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Military Considerations on Spotted Fever Group *Rickettsia*:

A Review

by

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Dedication

To my wife, mother, and father.

To the soldiers who came before me and will come after me.

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Abstract

Military Considerations on Spotted Fever Group *Rickettsia*: A Review

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American military operations are impacted by infectious disease. A cryptic bacterial threat of growing military importance is spotted fever group (SFG) *Rickettsia*. SFG *Rickettsia* are a national security concern due to their ability to detract from Department of Defense (DoD) mission readiness. Many service members risk exposure to debilitating SFG rickettsiosis (SFGR) by spending prolonged periods in SFG *Rickettsia*-infected tick habitats throughout the world. Indeed, there are few deployment locations where troops are not at risk for SFGR. Should a soldier contract an SFGR, immediate issues arise for their health and the greater DoD mission. Firstly, SFGR is difficult to differentiate and has a high case fatality rate (CFR) if not treated quickly. Secondly, mission success and the individual soldier's health outcomes are impacted. This review will synthesize the current understanding of SFG *Rickettsia* in the published literature and discuss it in the context of areas of military concern.

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General Introduction on SFG *Rickettsia*

Due to their distribution and emerging potential as causative agents of spotted fever group rickettsiosis (SFGR), SFG *Rickettsia* are typically identified as pathogens of interest by the United States Department of Defense (DoD) (23; 51). SFG *Rickettsia* are obligate intracellular, Gram-negative bacteria that are transmitted to humans exclusively by ticks; other rickettsial species can be transmitted by fleas, mites, and lice (3; 64). Organisms belonging to the genus *Rickettsia* are classified as either SFG, typhus group, transitional group, or ancestral group (2). Ancestral *Rickettsia* are known to be primarily benign to humans (13). In contrast, the other three *Rickettsia* groups exhibit pathogenic traits and have undergone substantial genomic reduction such that they now have genomes of around 1.5 MB in size (13). The diseases caused by SFG *Rickettsia* are numerous and increasing (Table 1). The predominant SFGRs include Rocky Mountain spotted fever (RMSF), Mediterranean spotted fever (MSF), and African tick bite fever (ATBF) (12). In addition, SFGR is known for causing crippling, incapacitating symptoms that are difficult to differentiate and a high fatality rate if left untreated (70; 76).

Due to the substantial duration of time that service members operate in tick habitats, both on and off duty, SFGR poses a uniquely cryptic threat to military operations because of its ability to directly detract from combat effectiveness (63). Naturally, the risk of tick-borne diseases (TBD) increases the longer one remains in tick environments (90). However, DoD personnel often have job requirements that necessitate outdoor activity (47). In addition, military members frequently spend off-duty leisure time in tick environments (65). Should a service member contract an SFGR while on or off duty, the disease's effects can lead to decreased unit operational readiness and can be

TABLE. Rickettsial disease agents, vectors, and geographic distributions

Disease	Agent	Vector	Geographical distribution
Spotted fever group			
Rocky Mountain spotted fever	*Rickettsia rickettsii*	Tick	North, Central, and South America
North Asian tick-borne rickettsiosis (Siberian tick typhus)	*R. sibirica*	Tick	North Asia
Queensland tick typhus	*R. australis*	Tick	Eastern Australia, Tasmania
Flinders Island spotted fever	*R. honei*	Tick	Australia and Southeast Asia
African tick bite fever	*R. africae*	Tick	Sub-Saharan Africa, Caribbean (French West Indies), and Oceania
Mediterranean spotted fever	*R. conorii*	Tick	Europe (Mediterranean basin), Middle East, Indian subcontinent, Africa
R. parkeri rickettsiosis (Maculatum disease)	*R. parkeri*	Tick	Southern U.S., South America
Japanese spotted fever	*R. japonica*	Tick	Japan and South Korea
Tick-borne lymphadenopathy	*R. slovac, R. raoultii*	Tick	Europe
364D-associated rickettsia (Pacific Coast tick fever)	*R.* species 364D	Tick	U.S.
Far Eastern spotted fever	*R. heilongjiangensis*	Tick	Eastern Asia
Transitional group			
Rickettsialpox	*R. akari*	Mite	U.S., Russia, Korea, Africa
Queensland tick typhus	*R. australis*	Tick	Eastern Australia, Tasmania
Typhus			
Epidemic typhus, Brill-Zinsser disease	*R. prowazekii*	Body lice, ectoparasites of flying squirrels	Worldwide
Murine typhus	*R. typhi, R.felis*	Rat flea, cat flea	Worldwide
Scrub Typhus			
Scrub typhus (tsutsugamushi disease)	*Orientia tsutsugamushi, O. chuto*	Trombiculid mite larvae (chiggers)	Asia-Pacific region, northern Australia, UAE, Africa, Chile

UAE, United Arab Emirates

Table 1: General information of SFG *Rickettsia*

The above information overviews SFG, transitional, and typhus group rickettsial agents, vectors, and distribution. Table taken with permission from Clark, 2019 under the Open Government License (26).

potentially fatal to the soldier, sailor, airman, marine, or guardian in question (47; 72; 84).

Due to changes in the global environment, travel, and trade, ticks and TBDs such as SFGR are increasing in occurrence and case rate. Furthermore, novel, and existing diseases continue to evolve (85). As the U.S. and its allied military forces conduct many unified operations in environments conducive to tick habitats, it is critical to understand the threat, properties, and future of SFG *Rickettsia* (63; 76; 100). Such an understanding will better equip the American warfighter to overcome the threat of SFGR on mission readiness and effectiveness.

Outside the continental United States (OCONUS) areas containing military bases show sustained and expanding reports of SFG *Rickettsia* cases (38; 48; 72; 76; 89) (Fig. 1; 2; 3). For example, 6% of U.S. military personnel participating in a post-deployment serological survey on return from Honduras were SFG *Rickettsia* positive IgG (20). Additionally, U.S. soldiers deployed to training environments in Botswana reported a 24% and 33% seroconversion to SFG *Rickettsia* in field and garrisoned environments respectively (83). Thus, in order to best protect service members, it is critical to track and identify trends in SFG *Rickettsia* endemism and expansion. This knowledge will inform military decision-making process and command decisions involving SFG *Rickettsia* risk and mitigation. Current integrated DoD pest management procedures are effective against a wide range of arthropod vectors, including ticks (32; 90). However, serological assays of deployed U.S. military personnel show exposure to SFG *Rickettsia*, suggesting that additional efforts may be needed to reduce SFG *Rickettsia* infection (47). Currently SFGR incidence remains low amongst U.S. military populations, though increasing, with

some reports stating 3 cases per 100,000 person-years, with 57% of SFGR being male and 46% serving on active duty (Fig. 4) (50).

In summary, military populations operate globally in every environment that humans naturally inhabit (38). Each of these militarily relevant regions has distinct species of ticks that are endemic to the respective area, and act as vectors for regionally specific or broadly distributed SFG *Rickettsia* species which pose various infectious risks. Therefore, it is necessary to understand SFGR infection and risk as it applies to military populations; such knowledge will help establish a foundational understanding of the vectors of SFG *Rickettsia* and how our military personnel become infected. Additionally, understanding the various SFGR risks that service members face globally, how this risk depends on the region they find themselves in, as well as how SFGR incidence applies to military operations, are each discussed below.

Military relevance

To understand precisely why SFG *Rickettsia* poses such a concern, it would be prudent to first establish a fundamental background on military operation. "Military Readiness" is a comprehensive term that can encompass levels as high as the President's office and anything below. For the purposes of understanding SFG *Rickettsia* impact, this term will be applied to the operational level, which primarily focuses on individual units of DoD/military personnel and does not directly involve massive planning operations. The three pillars of military readiness consist of building, increasing, and sustaining readiness, and each broadly impacts mission success in training and deployment environments (18; 42). In a simplified regard, military readiness focuses on personnel, missions, and tasks that are assigned or completed at the unit level (42).

Figure 1: Global presence of U.S. military activity

The above figure displays U.S. military installations across the globe. In comparison to previous figures displaying SFG Rickettsia presence, the overlap of militarily relevant location and SFG Rickettsia overlap is substantial. Map taken with permission from Vine, 2019 as shown by license: 1152209-1 (96).

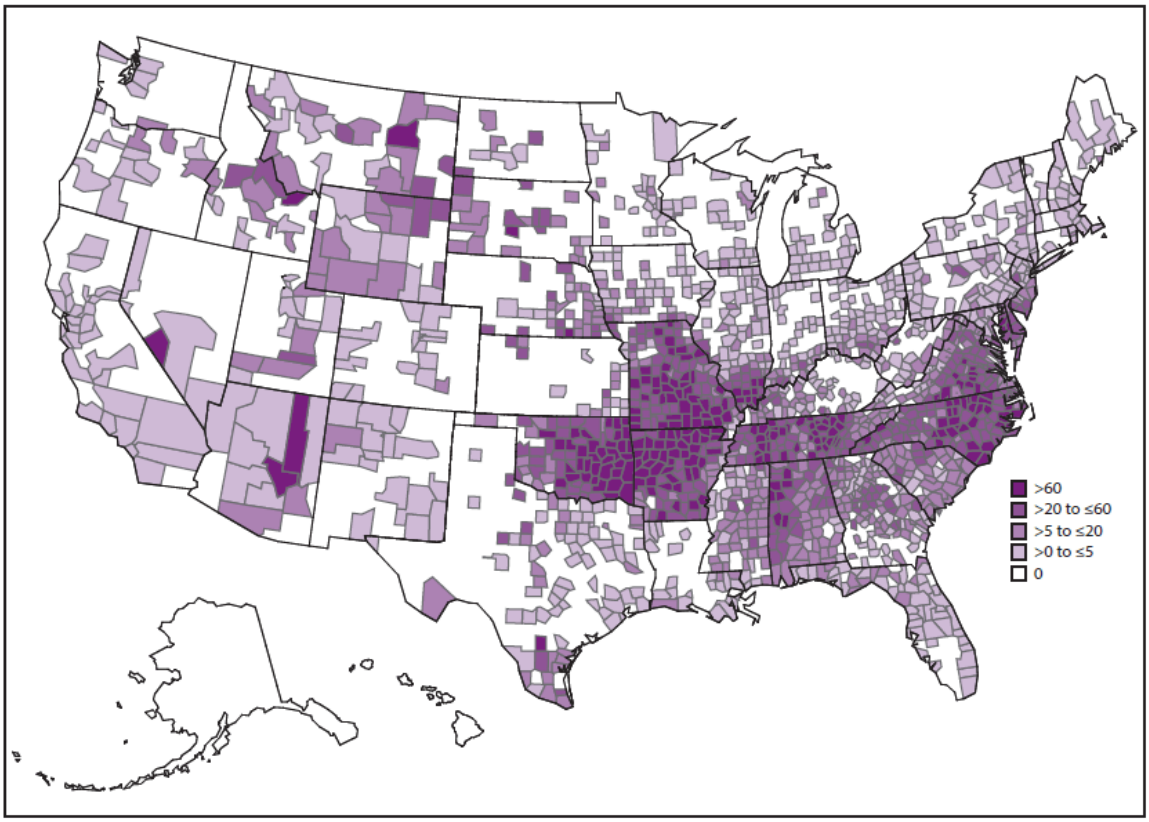

Figure 2: Reported incidence of SFGR by U.S. county

Geographical representation of SFGR by county per 1,000,000 people per year. SFGRs included in the national surveillance graph consist of RMSF and other SFGR. Figure reproduced from Biggs, 2016 under the Creative Commons Attribution 4.0 international license (12).

