

Chapter 5

Emergence of New Tickborne Infections

5.1 Introduction

Ticks are responsible for the most diverse range of microbial pathogens transmitted by vectors and are second only to mosquitoes as the most frequent vector of human infectious diseases. These hematophagous arthropods parasitize every class of vertebrates in nearly all areas of the world [1] and represent one of the most important mediators of zoonoses to humans. In the past decade or more, there have been global expansion and emergence of several tickborne diseases: Lyme disease, ehrlichiosis, anaplasmosis, babesiosis, and others. Moreover, new tickborne diseases continue to be discovered in recent years, such as novel phleboviruses of the *Bunyaviridae* family emerging separately in Asia, Europe, and North America. One of these viruses was first recognized in China in 2010, presenting in patients with fever, leucopenia, thrombocytopenia, and organ dysfunction, called severe fever with thrombocytopenia syndrome virus [SFTSV] [2]. A closely related phlebovirus, called Heartland virus, was subsequently discovered in 2012 to cause a similar clinical syndrome in 2012 in Missouri, United States [US] [3].

Recently described new emerging tickborne infections also include previously unrecognized species of rickettsia causing human diseases, such as *Rickettsia slovaca*, first clinically recognized in 1997 in Europe as causing tickborne lymphadenopathy [TIBOLA] [4], and newly described spirochete causing relapsing fever-type illness, first appearing in humans in Russia in 2011 [5], and subsequently reported in the US in 2013, known as *Borrelia miyamotoi* [6]. In 2010 a new member of the *Anaplasmataceae* family, *Candidatus neohrlichia mikurensis*, was recognized to cause a septicemic illness in Europe [7].

5.2 Ticks

There are at least 869 species or subspecies of ticks recognized [1]. These comprise of two major families: the Ixodidae or hard-ticks because of their sclerotized, hard dorsal plate, which represent the most important group medically and numerically, and the Argasidae or soft-ticks because of their flexible cuticle. A third family consisting of a single species, the Nuttalliellidae, is confined to southern Africa [1]. Ticks have three stages in their life cycle: the larval, nymphal, and adult forms. Hard-ticks and soft-ticks differ morphologically and in their life cycle and ecology. Ixodid ticks have several advantageous attributes for transmitting infectious pathogens as vectors more than soft-ticks or argasids. Ixodid ticks feed for long periods [several days], firmly attached to the host, and remain unnoticed as their bite is painless, and their feeding hosts include a large variety of vertebrates in diverse habitats [1]. Argasids, conversely, feed briefly and often on a single host species, and they live in dry areas, mostly in sheltered habitat near their hosts [1]. It is believed that ticks evolve about 225 million years ago in the Paleozoic or early Mesozoic eras, initially parasitizing reptiles [8].

Ixodids or hard-tick adult size averages 20–30 mm, females longer than males, and the larva is about 2 mm with progressive increase in size to the nymph, which is under 1 cm, and then to the adult form [1]. Each stage of the tick attaches and feeds on a single host over several days; then once satiated it detaches and finds a resting place to molt to the next stage. The larva and nymph are the main transmitters of diseases, as the adult feeds only briefly and the males may not feed at all. Ixodid ticks actually spend >90% of their life cycle unattached from the host and mainly live in open areas of meadows but are transported to woods by their hosts [9]. These ticks exhibit active host-seeking behavior by climbing plants and attaches to passing animal hosts or emerge from their habitat and attack nearby animals [1].

The life cycle of the ixodid ticks usually extends to 2 years but may vary from 6 months to 6 years, depending on the environmental conditions such as temperature and humidity [1]. Some tick species are host specific, feeding only a limited variety of animals, but this may vary with the stage, and some ticks have different host for each feeding stage. In general the animals in their habitat influence host selection and diverse species of ticks have different affinities for attacking humans [10]. The relationship with ecology or environment, natural animal inhabitants and host specificity or range, and geographic distribution are all interrelated with respect to the life cycle of these ticks. The brown dog tick, *Rhipicephalus sanguineus*, for instance, is well adapted to the vegetation and climate in the Mediterranean region and other areas with similar conditions such as Mexico, Arizona, California, Texas, and parts of Brazil, all regions where the brown dog tick can transmit rickettsial pathogens. Each feeding stage of *R. sanguineus* has high specificity, and it readily feeds on dogs and is important vectors for Mediterranean spotted fever in the Mediterranean region, and occasionally Rocky Mountain spotted fever in southwestern US and Mexico. In contrast, *Ixodes scapularis* [black-legged deer tick] in the US and *Ixodes ricinus* [European sheep tick] in Europe are adapted to the woods

and forests with relative high humidity and are sensitive to desiccation from dry places. Ticks inhabiting wooded areas, *I. scapularis*, *I. ricinus*, *Amblyomma americanum* [lone star tick], and others, feed on different host species, such as small and large mammals and birds [1, 11].

The life cycle of ixodid wooded ticks, as exemplified by *I. ricinus*, starts with the gravid female laying 100–1000 of eggs on the grass, and the larvae hatch in 4–6 weeks, which attach and feed on small mammals [rodents] and birds, become engorged, detach from grass, and molt to adults in 10–20 weeks and which then attach and feed on small or large mammals and birds, then the engorged female mates with males to continue the cycle [11]. Soft-bodied ticks [argasids] are inhabitants of sheltered environments, nests, caves, burrows, and primitive man-made shelters. Unlike ixodids, they lack cement from the salivary glands for firm attachment but produce anticoagulants and cytolytic substances to facilitate brief multiple feeding times [1]. Thus, the time spent on the host is relatively short, up to a few hours, and after meals they can be found in cracks and crevices or below the soil surface in their home environment.

5.3 Historical Aspects

Ticks have been recognized to inflict bites on humans for thousands of years by ancient Greek scholars [1]. Their ability to transmit infectious disease was first recognized in animals; cattle fever caused by tickborne protozoan [*Babesia bigemina*] was described in Texas at the end of the nineteenth century [12]. Subsequently, within the first decade of the twentieth century, ticks were implicated in the transmission of microbes to humans. *Borrelia duttoni*, transmitted by a soft-bodied tick [*Ornithodoros moubata*], was found to cause relapsing fever in 1905 [13], and in 1909 Rocky Mountain spotted fever agent, *Rickettsia rickettsii*, was shown to be transmitted by the wood hard-tick *Dermacentor andersoni* [14]. Although Mediterranean spotted fever was first described in Tunis in 1910 [15], it was not until the 1930s that the brown dog tick [*Rhipicephalus sanguineus*] was recognized as the vector of the causative *Rickettsia conorii* [16].

Tularemia was recognized in squirrels in 1911 and the first human case may have been described in Japan in 1837 [11], but the first documented human case was reported in 1914 [17]. However, the epidemiology and the role of diverse ticks in the transmission of *Francisella tularensis* were not reported until 1929 [18]. In the era following World War II, many tickborne diseases of animal and humans were discovered of bacterial, viral, and protozoan etiologies [19]. Q-fever, caused by *Coxiella burnetii*, was first described in Australia in 1935 [20], and although the organism can be found in >40 species, it rarely is a vector-transmitted disease [most commonly transmitted by inhalation or ingestion], and tick transmission was initially reported in 1947 [21].

Lyme borreliosis, named after the town Old Lyme in Connecticut [US] in 1995, was discovered to be caused by *Borrelia burgdorferi* in 1982 and transmitted by ixodid ticks [22], the black-legged deer tick *I. scapularis* in the northeastern and

upper midwestern US and the western black-legged tick [*I. pacificus*] along the Pacific coast. A similar condition was described in Europe more than 100 years before, with a rash of “erythema chronicum migrans,” which was subsequently found to be caused by *Borrelia garinii*, *B. burgdorferi*, and *Borrelia afzelii* and transmitted by ixodid ticks [*I. ricinus*] [23]. Lyme borreliosis is the most common vector-transmitted disease in the US and Europe, but also occurs widely in other countries of the former Soviet Union, China, Japan, Australia, and probably in North Africa [11]. The main reservoir of Lyme borreliosis are small mammals, especially rodents such as the white-footed mouse [*Peromyscus leucopus*] in northeastern US and *Apodemus* species in Europe [24, 25]. Although deer are hosts for the black-legged ticks, they are not reservoirs for Lyme borreliosis. In Europe *B. afzelii* life cycle is maintained in rodents and *B. garinii* in avian reservoirs [26].

