Chapter 8

GLANDERS

INTRODUCTION

MILITARY RELEVANCE

HISTORY

INFECTIOUS AGENT

DISEASE
  Epidemiology
  Transmission
  Pathogenesis
  Clinical Disease in Animals
  Clinical Disease in Humans
  Laboratory Diagnosis
  Treatment
  Prophylaxis

SUMMARY
INTRODUCTION

Glanders is a debilitating and often fatal zoonotic disease of solipeds including horses, mules, and donkeys caused by infection with the bacterium *Burkholderia mallei*. It is characterized by ulcerating granulomatous lesions of the skin and mucous membranes. Disease progression and pathology in humans and horses are similar, yet the clinical presentation of any two cases in the same species—even if related by direct transmission—may vary significantly.1–5

Generalized symptoms include fever, myalgia, headache, fatigue, diarrhea, and weight loss. After infection, the organism travels through lymph channels first to regional lymph nodes often causing irritation (lymphangitis, lymphadenitis) en route. Unchecked, organisms may enter the bloodstream and be carried throughout the body. Without effective treatment, the course of disease may range from one that is acute and rapidly fatal to one that is very slow and protracted with alternating remissions and exacerbations.

Glanders is an old disease, having been described toward the beginning of recorded history. It is less commonly known by other names, including equinia, malleus, droes, and farcy. Farcy is an ancient term given to a particular cutaneous manifestation of glanders that at the time (before 1882) was believed to be a completely separate disease in horses. With this cutaneous manifestation of glanders, nodular abscesses (farcy buds) became ulcerated, and regional cutaneous lymphatic vessels became thickened and indurated (farcy pipes) and oozed a glanders-typical yellow-green gelatinous pus (farcy oil).6 Pure farcy without ulceration of the mucous membranes is rare, if not just a temporary stage, as is vice versa.7 Humans, goats, dogs, cats, rabbits, and carnivorous predators living in close proximity to infected equids or carcasses have been naturally infected.7–9 Camels have also been infected and are associated with human disease.7 Naturally occurring glanders has been eradicated in most countries, but is still found in parts of Africa, the Middle East, Eastern Europe, Asia, and South America. Glanders has drawn interest as a possible warfare agent in the biological weapons programs of several countries.

MILITARY RELEVANCE

*B mallei* was one of the first biological warfare agents used in the 20th century. Germany used an ambitious biological sabotage campaign in several countries, including the United States, Russia, Romania, France, and Mesopotamia, on both the western and eastern fronts during World War I. Additionally, cattle, horses, mules, and other livestock being shipped from the United States to the Allies were bebeuged and inoculated with cultures of *B mallei*.8 In 1914, a member of the German army named Anton Dilger, an American-educated surgeon, was sent home to live with his parents in Virginia after a nervous breakdown. He brought strains of *Bacillus anthracis* and *B mallei* and set up a laboratory with his brother’s help to grow the organisms in a private home in Chevy Chase, Maryland. Organisms were delivered to another contact from Germany waiting in Baltimore, who then inoculated horses awaiting shipment to the Allies in Europe.

German agents also infected 4,500 mules in Mesopotamia with glanders, a German agent was arrested in Russia with similar intentions in 1916, and French cavalry horses were also targets for intentional glanders infection.8 Germany and its allies infected many mules and horses on Russia’s eastern front, and this action successfully impaired artillery movement and troop and supply convoys. Concurrent with this rise in animal cases during and after the war, human cases increased in Russia. Attempts to contaminate animal feeds also occurred in the United States. Between 1932 and 1945 the Japanese used *B mallei* to deliberately infect horses, civilians, and prisoners of war at the Ping Fan Institute, also known as Unit 731, in occupied Manchuria. Two laboratory workers accidentally exposed to *B mallei* died at the institute in 1937.10

