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# *Rickettsia rickettsii* and Other Members of the Spotted Fever Group as Potential Bioweapons

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## 1. INTRODUCTION

Since the anthrax attacks in the fall of 2001, there has been an increase in the level of anxiety felt throughout society in general about the use of biological agents as weapons. Although much of the nation's and the world's attention has been focused upon anthrax, botulinum toxin, and Ebola as weapons,[1] there are other microbes that also pose a significant threat due to their potential as bioweapons. Characteristics that are shared by each of these organisms are that they are easily obtainable in nature, difficult to detect, and highly infectious at a low dose, can be easily transmitted by aerosol, and have a short incubation period. One frequently overlooked genus of bacteria that fulfills these criteria and also poses a significant threat is the *Rickettsia*. Members of this genus, such as *Rickettsia rickettsii*, a spotted fever group (SFG) rickettsia, have long been recognized as inherently dangerous with many reports of accidental infections and even deaths because of inhalational transmission to scientists who have worked with these organisms.[2–5] *Rickettsia prowazekii*, a member of the typhus group, not only occurs in periodic outbreaks,[6,7] but also has the infamous history of having been developed as a bioweapon by both the Japanese Army during World War II and the former Soviet Union.[8,9] Because of its history and reputation, many scientists suspect that *R. prowazekii* is the most probable

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*Rickettsia* to be utilized as a bioweapon. There is also a substantial possibility that *R. rickettsii* or another member of the SFG would be utilized and that we should also prepare for that scenario. *R. rickettsii* is the most pathogenic rickettsia, and other pathogenic members of the SFG are formidable in their own right and could have a potential devastating effect if loosed upon an unsuspecting society and an unprepared medical and public health system.

## 2. SFG RICKETTSIAE WITH BIOWEAPON POTENTIAL

One of the misconceptions accepted by the general public when considering the "bioweapon potential" is that the microbial agent will cause mass numbers of deaths. While that is a real concern, one should not overlook the "terror impact" of a potential agent. The effectiveness of a bioweapon can be also measured in its impact upon altering or inhibiting the daily routines of a community. For example, if clusters of patients with general symptoms of fever, headache, and myalgia begin to appear in clinics or hospitals a few days after the aerosol release from a small airplane, not only would emergency services be overwhelmed, but media coverage would also launch the populace into panic. If the disease is difficult to diagnose rapidly and it causes a range of clinical severity from life threatening to temporarily incapacitating, one could easily imagine the chaos that would ensue. The above scenario could easily be caused through the utilization of any of the pathogenic members of the SFG (Table I). The pathogenic members of the SFG are *R. rickettsii*, *R. conorii*, *R. australis*, *R. akari*, *R. africae/R. parkeri*, *R. japonica*, *R. honei*, and *R. sibirica*.[10] The members of this group have world-wide distribution with continuous reporting of new endemic areas.[11] The most devastating disease caused by a *Rickettsia* is Rocky Mountain spotted fever (RMSF). In the pre-antibiotic era, case fatality rates were 23%, with 80% fatalities in some regions and outbreaks.[10] Its case fatality rate is similar to that of bubonic plague and tularemia and greater than that of Lassa fever and Rift Valley fever, which are Category A agents. In nature it is transmitted through the bite of *Dermacentor varabilis*, *D. andersoni*, *Rhipicephalus sanguineus*, or *Amblyomma cajennense* ticks. *Rickettsia conorii* causes boutonneuse or Mediterranean spotted fever (MSF) and is transmitted by *Rhipicephalus sanguineus* ticks and has a case-fatality rate of 1–5%.[10] Rickettsial diseases caused by *R. australis* (Queensland tick typhus), *R. honei* (Flinders Island spotted fever), *R. akari* (rickettsialpox), *R. africae* (African tick bite fever), and *R. japonica* (Japanese spotted fever) are less severe than that of RMSF.[10] As stated previously, these less severe rickettsial diseases are still of importance because an outbreak would temporarily overwhelm the health care system and inhibit the normal daily activities of society.