Rickettsiosis, caused by intracellular bacteria, has been recognized to be expanding globally for several decades and represent one of the major zoonoses [27]. Prior to 1974 only four tickborne rickettsiosis were known with one tickborne spotted fever group identified in separate regions: Rocky Mountain spotted fever due to *R. rickettsii* was first described as a clinical entity in 1899 in the Americas; *R. conorii* present in Europe, southeast Asia, and North Africa causing Mediterranean spotted fever; *R. siberia* recognized in Siberia and western Russia; and *R. australis* found in Australia [11]. The members of the *Rickettsia* genus are classified into four groups: the spotted fever group rickettsiae, typhus group rickettsiae, *Rickettsia bellii* group, and *Rickettsia Canadensis* [27]. The recognition and expansion of pathogenic *Rickettsia* have been facilitated by molecular techniques in recent times. Rickettsiae once considered nonpathogenic to humans and new species have been identified in the past 25 years. There are now 26 *Rickettsia* species validated in addition to subspecies mentioned. Some new species identified since 2005 include *Rickettsia asiatica*, *Rickettsia heilongjiangensis*, *Rickettsia hoogstraalii*, *Rickettsia raoulti*, and *Rickettsia tamourae* [27]. Tickborne rickettsiosis is now present in all continents of the world and some Caribbean and Pacific islands. *Rickettsia africae* which causes African tick-bite fever is believed to have been exported from Africa during the slave trade of the 1800s to several Caribbean islands, Guadeloupe, St. Kitts, Nevis, Dominica, US Virgin Islands, Montserrat, St. Lucia, Martinique, and Antigua [27].

5.3.1 Tickborne Zoonoses: General Background

Tickborne infections of humans, farm, and domestic animals are primarily associated with wildlife animal reservoirs. Humans and domestic animals are incidental hosts that result from infringement of the usual circulation between wildlife and tick vectors in their natural habitats. The risks of tickborne diseases vary geographically and are determined by the climate, environment, presence of rodents and other vertebrate reservoirs, and the species of ticks parasitizing wild and domestic animals [28]. These zoonoses can emerge in previously non-endemic areas when climate conditions and circumstances favorable to the maintenance and transmission arise.

This may include displacement of the hosts of vectors and reservoir animals by human expansion and development. Birds are important reservoirs for several pathogens and act as vehicles for infected ticks and probably play an important role in the dispersal of tickborne zoonoses [29]. Although for each pathogen one or more tick vectors and several animal reservoirs may exist, various combinations of coinfection with different microbes may coexist, and studies have demonstrated mixed infection in ticks of 7–10.9% [29, 30]. For example, the black-legged deer tick [*I. scapularis*], from the northeastern and upper midwestern US, can be coinfecting and transmit multiple pathogens to humans such as Lyme and myamotai borreliosis, anaplasma, babesia, and Powassan virus. Concurrent infection by two or more tickborne microbes is fairly common and frequently detected in clinical and veterinary practice [31]. Coinfection with multiple pathogens may be transmitted by ticks simultaneously during the same blood meal or at separate times. The natural reservoir hosts of many microbes [*B. burgdorferi*, *Anaplasma phagocytophilum*, and *Babesia microti*] transmitted by Ixodes ticks are rodents, which are often coinfecting with multiple organisms and may transfer the microbes to the larvae or nymphs and then to animals and humans [32]. Simultaneous coinfection of several pathogens may result in diverse host responses, which can conflict and antagonize each other, allowing the pathogens to synergistically infect the host more successfully, with more prolonged and severe disease with multitude of clinical manifestations, than normally reported with a single pathogen infection [33]. Sequential infection with a new microbe may allow the establishment of a new infection which otherwise may not have occurred because of elimination by the host immune defense mechanisms [31].

The majority of tickborne infections are transmitted during the course of the blood meal from contaminated tick saliva [i.e., *B. burgdorferi*, relapsing fever borreliae, and spotted fever rickettsiae], regurgitated midgut contents [*B. burgdorferi*], feces [*Coxiella burnetii*], or coxal fluid in argasid ticks with relapsing fever borreliae [27]. Transmission from contamination of abraded skin or mucosa of the eye following crushing of the ticks with the fingers is possible. Transmission by accidental ingestion of the arthropods is well known in animals and is possible to occur in humans. Transmission of several tickborne zoonoses can occur by blood transfusion and sharing of needles and syringes from an unsuspected blood donor or drug abuser. Different *Babesia* species, for instance, have been reported to be transmitted from blood transfusions in both animals [dogs] and humans [31].

5.4 New Tickborne Bunyaviruses

Tickborne viruses that cause human diseases belong to three main families: *Bunyaviridae*, *Flaviviridae*, and *Reoviridae*. The most common tickborne viruses belong to the *Flaviviridae* family and are endemic in Europe and Asia: tickborne encephalitis virus [TBEV] being the most common in Europe, louping illness virus [LIV], Omsk hemorrhagic fever virus [OHFV], and Kyasanur Forest disease virus [KFDV]; in the Middle East Alkhurma hemorrhagic fever virus [AHFV]; and in

North America only the Powassan virus from this group is present [34]. TBEV is widely distributed in Europe and the vector is *I. ricinus*, the sheep dog tick. Prior to 2009–2010, the only recognized members of the *Bunyaviridae* family causing tickborne infection in humans were the Crimean-Congo virus and the Bhanja virus. Since then two similar bunyaviruses of the *Phlebovirus* genus have emerged in China in 2009, severe fever with thrombocytopenia syndrome virus [SFTSV], and the Heartland virus in the US in 2012.

The *Bunyaviridae* family of viruses constitutes the largest group of RNA viruses with more than 350 identified [35]. They are enveloped, spherical virions with a diameter of 80–120 µm, containing three single-stranded RNA segments [negative sense], with no matrix proteins [35]. The family of *Bunyaviridae* contains five genera: *Hantavirus* genus, type species Hantaan virus; *Nairovirus* genus, type species Crimean-Congo hemorrhagic fever virus [CCHFV]; *Orthobunyavirus* genus, type species Bunyamwera virus; *Phlebovirus* genus, type species Rift Valley fever virus [RVFV]; and *Tospovirus* genus, type species Tomato spotted wilt virus [36, 37]. Presently there are at least 40 viruses of the *Bunyaviridae* family not assigned a genera or species, including Bhanja virus, Forecariah virus, and the Kismayo virus [38]. The Bhanja serogroup viruses are most closely related to SFTSV and the Heartland virus, and members of this group have been isolated from ticks on livestock and wild animals in India, Southern Europe, and Africa [38].

The *Bunyaviridae* are generally found in arthropods and wild animals, especially rodents, and some members can infect humans. The Hantavirus is the only member of this family not transmitted by vectors, but by rodent excreta [36]. The *Orthobunyavirus* [i.e., La Crosse virus] infects only mosquito vectors, the *Nairovirus* [i.e., CCHFV] is largely limited to ticks, and the *Phlebovirus* [i.e., sandfly fever] is transmitted by sandflies and midges, but RVFV which is a member of this group can infect a wide range of arthropods and is primarily transmitted by mosquitoes [36].

5.4.1 Severe Fever with Thrombocytopenia Syndrome [SFTS]

SFTS was first recognized as a distinct clinical entity in Henan province of China in 2007. Patients presented with high fever, gastrointestinal bleeding, abdominal pain, bloating, nausea, and vomiting, with low platelets and white blood count, elevated alanine and aspartate transaminases, and proteinuria [39]. In 2010, a novel bunyavirus designated Huaiyangshan virus and subsequently changed to SFTSV was isolated from patients with this syndrome [40].

It is believed that the SFTSV actually originated in China about 50–150 years ago [41]. The SFTSV and Heartland virus are assigned in the *Phlebovirus* genus, but they are different from other known phleboviruses. The phleboviruses consist of about 70 antigenically distinct serotypes classed into two groups. The *Phlebovirus* fever group is transmitted by the phlebotominae sandflies or mosquitoes and the Uukuniemi group by ticks [42]. Although SFTSV have limited sequence similarities to other viruses of the Uukuniemi group, it was assigned to this subtype due to

serological similarities and lack of small nonstructural protein on the M segment, and ticks are also the common vector [43]. Isolates of SFTSV from different geographical regions share 90% genetic sequence similarity and are grouped into five sublineages A–E [44]. Lineage A constitutes isolates from animals, dogs, cats, goats, buffaloes, and cattle, with no geographical clustering pattern [41].