In response to perceived biological warfare threats from Japan and Germany, the United States began work on biological warfare agents at Camp Detrick, Maryland (now Fort Detrick) in 1942. *B mallei* was studied for potential use but was not weaponized. Between November 1944 and September 1953, seven laboratory-acquired human infections from *Malleomyces mallei* (the taxonomic name of glanders at that time) occurred in Camp Detrick employees. Howe and Miller reported the first six of these infections in a case series, which remains the largest reported human case series in US medical literature.5 Information on the seventh case was not published before 2005. All seven original case files were thoroughly reviewed for this chapter. An eighth laboratory-acquired infection occurred in March 2000 during US defensive research on *B mallei*.11 Also, the Soviets were alleged to have used weaponized *B mallei* against opposition forces in Afghanistan between 1982 and 1984.12
The United States signed the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological and Toxin Weapons, which banned development, production, stockpiling, acquisition, and retention of biological agents, toxins, and the weapons to deliver them in 1972. All offensive biological warfare work at Fort Detrick had ceased by this time; any remaining biological weapons were destroyed by 1973. Biodefense related research aimed toward the development of countermeasures to combat *B mallei* infections, however, continues to be conducted in the United States. A report by the Monterey Institute of International Studies states that between 1931 and 1945 Japan developed *B mallei* as a biowarfare agent. There are no known current attempts for acquisition and use by terrorists.13

*B mallei* was considered a potential biothreat agent in 1947 because of its high infectivity, high degree of incapacitation among those infected, and agent availability.14 It could be a more significant threat if weaponized. As exemplified by past clusters of laboratory-acquired infections, *B mallei* is particularly infectious by the respiratory route. It is not considered to be highly contagious among humans, and reports of person-to-person transmission are rare. If a determined bioterrorist gained access to the agent, whether from an infected animal, laboratory culture, or commercial culture, the consequences could be severe. Because the clinical symptoms of glanders are protean and nonspecific, and most physicians in the west are not familiar with the disease, diagnosis and treatment may be delayed postattack, even in regions with the most advanced medical facilities. Delayed diagnosis and treatment would likely result in significant morbidity and mortality. In addition, treatment may be complicated by the relative scarcity of knowledge and experience in therapy. As equids and some other animals are susceptible, further spread from animals to humans may continue long after an initial attack. Fortunately, glanders is curable and postexposure prophylaxis may be an option if an attack was rapidly confirmed. As with other agents, genetic engineering could be used to produce a strain with unpredictable virulence and atypical antibiotic resistance. Thus, if *B mallei* was cultured, concentrated, and delivered as an infectious aerosol, significant casualties could result.15

HISTORY

Glanders is one of the oldest documented infectious diseases with symptoms being recorded by Hippocrates as early as 425 BCE. Aristotle described the disease in horses in 330 BCE and named it “malleus,” meaning hammer or mallet. It was associated with clustered horses around the globe, particularly army horses and mules. The occurrence of glanders in domesticated equids was so familiar that horses and their glanders commonly appeared together in early literature. By about the 4th century, Aspyrtus and Vegetius recognized the contagious nature of the disease and recommended isolation of affected animals. Glanders was not studied in a systematic matter until centuries later. The first veterinary school was established in Lyon, France, in the mid-1700s to deal with the serious problems of rinderpest and glanders. Many researchers at the school became infected and died of glanders during their studies. The first account of humans glanders was not published until 1821.1 In 1837, Rayer proved the transmissibility of the disease by successfully infecting a horse using material taken from a pustule of a human glanders patient.17,18

In 1882, Loeffler and Schutz isolated the causative agent, now called *Burkholderia mallei*, in pure culture from the liver and spleen of a glanders horse.1,2

Up until the industrial revolution, horses and mules were the primary modes of transportation in all developing economies. Particularly in urban locations, these animals were housed under crowded conditions, and glanders was passed from the infected to the uninfected. Horses and mules were in high demand during the American Civil War. Thousands of animals passed through remount stations where glanders was found in epidemic proportions. The problem was exacerbated after the American Civil War when infected military stock was sold to civilians, which facilitated spread of the disease to communities. Heavy losses of horses and the infrequent but deadly transmission to humans in the late 19th century led several countries to consider glanders control and eradication programs. Early programs in some countries involved destroying only clinically ill equids, with compensation, and meticulous disinfection of the premises of such cases. Despite these tactics, glanders would reemerge in new or remaining animals in stables and barns that once housed infected animals and the number of countrywide cases increased. The notion of a carrier-state began to be accepted. In spite of epidemic disease in equine populations, there were no simultaneous epidemics in humans.