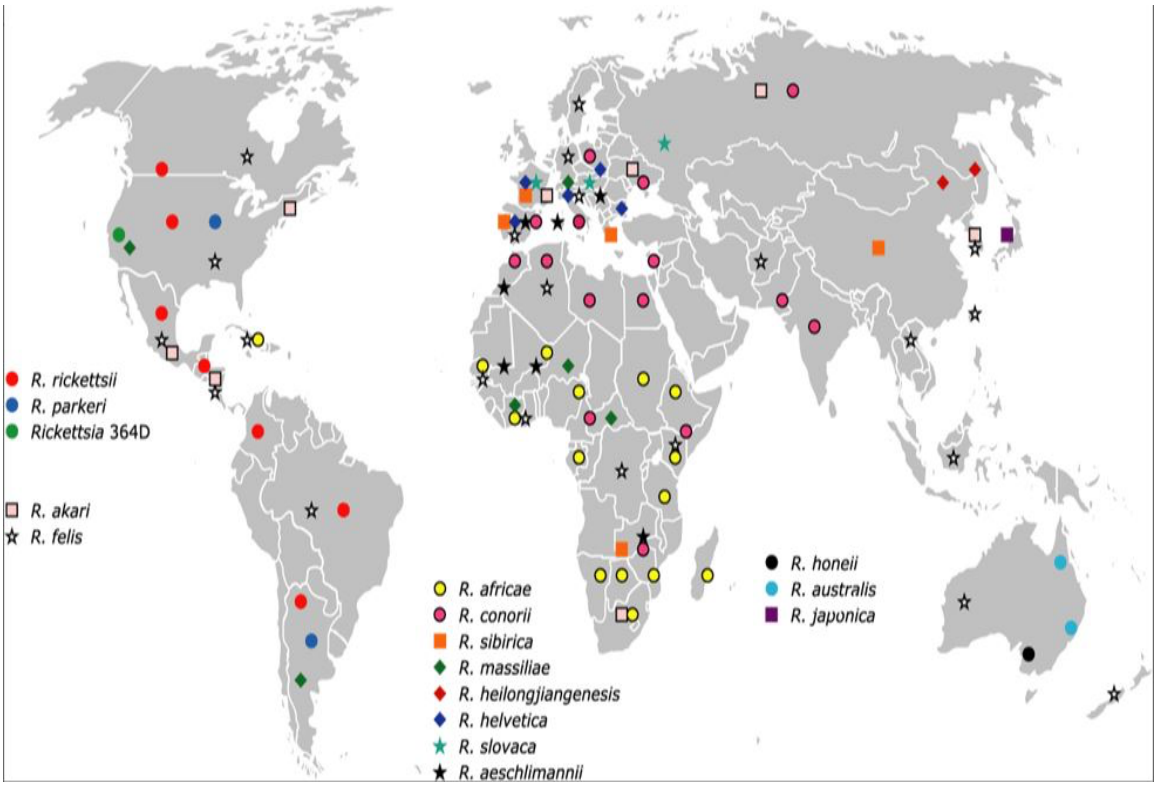

Figure 3: Geographical distribution of SFG *Rickettsia*

Above is shown the global distribution of SFG *Rickettsia*. Species are grouped in clusters according to continent. The top left shows American SFG *Rickettsia* species, the bottom left shows two non-fatal SFG and transitional *Rickettsia* species, the center displays African and European species, with the right showing Asian species. Figure taken with permission from Wood, 2012 shown by license number 5162170811091 (98).

Units in the military comprise groups that range from as few as three individuals, such as a fireteam in a squad, to as many as thousands, such as a brigade (6; 9; 18; 33). If one individual falls, fellow unit-members must care for that individual and assume their responsibilities. SFG *Rickettsia* and other infectious disease are especially effective at quickly diminishing military readiness because infection can remove individuals of a unit from being mission effective, i.e., being able to do their job. At a small unit level, incapacitation of one or two individuals could lead to mission failure of the entire unit. Should this occur in enough small units, the cumulative effect could be that entire larger units also fail their missions.

U.S. operatives are at risk of SFG *Rickettsia* exposure, which is substantiated through a combination of epidemiological and serological data from DoD and allied personnel, as well as surveillance of tick populations in current or future deployment regions (47; 51; 76). SFG *Rickettsia* are found everywhere troops currently are, or possibly are going to be. Indeed, distribution of SFG *Rickettsia* overlaps heavily with global military base location (7; 65). For example, serological data from U.S. forces in Korea, and other regions that have endemic SFG *Rickettsia*, show that service members are being infected with SFG *Rickettsia* (35; 47; 72). Moreover, various branches of the American and allied militaries have reported SFG *Rickettsia*-related illness throughout the world (35; 76).

One of the primary roles of the Army Health System (AHS) is to implement force health protections that encompass all aspects of a soldier's physical and mental health during any point in service (9). Whether it be in garrisoned training, or while conducting missions in a deployed environment, the AHS must track and be prepared for any health

issue that service members may face (33). This is due to the ever-evolving global landscape that the military must constantly be prepared to face. Threats of infectious disease are a health and mission outcome variable that can be mitigated and ameliorated through constant vigilance and forward planning. That being the case, it is important that current AHS health considerations for vector-borne diseases further emphasize the importance of SFG *Rickettsia* surveillance and control.

Vector and transmission overview

SFG *Rickettsia* are exclusively transmitted by a tick vector (10). Over time, knowledge of which particular tick species are capable vectors of SFGR has grown. This has added on understanding of transmission dynamics and revealed the sheer quantity of vectorially capable tick species. In the early 1900s, it was shown that the transmission of *Rickettsia rickettsii*, the etiologic agent of RMSF, occurred through *Dermacentor andersoni*, which is commonly referred to as the Rocky Mountain wood tick (77; 97). Later, the brown dog tick, *Rhipicephalus sanguineus*, was found to be implicated in the spread of *Rickettsia conorii*, the causative agent of MSF (87).

While many hard tick species have been found to carry various species of SFG *Rickettsia,* the general infection and transmission process is relatively conserved between species (81). After a female tick lays her eggs, the eggs hatch into larval ticks, which then mature and take their first blood meal (Fig. 5) (90). Depending on the tick species and sex, the tick may take two or more blood meals from a vertebrate host within their lifetime (81). Primary infection of non-SFG *Rickettsia*-infected ticks occurs when the tick takes a blood meal on a host with bacteremic rickettsial infection (29; 81). At any life stage after initial infection, the ticks are then capable of passing on the infection to

subsequent primary hosts, e.g., small rodents, or to accidental hosts, e.g., humans (57; 90). *Rickettsia rickettsii* and other SFG *Rickettsia* have been isolated from rodents in nature, which supports the notion that rodents are one of the key environmental reservoirs from which ticks acquire the infection (29; 89).

A critical mechanism of SFG *Rickettsia* maintenance in wild tick populations is through transovarial and transstadial transmission; these processes are essential for ticks to maintain and spread infection to their offspring (29; 81). Transstadial transmission is the passage of a pathogen, in this case SFG *Rickettsia*, from one life stage to the next (29). For example, a larval tick that becomes infected would maintain that infection throughout its life cycle (29). Transovarial transmission is passage of a pathogen from mother to offspring (29; 81). While the rate of transovarial transmission varies by tick species, an infected female *Dermacentor andersonii* shows a transmission range rate of above 35% to nearly 100%, indicating that many of the newly hatched larval ticks will begin their life already infected with SFG *Rickettsia* (29; 81). This process of transovarial-transstadial transmission maintains SFG *Rickettsia* in wild tick populations (29; 81).

Mechanism of infection overview

The initial step of human *Rickettsia* infection starts with entry into a human from an infected feeding tick (80). Once inside the human, the *Rickettsia* migrate to cells, primarily targeting epithelial cells; however immune cells, such as dendritic cells and macrophages, can also be targeted (53). The infected immune cells then disseminate the *Rickettsia* through the lymph nodes and lymphatic vessels (53). Once spread throughout the host they begin their targeted attack on vascular endothelial cells, and may also bind

to lung, heart, and liver tissues (80). This disseminated invasion of endothelial cells triggers an innate chemokine and cytokine signaling cascade that recruits significant CD4+ and CD8+ T cell defenses (30). Cases of severe disease correlate with rickettsial ability to proliferate within host macrophages, thereby surviving to cause more severe symptoms (30). Though the host immune system has been shown to play a role in infection dissemination, the underlying pathogenic causes of severe rickettsial disease and symptoms are not entirely understood (80).

Clinical symptoms overview

SFG *Rickettsia* infection ranges widely in clinical manifestations and outcomes (44). Some *Rickettsia* species, such as *R. rickettsii*, have a high CFR (2). Conversely, other species, such as the SFG/transitional group species *R. akari,* are considered almost uniformly self-limiting (3). A significant challenge posed with specific SFG *Rickettsia* diagnosis is the broad similarity of symptoms as well as the overall lack of symptoms. Spotted fever rickettsioses derive their name from the telling symptom of a spotted rash developing from limbs to the trunk of the patient with an accompanying fever (12). An additional symptom that is relatively unique to SFGR is the formation of a dark eschar that is coupled with non-specific febrile symptoms (Fig. 6) (86). The contrast of eschar formation combined with febrile illness, a nearly ubiquitous viral symptom, contributes to difficulties with quick and reliable SFGR diagnosis (12). Furthermore, multiple SFG *Rickettsia* species are the etiologic agents for multiple SFGRs (86). This fact can make purported or exact diagnosis difficult without exact species knowledge. For example, the primary SFG *Rickettsia* that cause disease in humans are *Rickettsia rickettsia*, the causative agent of RMSF, *Rickettsia conorii*, responsible for MSF, and *Rickettsia africae,*

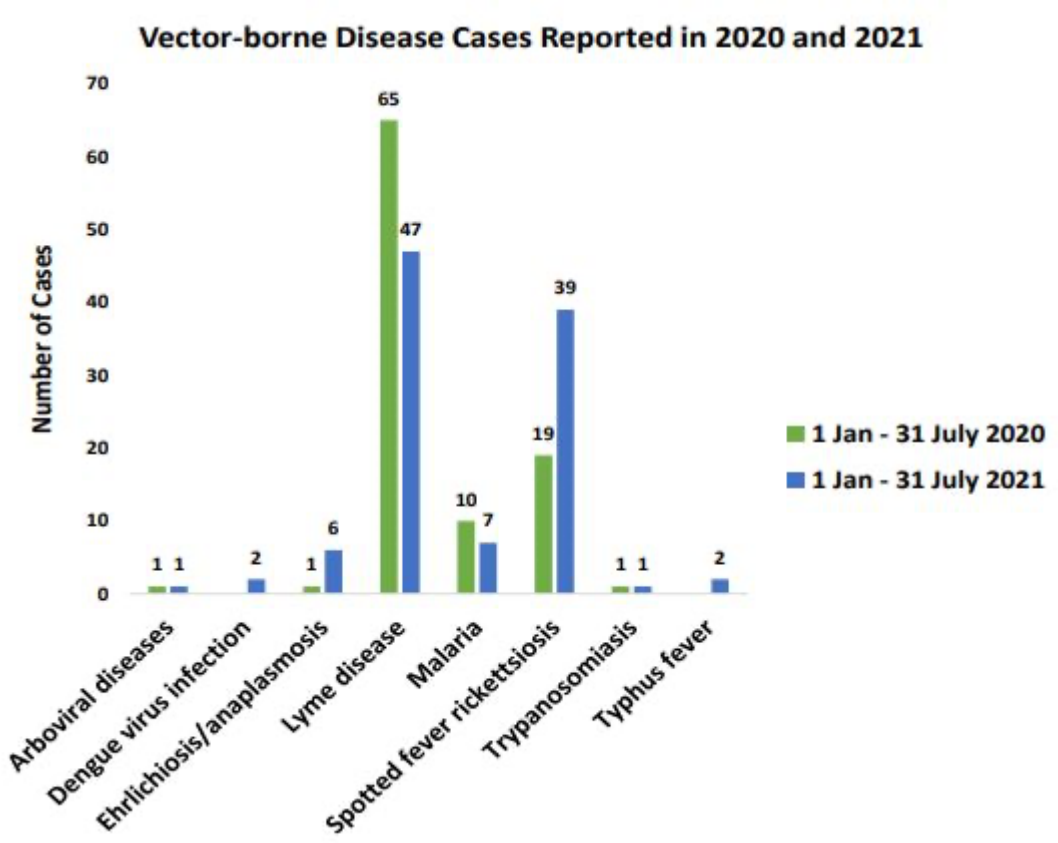

Figure 4: Vector-borne disease cases reported in 2020 and 2021

Above displays the U.S. Army Disease Reporting System internet (DRSi) surveillance of all reported vector-borne disease cases of active duty and reserve Army personnel and beneficiaries at all Army locations and health installations. Graph taken with permission from Army vector borne disease report 27-July 2021 under public release approve and unlimited distribution (8).