Segmented-genome viruses such as the bunyaviruses are capable of rapid recombination, which is associated with pathogenicity and transmissibility among vectors and hosts and increases the risk for new outbreaks [45]. Recently two strains of SFTSV were found to have reassortment in the small RNA segment, which may drive rapid changes in the virus [46]. The basis of genetic diversity of SFTSV, although not completely understood, may be explained by lack of proofreading function of its RNA-dependent RNA polymerase, with high mutation rate [about 10^{-4} substitution per site each year] during its replication [41].

5.4.1.1 Epidemiology of SFTSV Infection

SFTS as a distinct syndrome was first reported in rural regions in Henan and Hubei provinces of Central China in 2009 [44]. Since then the disease has been found in 11 province of China with over 2500 reported cases. There is evidence that the microbe is widely distributed in China and only a small proportion of infected subjects develop clinical disease. Serosurveillance studies of populations in hilly regions of China showed that 1.0–3.8% of people had SFTSV antibodies [47–49]. There is also evidence that the virus has been circulating naturally in some regions of China showing seasonal variance with most cases occurring from May to July, and clinical disease occurred mainly in older subjects [92%] [50]. It has been estimated that the annual incidence of disease is about 5 per 100,000 of the rural population [49]. Seroprevalence and asymptomatic viremia of SFTSV in blood donors from endemic regions have also been reported. In a study of 17,208 blood donors, seropositivity ranged from 0.27% to 0.54%, but very low-grade viremia was detected in only two subjects [51]. Overall, SFTS is mainly reported from rural areas of central and northeastern China from May to September, targeting farmers >50 years of age [52]. Subjects were predominantly affected from farming-related exposures and numerous domestic and wild animals were infected by SFTSV [53].

SFTS was first reported outside China in North Korea in 2009 [54] and a subsequent fatal case was reported from South Korea in 2012 [55]. During 2013 SFTS was diagnosed in 35 patients in South Korea, and phylogenetic analysis of SFTSV isolates from South Korea and China was closely related [56]. Locally transmitted cases have also been identified in Japan with 11 cases recognized by 2013, all living in western Japan, and were >50 years of age, and there were six fatalities [57]. Phylogenetic analysis of the Japanese isolates indicated that the genotype was independent from those in China. It has been estimated that SFTSV was likely circulating unrecognized in animals and humans in Korea and Japan for sometimes before these reports. In 2014, there were 108 suspected cases of SFTS in Japan and 41 were confirmed cases by PCR [58].

5.4.1.2 Vector and Ecology

The tick vector of SFTSV is considered to be a widely distributed hard-tick of the Ixodidae family, *Haemaphysalis longicornis*, present in China and other countries of Asia [59]. In endemic areas of China, the prevalence of SFTSV in these ticks collected from domestic animals ranged from 2.1% to 5.4%, and the RNA sequences of viral isolates were very closely related to those in patients [39, 59]. The virus is also detected at a lower rate [0.6%] of another widely distributed tick, *Boophilus (Rhipicephalus) microplus*, in endemic and non-endemic areas [44]. Viral RNA of SFTSV was also detected in *H. longicornis* ticks at all stages of its life cycle, indicating both transstadial and transovarial routes of transmission and the ability of ticks to play a role as a vector and a reservoir of the virus [60]. In a study from South Korea, 13,000 ticks were examined for SFTSV from nine provinces, and the minimum detection rate in *H. longicornis* was 0.46%, with the highest prevalence in the southern region [61]. In South Korea SFTSV was also detected in other hard-ticks at lower rates, *Ixodes nipponensis* and *Amblyomma testudinarium* [62], and in China the virus was also detected in mites from field mice and goats in endemic regions [44]. Common with many tickborne infections, not all patients with clinical disease have a history of tick bites, and in patients from China only 52% with SFTS recall tick exposure [48].

5.4.1.3 Reservoir Hosts

A variety of domestic and wild animals have been found to carry SFTSV, at different rates in various endemic regions of China. Seroprevalence in domestic animals ranged from in cattle 32–80%, goats 57–95%, chickens 1–36%, dogs 6–55%, and pigs 2–6% [44, 52, 63, 64]. Low levels of viral RNA were found in a small fraction [1.7–5.3%] of the animals studied [48, 65]. The viruses from animals and those from patients and ticks shared 95.4% of the genomic sequences [65]. Many wild animals such as deer, hedgehog, weasel, possum, and some bird species are hosts for the vector ticks and could carry the virus. However, rodents are known reservoirs of many bunyaviruses, but the rates of infection of rats [3.03–8%] with SFTSV are lower than in livestock [48, 66]. Hence, it appears that domestic animals may be the amplifying hosts of SFTSV and are probably the main reservoir of the virus.

Transmission of SFTSV is considered mainly from tick bites, but there is also evidence from multiple reports that the virus can be transmitted from human to human by direct contact with blood of infected patients [67–71]. A cluster of cases in families/households have been reported to be transmitted by blood contact, and blood transfusion and laboratory accidents from handling infected blood are potential means of transmission of SFTSV.

5.4.1.4 Pathogenesis and Immunity of SFTSV Infection

Although the pathogenesis of SFTS is not fully understood, major strides have been made in the few years since its description in understanding the mechanisms of the disease. Like other severe viral infections, cytokine and chemokine imbalance appears to be important in the pathogenesis, and severe, fatal cases of SFTS usually demonstrate “cytokine storm.” Increased levels of tumor necrosis factor-alpha [TNF- α], interferon-gamma [IFN- γ], and IFN-induced protein-10 were associated with disease severity [72]. SFTSV gain entry to many human and animal cells, including macrophages and dendritic cells, by binding of the virus glycoprotein [GN/GC] to a receptor, the C-type lectin DC-SIGN [73].

Dynamic changes in viral load, T-cell subsets, and cytokines have been measured and analyzed in patients with SFTS and correlated with outcome. High levels of peak viral RNA load, serum liver enzymes, and serum interleukin [IL]-6 and IL-10 were associated with higher fatality rates [74]. These markers declined within 2 weeks of onset in survivors, and CD69+ T-cells were elevated early after infection, while HLA-DR+ and CTLA4+ T-cells elevated during the recovery phase of survivors [74]. Hence, high SFTSV viral load, very low platelets, high transaminases, marked elevation of proinflammatory and anti-inflammatory cytokines, and activation of CD 69+ T-cells were markers of severe disease and poor outcome. Cytokine storm with suppression of CD4+ and CD3+ lymphocytes with progressive decline but higher B lymphocytes has also been reported in severe and fatal cases of SFTS [75, 76].

Analysis of the immune response during the course of illness may also assist in understanding the pathogenesis of disease. In a study of 298 confirmed cases of SFTS and 55 followed after convalescence, during the first week of illness, there was a loss of T, B, and NK lymphocytes which were subsequently restored, but severe disease was associated with slower recovery and lower humeral immunity [77]. SFTSV-specific IgM antibody could be detected within 9 days, peaked at 4 weeks, and persisted for 6 months. IgG antibody could be detected in most patients within 6 weeks, peaked at 6 months, and persisted for at least 3 years [77]. There is also evidence that SFTSV is capable of infecting monocytes and suppresses IFN-beta and NF-kappa B promoter activities, facilitating the virus replication in human monocytes by restricting the innate immune response [78]. The virus can also disrupt type 1 interferon signaling by the nonstructural protein-mediated sequestering of signal molecules [STATS I and II] into inclusion bodies [79]. IFN- β production is an important host defense mechanism against viral pathogens, and inhibition of this response has been reported with other bunyaviruses [80, 81].

Limited pathological studies of fatal SFTS cases have been reported and the characteristic feature is the presence of necrotizing lymphadenitis of lymphoid tissues [82]. Leucopenia and thrombocytopenia which are hallmarks of the disease were not due to bone marrow suppression or aplasia, but are likely related to peripheral destruction or sequestration [83].