Vaccines and therapeutic agents were developed but they did not reduce the glanders burden. By 1890, the mallein diagnostic skin test was developed. Control and eradication programs would soon incorporate the testing of all contact equids, followed by quarantine and a recommendation for slaughter of all skin test-positive animals. This program failed in some locales at first because of lack of enforcement and lack
of incentive to owners for killing their nonclinically ill animals. Some horse owners would deliberately hide contact animals to avoid testing, or they would sell these and asymptomatic test-positive animals to unsuspecting individuals to salvage economic loss. Inexpensive steam transportation helped the disease spread by shipping B mallei-infected animals to other regions and countries. The United States was blamed for the import of glandereous horses to Cuba in 1872 and for the great increase of glanders cases in Canada near the turn of the 20th century, where tens of thousands of US horses were shipped annually.

Once control programs offered indemnity to test-positive and contact animals, and popular belief accepted the existence of a carrier-state, glanders eradication progressed more rapidly. Eliminating glanders in livestock effectively also eradicated the disease in humans in countries with such programs. Great Britain’s experience with the rise and fall of glanders outbreaks in equids typifies many countries, and is shown in Figure 8-1. Eradication of glanders was achieved in Great Britain by 1928 and in Canada by 1938, about 30 years after eradication programs were initiated. Glanders was successfully eradicated in the United States by 1942; the last naturally occurring human case was recorded in 1934.

Glanders is a zoonotic disease of concern internationally and is notifiable to the 164-member Office International des Epizooties in accordance with the International Animal Health Code. Eradication programs still exist for several countries attempting to eliminate the disease. In more than 500,000 equids tested in Turkey between 2000 and 2001, for example, less than 2% tested positive and were destroyed. Only one of these, a mule, showed clinical signs of infection. Over the past two decades, glanders in livestock was reported in Afghanistan, Bahrain, Belarus, Bolivia, Brazil, China, Eritrea, Ethiopia, India, Iran, Iraq, Kuwait, Latvia, Lebanon, Mongolia, Myanmar, Pakistan, Russia, Turkey, and the United Arab Emirates.

Between 1996 and 2003 glanders in humans was reported in Cameroon, Curacao, Sri Lanka, Turkey, and the United States (laboratory acquired). Exhibit 8-1 depicts the year equine glanders was last reported to

---

Figure 8-1. Glanders cases and outbreaks reported to the Department for Environment, Food, and Rural Affairs in Great Britain, 1877–1928. Glanders was eradicated in Great Britain in 1928.


180
the Office International des Epizooties among coun-
tries and territories that have been without glanders
activity (by Office International des Epizooties report)
since 1996. Given the recent outbreaks in horses,
donkeys, and dromedaries in some regions of India,
Bahrain, Brazil, Lebanon, Pakistan, and the United
Arab Emirates, glanders is currently considered a re-
emerging infectious disease in these areas. 24–26,30–32,34–37
Bioterrorism should be considered as a possible source
in the event that confirmed human glanders occurs, in
the absence of infected animals, in the countries and
territories listed in Exhibit 8-1.