**TABLE I**
**Pathogenic Members of the Spotted Fever Group of Rickettsiae**

| Organism | Vector(s) | Distribution |
|---|---|---|
| *Rickettsia rickettsii* | *Dermacentor andersoni, Dermacentor varabilis, Rhipicephalus sanguineus, Amblyomma cajennense* | The Americas |
| *R. africae/R. parkeri* | *Amblyomma* species | Africa, the Americas |
| *R. akari* | *Liponysossides sanguineus* | Northern Hemisphere temperate zones |
| *R. australis* | *Ixodes holocyclus* | Australia |
| *R. conorii* | *Rhipicephalus sanguineus* | Mediterranean, sub-Saharan Africa, India |
| *R. japonica* | Presumably *Haemaphysalis flava, Dermacentor taiwanensis* | Asia |
| *R. honei* | *Aponomma hydrosauri* | Australia, Asia |
| *R. sibirica* | *Dermacentor, Haemaphysalis,* and *Hyalomma* species | Asia, Eastern Russia |

## 3. FEASIBILITY OF OBTAINING, PROPAGATING, STABLIZING, AND WEAPONIZING SFG RICKETTSIAE

Scientists tend to believe that in order to create a weapon, the newest and most recent technology is required. Among legitimate scientists, select agent research is regulated by myriad rules and governing bodies. However, in order to protect society against future threats, we will need to understand the mindset of terrorists, evaluate all possibilities, and recognize what work could be accomplished in a basement, garage, or warehouse laboratory.

If one takes a critical analysis of the requirements as set forth by CDC and NIH for an organism to be considered as a potential bioterror weapon, it is easy to see how *R. rickettsii* could be converted into a weapon. First, let us examine the feasibility of obtaining the etiologic rickettsia. One method to obtain a SFG rickettsia would be to criminally obtain it from an existing source. However, most laboratories that conduct research on SFG rickettsiae are heavily regulated, and their stocks are secured and constantly monitored by authorities. Another potential source is economically desperate scientists engaged in bioweapons research in the former Soviet Union. Biopreparat was engaged in research on *R. prowazekii,* and joint efforts are underway to secure these agents.[12] Another scenario would involve collecting a large number of ticks from an endemic region and isolating rickettsiae in guinea pigs, cell culture, or embryonated eggs. All the potential terrorist would need for this is a tub to hold the animals or an incubator for eggs or flasks of cells. This level of laboratory safety is in stark contrast to what is done in laboratories in developed countries. The cumulative result of incidents involving laboratory exposure is that all present-day rickettsial research must be conducted in a biosafety level 3

laboratory that adheres to strict safety procedures to prevent aerosol-transmitted infections.[1] In order to combat self-infections by aerosolized rickettsiae, the terrorist could self-medicate with tetracycline.

The drawback to such an approach is that because highly virulent strains of RMSF kill their tick hosts, the overall tick infection rate is less than 0.1%, making collection of thousands of ticks likely to be necessary for isolation of *R. rickettsii.*[13–15] Niebylski and others reported that 94% of infected *D. andersoni* larvae died before molting into the nymphal stage of development.[15] Nymphs that acquired *R. rickettsii* during feeding fared slightly better with only 35% of the ticks dying before molting into the adult stage. Vertical transmission of rickettsiae from the mother tick was only observed in 39% of the offspring.[15] Although the tick lethality property may limit the incidence of RMSF in nature, *R. rickettsii* isolations from ticks are regularly achievable.

One aspect of potential RMSF and SFG rickettsial ecology that has been neglected is whether SFG rickettsiae have the ability to exist in a stable, extracellular, dormant form in arthropod feces. *R. prowazekii* has such a dormant form and has been observed in louse feces, and *R. typhi* in flea feces,[16] and it stands to reason that a similar ability could be shared by rickettsiae of the SFG. This hypothesis needs to be further investigated.

Once the starting material is obtained, the next step would be to propagate sufficient amounts of the organism to be utilized in an attack. The primary methods to multiply the rickettsial agent would be to use either embryonated eggs or cell culture. All that the potential bioterrorist would need to successfully propagate a rickettsial agent is a cell culture hood to prevent contamination of their cultures and an incubator. For growth in embryonated eggs, only an incubator would suffice.

Since all *Rickettsia* species are obligately intracellular organisms, the bioterrorist would need a method to stabilize the agent.[1] A stable form can be accomplished by lyophilization of the cultures. This material could then be milled to particles between 1–5 μm diameter. Particles of such a small size travel deep into the pulmonary alveoli and are retained in the lungs.[17] To complete the weaponization process, the agent could be treated chemically to prevent clumping by electrostatic forces.

