Pathogenic Rickettsiae as Bioterrorism Agents

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Because of their unique biological characteristics, such as environmental stability, small size, aerosol transmission, persistence in infected hosts, low infectious dose, and high associated morbidity and mortality, *Rickettsia prowazekii* and *Coxiella burnetii* have been weaponized. These biological attributes would make the pathogenic rickettsiae desirable bioterrorism agents. However, production of highly purified, virulent, weapon-quality rickettsiae is a daunting task that requires expertise and elaborate, state-of-the-art laboratory procedures to retain rickettsial survival and virulence. Another drawback to developing rickettsial pathogens as biological weapons is their lack of direct transmission from host to host and the availability of very effective therapeutic countermeasures against these obligate intracellular bacteria.

Epidemics of louseborne typhus, plague, influenza, and dysentery have been important in the shaping of human destiny and have caused more deaths than all the wars throughout history [1–4]. The ease with which these epidemics can spread within crowded human populations has made their causative agents attractive to nations, for use as battlefield weapons, or to terrorists, for use as weapons of mass destruction or civil disruption [1–4]. Several biological agents, including *Rickettsia prowazekii* and *Coxiella burnetii*—the causative agents of louseborne typhus and Q fever, respectively—have been weaponized and field-tested, with variable effectiveness, by the former Soviet Union, Japan, and the United States [2, 3]. Because the process of weaponization of biological agents is intended to maximize the spread and lethality of disease after human and/or animal exposure, the weaponized pathogens must be modified genetically and structurally to make them environmentally stable, invisible to rapid detection, resistant to available effective therapy, and capable of evading host immune responses. The impact of the use of such a biological weapon would be beyond imagination, because scores of innocent and unsuspecting people would be affected. In the present article, the pros and cons of weaponizing rickettsial pathogens and the potential of these pathogens in the hands of bioterrorists are discussed, taking into consideration the biological attributes of this group of gram-negative, obligate intracellular bacteria.

**THE CHALLENGE OF RICKETTSIAL PATHOGENS**

Rickettsioses are a good example of diseases whose importance is not adequately appreciated, except by patients. Even today, many cases of rickettsial diseases will never be diagnosed. Rickettsial diseases are widely distributed throughout the world as zoonotic cycles in foci of endemicity, with sporadic and often seasonal outbreaks developing [5, 6]. However, from time to time, these infections have reemerged in epidemic form in human populations (e.g., infection with *R. prowazekii* was responsible for >30 million cases of typhus during and immediately after World War I, causing ~3 million deaths) [7]. The recent outbreak of louseborne typhus in refugee camps in Burundi, which involved thousands of cases in humans (with the associated mortality rate exceeding 10%), is a reminder that rickettsial diseases can reemerge in epidemic forms as a result of the catastrophic breakdown of social conditions [1, 7, 8]. In contrast to the explosive nature of outbreaks of louseborne typhus, sporadic but limited outbreaks of louseborne typhus and other rickettsial diseases have been reported throughout the world. In the United States,
drastic increases in the number of cases of murine typhus in the 1940s, Rocky Mountain spotted fever (RMSF) in the late 1970s, and human ehrlichioses in the 1990s attest to the potential emergence of these infections in at-risk populations [1, 6].

The clinical presentation of rickettsial diseases can vary from mild to very severe, with the case-fatality rate for highly virulent rickettsiae ranging from 2% to >30%. However, a mortality rate as high as 66% was reported for disease due to *R. rickettsii* occurring prior to 1920, before the discovery of antibiotics. In recent years, increased awareness and proper treatment and care have had a great impact on reducing case-fatality rates associated with rickettsial diseases. The severity of rickettsial disease has been associated with pathogen virulence and host-related factors (e.g., age, delayed diagnosis, hepatic and renal dysfunction, CNS abnormalities, and pulmonary compromise) [1, 9]. Despite the variability in their clinical presentation, pathogenic rickettsiae cause debilitating disease, and any one of the highly virulent rickettsial species listed in table 1 could be used as a potential biological weapon. Classic epidemic typhus is the most severe rickettsial disease, with symptoms including a high fever (temperature, ∼42°C), extreme pain in the muscles and joints, stiffness, and the appearance of cerebral impairment ∼10 days after infection. During later stages of infection, patients appear to be delirious and exhibit neurologic symptoms, including stupor. Thrombosis of the small vessels in the extremities may produce gangrene and necrosis. The mortality rate among untreated patients is ∼20%. In severe epidemics, the mortality rate can often be as high as 40%.

The human body louse, *Pediculus humanus corporis*, is the principal vector for *R. prowazekii*. Although the head louse *P. humanus capitis* is capable of maintaining *R. prowazekii* experimentally, its role in the transmission of this rickettsiosis is not well established. Body lice are intermittent feeders with a preference for a mean temperature (±SD) of 20°C ± 2°C, which limits their resting sites to areas away from direct contact with the patient’s skin, such as the folds of clothing. The body louse will abandon a patient with a fever and seek another host. This one attribute is a major factor in the transmission of typhus and is responsible for the flaring of an epidemic in a susceptible population. However, there is another face of typhus, a recrudescent typhus (i.e., Brill-Zinsser disease) that has not been studied in any significant depth. The symptoms of recrudescent typhus are less pronounced, and the associated mortality rate is <1% [1, 7]. Patients with this disease could serve as a long-term source of *R. prowazekii*, permitting transmission of rickettsiae via lice to occur months to years after the primary infection. Recrudescent typhus may have been the mechanism for the dissemination of *R. prowazekii* in many parts of the world.

