., C.W. Tibbs, M.E. Dobson, S. Paparello, and G.A. Dasch, *Diagnosis of acute Typhus infection using the polymerase chain reaction.* The Journal of infectious diseases, 1990. **161**(4): 791-793. DOI: 10.1093/infdis/161.4.791.
205. Wood, D.E., J. Lu, and B. Langmead, *Improved metagenomic analysis with Kraken 2.* Genome Biol, 2019. **20**(1): 257. DOI: 10.1186/s13059-019-1891-0.

206. Sundell, D., C. Öhrman, D. Svensson, E. Karlsson, B. Brindefalk, K. Myrtennäs, J. Ahlinder, M.H. Antwerpen, M.C. Walter, M. Forsman, P. Stenberg, and A. Sjödin, *FlexTaxD: flexible modification of taxonomy databases for improved sequence classification.* Bioinformatics, 2021: 1-2. DOI: 10.1093/bioinformatics/btab621.
207. Koster, J. and S. Rahmann, *Snakemake-a scalable bioinformatics workflow engine.* Bioinformatics, 2018. **34**(20): 3600. DOI: 10.1093/bioinformatics/bty350.
208. Bankevich, A., S. Nurk, D. Antipov, A.A. Gurevich, M. Dvorkin, A.S. Kulikov, V.M. Lesin, S.I. Nikolenko, S. Pham, A.D. Prjibelski, A.V. Pyshkin, A.V. Sirotkin, N. Vyahhi, G. Tesler, M.A. Alekseyev, and P.A. Pevzner, *SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing.* J Comput Biol, 2012. **19**(5): 455-477. DOI: 10.1089/cmb.2012.0021.
209. Walker, B.J., T. Abeel, T. Shea, M. Priest, A. Abouelliel, S. Sakthikumar, C.A. Cuomo, Q. Zeng, J. Wortman, S.K. Young, and A.M. Earl, *Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement.* PLoS One, 2014. **9**(11). DOI: 10.1371/journal.pone.0112963.
210. Parks, D.H., M. Imelfort, C.T. Skennerton, P. Hugenholtz, and G.W. Tyson, *CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes.* Genome Res, 2015. **25**(7): 1043-1055. DOI: 10.1101/gr.186072.114.
211. Seppey, M., M. Manni, and E.M. Zdobnov, *BUSCO: Assessing genome assembly and annotation completeness.* Methods Mol Biol, 2019. **1962**: 227-245. DOI: 10.1007/978-1-4939-9173-0_14.
212. Kolmogorov, M., J. Yuan, Y. Lin, and P.A. Pevzner, *Assembly of long, error-prone reads using repeat graphs.* Nat Biotechnol, 2019. **37**(5): 540-546. DOI: 10.1038/s41587-019-0072-8.
213. Katoh, K., K. Misawa, K. Kuma, and T. Miyata, *MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform.* Nucleic Acids Res, 2002. **30**(14): 3059-3066.
214. Kumar, S., G. Stecher, and K. Tamura, *MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets.* Mol Biol Evol, 2016. **33**(7): 1870-1874. DOI: 10.1093/molbev/msw054.
215. Thompson, J.D., D.G. Higgins, and T.J. Gibson, *CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice.* Nucleic Acids Res, 1994. **22**(22): 4673-4680. DOI: 10.1093/nar/22.22.4673.
216. Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura, *MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms.* Mol. Biol. Evol., 2018. **35**(6): 1547-1549. DOI: 10.1093/molbev/msy096.
217. Langenwalder, B., S. Schmidt, U. Gilli, N. Pantchev, M. Ganter, C. Silaghi, M.L. Aardema, and F.D. von Loewenich, *Genetic characterization of Anaplasma phagocytophilum strains from goats (Capra aegagrus hircus) and water buffalo (Bubalus arnee) by 16S rRNA gene, ankA gene and multilocus sequence typing.* Ticks and Tick-Borne Diseases, 2019. **10**(6): 101267. DOI: 10.1016/j.ttbdis.2019.101267.