## 4. METHODS OF DISPERSAL

The weaponized *R. rickettsii* could now be placed in a mechanism for aerosol dispersal, such as a crop duster sprayer, and dispersed over a location by plane or dispersed in a semi-enclosed space, such as a subway or in the air conditioning ducts of a building, where it could be spread by the environmental circulation of air.

Once in aerosol form, a person would only need to inhale a few organisms for the disease to occur.[3,4] Aerosol studies where target animals were exposed

**TABLE II**
**Comparison of the Infectivity of SFG Rickettsiae and Other Bacterial Select Agents**

| Bacteria | Infectious dose by aerosol | Incubation period | Transmission routes |
|---|---|---|---|
| *Bacillus anthracis* | 8,000–50,000 organisms | 2–45 days | Aerosol |
| *Burkholderia mallei* | Unknown (apparently low) | 1–14 days | Aerosol, direct contact with nasal secretion of infected equines |
| *Coxiella burnetii* | 10 organisms | 2–3 weeks | Aerosol, direct contact infected animals |
| *Francisella tularensis* | 5–10 organisms ($10^6$–$10^8$ by ingestion) | 1–14 days depending on route of transmission | Aerosol, handling of infected animals |
| *Yersinia pestis* | 100 organisms | 2–6 days | Aerosol |
| *Rickettsia rickettsii* and pathogenic members of the SFG | <10 organisms | 3–14 days | Aerosol |

to various doses of *R. rickettsii* show that as few as 0.8 rickettsia are required to cause disease in guinea pigs[18] and 1.5 yolk sac $LD_{50}$ can infect rhesus and cynomolgus monkeys.[19,20] Male guinea pigs were exposed via different routes (aerosol, nasal, conjunctival, gastric, and subcutaneous) to dilutions of *R. rickettsii* and monitored for clinical signs of infection. All animals that inhaled at least 80 organisms by aerosol became ill with a mortality rate of 75%, and 25% of animals that received a dose that was calculated as 0.8 rickettsia became ill.[18] Saslaw and Carlise observed in their nonhuman primate aerosol-model that 93% (56 of 60) of the animals developed clinical signs of RMSF and 75% of the monkeys that had clinical infection died within 7–24 days post-exposure.[19] The animals became febrile 5–7 days after exposure with the appearance of other symptoms such as rash, lethargy, and anorexia 1–2 days after onset of fever.[19] The quantity of rickettsiae needed for infection by aerosol is comparable to that of other potential biological weapons (Table II).

## 5. PATHOGENESIS OF AEROSOL TRANSMISSION

Numerous studies of accidental laboratory infections and experimental animal studies provide a detailed analysis of the pathology of RMSF transmitted by aerosol.[2–4,18–20] All of the studies found that RMSF infections acquired by aerosol could not be distinguished from naturally occurring infections. Laboratory workers who were infected by aerosol had fever, chills, myalgia, headache, and rash on their extremities.[2,3] In the study by Pike,[4] the number of

laboratory infections with *R. rickettsii* attributed to aerosols (217) was greater than that of parenteral (45 cases) or animal/ectoparasite exposure (66).

Aerosol-infected monkeys developed[19,20] typical lesions with perivascular infiltration of lymphocytes.

## 6. AVAILABLE METHODS FOR DIAGNOSIS, TREATMENT, AND PREVENTION

It is easy to envision difficulties in diagnosis arising from the utilization of SFG rickettsiae in a bioterrorist attack. Rickettsioses are notoriously difficult to diagnose and are under- and misdiagnosed even under normal clinical situations.[21–23] It is not difficult to imagine that during an attack out of the tick season with patients presenting nonspecific systemic symptoms, a rickettsial disease would very likely not be considered even by physicians convinced of a bioterror attack. Anthrax or one of the hemorrhagic fever viruses would be more likely to be included in the differential diagnosis.