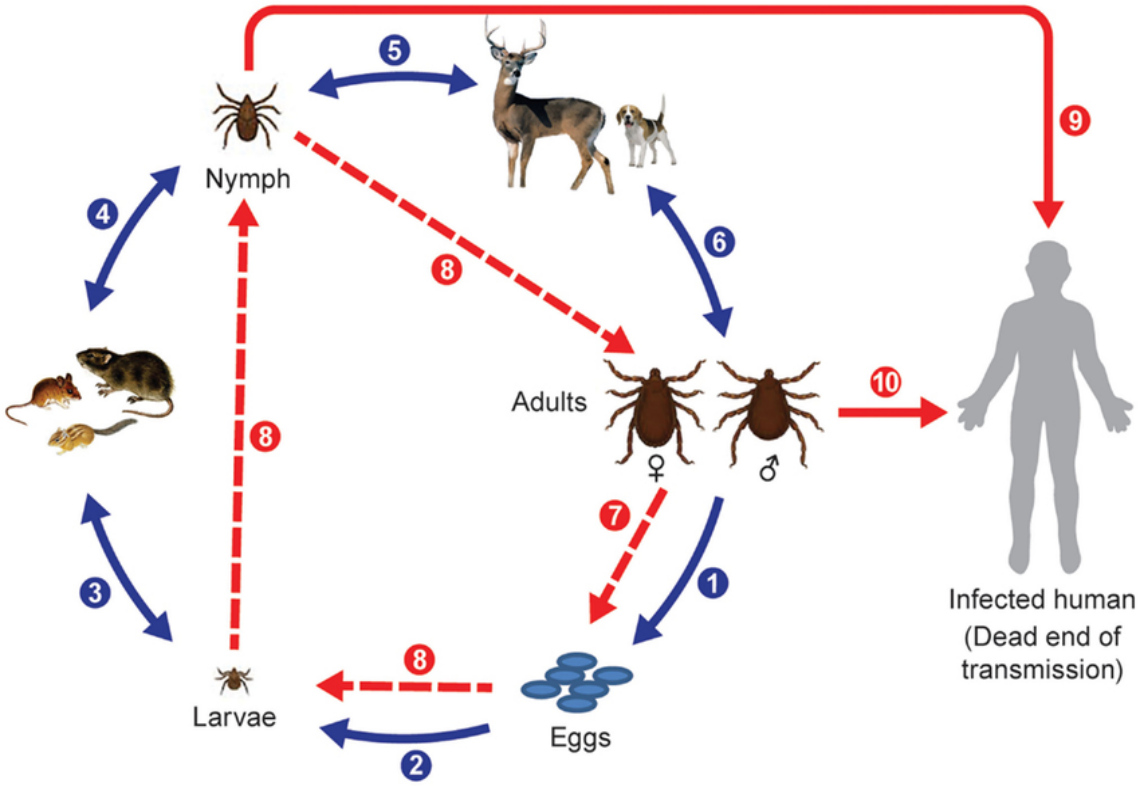

Figure 5: Standard Ixodid tick life cycle and SFG *Rickettsia* transmission
Blue arrows signify the primary progression of a tick life cycle: 1. Female oviposition, 2. egg hatching, 3. larvae take their first blood meal on a small rodent or animal, 4. larvae develop into nymphs, 5. nymphs take blood meals on a larger animal, and 6. nymphs develop into adults, where mating and oviposition occurs. Dashed red arrows show the transstadial-transovarial pathway transmission. Solid red lines show SFG *Rickettsia* transmission from an infected tick to a human. Figure taken from Eremeeva, 2015 with permission under the Creative Commons Attribution 4.0 international license (29).

cause of ATBF. Other SFGRs of emergent and rare occurrence are often noted to have similar symptoms to RMSF with slight, but substantial, differences (44). This may also be the case for the other common SFGR-associated diseases.

Symptoms of SFGR infection are variable within and between host species. For example, a rash occurs in a variable percentage of individuals depending on the etiologic species (12; 28). Similarly, a variable is the percentage of patients that exhibit the dark eschar at the tick bite site (12; 28). Despite the fact that eschar formation is frequently seen as a critical differential symptom of SFG *Rickettsia* infection, its formation varies widely depending on the species of *Rickettsia* causing the infection (2). For example, in patients with MSF, around 70% exhibit an eschar. This number rises to 90% in patients with ATBF. Conversely, eschar formation is extremely anomalous in RMSF (12; 13). Another concrete example that illustrates the variable symptoms of SFGR is that around 90% of patients with RMSF exhibit the characteristic maculopapular rash at some point during the course of infection (12). However, tick-borne lymphadenopathy (TIBOLA) causes a rash in fewer than 2% of all patients recorded thus far (14).

Section 2: Vectors and Transmission

Infection of the tick vector

Ticks have long established themselves as highly competent vectors of human disease (91). Their vector competence is defined as the tick's ability for a pathogen to infect, replicate, and then be transmitted into another host (91). These behaviors are influenced and modified by host tick biology, vertebrate host-seeking characteristics, and ambient and fluctuating environmental temperatures (Fig. 7) (61; 89). Temperature impacts tick replication, feeding habits, and life cycle development (34). As such,

temperature ranges that exceed the optimal zone for ticks may result in decreased host-seeking behavior (71).

Since various tick species inhabit many different segments of the globe, certain tick species actively search for hosts (quest) during warm weather, while others hide and wait for cooler temperatures (81). To this end, some tick species are adapted for survival in colder environments or regions of seasonal temperature fluctuations (34). Thus, temperature affects tick survival as well as survival of the rickettsial species these ticks may host. Finally, fluctuations in temperature do not just manipulate tick survival and behavior, but also tick physiology (36; 52). Overall, temperature plays a significant role in infection of the tick, rickettsial replication within the tick, and transmission from the tick into a suitable host (52; 71).

Overview of tick vector transmission

Ticks are arachnids in the order parasitiformes of the subclass Acari (64). A notable divergence from other arachnids is that ticks contain mouthparts on the capitulum, which is the central body region (64). In addition, a tick's eight legs are joined at the podosoma, which forms the body, or idiosoma. *Argasidae*, soft ticks, and *Ixodidae*, hard ticks, are the two prominent families of ticks (64). Hard tick primary host-seeking is a process called questing (64). This consists of climbing plants in correspondence with the life cycle stage (55). Larva and nymphs frequently quest small plants that are close to the ground; thus, their target hosts are normally small rodents (57; 55). Frequently these rodents are a main mammalian reservoir of *Rickettsia* (29). Therefore, when a larva or nymph feeds on the infected rodent, the tick is infected and can transmit the infection to new hosts (55; 57). The ability of individual tick or tick species to be a competent vector

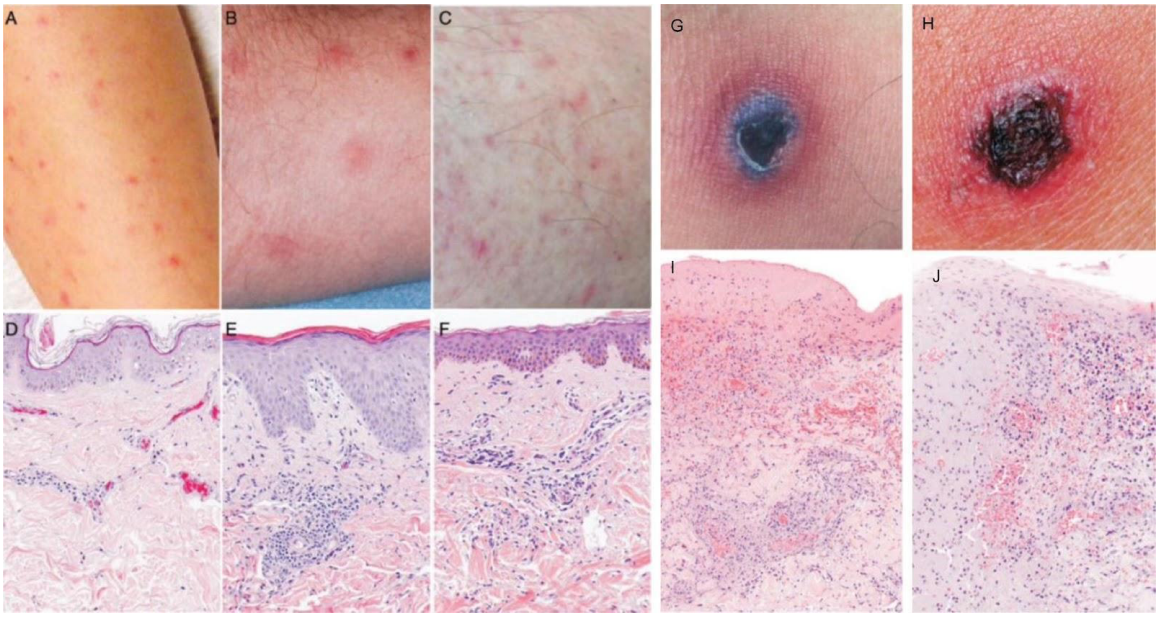

Figure 6: Clinical epidermal presentation of SFG *Rickettsia* infection

Images A, B, and C display the clinical presentation of an SFG *Rickettsia*-derived maculopapular rash. G and H display the characteristic black eschar formation. *R. akari* infection is shown in C, F, G, I. *R. rickettsii* infection is shown in image A and D. *R. parkeri* infection is B, E, H, J. Figure taken with permission from Adem, 2019 shown by Elsevier license number: 5127231275535 (2).