5.4.1.5 Animal Models of SFTSV Infection

Animal models are useful for elucidating the pathogenic mechanisms and development of new therapies for many human diseases. Newborn mice and rats, especially Kunming mice, are highly susceptible to SFTSV, and infected mice demonstrate pathological changes with large areas of necrosis only in the liver, but the virus can be detected in numerous organs [84]. Interferon- α /interferon- β knockout mice are also highly susceptible to infection with the virus, with 100% mortality in 3–4 days after inoculation [85]. The virus is found in numerous organs with heavy viral burden in mesenteric lymph nodes and spleen but no detectable histological changes. It appears that C57/BL6 mouse may be a better model for SFTSV infection, as the animals demonstrate leucopenia and thrombocytopenia similar to humans [86]. Moreover, histopathological changes were found in the spleen, liver, and kidney, but the spleen appears to be the primary target as viral replication could be demonstrated in this organ. The thrombocytopenia is probably caused by virus-bound platelets that underwent phagocytosis by splenic macrophages [86]. Nonhuman primates are considered the gold standard animal models for studying human disease pathogenesis, and a recent study has been reported by infecting rhesus macaques. SFTSV infection of *Macaca mulatta* did not result in severe disease symptoms or death but caused fever, thrombocytopenia, leucopenia, and elevated transaminases and cardiac enzymes [87]. Minor pathological lesions were found in the liver and kidney during the late stages of infection, and elevation of inflammatory cytokines was present in the blood. Thus, infection of this primate model resembles mild SFTS in humans.

Other animal studies assessed potential therapeutic and preventative interventions. The nonstructural protein of the S segment [SFTSV/NSs] fraction of SFTSV appears to antagonize interferon and suppress the host's innate immunity to facilitate infection. However, immunization with recombinant SFTSV/NSs was ineffective in promoting virus clearance in infected C57L/6J mice [88]. In another study using a mouse model, antiserum from a recovered patient with SFTS prevented lethal infection with the virus and improved clinical signs in nonlethal infection [89]. Other agents tested including steroids, ribavirin, and a site-I protease inhibitor were ineffective.

5.4.1.6 Clinical Aspects of SFTS

The incubation period of SFTS after a tick bite is estimated to be 5–14 days [44, 90] and after exposure to infected blood as 7–12 days [67]. The major stages of the disease recognized include an initial febrile flu-like illness, a second stage of multi-organ failure, and a subsequent convalescent phase [44, 52]. The first stage of the disease is characterized by sudden onset of fever from 38 to 41°C, headache, fatigue, myalgia, nausea, vomiting, and diarrhea, associated with high viral load, leucopenia, thrombocytopenia, lymphadenopathy, and elevation of transaminases and creatine phosphokinase. This stage may last for 5–11 days but may resolve after a week and enter a convalescent phase in patients with mild disease. The second stage occurs after 5 days and in more severe disease progress to develop multiorgan failure, first involving the liver, then the heart, lungs, and kidneys, which can persist for

7–14 days or lead to death [44, 90]. Coma may occur in about 6% but as high as 27% and confusion is seen in 22–36% [44, 52]. A recent report of 538 patients with SFTS described development of encephalitis in 19% of cases, with a fatality rate of 44.7% in this subgroup [91]. In nonfatal cases the biomarkers start to decrease with decline of the viral load and improvement of the platelet count by day 9–11, whereas in fatal cases the viral load, biomarkers, and thrombocytopenia continue to increase. The overall case fatality rate of 2500 reported cases in China averages at 7.3% [44], but the initial report was 12–30% [39].

Convalescence in survivors varies from 11 days to 19 days after onset of illness but in most severe cases occurred after 14 days [40, 52]. Clinical symptoms improve and the laboratory abnormalities gradually return to normal after 3–4 weeks [89]. Prognostic factors for disease severity and outcome of SFTS are related to host factors, clinical manifestations, and laboratory parameters. Age is a key factor in the severity of disease and outcome, with older age carrying a worst prognosis [44, 52, 90]. Children rarely become infected [possibly from less tick exposures and more robust immunity] and those who become infected manifest mild symptoms with fever, malaise and gastrointestinal symptoms, and minor laboratory abnormalities [92]. Clinical features associated with adverse prognosis include neurological manifestations, acute respiratory distress syndrome, and disseminated intravascular coagulopathy [93]. Host immune responses and viral replication are important factors in determining clinical severity and outcome, and high viral load in blood at admission is associated with a worst prognosis and fatality [93]. Laboratory parameters associated with poor prognosis and fatality include hypoalbuminemia, hyponatremia, coagulation disturbance, elevated transaminases, elevated creatinine, decreased lymphocyte count, and elevated lactic dehydrogenase levels in the late stage [94, 95].

5.4.1.7 Diagnosis of SFTS

Diagnosis of SFTS is based on epidemiological exposure risk and clinical features with presence of fever, thrombocytopenia, and leucopenia. Laboratory diagnosis can be confirmed by viral nucleic acid test with polymerase chain reaction [PCR] or by serology. Rapid diagnosis in acute illness is best accomplished with a reverse transcriptase [RT] PCR which is highly sensitive and specific [44]. Potential detection limit of 10 viral RNA copies/ μL was achieved using quantitative real-time PCR with 98.6% sensitivity and over 99% specificity [96]. Moreover, the quantitative PCR at acute diagnosis can assist in the prognosis based on the viral load [58].

Serological methods for diagnosis include conventional immunofluorescence and serum neutralization assays which are not helpful in acute cases or early diagnosis, and they are costly and require well-trained personnel [52]. Indirect enzyme immunoassay [EIA] and double-antigen sandwich EIA have been used to detect total antibodies or viral-specific IgM and IgG [52]. A highly sensitive and specific EIA utilizing a glycoprotein N from the nucleocapsid has been developed [63]. In serological diagnosis acute and convalescent sera are best tested, thus providing a delayed diagnosis, although the presence of specific IgM antibodies in acute disease can be diagnostic.

5.4.1.8 Treatment of SFTS

Management of patients with SFTS is largely supportive with correction of fluid and metabolic disturbances and blood transfusion if necessary for significant blood losses. Platelet transfusion may be required for severe thrombocytopenia [$<30 \times 10^9/L$] with significant bleeding, and appropriate antibiotics for suspected or proven secondary bacterial infections [44]. Mechanical ventilation may be needed in severe cases with ARDS or coma for airway protection and hemodialysis for severe renal failure.

No specific therapy has been shown to be effective for SFTSV infection. Ribavirin was of interest as it had been considered effective or approved for treatment of other bunyavirus infections, such as Rift Valley fever virus and Crimean-Congo hemorrhagic fever virus [97, 98]. While ribavirin has some in vitro activity against SFTSV, it did not effectively reduce virus replication in pre-infected cells [99]. Moreover, ribavirin treatment of patients with SFTS produced no significant clinical benefit and had no effect on platelet counts or viral loads [100]. Convalescent sera from recovered patients infected with SFTSV have high neutralizing antibodies against the virus and may have a role in treatment of severe cases or postexposure prevention after contact with infected blood [89, 101].

5.4.2 Heartland virus

Heartland virus infection was first described from a patient in Missouri [US] in 2012 and the agent is a phlebovirus closely related to SFTSV [3]. Phylogenetic and serological analysis revealed that Heartland virus should belong to the Bhanja group of viruses in the *Phlebovirus* genus [38]. Bhanja viruses have been isolated from various species of hard-ticks and are divided into the African and Eurasian lineages. *Amblyomma americanum* [lone star tick] is the vector of Heartland virus [HRTV]. Investigations had revealed the presence of viable virus in *A. americanum* nymphs in a patient's farm and nearby conservation area, with >97.6% sequence identity to human strains [102]. Ticks probably become infected by feeding on viremic hosts during their larval stage and may transmit the virus to humans during the spring and early summer when nymphs are plentiful.

Serological investigations in wild and domestic animals in Missouri have detected high antibody prevalence to HRTV in raccoon [42.6%], horse [17.4%], and white-tailed deer [14.3%], which suggest that these species are possible candidate reservoir hosts [103]. Antibodies were also found in dogs [7.7%], but no HRTV was isolated from any animal sera or ticks.