EXHIBIT 8-1
YEAR EQUINE GLANDERS LAST REPORTED TO OIE BEFORE 1996*

<table>
<thead>
<tr>
<th>Country or Territory</th>
<th>Year</th>
<th>Country or Territory</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>1891</td>
<td>Moldavia</td>
<td>1957</td>
</tr>
<tr>
<td>Austria</td>
<td>1952</td>
<td>Nambia</td>
<td>1925</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>1954</td>
<td>Netherlands</td>
<td>1957</td>
</tr>
<tr>
<td>Canada</td>
<td>1938</td>
<td>Northern Ireland</td>
<td>1910</td>
</tr>
<tr>
<td>Croatia</td>
<td>1959</td>
<td>Norway</td>
<td>1889</td>
</tr>
<tr>
<td>Denmark</td>
<td>1928</td>
<td>Poland</td>
<td>1957</td>
</tr>
<tr>
<td>Egypt</td>
<td>1928</td>
<td>Portugal</td>
<td>1952</td>
</tr>
<tr>
<td>Estonia</td>
<td>1945</td>
<td>Romania</td>
<td>1960</td>
</tr>
<tr>
<td>Finland</td>
<td>1943</td>
<td>Serbia and Montenegro</td>
<td>1959</td>
</tr>
<tr>
<td>France</td>
<td>1965</td>
<td>Slovakia</td>
<td>1954</td>
</tr>
<tr>
<td>Georgia</td>
<td>1960</td>
<td>South Africa</td>
<td>1945</td>
</tr>
<tr>
<td>Germany</td>
<td>1955</td>
<td>Spain</td>
<td>1956</td>
</tr>
<tr>
<td>Great Britain</td>
<td>1928</td>
<td>Sudan</td>
<td>1989</td>
</tr>
<tr>
<td>Greece</td>
<td>1965</td>
<td>Sweden</td>
<td>1943</td>
</tr>
<tr>
<td>Hungary</td>
<td>1956</td>
<td>Switzerland</td>
<td>1937</td>
</tr>
<tr>
<td>Ireland</td>
<td>1920</td>
<td>United States of America</td>
<td>1942</td>
</tr>
<tr>
<td>Israel</td>
<td>1951</td>
<td>Yug Rep of Macedonia (former)</td>
<td>1957</td>
</tr>
<tr>
<td>Japan</td>
<td>1935</td>
<td>Zimbabwe</td>
<td>1911</td>
</tr>
</tbody>
</table>

*The most recent year evidence of equine glanders was reported to the OIE among countries and territories free of equine glanders for at least 5 years (between 1996 and 2013). Included only are territories for which data exist on the reporting of equine glanders to the OIE. OIE: Office International des Epizooties

**INFECTIOUS AGENT**

Glanders is caused by *B. mallei*, a gram-negative bacillus that is a close relative to *Burkholderia pseudomallei*, the etiologic agent of melioidosis. Whole-genome comparisons of *B. pseudomallei* and *B. mallei* in combination with multilocus sequence typing (MLST) analyses suggest that *B. mallei* is a clonal descendant of *B. pseudomallei* that has evolved through genome downsizing. 38 Unlike *B. pseudomallei*, which can be isolated from tropical soil, *B. mallei* is an obligate animal pathogen and has not been found free-living in the environment. 39 The lack of flagellar-based motility is a primary means by which *B. mallei* can be differentiated from *B. pseudomallei*. Growth requirements are not complex and *B. mallei* can be cultivated on basic nutrient medium. However, glycerol or glucose can be added to the medium to enhance growth. When stained, the cells typically exhibit bipolar staining.

*B. mallei* is well traveled taxonomically. Since its discovery, this microorganism has been placed in several genera, including *Bacillus*, *Corynebacterium*, *Mycobacterium*, *Loefflerella*, *Pfeifferella*, *Mallokomyces*, *Actinobacillus*, and *Pseudomonas*, and was finally assigned to the genus *Burkholderia* in 1992. 40,41 This microorganism is not particularly hardy in the environment. 40 *B. mallei* is susceptible to drying, heat, and sunlight. In warm and moist environments, the organism may survive a few months and can survive in room temperature water as long as 1 month. 1,19,42 Experimentally and under the most favorable temperature and moisture conditions, *Loeffler* was able to extend the viability of *B. mallei* to 100 days. Survival of *B. mallei* in distilled water in the laboratory was determined to be less than 30 days. 43 In nature, viability of the organism is unlikely after 90 days, and most infectivity is lost within 3 weeks.
Particularly in culture, *B. mallei* is easily aerosolized as demonstrated by at least seven of the eight laboratory-acquired infections in the United States since 1944. Given its high infectivity by aerosol, laboratory studies on this Centers for Disease Control and Prevention Tier 1 select agent are performed at biosafety level 3 (BSL-3) facilities. Varying degrees of virulence among strains have been shown in the laboratory and in nature.4,5,7 The infectious dose is considered to be low, depending on the route of infection, susceptibility, and strain virulence. One to 10 organisms of some strains by aerosol are lethal to hamsters.3 Inhaling only a very few organisms may cause disease in humans, equids, and other susceptible species.4,4,46

**DISEASE**

**Epidemiology**

Naturally acquired cases of glanders in humans or equines are sporadic and rare; most countries have eradicated glanders. Glanders is still infrequently reported in northern Africa, the Middle East, South America, Asia, and eastern Europe.21 Serologic cross-reactivity with *B. pseudomallei* precludes the accurate distribution and prevalence of *B. mallei* by serologic means alone. However, new reagents and assays potentially leading to improved serodiagnosis of human glanders have been described, as detailed below. Although human outbreaks have been reported in Austria and Turkey, no human epidemic has been recorded.47