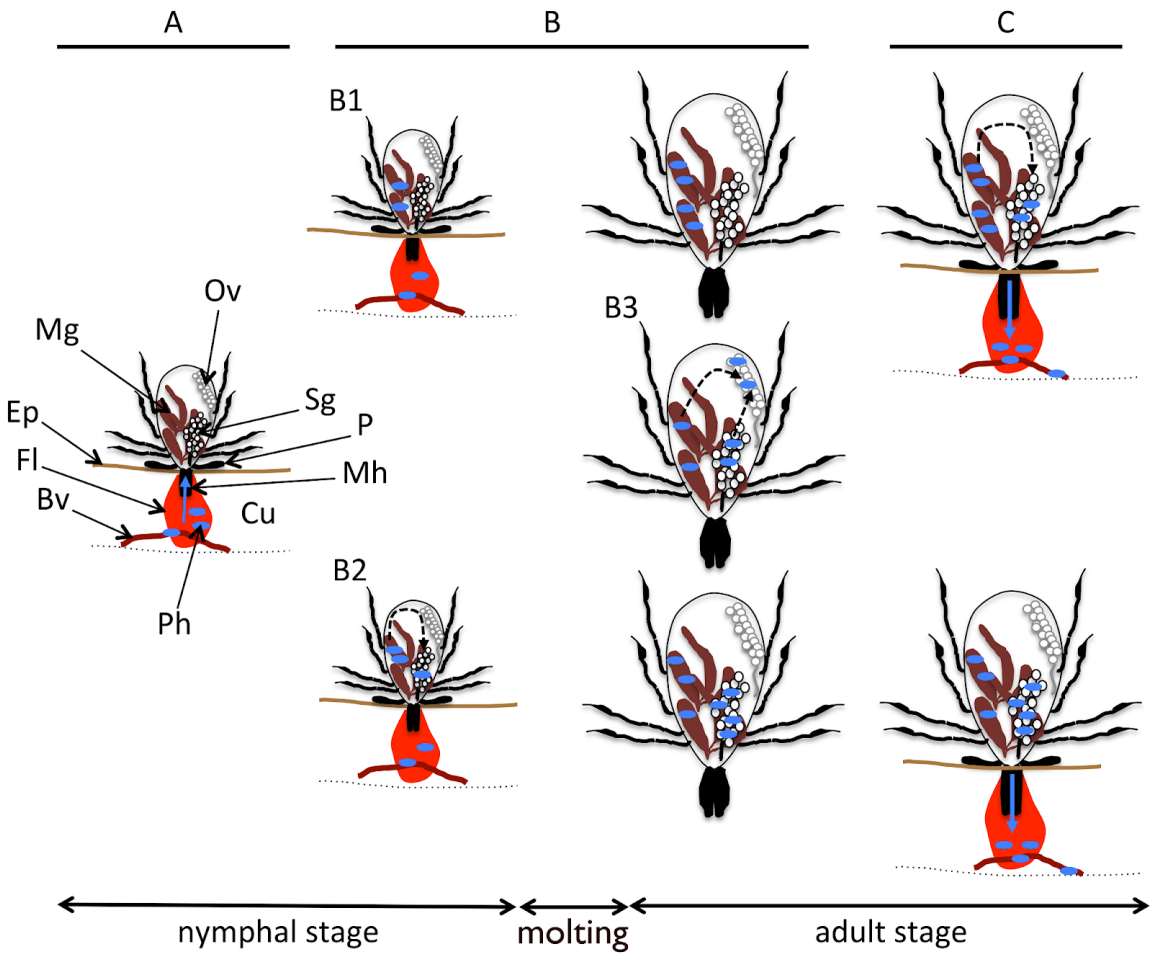

Figure 7: General tick-borne pathogen transmission route

Segment A displays a nymph stage tick becoming infected with a pathogen post-blood meal. Segment B shows the pathogen developing inside the tick midgut (B1), invasion into the hemolymph (B2), and migration into ovaries (B3). Segment C displays transmission from an adult tick into a new host during the blood meal. Abbreviations of tick and host anatomy are as follows: Ov: ovaries, Sg: salivary glands, P: palp Mh: mouthparts, Cu: cutis, Bv: blood vessel, Fl: feeding lesion, Ep: epidermis, Mg: midgut, Ph: pharmacological armament. Figure obtained with permission from Liu, 2014 under the Creative Commons Attribution 4.0 international license (55).

can be understood by studying the life cycle, behavior, and vector potential of the tick in question (81).

Not all ticks are capable of transmitting disease. Conversely, other ticks can transmit disease but are not readily infectable by certain pathogens (81). Their potential as vectors stems from key traits that separate ticks from other arthropod vectors (55). Firstly, ticks as a species are particularly resilient and tough creatures with a long lifespan (64). Depending on the species, these creatures can live several years despite the tick taking only two to three blood meals (64). Unlike many arthropods that take a blood meal as quickly as possible, ticks attach to their host for substantially longer periods of time (93). Indeed, since the blood meal often takes days, the likelihood that the tick will acquire pathogens is much higher than that of other vectors (93). Hosts also move, and an attached tick moves with them (99). This helps to spread tick populations across their immediate environment and even the globe; this process also inherently spreads the pathogens that the ticks carry (99). Ticks are also prolific reproducers, maintaining a high population in most favorable environments, which furthers interactions with vectors and hosts (93).

Dermacentor **vectorial activity**

Dermacentor species have been shown to have a wide distribution range and the ability to act as vectors for multiple SFG *Rickettsia* and associated diseases, most notably, *Dermacentor*-borne necrosis erythema and lymphadenopathy (DEBONEL), TIBOLA, MSF-like illness, and *R. slovaca* (Fig. 8) (10; 66; 91). *Dermacentor* ticks are a hard tick species that is noted for its extreme hardiness in adverse conditions, short life cycle, broad host range, and prolific reproduction (34). These ticks favor temperate

forests and river basins (66). Of note, in Italy and Spain, up to 36% and 35%, respectively, of questing *Dermacentor* ticks tested positive for at least one species of SFG *Rickettsia* (37).

*Rhipicephalus v*ectorial activity

Rhipicephalus sanguineus is one of the most geographically widespread tick species (Fig. 9) (79). However, even though *Rh. sanguineous* is globally dispersed, its associated disease, MSF, is still mainly localized to Northern Africa and Mediterranean regions of Europe (40; 79). *Rh. sanguineous* ticks mainly inhabit warmer climates and most commonly feed on wild or domestic animals, and uncommonly humans (40).

Amblyomma vectorial activity

Amblyomma is an essential vector of numerous types of tick-borne encephalitis and other bacterial infections (Fig. 10). This tick also carries *Rickettsia amblyommii*, an SFG *Rickettsia* of currently undefined pathogenicity (5). In addition, *Amblyomma* may be vectors for this emergent species; patients with unknown tick-borne illness demonstrated increases in IgG that react with *R. amblyommii* (5). *Amblyomma* species are also known to transmit numerous SFG *Rickettsia* across the Americas and Africa and exhibit one of the greatest proficiencies in host-seeking behavior; they actively hunt for hosts instead of only questing (11; 73).

Section 3: Bacterial Agents of SFGR

Foundational information on etiologic agents

Rickettsia derive their name from Howard Ricketts, a researcher from the University of Chicago, who devoted years to the investigation of RMSF (77). The process of SFG *Rickettsia* infection begins upon the attachment and subsequent blood

meal on an uninfected host by a *Rickettsia*-infected tick (97). Upon entry to the mammalian host's body, the *Rickettsia* begin to spread throughout the bloodstream (80). These bacteria then target vascular endothelial cells and induce receptor-mediated endocytosis via targeted binding to surface receptor proteins (80; 95). From this stage, these bacteria develop and reproduce in the cytosol of the host cell. Eventually, *Rickettsia* polymerize host cell actin filaments as a means to escape the current host cell and infect directly adjacent cells (16; 56).

R. rickettsii

R. rickettsii is the causative agent of RMSF and is one of the longest-studied rickettsial species (99). It is distributed in low frequency throughout much of the Americas and confirmed infection of hard tick populations occurs throughout the United States, Mexico, Canada, and Central America (11; 12; 14; 28). A major vector of *R. rickettsii* is the *Rh. sanguineus* tick, also called the brown dog tick (97). This tick lives throughout the Americas (97). The majority of all U.S. cases of RMSF occur in Southern and Midwestern states (97). Cases of human disease throughout the U.S. from 1999 to 2007 described a significantly higher CFR in Native American populations, likely resulting from slow RMSF identification and treatment (12). Additionally, increased incidence in Mexico is noted, where uncontrolled dog populations serve as hosts for the *Rhipicephalus* ticks (12). *R. rickettsii* is found in many tick species throughout Central and North America; epidemiologically relevant vectors other than *Rhipicephalus* include *Dermacentor variabilis* and *Dermacentor andersoni*, *Amblyomma cajennense*, and uncommonly *Amblyomma americanum* (14; 28; 66; 73). Unlike many SFG *Rickettsia*, *R. rickettsii* rarely cause an identifiable eschar at the bite site (14). Common SFGR

Figure 8-1: Unengorged adult female *Dermacentor variabilis*
Figure obtained with permission from Biggs, 2016 Creative Commons Attribution 4.0 international license (12).

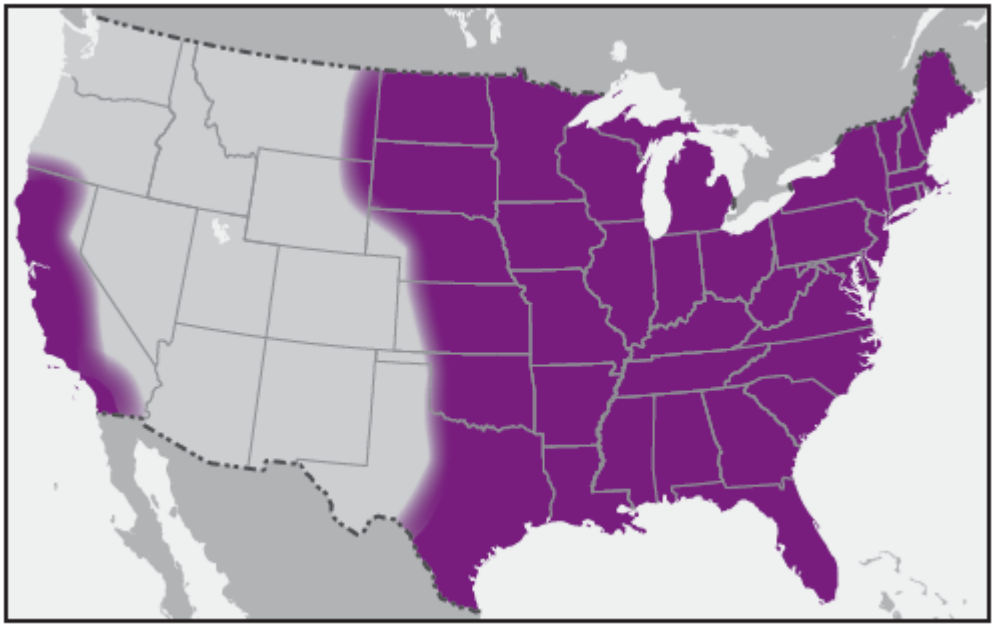

Figure 8-2: *Dermacentor variabilis* distribution across the U.S.
Figure obtained with permission from Biggs, 2016 Creative Commons Attribution 4.0 international license (12).

Figure 9-1: Unengorged adult *Rhipicephalus sanguineus*
Figure with permission obtained from Biggs, 2016 Creative Commons Attribution 4.0 international license (12).

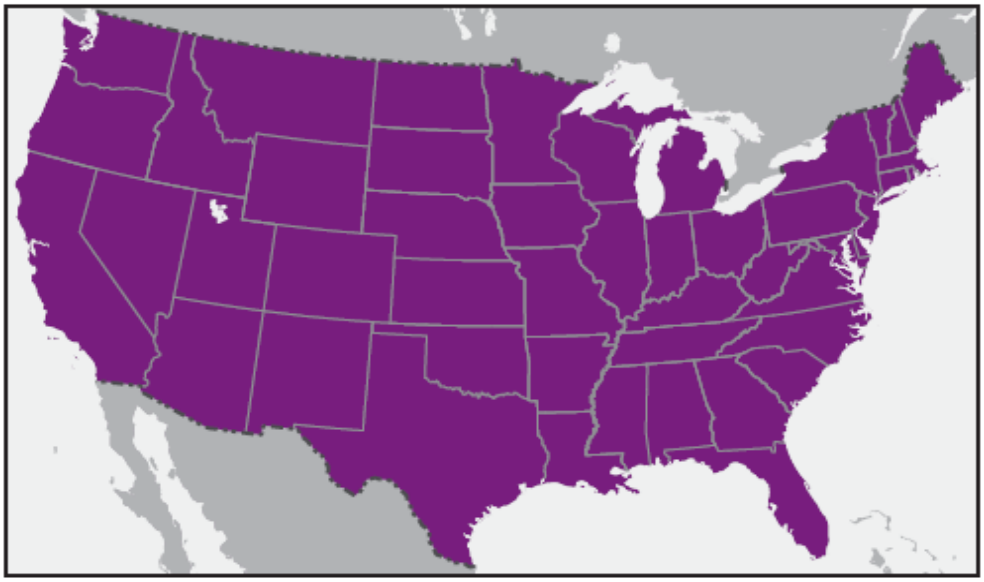

Figure 9-2: *Rhipicephalus sanguineus* distribution across the U.S.
Figure obtained with permission from Biggs, 2016 Creative Commons Attribution 4.0 international license (12).