5.4.3 Clinical Features of Heartland virus Infection

The original two index cases were male farmers, aged 57 and 67 years, who presented with fever, leucopenia, and thrombocytopenia [3]. Both patients survived without hemorrhagic complications or multiorgan failure. An additional five

nonfatal cases were subsequently identified through active surveillance in Missouri [104]. More recently a fatal case of HRTV infection was described in 80-year-old male on a farm in Tennessee [100]. The patient had a history of multiple tick bites, with detectable tick on his body 2 weeks prior to onset of illness, and had comorbid conditions of chronic obstructive lung disease and alcoholism. He presented with weakness, fever, and altered mental status, and tests showed persistent leucopenia, progressive thrombocytopenia, anemia, and elevated transaminases. His clinical course deteriorated on broad-spectrum antibiotics and subsequent hypotension, hypoxia, and renal dysfunction occurred before his death. Autopsy findings were largely nonspecific and the bone marrow revealed myeloid hyperplasia and trilineage hematopoiesis. Although this fatal case resembles severe cases of SFTS, there was no evidence of necrotizing lymphadenitis as described in fatal cases of infection with SFTSV, but the spleen demonstrated white-pulp depletion and scattered immunoblasts [105]. The differential diagnosis of HRTV infection would include infections with borrelia, rickettsia, ehrlichia, anaplasma, viruses, thrombotic thrombocytopenia, and hematological malignancies.

A subsequent fatal case has been described in Oklahoma [105], bringing the total number of HRTV infection detected to date to nine cases with two fatalities from three states. However, more cases are likely to be recognized in the future from other regions of the US, as wildlife serological studies have determined that HRTV is widespread within the central and eastern US [106]. Of 1428 animals tested, 103 were seropositive for the HRTV including 55 deer, 33 raccoon, 11 coyote, and 4 moose. Thirteen states had seropositive animals from Florida, Georgia, Illinois, Indiana, Kansas, Kentucky, Maine, Missouri, New Hampshire, North Carolina, Tennessee, Texas, and Vermont [106].

Similar to SFTS, there is no known specific therapy for HRTV infection. Management is primarily supportive, but doxycycline should be used initially in severe cases for treatable differential diagnoses [borreliosis, rickettsiosis, ehrlichiosis, etc.] until these conditions are excluded. There is no data yet on pathogenesis and animal models of HRTV infection. However, the mechanisms in disease pathogenesis maybe similar to that of SFTSV since the two viruses are closely related.

5.4.4 *Borrelia miyamotoi Disease*

Borrelia miyamotoi is a spirochete that is closely related to species that cause relapsing fever. It was first discovered in *Ixodes persulcatus* ticks in Japan in 1994 and subsequently documented in ticks and rodents in Europe and North America, but was not recognized to cause human infection until reported from Russia in 2011 [5]. In 2013, the first case of *B. miyamotoi* infection was described in the US with clinical manifestation of meningoencephalitis [6]. Subsequently two more cases were reported in the US presenting like human granulocytic anaplasmosis [107]. Cases of *B. miyamotoi* infection were also reported from New England [108], the Netherlands [109], and Japan [110].

The largest case series of *B. miyamotoi* disease [BMD] was recently reported from northeastern US. Blood samples from acutely febrile patients presenting to primary care offices, emergency departments, or urgent care clinics in 2013–2014, requesting testing for tickborne infections, were sent to IMUGEN [Norwood, Massachusetts] [111]. Whole blood PCR for presence of specific DNA sequences of common tickborne pathogens and BMD were performed. Among 11,515 patients tested, 97 BMD cases were identified by PCR. Most of the patients presented with fever, chills, marked headache, myalgia, or arthralgia, and 24% required hospitalization [111]. Elevated liver enzymes [82%], leucopenia, and thrombocytopenia were common, and symptoms resolved after doxycycline and patients treated with amoxicillin or ceftriaxone also improved. Serology for the acute diagnosis of BMD was poor, as only 1 of 39 cases with circulating DNA had *B. miyamotoi* IgM and none with IgG, but convalescent sera were reactive in 78%. In this study of acute febrile patients, BMD was confirmed less frequently than babesiosis and human granulocytic anaplasmosis.

B. miyamotoi infection is transmitted by the black-legged deer ticks [*I. scapularis*] and has a similar range and distribution as Lyme borreliosis. Most cases of BMD occurred in July and August, suggesting transmission by larval ticks which have their peak activity in these months. Lyme disease, babesiosis, and human anaplasmosis mainly occur in June and early July, when the nymphal deer ticks are most abundant [112]. *B. miyamotoi* had been shown to undergo transovarial transmission and can be transmitted experimentally by larvae [113].

In regions where tickborne infections are possible, with or without known tick bites/exposure such as in many parts of northeastern, north-central, and far western US, empiric treatment with doxycycline may be reasonable for acutely sick, febrile patients for possible borrelia, rickettsia, ehrlichia, and anaplasma infections until a diagnosis is confirmed.

5.5 Conclusion and Future Perspectives

Novel tickborne infections continue to emerge in distant unrelated regions of the world with worrisome frequency. New, previously unrecognized, pathogens from tick exposure will continue to be discovered in the future. This is further exemplified by the recent detection of a novel *Orthomyxovirus* of the genus *Thogotovirus*, labeled Bourbon virus, associated with a fatal infection in a previously healthy male from Kansas after tick bites in 2014 [114]. The differential diagnosis of acute febrile illness after tick exposure is quite diverse and largely depends on the local region or geography at the time of exposure. For instance, in the US, there are 14 tickborne diseases listed by CDC, and now there will be 15 with the addition of Bourbon virus [see Table 5.1]

Tickborne diseases are theoretically preventable with appropriate clothing [long sleeve shirts and pants], avoidance of high-risk exposures, use of insecticides or acaricides to control tick population, or use of personal insect repellants. Despite these known preventative measures, for decades tickborne diseases have continued to

Table 5.1 Tickborne diseases of North America, mainly United States

Disease	Pathogen	Vector	Distribution
Anaplasmosis	<i>Anaplasma phagocytophilum</i>	<i>I. scapularis</i>	Eastern and upper-middle US
		<i>I. pacificus</i>	Pacific coast/Western US
Babesiosis	<i>Babesia microti</i>	<i>I. scapularis</i>	Northeast/upper midwestern US
BMD	<i>Borrelia miyamotoi</i>	<i>I. scapularis</i>	Northeastern/midwestern US
Bourbon virus disease	<i>Thogotovirus</i>	Unconfirmed tick	Kansas
Colorado tick fever	Colorado tick fever virus	<i>Dermacentor andersoni</i>	Rocky Mountain states
Ehrlichiosis	<i>Ehrlichia chaffeensis</i> / <i>E. ewingii</i>	<i>Amblyomma americanum</i>	Southcentral/Eastern US
Heartland virus	<i>Phlebovirus/Bunyavirus</i>	Lone star tick [<i>A. americanum</i>]	Missouri, Tennessee, Oklahoma
Lyme disease	<i>Borrelia burgdorferi</i>	<i>I. scapularis</i>	Northeast/upper midwestern US
		<i>I. pacificus</i>	Pacific Coast
Powassan disease	Powassan virus	<i>I. scapularis</i> and Groundhog tick	Northeast, Great Lakes area
R. parkeri rickettsiosis	<i>Rickettsia parkeri</i>	Gulf Coast tick	Gulf Coast, Southwestern US
RMSF	<i>Rickettsia rickettsii</i>	Am. dog tick	Eastern US [most common]
		RM wood tick	Western US
		Brown dog tick	Arizona, Texas, and Mexico
364D rickettsiosis	<i>Rickettsia phillipi</i>	Pacific Coast tick	California
STARI	Unknown agent	Lone star tick	Eastern, Southeastern US
[Southern tick-associated rash illness]			
Tickborne relapsing fever	<i>Borrelia hermsii</i>	<i>O. hermsii</i> [soft-tick] of squirrel, chipmunk	Western US [15 states]
Tularemia	<i>Francisella tularensis</i>	Dog tick, wood tick, and lone star tick	Throughout US

BMD *Borrelia miyamotoi* disease, *I* ixodes, *Am* American, *O* *Ornithodoros*, *RM* Rocky Mountain, *SF* spotted fever

flourish with worldwide expansion and emergence. Thus, new approaches for prevention of tickborne infections are needed. Directed mass education of targeted at-risk populations [farmers, outdoor campers, hikers, etc.] in endemic areas may be of benefit to inform the public of the risk of these diseases and preventative measures, including large visible signs in these areas. Development of specific vaccines for these various pathogens is not feasible nor would be cost-effective due to their relatively low frequency. However, it may be worthwhile to evaluate new strategies such as a vaccine to prevent tick bites that could be used to prevent many tickborne diseases. Anti-tick vaccines could potentially reduce tickborne infections by reducing the tick burden or interference in host-tick-human transmission by vaccination of the vertebrate hosts [115]. Anti-tick vaccines would be environmentally safe, unlikely to select resistance as compared to insecticides, and can include multiple antigens to target a broad range of ticks [116].