218. Dayhoff, M.O., R.M. Schwartz, and B.C. Orcutt, *A model of evolutionary change in proteins*. Atlas of protein sequence and structure. Vol. 5. 1978, Washington, D.C.: National Biomedical Research Foundation.

219. Darling, A.E., B. Mau, and N.T. Perna, *progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement.* PLoS One, 2010. **5**(6): e11147. DOI: 10.1371/journal.pone.0011147.
220. Larkeryd, A., K. Myrtennas, E. Karlsson, C.K. Dwibedi, M. Forsman, P. Larsson, A. Johansson, and A. Sjodin, *CanSNPer: a hierarchical genotype classifier of clonal pathogens.* Bioinformatics, 2014. **30**(12): 1762-1764. DOI: 10.1093/bioinformatics/btu113.
221. Minh, B.Q., H.A. Schmidt, O. Chernomor, D. Schrempf, M.D. Woodhams, A. von Haeseler, and R. Lanfear, *IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era.* Mol Biol Evol, 2020. **37**(5): 1530-1534. DOI: 10.1093/molbev/msaa015.
222. Weinert, L.A., J.H. Werren, A. Aebi, G.N. Stone, and F.M. Jiggins, *Evolution and diversity of Rickettsiabacteria.* BMC Biology, 2009. **7**: 6. DOI: 10.1186/1741-7007-7-6.
223. Ciloglu, A., O. Ibis, A. Yildirim, M. Aktas, O. Duzlu, Z. Onder, E. Simsek, G. Yetismis, V.A. Ellis, and A. Inci, *Complete mitochondrial genome characterization and phylogenetic analyses of the main vector of Crimean-Congo haemorrhagic fever virus: Hyalomma marginatum Koch, 1844.* Ticks Tick Borne Dis, 2021. **12**(5): 101736. DOI: 10.1016/j.ttbdis.2021.101736.
224. Pritchard, L., R.H. Glover, S. Humphris, J.G. Elphinstone, and I.K. Toth, *Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens.* Anal. Methods 2018. **8**: 12-24. DOI: 10.1039/C5AY02550H.
225. *Handbook of birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic. Terns to woodpeckers*, ed. S. Cramp. Vol. 4. 1985, Oxford: Oxford University Press.
226. *Handbook of the birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic. Waders to gulls*, ed. S. Cramp and K.E.L. Simmons. Vol. 3. 1983, Oxford: Oxford University Press.
227. *Handbook of the birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic. Hawks to bustards*, ed. S. Cramp and K.E.L. Simmons. Vol. 2. 1980, Oxford: Oxford University Press.
228. Allaby, M., *A dictionary of zoology*. 5 ed. 2020: Oxford University Press.
229. Wilson, J.B., *Guilds, functional types and ecological groups.* Oikos, 1999. **86**: 507-522.
230. Hoffman, T., L.G. Carra, P. Öhagen, T. Fransson, C. Barboutis, D. Piacentini, J. Figuerola, K. Kiat, A. Onrubia, T.G.T. Jaenson, K. Nilsson, Å. Lundkvist, and B. Olsen, *Association between guilds of birds in the African-Western Palaearctic region and the tick species Hyalomma rufipes, one of the main vectors of Crimean-Congo hemorrhagic fever virus* One Health, 2021. **13**: 100349. DOI: 10.1016/j.onehlt.2021.100349.
231. Uspensky, I., *Preliminary observations on specific adaptations of exophilic ticks to forest or open country habitats.* Experimental and Applied Acarology, 2002. **28**: 147-154. DOI: 10.1023/A:1025303811856.