Figure 10-1: Unengorged adult female *Amblyomma americanum*
Figure obtained with permission from Biggs, 2016 Creative Commons Attribution 4.0 international license (12).

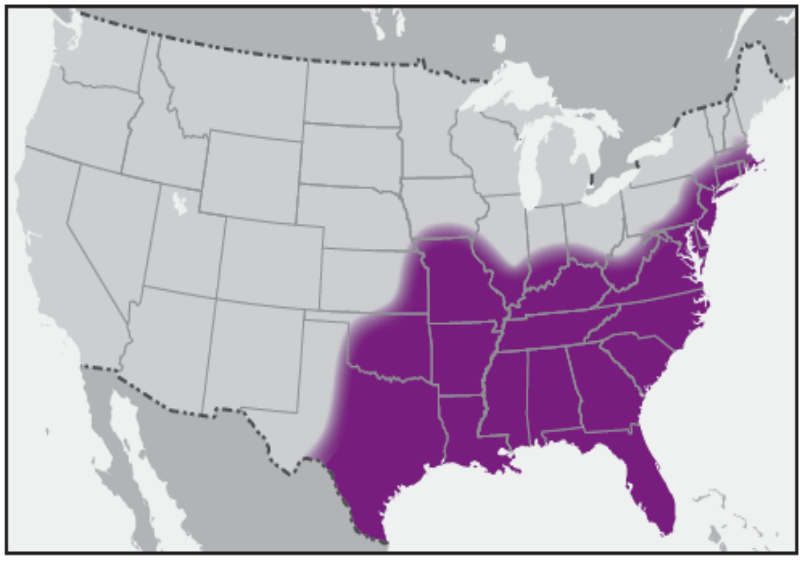

Figure 10-2: *Amblyomma americanum* distribution across the U.S.
Figure obtained with permission from Biggs, 2016 Creative Commons Attribution 4.0 international license (12).

symptoms occur abruptly in RMSF disease progression, and include nausea, headache, and general malaise triggered after a high fever (70). After the fourth day of symptom onset, a rash tends to form around the limbs, progressing to a maculopapular rash (12).

R. parkeri

From the first case reported in 2004, until 2015, *R. parkeri* caused SFGR in at least 40 patients across 10 U.S. states (12; 68). *Amblyomma maculatum* is the primary vector of *R. parkeri* and is distributed throughout the southeastern U.S. from Maryland and Virginia to Texas (12). It is also found throughout Midwest states, such as Oklahoma and Kansas (12). Infections occur in middle aged adults and not children; to date, *R. parkeri* has not been implicated in any fatalities (12; 68). Currently, the reasons for the lack of mortality and the demographic constraints remain unclear.

R. japonica

Japanese spotted fever is endemic to southern Japan, has spread throughout southeast Asia and Korea and is caused by *R. japonica* (89). Common *R. japonica* symptoms include the typical rickettsial manifestations: eschar, rash, headache, and fever (22). Sequenced genomes of *Rickettsia* in those regions revealed strains that were closely related to *R. japonica* from captured *Haemaphysalis* ticks, which serves as the primary vector for *R. japonica* (22).

R. conorii

Known to be the agent of MSF, *R. conorii* is found throughout northern Africa and the Mediterranean regions (21; 79). It is thought that changing ecosystems and global temperatures are driving changes to the *R. conorii* vector; *Rh. sanguineus* alterations in

feeding and host-seeking behaviors may be contributing to recent deviations from the established epidemiology of MSF (71). *R. conorii* infection exhibits a CFR of around 4% in patients with non-severe disease and 50% or greater in patients that are hospitalized with severe disease (48). Patients with severe disease can exhibit atypical neurological complications and multi-organ infection (14). Other clinical manifestations include common rickettsial symptoms, with the addition of multiple eschars on the patient (40). Similar to other SFG *Rickettsia*, *R. conorii* infection is associated with mammalian reservoirs such as feral dogs. In regions where MSF is highly prevalent, serological surveys of wild dogs showed 15-72% seropositivity (14). *R. conorii* is found at a low frequency in wild *Rhipicephalus sanguineus* ticks (40). This, coupled with spikes of MSF mainly during the summer months, suggests that infected ticks are likely unable to survive colder temperatures (71).

R. africae

ATBF is caused by *R. africae*, which is carried throughout Africa by its two primary vector species, *Amblyomma variegatum* in eastern, western, and central Africa, and *Amblyomma hebraeum* in southern Africa (100). It has been detected throughout Africa, including in Madagascar and the Union of Comoros (100). In certain regions of Africa, such as Tanzania and Comoros, infection rates of *R. africae* in *Amblyomma variegatum* were 77% and 90%, respectively; these high numbers suggest endemicity of this species even on African islands (100). In certain regions of Africa, the percentage of ATBF IgG-positive patients exhibiting a febrile illness is around 63% (82). Thus, these bacteria are a significant concern for all African regions (82). *R. africae* elicit symptoms extremely similar to *R. conorii*. The resemblance is so striking that, for many years, *R.*

conorii was believed to be the etiologic agent of ATBF (82). *R. africae* infection routinely results in a lower CFR than *R. conorii*, with common SFGR symptoms being a generalized febrile illness resulting in a scattered rash with multiple eschars (82).

Additional emergent SFG *Rickettsia* of military concern

Rickettsia akari

In recent years, *R. akari* has been classified in both the SFG *Rickettsia* and transitional group *Rickettsia* categories. However, this organism is included herein as a *Rickettsia* that elicits SFG *Rickettsia* symptoms (3). Interestingly, *R. akari* is the only currently known SFG *Rickettsia* not transmitted by a tick vector. Instead, these bacteria are transmitted by the mouse mite, *Liponyssoides sanguineus* (2). Like with other SFG *Rickettsia*, humans are not competent reservoirs for *R. akari. R. akari* is found mainly in urban environments, especially in the Balkan states, Ukraine, and Korea (2). Transmission occurs when an infected mite fails to locate its preferred host, the typical house mouse *Mus musculus*, and instead feeds on a human (3). Infection results in the common rickettsial rash, eschar, and fever; this symptom triad occurs in more than 90% of patients (3). The disease is primarily self-limiting and resolves around ten days after symptom onset, but around a third of patients may require hospitalization and treatment with doxycycline (2). Death is an extreme rarity in *R. akari* infection, and almost all patients recover fully within two weeks (3).

Rickettsia raoultii

Found to be transmitted by *Dermacentor* species, *R. raoultii* is an emergent SFG *Rickettsia* species that causes the disease TIBOLA, which is also known as DEBONEL (88). Patients with TIBOLA/DEBONEL exhibit thrombocytopenia and leukopenia, in

conjunction with enlarged lymph nodes (88). Case reports describe successful treatment with doxycycline and ceftriaxone to ameliorate symptoms (10; 12; 88). Little about *R. raoultii* pathogenicity and distribution is reliably established (60). However, this species has been detected throughout Poland, Kazakhstan, Spain, France, and China (12; 31; 87).

Rickettsia slovaca

Also known to cause TIBOLA/DEBONEL, *R. slovaca* is a rickettsial agent that is transmitted by *D. reticulatus* and *D. marginatus ticks* (91). Infection occurs throughout Europe (10). TIBOLA symptoms are common in *R. slovaca* infected individuals, as is the exhibition of the disease Scalp Eschar and Neck Lymphadenopathy after Tick bite (SENLAT) (10). In addition, long-lasting SENLAT symptoms, which are characterized as chronic malaise, alopecia, and scarring at the tick bite location, have been noted in both treated and untreated patients (10).

Section 4: Human Immune Response

Introduction to human immune response to SFGR infection

Mammalian host immune systems recognize tick attachment and then trigger phagocyte activation, which includes macrophages, neutrophils, eosinophils, and monocytes (53). In order to mitigate foreign body invasion, reactive oxygen ions are also produced and released around the bite site (25). Ticks themselves are resistant to reactive oxygen species (ROS), which are delivered via phagosome nicotinamide adenine dinucleotide phosphate oxidases (NOX) production of superoxide ions like hydrogen peroxide (25; 41). Interestingly, the process of tick feeding produces ROS, which then leads to immune interference and tissue degradation (25; 41; 49). This, in turn, likely aids in vascular permeability, further enhancing blood-feeding (25; 41; 49).

Immunopathology of SFGR

The bite site functions as the initial staging area for infection and dissemination (84). After successful passage from the tick into the mammalian host, *Rickettsia* induce receptor-mediated endocytosis into vascular endothelial cells (54; 95). Advances in the development of molecular techniques to study *Rickettsia* and increased interest in SFG rickettsial infections have recently propelled our understanding of the molecular immunopathological mechanism of infection (54). The receptors and processes for receptor-induced endocytosis and phagocytosis have not been fully identified, but it is known that the small GTPase Cdc42, phosphoinositide 3-kinase (PI3K), focal adhesion kinase (FAK), and others, are involved (56). It has also been shown that recruitment of Ku70 subunit, a DNA-dependent protein kinase, to SFG *Rickettsia* entry sites occurs (43). In addition, rickettsial OmpB, an outer membrane protein, and Ku70 interactions have been experimentally identified as a pathway for rickettsial cell invasion (43).

Auto-transporting outer membrane proteins perform important roles in *Rickettsia*-endothelial adhesion; these include members of the surface cell antigen protein family (Sca) and the outer membrane protein (Omp) families (16). Sca1, Sca2, and OmpA are important for binding to host cells across SFG *Rickettsia* infection, and these proteins are found in many Gram-negative bacteria (16; 54; 92; 95). The actual function of all Sca family proteins is not entirely known, but it is believed to be important for host receptor specificity (16).

After binding to a proper receptor and subsequent phagocytosis, *Rickettsia* enter the cytosol in a phagolysosome (67). Host cell entry is a necessary step in SFG *Rickettsia* reproduction (19). However, phagolysosome-mediated entry comes with an additional danger: degradation of foreign bodies such as invading bacteria (80). *Rickettsia* escape

the phagosome by upregulating *thyC* and *pld*, which encode hemolysin C and phospholipase D, respectively (80). These two bacterial products destroy the phagolysosome membrane, allowing the *Rickettsia* to enter the cytosol; here, these bacteria are surrounded by nutrients, amino acids, and ATP without the immediate threat of destruction (19; 80). Once inside the cell, *Rickettsia* infection leads to bacterial injury of the infected tissues (67). While cellular invasion undoubtedly causes cellular distress, the actual molecular mechanism(s) of host cell damage are not well understood (19). However, certain probable molecular players have been identified and will be discussed in the following section (67).

Human immunological defenses against SFG *Rickettsia*

Rickettsia are able to reliably infect and subsequently replicate in a variety of cell types *in vitro.* In natural, *in vivo* infections, SFG *Rickettsia* primarily target vascular endothelial cells within medium and small blood vessels (80). However, to a lesser extent, SFG *Rickettsia* also target human immune cells such as macrophages, hepatocytes, and monocytes (24). The targeted attack of these immune cells allows the infection to persist and spread (67). This destruction of human vascular endothelial and immune cells does not go unnoticed by the host immune system an acute infection response is triggered, including changes in circulating CD4+ T cells (24). In an effort to contain and destroy the infectious threat, a culmination of the infection response leads to immune-mediated vascular infiltration of macrophages, B cells, and CD4+ and CD8+ T cells (24). At this stage of the early immune response, the immune system has recruited and begun differentiation of various cell types needed to sequester and destroy the infection (Fig. 11) (56).