References

1. Sonenshine DE (1991) *Biology of ticks*, vol 1. Oxford University Press, New York
2. Xu B, Liu L, Huang X et al (2011) Metagenetic analysis of fever, thrombocytopenia and leucopenia syndrome [FTLS] in Henan Province, China: discovery of a new bunyavirus. *PLoS Pathog* 7:e1002369
3. Mc Mullan LK, Folk SM, Kelly AJ et al (2012) A new phlebovirus associated with severe febrile illness in Missouri. *N Engl J Med* 367:834–841
4. Ibarra V, Oteo JA, Portillo A et al (2006) *Rickettsia slovaca* infection: DEBONEL/TIBOLA. *Ann N Y Acad Sci* 1078:206–214
5. Platonov AE, Karan LS, Kolyasnikova NM et al (2011) Humans infected with relapsing fever spirochete *Borrelia miyamotoi*, Russia. *Emerg Infect Dis* 17:1816–1823
6. Gugliotta JL, Goethert HK, Berardi VP et al (2013) Meningoencephalitis from *Borrelia miyamotoi* in an immunocompromised patient. *N Engl J Med* 368:240–245
7. Fehr JS, Bloemberg GV, Ritter G et al (2010) Septicemia caused by tickborne bacterial pathogen *Candidatus Neoehrlichia mikurensis*. *Emerg Infect Dis* 16:1127–1129
8. Klompen JSH, Blank WC IV, Kierens JE, Oliver JH Jr (1996) Evolution of ticks. *Annu Rev Entomol* 41:141–161
9. Needham GR, Peel PD (1991) Off-host physiological ecology of ixodid ticks. *Annu Rev Entomol* 36:659–681
10. Estrada-Pena A, Jongejans E (1999) Ticks feeding on humans: a review of records on human biting Ixodoidea with special reference to pathogen transmission. *Exp Appl Acarol* 23:685–715
11. Parola P, Raoult D (2001) Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. *Clin Infect Dis* 32:897–928
12. Smith T, Kilbourne FL (1893) Investigations into the nature, causation and prevention of Texas or southern cattle fever. *Bull Bur Anim Ind US Dept Agric* 1:301
13. Dutton JE, Todd JL (1905) The nature of tick fever in the eastern part of Congo Free State, with notes on the distribution and bionomics of the tick. *Br Med J* 2:1259–1260
14. Ricketts HT (1909) Some aspects of Rocky Mountain spotted fever as shown by recent investigations. *Med Rec* 16:1843–1855
15. Connor A, Bruch A (1910) Une fièvre eruptive observe en Tunisie. *Bull Soc Pathol Exot Filial* 8:492–496
16. Brumpt E (1932) Longevite du virus de la fièvre boutonneuse [*Rickettsia conori*, n.sp.] chez a la tique *Rhinicephalus sanguineus*. *CR Soc Biol* 110:1197–1199
17. Wherry WB, Lamb BH (1914) Infection of man with *Bacterium tularense*. *J Infect Dis* 15:331–340
18. Francis E (1929) A summary of the present knowledge of tularemia. *Medicine (Baltimore)* 7:411–432
19. Sonenshine DF (1993) *Biology of ticks*, vol 2. Oxford University Press, New York
20. Mauvin M, Raoult D (1999) Q-fever. *Clin Microbiol Rev* 12:518–553
21. Ecklund CM, Parker RR, Lackman DB (1947) Case of Q-fever probably contracted by exposure to ticks in nature. *Public Health Rep* 62:1413–1416
22. Johnson RC, Schmid GP, Hyde FW, Steigerwalt AG, Brenner DJ (1984) *Borrelia burgdorferi* sp. nov.: etiological agent of Lyme disease. *Int J Syst Bacteriol* 34:496–497
23. Rijpkema SGT, Tazelaar D, Molkenboer M et al (1997) Detection of *Borrelia afzelii*, *Borrelia burgdorferi* sensu stricto, *Borrelia garinii*, and group VS 116 by PCR in skin biopsies of patients with erythema migrans and acrodermatitis chronica atrophicans. *Clin Microbiol Infect* 3:109–116
24. Cook V, Barbour AG (2015) Broad diversity of host responses of the white-footed mouse *Peromyscus leucopus* to *Borrelia* infection and antigens. *Ticks Tick Borne Dis* 6:549–558
25. Gern L, Estrada-Pena A, Frandsen F et al (1998) European reservoir hosts of *Borrelia burgdorferi* sensu lato. *Zentralbl Bakteriologie* 287:196–204

26. Gern L, Humair PF (1998) Natural history of *Borrelia burgdorferi* sensu lato. Wien Klin Wochenschr 110:856–858
27. Parola P, Paddock CD, Socolovschi C et al (2013) Update on tickborne rickettsiosis around the world: a geographic approach. Clin Microbiol Rev 26:657–702
28. Fritz CL (2009) Emerging tickborne diseases. Vet Clin North Am Small Anim Pract 39:265–278
29. Franke J, Fritzsich J, Tomaso H, Straube E, Doprn W, Hiodebrandt A (2010) Coexistence of pathogens in host seeking and feeding ticks within a single natural habitat in Central Germany. Appl Environ Microbiol 76:6829–6836
30. Eshoo MW, Crowder CD, Carolan HE, Rounds NA, Ecker DJ, Jaag H, Mottes B (2014) Broad-range survey of tickborne pathogens in southern Germany reveals a high prevalence of Babesia microti and a diversity of other tickborne pathogens. Vector Borne Zoonotic Dis 8:584–591
31. Banneth G (2014) Tickborne infections of animals and humans: a common ground. Int J Parasitol 44:591–596
32. Johnson RC, Kodner C, Jarnefeld J, Eck DK, Xu Y (2011) Agents of human anaplasmosis and Lyme disease at Camp Ripley, Minnesota. Vector Borne Zoonotic Dis 11:1529–1534
33. Krause PJ, Mc Kay K, Thompson CS et al (2002) Disease-specific diagnosis of co-infecting tickborne zoonoses: babesiosis, human granulocytic ehrlichiosis, and Lyme disease. Clin Infect Dis 34:1184–1191
34. Lani R, Mopghaddam E, Haghani A, Chang LY, Abubaker S, Zandi K (2014) Tick-borne viruses: a review from the perspective of therapeutic approaches. Ticks Tick-borne Dis 5:457–465
35. Plyusnin A, Beaty BJ, Elliott RM et al (2011) Family *Bunyaviridae*. In: Ninth report of the International Committee on Taxonomy of Viruses, 1st edn. Elsevier, London, pp 725–741
36. Mertz GJ (2009) Bunyaviridae: Bunyavirus, Phlebovirus, Nairoviruses, and Hantaviruses. In: Richman DD, Whitley RJ, Hayden RG (eds) Clinical virology, 3rd edn. ASM Press, Washington, DC, pp 977–1007
37. Anonymous. *Bunyaviridae*. Wikipedia. <https://en.wikipedia.org/wiki/Bunyaridae>
38. Matsuno K, Weiserd C, Travassos de Rosa APA et al (2013) Characterization of the Bhanja serogroup viruses [*Bunyaviridae*]: a novel species of the genus *Phlebovirus* and its relationship with other emerging tickborne phleboviruses. J Virol 87:3719–3727
39. Yu XJ, Liang MF, Zhang SY et al (2011) Fever with thrombocytopenia associated with novel bunyavirus in China. N Engl J Med 364:1523–1532
40. Xu B, Liu L, Huang X et al (2011) Metagenomic analysis of fever, thrombocytopenia and leucopenia syndrome [FTLS] in Hennan Province, China: discovery of a new bunyavirus. PLoS Pathol 364:e1002369
41. Lam TT, Liu W, Bowden TA et al (2013) Evolutionary and molecular analysis of the emergent severe fever with thrombocytopenia syndrome virus. Epidemics 5:1–10
42. Bishop DH, Calisher CH, Casals J et al (1980) Bunyaviridae. Intervirology 14:125–143
43. Palacios G, Savji N, Travassos de Rosa A et al (2013) Characterization of the Uukuiemi virus group [Phlebovirus: Bunyaviridae]: evidence of seven distinct species. J Virol 87:3187–3195
44. Liu Q, He B, Huang SY, Wei F, Zhu XQ (2014) Severe fever with thrombocytopenia syndrome, an emerging tickborne zoonosis. Lancet Infect Dis 14:763–772
45. Horne KM, Vanlandingham DL (2014) Bunyavirus—vector interactions. Viruses 6:4373–4397
46. Ding NZ, Luo ZF, Niu DD et al (2013) Identification of two severe fever with thrombocytopenia syndrome virus strains originating from reassortment. Virus Res 178:543–546
47. Cui F, Cao HX, Wang L et al (2013) Clinical and epidemiological study on severe fever with thrombocytopenia syndrome in Yiyuan County, Shandong Province, China. Am J Trop Med Hyg 88:510–512
48. Zhao L, Zhai S, Wen H et al (2012) Severe fever with thrombocytopenia syndrome virus, Shandong Province, China. Emerg Infect Dis 18:963–965
49. Zhang X, Liu Y, Zhao L et al (2013) Emerging hemorrhagic fever in China caused by a novel bunyavirus SFTSV. Sci China Life Sci 56:697–700
50. Zhang L, Ye L, Ojcius DM et al (2014) Characterization of severe fever with thrombocytopenia syndrome in rural regions of Zhejiang, China. PLoS One 9:e111127