232. Walker, A.R., A. Bouattour, J.-L. Camicas, A. Estrada-Peña, I.G. Horak, A.A. Latif, R.G. Pegram, and P.M. Preston, *Ticks of domestic animals in Africa: a guide to identification of species*. 2003, Atalanta, Houten, The Netherlands: Bioscience Reports, Edinburgh, Scotland, U.K.
233. Grandi, G., L. Chitimia-Dobler, P. Choklikitumnuey, C. Strube, A. Springer, A. Albihn, T.G.T. Jaenson, and A. Omazic, *First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden.* Ticks Tick Borne Dis, 2020. **11**(3): 101403. DOI: 10.1016/j.ttbdis.2020.101403.

234. Hornok, S. and G. Horvath, *First report of adult Hyalomma marginatum rufipes (vector of Crimean-Congo haemorrhagic fever virus) on cattle under a continental climate in Hungary.* Parasit Vectors, 2012. **5**: 170. DOI: 10.1186/1756-3305-5-170.
235. Hansford, K.M., D. Carter, E.L. Gillingham, L.M. Hernandez-Triana, J. Chamberlain, B. Cull, L. McGinley, L.P. Phipps, and J.M. Medlock, *Hyalomma rufipes on an untraveled horse: Is this the first evidence of Hyalomma nymphs successfully moulting in the United Kingdom?* Ticks and Tick-Borne Diseases, 2019. **10**: 704-708.
236. Chitimia-Dobler, L., S. Schaper, R. Riess, K. Bitterwolf, D. Frangoulidis, M. Bestehorn, A. Springer, R. Oehme, M. Drehmann, A. Lindau, U. Mackenstedt, C. Strube, and G. Dobler, *Imported Hyalomma ticks in Germany in 2018.* Parasit Vectors, 2019. **12**(1): 134. DOI: 10.1186/s13071-019-3380-4.
237. *Handbook of the birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic. Tyrant Flycathers to Thrushes.*, ed. S. Cramp. Vol. 5. 1988, Oxford: Oxford University Press.
238. *Handbook of the birds of Europe, the Middle East, and North Africa. The birds of the Western Palearctic. Flycatchers to Shrikes*, ed. C. Perrins and S. Cramp. Vol. 7. 1993, Oxford Oxford University Press.
239. Hoffman, T., M. Lindeborg, C. Barboutis, K. Erciyas-Yavuz, M. Evander, T. Fransson, J. Figuerola, T.G.T. Jaenson, Y. Kiat, P.E. Lindgren, A. Lundkvist, N. Mohamed, S. Moutailler, F. Nyström, B. Olsen, and E. Salaneck, *Alkhurma hemorrhagic fever virus RNA in Hyalomma rufipes ticks infesting migratory birds, Europe and Asia Minor.* Emerg Infect Dis, 2018. **24**(5): 879-882. DOI: 10.3201/eid2405.171369.
240. Labuda, M., L. Jones, T. Williams, V. Danielova, and P. Nuttal, *Efficient transmission of tick-borne encephalitis virus between cofeeding ticks.* J Med Entomol, 1993. **30**(1): 295-299.
241. Papageorgiou, D., C. Barboutis, C. Kassara, and S. Giokas, *Habitat selection of woodchat shrikes Lanius senator during spring stopover is related to foraging strategy.* Current Zoology, 2017. **63**(2): 139-149.
242. Jaarsma, R.I., H. Sprong, K. Takumi, M. Kazimirova, C. Silaghi, A. Mysterud, I. Rudolf, R. Beck, G. Foldvari, L. Tomassone, M. Groenevelt, R.R. Everts, J.M. Rijks, F. Ecke, B. Hornfeldt, D. Modry, K. Majerova, J. Votypka, and A. Estrada-Peña, *Anaplasma phagocytophilum evolves in geographical and biotic niches of vertebrates and ticks.* Parasit Vectors, 2019. **12**(1): 328. DOI: 10.1186/s13071-019-3583-8.