The first steps in elimination of the rickettsial threat begins with endothelial activation, a process wherein infected endothelial cells substantially modulate gene transcription and cellular responses (56). These processes are suggested to play an integral part in the immunological early warning system against SFG *Rickettsia* infection (56). One of the most effective methods to limit bacterial spread is simply through apoptosis (programmed cell death) (4). As an obligate intracellular pathogen, *Rickettsia* are entirely dependent on the host cell for survival (4). Thus, if the host cell undergoes apoptosis, the pathogen will no longer be in an environment in which it can survive and replicate, so therefore it will be killed (4). However, *Rickettsia* have developed mechanisms to hinder host cell apoptosis (30). Indeed, *Rickettsia*-associated anti-apoptotic activity of infected cells might be achieved by translocating Ku70 into the cytoplasm, though this process has yet to be conclusively uncovered (30).

Once rickettsial epitopes are recognized, the host cell utilizes interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and macrophages as a means to kill intracellular *Rickettsia* (30). Additionally, mouse models suggest autophagolysosome-mediated killing of *R. conorii* upon activation of IFN-γ and TNF-α (30). In human cells, IFN-γ and TNF-α are similarly activated (30). However, it is still uncertain if autophagy truly plays a significant step in clearance (30).

Additionally, it has been shown in mice that upon cytokine production, the immune system initiates hydrogen peroxide (H_2O_2) and ROS production. This manipulates tryptophan availability and nitric oxide (NO) production to hinder the infection (Fig. 12) (30; 92). *In vivo* mechanisms for the killing of rickettsiae in humans are noted (25). As with many human immune mechanisms of intracellular infection

defense, IDO likely does not work alone nor carries the sole responsibility of protection (25).

Human innate immune response to SFG *Rickettsia*

The innate immune response plays an important role against SFG *Rickettsia* infection. Innate immune system signaling proteins, such as the nod-like receptors (NLR) and toll-like receptors (TLR) are the major factors in this response (4; 92). NLR are a class of proteins known as pathogen recognition receptors (PRR) that are responsible for recognizing pathogen-associated molecular patterns (PAMP) (4; 92). These proteins are characterized by their specialized ability to recognize foreign bodies, particularly binding to components that the immune system recognizes as pathogen-specific (4; 56). Depending on the immune cell type, each TLR and NLR can specifically bind to a single protein or to an entire protein family (4; 56). When a TLR or an NLR interacts with a PAMP that it is designed to recognize, it binds the PAMP (4; 56). Specific, targeted binding of NLRs and TLRs to PAMPs triggers pro-inflammatory and antimicrobial activity (4; 56). Due to their ability to bind to and oligomerize with inflammasomes, NLRs are primarily involved in triggering inflammation (4). Various NLR and TLR families are able to recruit particular, discrete inflammasomes (4). Upon inflammasome activation, a cascade of apoptosis-inducing cytokines, such as interleukin-18 (IL-18), IL-1β, gasdermin D (GSDMD), among others, are released (92). In addition, IL-1β and IL-18 promote inflammation and Th1 cell activation, which in turn enhances NK and CD8+ cytotoxic T cell activities (80; 92). The combination of CD8+ T cells and inflammatory-mediated recruitment of other immune cells is an essential host immune response against persistent and disseminated infections (80).

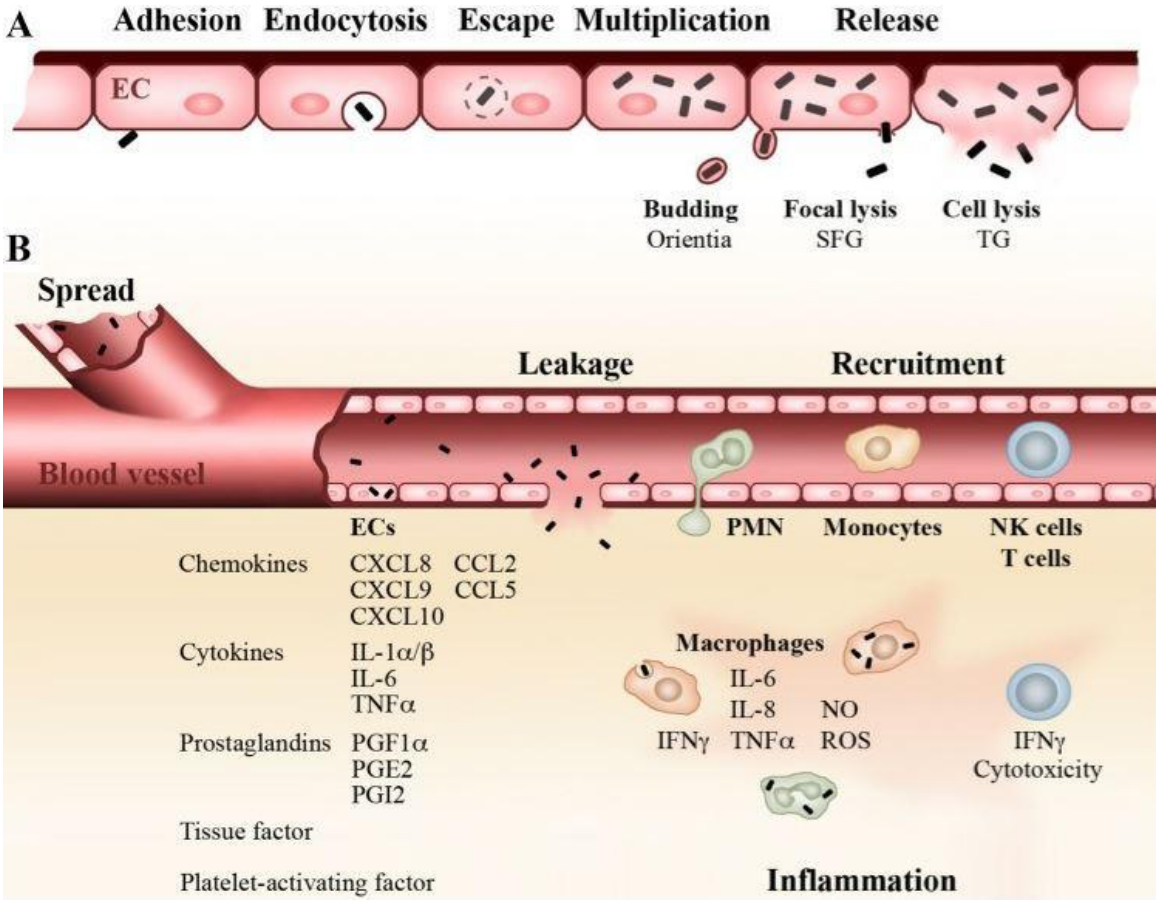

Figure 11: *Rickettsia* infection, replication, basic immune response

Rickettsia infect endothelial cells and begin replicating in the cytosol of the host cell (67). Rickettsial escape mechanisms can occur in several manners (67). SFG *Rickettsia* escape by focal lysis of the host cell (67). The top panel (A) displays *Rickettsia* infecting adjacent cells. The bottom panel (B) illustrates how release of the *Rickettsia* triggers cell-damage signaling chemokines that recruit macrophages, natural killer (NK) cells, and other immune cells to the infection site (67). Figure taken with permission from Osterloh, 2017 Creative Commons Attribution 4.0 international license (67).

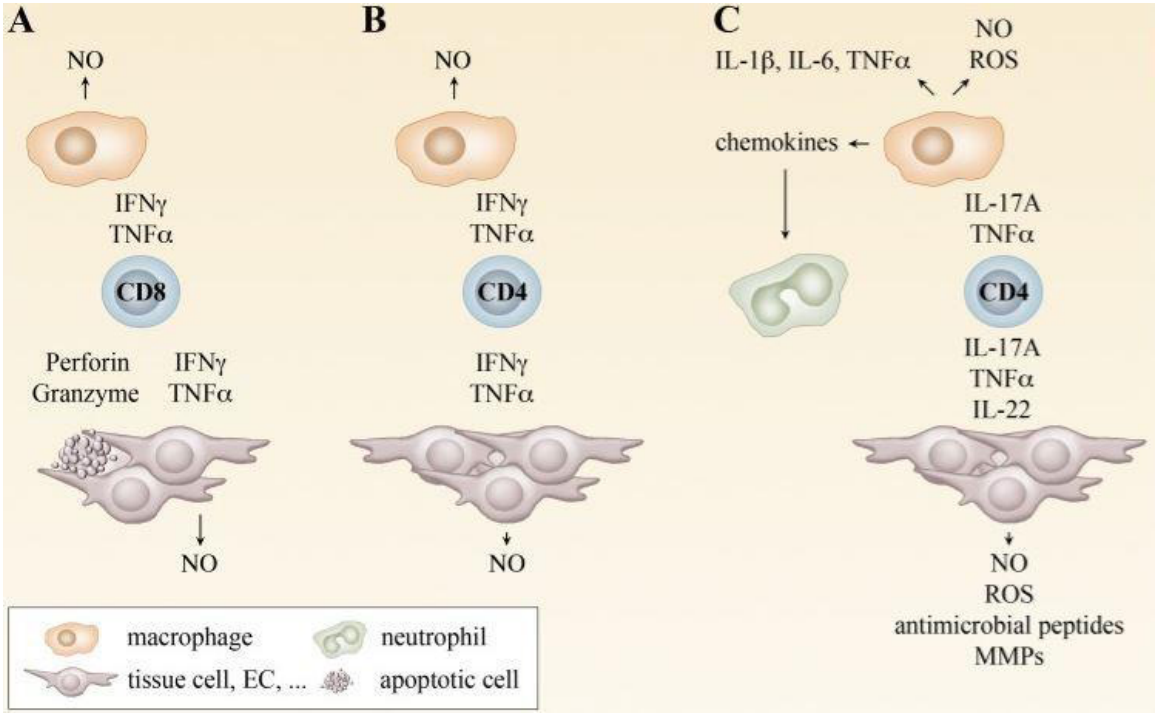

Figure 12: Immune cell response to *Rickettsia* infection

Upon infection recognition, T cells differentiate to CD8+ T cells, which release TNFα and IFNγ to induce NO production. Panel A shows the differentiation into Th1 T cells that release TNFα, IFNγ, and NO. Panel B displays CD4+ T cells differentiating into Th17 cells that release TNFα, IL-17, and IL-22, all of which induce ROS and NO production and inflammation. Panel C displays IL-17-induced recruitment of neutrophils to the site of infection, as well as the production of other pro-inflammatory and antimicrobial chemokines. Figure taken with permission from Osterloh, 2017 under the Creative Commons Attribution 4.0 international license (67).

In addition to the aforementioned innate systems, another critical component to fight rickettsial infection is the direct role of NK cells, as well as their involvement in IFN-γ secretion (80). IFN-γ, which is secreted predominantly by NK cells and activated T cells, acts as an enhancer of macrophage activation, antigen presentation, and innate immune activation (24; 92). In terms of *Rickettsia* clearance, IFN-γ promotes antibacterial mechanisms via tryptophan restriction and depletion, enhanced major histocompatibility complex (MHC) type 1 and type 2 presentation, and increased activity of NK cells (80; 92). Increased IFN-γ and NK cell presence are often noted in SFG *Rickettsia*-infected tissue. However, recent reports have suggested that the activity of IFN-γ and NK cells in fighting SFG *Rickettsia* may not be critical to clearing the infection (80).