Another problem in the diagnosis of rickettsial diseases is that there is currently no widely available test that is reliably diagnostic during the acute stage of the diseases. For the diagnosis of rickettsial diseases, most clinical laboratories use the immunofluorescence assay (IFA), which is considered the "gold standard" for the detection of antibodies to rickettsiae.[24] The advantage of the IFA is that the antigen contains all the rickettsial conformational proteins and group-shared lipopolysaccharide antigens. Current recommendations are that a diagnostic titer for RMSF is $\geq$64, or for a more confident laboratory confirmation a four-fold change between paired acute and convalescent serum specimens is required.[24,25] Other serologic tests that could be used for diagnosis are the indirect immunoperoxidase assay, Western immunoblotting, and enzyme immunoassays (EIA).[26–29] The problem with these serological assays is that antibodies to most SFG rickettsiae are not detected until the second week of illness, considerably after appropriate therapeutic decisions should have been made.[24] Currently, the best method for diagnosis of acute rickettsial infections is PCR amplification of selected rickettsial genes such as the 17-kDa lipoprotein gene, citrate synthase, 16S rRNA (*rrs*), *omp*A, and *omp*B.[24] Unfortunately, there are only a few laboratories in the United States that perform molecular diagnosis of rickettsioses on a regular basis. There is also a lack of commercially available kits for rickettsial molecular diagnostics.

In the case of rickettsial diseases, doxycycline or another tetracycline is the recommended treatment of choice, with chloramphenicol as the alternative drug. However, there are reports of rickettsiae being rendered resistant to antibiotics by experimental or naturally occurring means.[9,30] With the real possibility of an antibiotic-resistant rickettsia being used in a bioterrorist attack, it is imperative that the development and evaluation of new antirickettsial drugs be emphasized by the scientific community.

There have been previous attempts to develop effective vaccines against RMSF.[20,31,32] Although the vaccines developed had some success in experimental settings, all were discontinued because of the occurrence of laboratory-acquired infections in vaccinated individuals.[3,34]

## 7. NEEDED COUNTERMEASURES

It is our opinion that we are woefully underprepared for a bioterrorist attack using *R. rickettsii* or any other member of the spotted fever group. We lack a widely available diagnostic test for rickettsioses that is effective during the acute stage of illness. There has been little development of therapeutics for tetracycline- and/or chloramphenicol-resistant rickettsiae; and no new vaccine has replaced the old vaccine that was withdrawn from the market.

Although there is reason for concern, we should acknowledge the recent advances in the field of rickettsiology that would allow us to rapidly address the biothreat of SFG rickettsiae. First, there have been two significant advances utilizing proteomics and real-time PCR to improve point-of care (POC) diagnostics for acute-stage rickettsial diseases. La Scola and Raoult[34] developed an antigen capture assay for detection of *R. conorii* in circulating endothelial cells. In this method, infected endothelial cells are "captured" using magnetic beads coated with monoclonal antibodies against a human endothelial cell surface antigen and stained for the presence of intracellular rickettsiae using immunofluorescence. This method has a sensitivity of 50% and a specificity of 94%.[34] Labruna and others recently developed a real-time PCR assay for the quantification of *Rickettsia* species in ticks that can detect 1 copy of *R. rickettsii*.[35] This assay has been shown to detect both spotted fever and the typhus group rickettsiae. There are also ongoing efforts to develop proteomic assays to detect and enhance the signal from rickettsia-specific protein antigens and to define the "biosignature" of the human innate immune response to rickettsial infections. All of the aforementioned tests focus on the acute stage of the disease in order to allow clinicians to begin effective treatment in a more expedited fashion than normally occurs.

There has also been an increased effort to develop improved vaccines by focusing on subunit vaccines instead of the whole organism approach of yesterday.[36,37] Experimental vaccines were developed using epitopes of outer membrane protein A and outer membrane protein B to stimulate protective immunity against *R. conorii*. A multivalent vaccine combination of DNA encoding the protective epitopes and booster immunization with its corresponding recombinant proteins provided protection against a lethal challenge with *R. conorii*. These antigens could potentially be components of an improved human vaccine. There are also efforts underway to utilize microarray technology and the ever-growing number of sequenced *Rickettsia* species genomes not only to identify new vaccine candidates, but also to evaluate the

mechanisms of rickettsial pathogenesis and to identify targets for therapeutic intervention.

In the current geopolitical climate, there is an increased possibility of attacks upon society using biological agents. It would be foolhardy to prepare only for 2–3 agents due to their notoriety especially when there are reports that many organisms have been weaponized throughout history. *R. rickettsii* and the other members of the spotted fever group are some of the organisms that fall in the true threat category. They are easy to obtain, weaponize, and use. It will be only through intense and continued research efforts to develop improved diagnostics, treatments, and vaccines that society can brace itself against attack.

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