243. Hoffman, T., P. Wilhelmsson, C. Barboutis, T. Fransson, T.G.T. Jaenson, P.-E. Lindgren, F.D. Von Loewenich, Å. Lundkvist, B. Olsen, and E. Salaneck, *A divergent Anaplasma phagocytophilum variant in an Ixodes tick from a migratory bird; Mediterranean basin.* Infection ecology & epidemiology, 2020. **10**(1): 1729653. DOI: 10.1080/20008686.2020.1729653.
244. M'Ghirbi, Y., H. Yaich, A. Ghorbel, and A. Bouattour, *Anaplasma phagocytophilum in horses and ticks in Tunisia.* Parasit Vectors, 2012. **5**. DOI: 10.1186/1756-3305-5-180.
245. Chastagner, A., X. Bailly, A. Leblond, S. Pradier, and G. Vourc'h, *Single genotype of Anaplasma phagocytophilum identified from ticks, Camargue, France.* Emerg Infect Dis, 2013. **19**(5): 825-827. DOI: 10.3201/eid1905.121003.

246. Langenwalder, D.B., S. Schmidt, C. Silaghi, J. Skuballa, N. Pantchev, I.A. Matei, A.D. Mihalca, U. Gilli, J. Zajkowska, M. Ganter, T. Hoffman, E. Salaneck, M. Petrovec, and F.D. von Loewenich, *The absence of the drhm gene is not a marker for human-pathogenicity in European Anaplasma phagocytophilum strains.* Parasit Vectors, 2020. **13**(1): 238. DOI: 10.1186/s13071-020-04116-z.
247. Kim, M., H.S. Oh, S.C. Park, and J. Chun, *Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes.* Int J Syst Evol Microbiol, 2014. **64**: 346-351. DOI: 10.1099/ijs.0.059774-0.
248. Pascucci, I., M. Di Domenico, G. Capobianco Dondona, A. Di Gennaro, A. Polci, A. Capobianco Dondona, E. Mancuso, C. Camma, G. Savini, J.G. Cecere, F. Spina, and F. Monaco, *Assessing the role of migratory birds in the introduction of ticks and tick-borne pathogens from African countries: An Italian experience.* Ticks Tick Borne Dis, 2019. **10**(6): 101272. DOI: 10.1016/j.ttbdis.2019.101272.
249. Raoult, D., P.E. Fournier, P. Abboud, and F. Caron, *First documented human Rickettsia aeschlimannii infection.* Emerg Infect Dis, 2002. **8**(7): 748-749. DOI: 10.3201/eid0807.010480.
250. Tosoni, A., A. Mirijello, A. Ciervo, F. Mancini, G. Rezza, F. Damiano, R. Cauda, A. Gasbarrini, G. Addolorato, and Internal Medicine Sepsis Study Group, *Human Rickettsia aeschlimannii infection: first case with acute hepatitis and review of the literature.* Eur Rev Med Pharmacol Sci, 2016. **20**(12): 2630-2633.
251. Di Lecce, I., C. Bazzocchi, J.G. Cecere, S. Epis, D. Sassera, B.M. Villani, G. Bazzi, A. Negri, N. Saino, F. Spina, C. Bandi, and D. Rubolini, *Patterns of Midichloria infection in avian-borne African ticks and their trans-Saharan migratory hosts.* Parasit Vectors, 2018. **11**(1): 106. DOI: 10.1186/s13071-018-2669-z.
252. Cabezas-Cruz, A., T. Pollet, A. Estrada-Peña, E. Allain, S. Bonnet, and S. Moutailler, *Handling the microbial complexity associated to ticks*, in *Ticks and Tick-Borne Pathogens*. 2018, IntechOpen.
253. King, J.L., A.D. Smith, E.A. Mitchell, and M.S. Allen, *Validation of droplet digital PCR for the detection and absolute quantification of Borrelia DNA in Ixodes scapularis ticks.* Parasitology, 2017. **144**(4): 359-367. DOI: 10.1017/S0031182016001864.