Interferon and inflammasome recruitment are chief infection fighting methods that are utilized by the innate immune system (15). Experimental *in vivo* analysis has shown that obligate intracellular bacteria elicit a strong, protective IFN-γ and interferon regulatory factor 1 (IRF1) response, whereas type 1 IFN is not protective (15). However, the host response to *Rickettsia* mimics that of a viral response; the exact reason for this is unknown but is possibly due to its obligate intracellular life cycle (36). Just as it does with many viruses, type 1 IFN limits *R. parkeri* infection (80). It has been suggested that type 1 IFN changes the cytosolic environment of the infected host cell, though the exact mechanism remains to be definitively uncovered (80).

Various SFG *Rickettsia* have additional targets other than endothelial cells, predominantly macrophages (24). In addition, other cell types are critical for disseminating *Rickettsia* as well as for combating the infection (24). When a macrophage

is infected, it has been shown to exhibit increased release of IL-1β, TNF-α, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) (24; 92). In addition to *Rickettsia* potentially using macrophages as a method of dissemination, it is possible that rampant chemokine secretion from infected macrophages leads to pathogenic immunological tissue damage (24; 92). Thus, maintaining a controlled immune response during a rickettsial infection is a crucial task of the host immune response (24).

Section 5: Vector and Disease Control

Current strategies

The fight to curtail disease is a struggle as old as humanity itself. Modern medicine allows its beneficiaries to enjoy treatments for a myriad of diseases. Unfortunately, vector-borne diseases frequently prove more challenging to eradicate. Aside from the laboratory or clinical departments of public health centric vector control programs, arthropod-borne disease control must comprehensively deal with humans, pathogens, and vectors, which are three constantly changing variables of immense complexity. Nonetheless, continuous efforts are underway in every facet of vector and disease control. To date, there are no approved vaccines for any SFG *Rickettsia* (74). However, promising vaccine candidates are potentially on the scientific horizon; breakthroughs in our understanding of tick salivary proteins, as well as pathogen-specific understanding, continue to advance (74). Fortunately, a vaccine may not be immediately needed so long as SFG *Rickettsia* remain susceptible to doxycycline and implemented SFGR mitigation techniques are effective. Until vaccines are available, the predominant methods to diminish the presence of SFG *rickettsia* are vector control and avoidance strategies.

Regardless of CONUS or OCONUS location, military personnel are frequently in tick habitats that could harbor SFG *Rickettsia* (29; 31; 38; 59; 94). In order to mitigate contact with ticks and other disease vectors, the DoD Armed Forces Pest Management Board (AFPMB), in conjunction with other expert government bodies, has outlined a series of procedures and critical skills that are designed to enable soldiers to prevent exposure as well as treat potentially infected compatriots (32; 90). Military-specific considerations revolve around avoidance and personal protective measures (PPM), education, surveillance and health information gathering, and tick habitat and host control (90). These combined efforts are utilized to minimize individual DoD members' exposure to potentially harmful tick populations, thereby maximizing mission readiness and effectiveness (27).

One of the most widely distributed protective methods is implementation of PPM against insect vectors of any variety, especially ticks (27). For example, U.S. Army soldiers are required to have pant legs bloused into boots (90). Shirts are also required to be tucked completely into trousers and secured with DoD-approved/issued belts (90). Headgear also helps to provide protection during crawling or crouching movements (90). When the approved clothing measures are properly implemented by a service member, it limits a tick's ability to latch onto and bite into the actual body of the individual (90). In addition to clothing protectants, the DoD authorizes the uses of DEET (N,N-diethyl-3-methylbenzamide or N,N-diethyl-m-toluamide) as the standard skin applied insect repellent (32; 90). When used in combination with permethrin-treated uniforms, this duo serves as an effective tick repellent (90). DEET is applied to an individual's skin by themselves, and permethrin is used to saturate a uniform using approved DoD methods

(32). DoD members who are expecting to enter tick habitats are also taught how to perform proper tick checks, such as searching the entire body and focusing on regions such as the groin, underarms, legs, neck, head, and other difficult-to-see areas of the body where ticks are likely to bite (32; 40).

Education holds a paramount role in individual and group readiness and willingness to abide by AFPMB guidelines (32). DoD members are taught about the variety of arthropod vectors that they might encounter in a particular environment, what methods to use to avoid them, and what procedures to implement should someone come in contact or be bitten (90). In addition to regular training while garrisoned, educational lectures are given to troops before and during movement to and within tick habitats (90). This strategy provides the individual with the necessary knowledge to maintain constant vigilance for signs of ticks on their person and their fellow service members. In military settings where individual training is applied to everyone, a positive feedback loop of self-policing is likely to occur, resulting in informed surveillance among units.

Larger-scale tick vector control can be seen in civilian and military installations' joint efforts to limit tick populations through habitat modification, integrated control, and host control (27; 90). Immediate action to limit tick populations is frequently conducted through habitat modification (90). This strategy is comprised of multiple techniques, often starting with vegetation management (27; 90). Integrated vector management (IVM) requires combining situationally dependent vector control methods, like acaricide usage and biological control, with continuous methods, such as surveillance and education (90). IVM poses an interesting means to effectively deal with tick populations

by attacking vector control problems though immediate and broad-sweeping approaches (90).

A tick's central habitat is under low and thick vegetation that allows little light to the bottom layers; here the tick waits for proper questing weather (93). The process of removing low brush eliminates the tick's needed habitat. As such, this process is noted to yield a marked decrease in tick bites (90). Tick habitat control also limits hiding places for rodents, which live in proximity to tick populations (90). Fewer small rodent hosts result in a smaller opportunity for ticks to receive the necessary blood meals required for their life cycle development (29; 55). Even more importantly for humans, decreased tick contact with rodents decreases contact with the suspected reservoirs of many tick-borne diseases (90).

Ticks quest in defined humidity and light conditions (93). If those conditions are not met, the ticks simply hide and wait for the proper conditions to arise (93). One process to artificially reduce proper questing conditions is to cut underbrush in such a way as to allow more sunlight to pass through the forest canopy and illuminate the ground (90). Methods for habitat control and modification include cutting grass below six inches in height, destroying leaf and stick piles, and removing excess vegetation (39). Additionally, controlled burns are an established and effective procedure to simultaneously eliminate unwanted vegetation and destroy ticks of all life stages (90). These burns eliminate much of the vegetation that ticks need for questing behavior and also scare away the rodent hosts (39).

Diagnosis and treatment

Even with the most rigorous vector mitigation actions, diseases will still occur. Thus, early detection via symptom identification at the field-medic level, and through laboratory confirmation at the medical microbiologist level, are absolutely necessary to properly treat military members that become ill with an SFGR. Frequently, the DoD personnel who have contracted an SFGR have likely been in a field environment; here, symptom identification would take a paramount role. As previously stated, the symptom similarities of various SFG *Rickettsia* species to each other and to other pathogens render a prompt and accurate diagnosis difficult. Nevertheless, with SFG *Rickettsia* infections, it is critical to accurately diagnose and treat patients as quickly as possible. The delay of an accurate diagnosis is a costly mistake (12). Therefore, the next section will describe challenges in SFGR diagnosis, the outcomes of such delays, and the proper treatment protocols.

Despite each SFGR being caused by distinct etiologic agents, one of the most challenging aspects for diagnosis and treatment of SFG *Rickettsia* is the wide variation in symptom presentation; this results in difficulties in the differential diagnosis (70). Patients infected with tick-borne rickettsial illness are noted to exhibit non-specific clinical symptoms, especially in the beginning stages of the disease (70). However, throughout the course of the illness, distinct clinical presentation differences do emerge between the various pathogens; these are often seen in the percentages of species-specific symptoms such as eschar formation, rash, and fever exhibited by ill patients (70). Considering the difficulty in diagnosis and the high level of exposure to potential SFG *Rickettsia*, it is essential for medics and corpsmen to be familiar with SFGR presentation to aid in their essential role of education, prevention, and referral.

One of the SFGR of most significant military concern is RMSF (63). This is, in part, due to its high CFR, which ranges from around 25% to 50% without treatment, and 5% to 10% even with timely treatment (12). Distribution of RMSF throughout much of the continental United States creates the potential for this disease to limit unit cohesiveness and effectiveness in units throughout the country (59). RMSF is characterized by an incubation period of three to twelve days after the initial attachment of an infected tick (12). Broadly, it has been noted that shorter incubation periods, fewer than five days, are associated with more severe disease (14).

Initial symptom presentation of RMSF commonly includes headache, chills, general myalgia and malaise, and a sudden fever (12). After two to four days, a rash appears (12). Less common symptoms include photophobia, abdominal pain, and vomiting (12; 23). A rash, fever, and tick bite are no longer considered the three-pronged necessity to diagnose RMSF, as only a minority of patients claim such events (26). When rash does develop, it starts with small, pink-colored macules on wrists, forearms, and ankles (12). Except for the face, the rash then spreads across most of the body (14). Notably, the absence of rash does not signify the absence of RMSF infection; a small percentage of patients never developed a rash, and fewer than 50% of patients develop a rash during their first three days of disease (12). In patients with sepsis and febrile illness caused by an unidentified etiological agent, it is crucial to include RMSF in the differential diagnosis (12). Indeed, since RMSF quickly develops from fever and rash into an intensive disease, the greatest cause of fatality is delayed diagnosis and treatment (12). Patients treated after their fifth or sixth day of symptom presentation are noted to have significantly higher mortality (12).

Due to the drastic range in mortality seen with treatment timing, the timeline for treatment is absolutely critical. If a SFGR is observed, or even suspected, antibiotics must be administered immediately (14). When dealing with *Rickettsia* infections, it is also necessary to recall that most antibiotics have either no effect or even a negative effect on the patient (14). For example, beta-lactamases and cephalosporins are documented to not affect patient health outcomes (12; 14). Conversely, the administration of sulfonamides is associated with adverse health outcomes for the patient (12). As such, clinicians are advised to administer tetracyclines as the standard SFG *Rickettsia* antibiotic; doxycycline is frequently the preferred antibiotic (14). Other antibiotics such as chloramphenicol can be experimentally used (12). However, chloramphenicol is not preferred since it has a greater association with longer illness than doxycycline (14). Doxycycline is commonly used as a preventive anti-malarial and is frequently administered in malarial endemic countries; this, in turn, could act as a preventative therapeutic for SFGR (45). Finally, azithromycin is a promising macrolide for treatment (14). In an instance where several children were sick with SFGR, a three-day course of azithromycin was shown to be as effective as a five-day course of doxycycline (14).

Laboratory confirmation of SFGR primarily relies on serology analysis and molecular detection techniques. Adding to the difficulties in differential diagnosis of SFG *Rickettsia*, antibodies generated against SFG *Rickettsia* and other pathogenic *Rickettsia* are frequently cross-reactive (78). This difficulty, nonetheless, can be circumnavigated by using a cross-absorption western blot. Different *Rickettsia* groups are frequently diagnosed using specific tests. For example, the reactivity of *Proteus vulgaris* OX-3 cells with SFG *Rickettsia* generated patient sera is used (78). Additionally, hemagglutination

testing is a reliably specific test for SFG *Rickettsia* sera; SFG *Rickettsia* antibodies can be detected in acute phase sera (12). Indirect hemagglutination tests have also been shown to be *Rickettsia* group specific; the ability to specifically bind to several SFG *Rickettsia*-specific antibodies with some cross-reactivity with antibodies from *R. akari* have been demonstrated (9). Likewise, enzyme-linked immunosorbent assay (ELISA) has reliably been shown to detect species specific SFG *Rickettsia* antibodies. Though exceptionally accurate, ELISAs are only useful after patient seroconversion, which generally occurs about a week past symptom onset (Fig. 13) (33; 78). Modern serological testing on SFG *Rickettsia* yields a binary diagnosis of infection, either having occurred or not. It will not, however, necessarily clearly delineate if the patient is currently infected; SFGR is known to cause a long-lasting antibody titer (12).