51. Zeng P, Ma L, Gao Z et al (2015) A study of seroprevalence and rates of asymptomatic viremia of severe fever with thrombocytopenia syndrome virus among Chinese blood donors. *Transfusion* 55:965–971
52. Liu S, Chai C, Wang S et al (2014) Systemic review of severe fever with thrombocytopenia syndrome: virology, epidemiology, and clinical characteristics. *Rev Med Virol* 24:90–102
53. Li Z, Hu J, Bao C et al (2014) Seroprevalence of antibodies against SFTS virus infection in farmers and animals, Jiangsu, China. *J Clin Virol* 60:185–189
54. Denic S, Janbeith J, Nair S, Conca W, Tariq WU, Al-Salam S (2011) Acute thrombocytopenia, leucopenia, and multiorgan dysfunction: the first case of SFTS Bunyavirus outside of China? *Case Rep Infect Dis* 2011:1–4
55. Kim KH, Yi J, Kim G et al (2013) Severe fever with thrombocytopenia syndrome, South Korea, 2012. *Emerg Infect Dis* 19:1892–1894
56. Park SW, Hun MG, Yun SM, Park C, Lee WJ, Ryou J (2014) Severe fever with thrombocytopenia virus, South Korea. *Emerg Infect Dis* 20:1880–1882
57. Takahashi T, Maeda K, Suzuki T et al (2014) The first identification and retrospective study of severe fever with thrombocytopenia syndrome in Japan. *J Infect Dis* 209:816–827
58. Yoshikawa T, Fukushi S, Tani H et al (2014) Sensitive and specific PCR systems for detection of both Chinese and Japanese severe fever with thrombocytopenia syndrome virus strains and prediction of patient survival based on viral load. *J Clin Microbiol* 52:3325–3333
59. Zhang YZ, Zhou DJ, Qin XC et al (2012) The ecology, genetic diversity, and phylogeny of Huaiyangshan virus in China. *J Virol* 86:2864–2868
60. Wang S, Li J, Niu G et al (2015) SFTS virus in ticks in endemic areas of China. *Am J Trop Med Hyg* 92:648–649
61. Park SW, Song BG, Shin EH et al (2014) Prevalence of severe fever with thrombocytopenia syndrome virus in *Haemaphysalis longicornis* ticks in South Korea. *Ticks Tick-borne Dis* 5:975–977
62. Yun SM, Lee WG, Ryou J et al (2014) Severe fever with thrombocytopenia syndrome virus in ticks collected from humans, South Korea, 2013. *Emerg Infect Dis* 20:1358–1361
63. Jiao Y, Zeng X, Guo X et al (2012) Preparation and evaluation of recombinant severe fever with thrombocytopenia syndrome virus nucleocapsid protein for detection of total antibodies in human and animal sera by double-antigen sandwich enzyme-linked immunosorbent assay. *J Clin Microbiol* 50:372–377
64. Ding S, Yin H, Xu X et al (2014) A cross-sectional survey of fever with thrombocytopenia syndrome virus infection of domestic animals in Laizhou City, Shandong Province, China. *Jpn J Infect Dis* 67:1–4
65. Niu G, Li J, Liang M et al (2013) Severe fever with thrombocytopenia syndrome virus among domesticated animals, China. *Emerg Infect Dis* 19:756–763
66. Ge HM, Wang QK, Li Z et al (2012) Investigation of severe fever with thrombocytopenia syndrome [SFTS] virus carrying situation in rodents in SFTS epidemic area of Donghai county, Jiangsu. *J Prev Med* 23:12–14 [in Chinese]
67. Tang X, Wu W, Wang H et al (2013) Human-to-human transmission of severe fever with thrombocytopenia syndrome Bunyavirus through contact with infectious blood. *J Infect Dis* 207:736–739
68. Gai Z, Liang M, Zhang Y et al (2012) Person-to-person transmission of severe fever with thrombocytopenia syndrome Bunyavirus through blood contact. *Clin Infect Dis* 54:249–252
69. Bao CJ, Guo XL, Qi X et al (2011) A family cluster of infection by a newly recognized Bunyavirus in eastern China, 2007; further evidence of person-to person transmission. *Clin Infect Dis* 53:1208–1214
70. Liu Y, Li Q, Hu W et al (2012) Person-to-person transmission of severe fever with thrombocytopenia syndrome virus. *Vector Borne Zoonotic Dis* 12:156–160
71. Chen H, Hu K, Zou J et al (2013) A cluster of cases of human-to-human transmission caused by severe fever with thrombocytopenia syndrome Bunyavirus. *Int J Infect Dis* 17:e206–e208
72. Deng B, Zhang S, Geng Y et al (2012) Cytokine and chemokine levels in patients with severe fever with thrombocytopenia syndrome virus. *PLoS One* 7:e41365