**BIOLOGICAL ATTRIBUTES OF RICKETTSIAL PATHOGENS**

Because of their unique biological characteristics (environmental stability, small size, aerosol transmission, persistence in infected hosts, low infectious dose, high morbidity, and substantial mortality), *R. prowazekii* and *C. burnetii* have been weaponized, and they very likely could serve as agents for bioterrorism. According to the World Health Organization’s estimate, if 50 kg of aerosolized *R. prowazekii* were released in a bioterrorist attack, the result would be >100,000 casualties (19,000 deaths and 85,000 incapacitated individuals) [10, 11]. Table 2 summarizes the characteristics of pathogenic rickettsiae that have the potential to be used as bioweapons, compared with the characteristics of a number of agents designated by the Centers for Disease Control and Prevention as “select” agents. Although the attributes of rickettsial pathogens that increase their potential for use as a biological weapon parallel those of other highly publicized, weaponized pathogens, realistically several compounding factors would make it very difficult to produce large quantities of highly purified weapon-quality rickettsiae. As obligate intracellular bacteria, rickettsiae require eukaryotic host cells for propagation, and the isolation and purification of rickettsiae without host cell contaminants would require highly skilled personnel and elaborate laboratory procedures. The virulent strains of both *C. burnetii* and *R. prowazekii* are highly infectious, and aerosolized organisms are often the source of the reported laboratory-acquired infections. Considering that the median infective dose of *R. prowazekii* is

**Table 1. Epidemiologic characteristics of selected highly pathogenic rickettsiae.**

<table>
<thead>
<tr>
<th>Rickettsia species</th>
<th>Disease</th>
<th>Vector(s)</th>
<th>Host animals</th>
<th>Geographic distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. prowazekii</em></td>
<td>Epidemic typhus</td>
<td>Human body lice</td>
<td>Humans</td>
<td>Worldwide</td>
</tr>
<tr>
<td></td>
<td>Recrudescent typhus</td>
<td>None</td>
<td>Humans</td>
<td>Worldwide</td>
</tr>
<tr>
<td><em>R. typhi</em></td>
<td>Murine typhus</td>
<td>Fleas</td>
<td>Rodents, opossums</td>
<td>Worldwide</td>
</tr>
<tr>
<td><em>R. rickettsii</em></td>
<td>RMSF</td>
<td>Ticks</td>
<td>Small mammals, dogs, rabbits, birds</td>
<td>North and South America</td>
</tr>
<tr>
<td><em>R. conorii</em></td>
<td>Boutonneuse fever</td>
<td>Ticks</td>
<td>Rodents, dogs</td>
<td>Africa, Southern Europe, India</td>
</tr>
<tr>
<td><em>R. sibirica</em></td>
<td>North Asia tick typhus</td>
<td>Ticks</td>
<td>Rodents</td>
<td>Eurasia, Asia</td>
</tr>
</tbody>
</table>

**NOTE.** RMSF, Rocky Mountain spotted fever.
<10 rickettsial particles, and considering the stability of R. prowazekii at room temperature, biological safety level 3 conditions are required when one works with these organisms and their propagation, purification, and molecular or biochemical manipulation. Furthermore, mass production of rickettsial pathogens and the procedures required for their aerosolization are highly hazardous. Another drawback to developing rickettsial pathogens as a biological weapon is their lack of direct transmission from host to host. Rickettsiae are maintained in nature in their arthropod vectors, via transovarial and transstadial transmission, and human infection occurs accidentally through either a bite from the infected arthropod or contact with Rickettsia-laden feces. Thus, the use of these agents for mass transmission requires a massive number of either infected arthropod vectors or kilograms of aerosolized rickettsiae. Because several rickettsial pathogens are on the Centers for Disease Control (intentional vs. natural transmission). Because the disease is still endemic in highlands and cold areas of Africa, Asia, and Central and South America, as well as in parts of Eastern Europe, determining the source of infection would be difficult. In the United States, where sylvatic typhus is endemic in the eastern states, it would be difficult to determine the source of infection immediately after the onset of disease. Humans serve as a host to R. prowazekii and human body lice and are reservoirs of the rickettsiae. In addition, humans serve as a mobile component of the louseborne typhus cycle, the behavior of which influences the pattern of typhus transmission. The conditions that allow for the coexistence of body lice and a susceptible population could be the starting point for a bioterrorism-initiated infection, because the release of the rickettsiae would set off a subsequent chain reaction. A similar situation could also exist for other rickettsial pathogens that are endemic in an affected area. Knowledge of the transmission cycle would play an important role in identifying the initial source of an epidemic. The sudden occurrence of a few human cases in areas of nonendemicity could set off the alarm.

Fortunately, immunity to pathogenic rickettsiae develops after recovery from infection and/or receipt of antibiotic treatment. Because it is difficult to diagnose rickettsial infection early after infection occurs, administration of antibiotic therapy involving tetracyclines (doxycycline, 200 mg/day for 5–10 days) before a definitive diagnosis is made would be the treatment of choice [12]. Preventive measures are complicated because of the lack of effective and safe rickettsial vaccines [11]. Surveillance of naturally or intentionally released pathogens is a first step in preventing the epidemics via rapid identification of causative agents and assessing the degree of their virulence. Initiating the proper treatment and curtailing outbreaks is the most crucial step, because prompt initiation of antibiotic treatment would prevent people from becoming ill and from dying.

Prophylactic measures are badly needed, because population...
growth and increased land use bring arthropods and their associated pathogens into human habitations. The availability of several rickettsial genomes and the development and usefulness of new molecular tools now allow more advanced drug design, as well as the generation of live attenuated and/or gene knockout rickettsiae as new-generation vaccines. In addition, analysis of rickettsial genomics and proteomics will enhance our clinical and field diagnostic capabilities, thus reducing misdiagnosis and false-negative results in routine surveillance studies.

Acknowledgments

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References