Molecular biological detection techniques for SFG *Rickettsia* have proven to be able to determine the existence of infection in the early stages of disease. These are remarkably useful for identification of infection preceding significant antibody production. Since its diagnostic properties rely on pathogen DNA, which can be extracted from host, vector, and patient, polymerase chain reaction (PCR) remains one of the "gold standards" for early detection (6). For SFGR, which does not cause significant bacteremia, eschar tissue samples are frequently used as a source of DNA for PCR (6). In patients without eschar formation blood samples also can be used (6). Naturally, since PCR amplification requires a gene target, the selection of said target is paramount for the ability to conclusively diagnose the etiological agent. Fortunately, *Rickettsia* have numerous suitable gene targets; rickettsial *ompA* and *ompB* are notably group

discriminating targets (44; 70). When used in conjunction with downstream amplification, an accurate determination of the causative species can be achieved.

Potential issues

On a global scale, the efforts to identify, contain, and control vectors and vector-borne disease are an ancient struggle. Luckily, innovations to previously utilized approaches are yielding positive results. In addition, novel therapeutics and strategies are nearing the translational precipice to actual implementation. The Health and Human Services tick-borne disease working group has a mission to proactively unveil how tick-borne disease impacts federal mission sets. Additionally, they seek to stop these infections and to lessen the burden of SFG *Rickettsia* (46). Additional improvements in DoD capacity to share tick surveillance information and case numbers rapidly and reliably within the organization and with allies might help for their mission (46).

Disease reporting for what to report, how to report, and when to report, is standardized throughout the country; this aids in proper epidemiological mapping (46). CDC-led efforts have established funding and subsequent guidance for U.S. tribal, state, and federal territories for tick surveillance stemming from the National Notifiable Disease Surveillance System (NNDSS) (1). Many diseases with a high fatality rate are declared as notifiable diseases, with SFGRs included on the list (1; 12). This data collection provides epidemiological background for decision making (1; 12). However, since serological testing is frequently a main method of SFG *Rickettsia* testing, and because SFG *Rickettsia* elicits long-term antibodies, it has been noted that a possible over-diagnosis of SFGR occurs (58).

The various departments of the DoD have joint task forces for pest management.

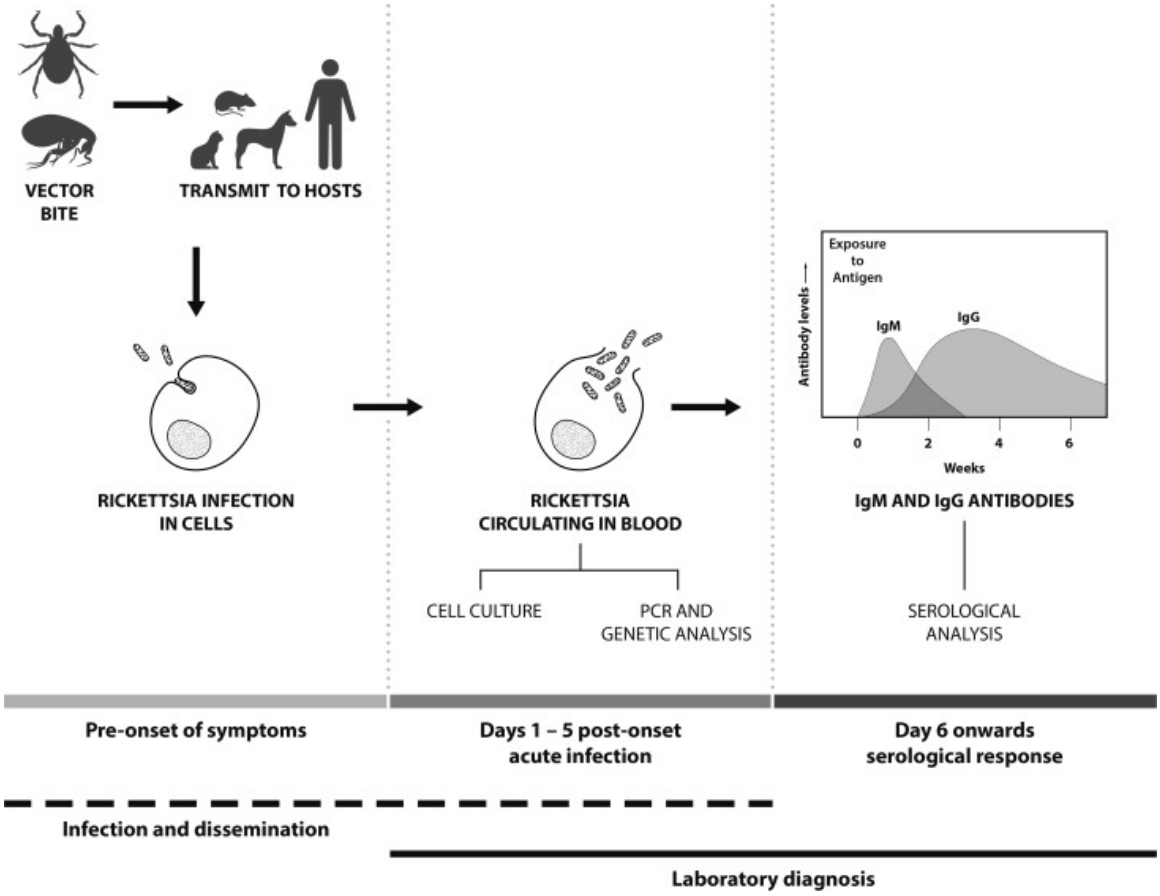

Figure 13: General timeline of *Rickettsia* diagnostic opportunities
Figure taken with permission from Robinson, 2019 under the Creative Commons Attribution 4.0 international license (78).

These aid in the unification and dissemination of reliable bio-intelligence (90). While military populations have their own mission set and personnel to contend with, nearly all non-forward deployed military personnel either live within or near civilian populations. Furthermore, almost all personnel frequent environments where ticks are heavily present (12; 61; 64; 78). Thus, the need for proper, standardized case reporting by U.S. states is imperative for the sustained health of the American warfighter.

The branches of the military have unified mission sets to curtail the spread of SFG *Rickettsia* and other diseases through the implementation of force health protection (FHP) (33; 62). FHP encompasses roles both immediately important to protect medical readiness, such as surveillance, education, training, and more prospective measures, such as research and development (33; 62). Joint DoD health surveillance efforts track infectious disease throughout the world, which collects bio-surveillance intelligence, aiding military and public health planning operations (62). FHP education and training endeavors cover degree-awarding programs to short training exercises; thereby improving on-site infectious disease preparedness (33; 62). Also critical, the research and development component of FHP ensures long-term health/mission readiness by investigating novel therapeutics, vaccines, and other prevention strategies, as well as emergent and existent pathogens; raising collective understanding and preparedness against targeted infectious disease threats (33; 62).

Because of the significant difference in CFR based on time of treatment, it is important for the DoD to continue developing methods to increase training of first-line healthcare, such as Army Medics and Naval Corpsman, in the differential diagnosis techniques needed to quickly and accurately identify the rickettsial agent. In addition to

field health care, there are no definitive criteria that differentiate which symptoms necessitate hospitalization (1; 12). This is a critical and relatively identifiable area that should be explored since it could drastically aid in a military member's health outcomes.

Future research

The tome of SFG *Rickettsia* knowledge is expanding as waxing interest is being paid to these bacteria, but still more research is needed to curtail the SFG *Rickettsia* threat to American military interests. The principal areas in which more study is needed begin first with developing commercially available rapid, and sensitive, diagnostic tests. Along with the development of species-specific antibody assays, these two advancements could drastically shorten the time from patient symptom exhibition to accurate diagnosis; leading to better health outcomes and greater mission readiness because of it. Another region of investigation worth expanding are serological surveys of DoD personnel. The implementation of DoD-wide pre-deployment and post-deployment antibody assays for SFG *Rickettsia* would provide exceptionally useful surveillance information; as well as provide feedback for what methods are most or least effective based on positive or negative serological results of troops. Future efforts also may benefit from identifying novel SFG *Rickettsia* vaccine candidates. For now, an SFG *Rickettsia* vaccine may not be immediately necessary due to effective treatments, however there may come a time where such a vaccine is mission critical. To date, doxycycline resistance has not been reported in SFG *Rickettsia*, however it may be beneficial to develop additional antimicrobial and vaccine therapies should resistance ever occur, however unlikely. From a cost-benefit standpoint, doxycycline treatment is reliable, safe, and affordable to administer; so, for the time being it may remain the chief SFGR treatment (6). In

conjunction with the aforementioned efforts, continued and expanded education of healthcare workers to consider SFGR diagnosis is a prudent method to limit SFGR effects by expediting treatment, enhancing surveillance, and sustaining SFGR reporting.

Section 6: Conclusion

SFG *Rickettsia* are obligate intracellular bacteria and the etiologic agent for debilitating spotted fever group rickettsiosis (28). Global distribution of the rickettsial tick vector necessitates CONUS and OCONUS action. SFG *Rickettsia* were once considered relatively rare in the continental United States, with SFGR incidence of around two per million people per year in 2000 (28). However, as of 2012, SFG *Rickettsia* prevalence has markedly increased, with SFG *Rickettsia* incidence recorded as high as 14 per million per year (28). Globally, cases of SFGR infection have also been increasing (85). SFG *Rickettsia* already posed a risk to service members due to their time spent in tick habitats (65). Now with SFG *Rickettsia* increasing worldwide, the potential impact on service members may be even more severe.

If treated quickly upon symptom presentation, the CFR remains low for most SFGR; however, the difficulty with accurate diagnosis plays a significant role in the measured fatality (12). SFGR symptoms are varied depending on the species; however, spotted fever manifestation and eschar formation are frequently considered telling symptoms of SFGR (44; 70). However, this is not always accurate since some SFG *Rickettsia,* such as *R. rickettsii*, are known to rarely result in eschar formation (12; 97). Thus, diagnoses may be incorrect because of the relatively non-specific clinical symptoms (12; 97). In military and civilian medical applications, patient recall plays a significant role in proper healthcare treatment. However, ticks are often not noticed by

patients, and they may erroneously deny any tick exposure, resulting in inopportune treatment administration (12).

Current tick and TBD control methods implemented by the military must be sustained in order to mitigate existing and future SFG *Rickettsia* threats. In addition to present measures, it would be beneficial for DoD mission success to investigate the feasibility of SFG *Rickettsia* vaccines. Though all still theoretical, once the rickettsial antigens that elicit strong CD4+ and CD8+ T cell responses are uncovered, it is possible that an efficacious vaccine could confer lifelong immunity (69). An additional need is the development of point-of-care tests for field use. Specific and rapid differential diagnostic tests would expediate appropriate care, especially in areas where multiple disease threats are present. While this would likely not eliminate the bio-threat, a quick, field-usable test would expedite accurate diagnoses and lead to positive outcomes since SFG *Rickettsia* are readily treatable.

Now, more than ever, SFG *Rickettsia* and other tick-borne pathogens have the potential to impact military operations. Given that surveillance shows an increasing incidence of SFG *Rickettsia*-positive human sera and increased numbers of SFG *Rickettsia*-infected ticks in nature, there is a clear need for additional attention to be directed to this agent (28; 63; 76; 86). Military bases, ergo service members, maintain a global presence and conduct operations of all varieties in tick habitats (38; 85). Should a service member fall ill, there is a potential for fatal outcomes, both in terms of human life, loss of military readiness, and mission failures (27; 32). As such, efforts must be made to develop proactive approaches to combat SFGR before these diseases begin to take a significant toll on America's preparedness.

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