73. Hofmann H, Li X, Zhang X et al (2013) Severe fever with thrombocytopenia syndrome virus glycoproteins are targeted by neutralizing antibodies and can use DC-SIGN as a receptor for pH-dependent entry into human and animal cell lines. *J Virol* 87:4384–4394
74. Li J, Han Y, Xing Y et al (2014) Concurrent measurement of dynamic changes in viral load, serum enzymes, T cell subsets, and cytokines in patients with severe fever with thrombocytopenia syndrome. *PLoS One* 9:e91679
75. Sun Y, Jin C, Zhang F et al (2012) Host cytokine storm associated with disease severity of severe fever with thrombocytopenia syndrome. *J Infect Dis* 206:1085–1094
76. Sun L, Hu Y, Niyansaba A, Tong Q, Lu L, Li H, Jie S (2014) Detection and evaluation of immunofunction of patients with severe fever with thrombocytopenia syndrome. *Clin Exp Med* 14:389–395
77. Lu QB, Cui N, Hu JG et al (2015) Characterization of immunological responses in patients with severe fever with thrombocytopenia syndrome: a cohort study in China. *Vaccine* 33:1250–1255
78. Qu B, Qi X, Wu X et al (2012) Suppression of the interferon and NF-Kappa B responses by severe fever with thrombocytopenia syndrome virus. *J Virol* 86:8388–8401
79. Ning YJ, Feng K, Min YQ, Cao WC, Wang M, Deng F, Hu Z, Wang H (2015) Disruption of type I interferon signaling by the nonstructural protein of severe fever with thrombocytopenia syndrome virus via hijacking of STAT2 and STAT1 into inclusion bodies. *J Virol* 89:4227–4236
80. Billecocq A, Spiegel M, Vialat P et al (2004) NSs protein of Rift Valley fever virus blocks interferon production by inhibiting host gene transcription. *J Virol* 78:789–806
81. Van Knippenberg I, Carlyon-Smith C, Elliott RM (2010) The N-terminus of *Bunyamwera orthobunyavirus* nss protein is essential for interferon antagonism. *J Gen Virol* 91:2002–2006
82. Hiraki T, Yoshi M, Suzuki T et al (2014) Two autopsy cases of severe fever with thrombocytopenia syndrome [SFTS] in Japan: a pathognomonic histological feature and unique complication of SFTS. *Pathol Int* 64:569–575
83. Quan Tai X, Feng Zhe C, Xiu Guang S, Dong GC (2013) A study of cytological changes in the bone marrow of patients with severe fever with thrombocytopenia syndrome. *PLoS One* 8:e83020
84. Chen XP, Cong ML, Li MH, Kang YJ, Feng YM, Plusnin A, Xu J, Zhang YZ (2012) Infection and pathogenesis of Huaiyanshan virus [a novel tickborne bunyavirus] in laboratory rodents. *J Gen Virol* 93:1288–1293
85. Liu Y, Wu B, Paessler S, Walker DH, Tesh RB, Yu XJ (2014) The pathogenesis of severe fever with thrombocytopenia syndrome virus infection in alpha/beta interferon knockout mice: insights into the pathogenic mechanisms of a new hemorrhagic fever. *J Virol* 88:1781–1786
86. Jin C, Liang M, Ning J et al (2012) Pathogenesis of emerging severe fever with thrombocytopenia syndrome virus in C57/BL6 mouse model. *Proc Natl Acad Sci U S A* 109:10153–10158
87. Jin C, Jiang H, Liang M et al (2015) SFTS virus infection in nonhuman primates. *J Infect Dis* 211:915–925
88. Liu R, Huang DD, Bai JY et al (2015) Immunization with recombinant SFTSV/NSs protein does not promote viral clearance in SFTSV-infected C57BL/6J mice. *Viral Immunol* 28:113–122
89. Shimada SS, Posadas-Herrera G, Aoki K, Morita K, Hayasaka D (2015) Therapeutic effect of post-exposure treatment with antiserum on severe fever with thrombocytopenia syndrome [SFTS] in a mouse model of SFTS virus infection. *Virology* 482:19–27
90. Gai ZT, Zhang Y, Liang MF et al (2012) Clinical progress and risk factors for death in severe fever with thrombocytopenia syndrome patients. *J Infect Dis* 206:1095–1102
91. Cui Liu R, Wang LY et al (2015) Severe fever with thrombocytopenia syndrome bunyavirus-related human encephalitis. *J Infect* 70:52–59
92. Wang LY, Cui N, Lu QB, Wo Y, Wang HY, Liu W, Cao WL (2014) Severe fever with thrombocytopenia syndrome in children: a case report. *BMC Infect Dis* 14:366
93. Deng B, Zhou B, Zhang S et al (2013) Clinical features associated with severe fever with thrombocytopenia syndrome Bunyavirus infection in Northeast China. *PLoS One* 6:e80802

94. Zhang YZ, He YW, Dai YA et al (2012) Hemorrhagic fever caused by a novel Bunyavirus in China: pathogenesis and correlates of fatal outcome. *Clin Infect Dis* 54:527–533
95. Cui N, Bao X-L, Yang ZD et al (2014) Clinical progression and predictors of death in patients with severe fever with thrombocytopenia syndrome in China. *J Clin Virol* 59:12–17
96. Sun Y, Liang M, Qu J et al (2012) Early diagnosis of novel SFTS Bunyavirus infection by quantitative real-time PCR assay. *J Clin Virol* 53:48–53
97. Debing Y, Jochmans D, Neyts J (2013) Intervention strategies for emerging viruses: use of antivirals. *Curr Opin Virol* 3:217–224
98. Tasdelen FN, Ergonul O, Doganci I, Tulex N (2009) The role of ribavirin in the therapy of Crimean-Congo hemorrhagic fever early use is promising. *Eur J Clin Microbiol Infect Dis* 28:929–933
99. Shimojima M, Fukushi S, Tani H et al (2014) Effects of ribavirin on severe fever with thrombocytopenia syndrome virus in vitro. *Jpn J Infect Dis* 67:423–427
100. Lu QB, Chui N, Li H et al (2013) Case fatality and effectiveness of ribavirin in hospitalized patients with severe fever with thrombocytopenia syndrome in China. *Clin Infect Dis* 57:1292–1299
101. Guo X, Zhang I, Zhang W et al (2013) Human antibody neutralizes severe fever with thrombocytopenia syndrome virus, an emerging hemorrhagic fever virus. *Clin Vaccine Immunol* 20:1426–1432
102. Savage HM, Godsey MS Jr, Lambert A et al (2013) First detection of Heartland virus [*Bunyaviridae: Phlebovirus*] from field collected arthropods. *Am J Trop Med Hyg* 89:445–452
103. Bosco-Lauth AM, Panella NA, Root JJ et al (2015) Serological investigation of Heartland virus [*Bunyaviridae: Phlebovirus*] exposure in wild and domestic animals adjacent to human case sites in Missouri 2012–2013. *Am J Trop Med Hyg* 92:1163–1167
104. Pastula DM, Turabelidze G, Yates KF et al (2014) Notes from the field: Heartland virus disease—United States, 2012–2013. *MMWR Morb Mortal Wkly Rep* 63:270–271
105. Oklahoma State Department of Health. Oklahoma State Health Department confirms first case and death of Heartland virus. <http://www.ok.gov/health/Organization/Office-of-Communication-News-releases/2014-New-Releases/Oklahoma-State-Health-Department-Confirms-First-Case-and-Death-of-Heartland-virus.html/>
106. Riemersma KK, Komar N (2015) Heartland virus neutralizing antibodies in vertebrate wild-life, United States, 2009–2014. *Emerg Infect Dis* 21:1830–1833
107. Chowdri HR, Gugliotta JL, Beradi VP, Goethert HK, Molloy PJ, Sterling SL, Telford SR III (2013) *Borrelia miyamotoi* infection presenting as human granulocytic anaplasmosis. *Ann Intern Med* 159:21–27
108. Krause PJ, Narasimham S, Worsler GP et al (2013) Human *Borrelia miyamotoi* infection in the United States. *N Engl J Med* 368:291–293
109. Hovius JW, Wever B, Sohne M et al (2013) A case of meningoencephalitis by the relapsing fever spirochete *Borrelia miyamotoi* in Europe. *Lancet* 382:658
110. Sato K, Takano A, Konnai S et al (2014) Human infections with *Borrelia miyamotoi*, Japan. *Emerg Infect Dis* 20:1391–1393
111. Molloy PJ, Telford SR III, Chowdri HR et al (2015) *Borrelia miyamotoi* disease in northeastern United States. A case series. *Ann Intern Med* 163:91–98
112. Bacon RM, Kugeler KJ, Mead PS, Center for Disease Control and [Prevention [CDC] (2008) Surveillance for Lyme disease United States, 1992–2006. *MMWR Surveill Summ* 57:1–9
113. Scoles GA, Papers M, Beati L, Fish D (2001) A relapsing fever group transmitted by *Ixodes scapularis* ticks. *Vector Borne Zoonotic Dis* 1:21–34
114. Kosoy OI, Lambert AJ, Hawkinson DJ, Pastula DM, Goldsmith CS, Hunt DC, Staples JD (2015) Novel Thogotovirus associated febrile illness and death, United States, 2014. *Emerg Infect Dis* 21:760–764
115. Liu XY, Bonnet SI (2014) Hard tick factors implicated in pathogen transmission. *PLoS Neglected Trop Dis* 8:e2566
116. Nuttall PA, Trimmell AR, Kazimirova M, Labuda M (2006) Exposed and concealed antigens as vaccine targets for controlling ticks and tick-borne diseases. *Parasite Immunol* 28:155–163