In nature, chronically infected horses are considered to be the reservoir of *B. mallei*, and they may also serve as amplifying hosts. A disease of primarily solipeds, donkeys are considered most prone to develop acute forms of glanders, whereas horses are more prone to develop chronic and latent disease. Mules, a crossbred animal resulting from the mating of horse and donkey, are susceptible to both acute and chronic disease as well as latent infections.40,48,49 A recent report indicates that Old World camels (dromedaries) can acquire glanders naturally when kept in close proximity to infected horses.35 Clinical disease in dromedaries closely resembled that seen in equids. Humans are an accidental host.

Zoonotic transmission of *B. mallei* from equid to human is uncommon, even with close and frequent contact with infected animals, which may be explained by low concentrations of organisms from infection sites and a species-specific difference in susceptibility to virulent strains. During World War II human glanders was rare despite a 30% prevalence in horses in China.50 Between 5% and 25% of tested animals in Mongolia were reactive, yet no human cases were reported. With successful transmission, however, humans are susceptible to infection.

Humans exposed to infected equids have contracted glanders in occupational, hobby, and lifestyle settings. Naturally infected humans have included veterinarians and veterinary students, farriers, flayers (hide workers), transport workers, soldiers, slaughterhouse personnel, farmers, horse-fanciers and caretakers, and stable hands. Subclinical or inapparent infections in horses and mules have posed a hidden risk to humans. Infection by ingesting contaminated food and water has occurred; however, it does not appear to be a significant route of entry for infections in humans.1,7,51 Laboratory workers have also been rarely and sporadically infected. In contrast to zoonotic transmission, culture aerosols are highly infectious to laboratory workers. The six infected workers in the Howe and Miller case series represented 46% of the personnel actually working in the laboratories during the year of occurrence.5

Different strains of *B. mallei* can now be discriminated by multiple-locus variable number tandem repeat analysis (MLVA), MLST, random amplified polymorphic DNA, and other fingerprinting methods. Such procedures have been shown to be useful in tracking the source and spread of an outbreak strain and in geographic/clonal relationship studies, and they have been described and summarized elsewhere.24,52 For example, Godoy et al used MLST with a set of seven loci in epidemiologic studies analyzing many isolates of *B. mallei*, *B. pseudomallei*, and *Burkholderia thailandensis* (a closely related but nonpathogenic environmental species) from diverse geographical locations that represented 71 sequence types; specific clones isolated from animals that were associated with disease in humans were identified.35,53 MLST was most useful for distinguishing strains of *B. pseudomallei* and *B. thailandensis*, which were clearly distinguished by the divergence between the alleles of seven loci. However, all the geographically diverse isolates of *B. mallei* analyzed had identical allelic profiles that clustered within the *B. pseudomallei* group of isolates; alleles at six of the loci in *B. mallei* were also present in *B. pseudomallei* isolates, and the allele at the seventh locus in *B. mallei* differed at only a single nucleotide site from *B. pseudomallei*. *B. mallei* was considered to cluster within and to be a clone of *B. pseudomallei* instead of a separate species. However, one recent analysis of camel-associated glanders used one *B. mallei*-specific sequence type to confirm the laboratory identification of glanders.35
MLVA has been found to be more useful for subtyping different strains of B mallei. For example, an MLVA analysis of a B mallei strain isolated from a diseased camel in Bahrain revealed close genetic proximity to a specific strain, which caused an earlier outbreak of glanders in horses in the United Arab Emirates in 2004.35 The MLVA was based on 23 different loci, as reported previously.28 Similar analyses focused on B mallei isolates from the Punjab region of Pakistan demonstrated that these strains were genetically distinct from isolates from other countries.28 In the event of a deliberate release of B mallei or a focal outbreak of glanders in humans or animals, these types of analyses would be critical tools for facilitating investigations aimed at determining the source of the organism.

Transmission

Transmission is direct by bacterial invasion of the nasal, oral, and conjunctival mucous membranes; by inhalation into the lungs; and by invasion of abraded or lacerated skin. Areas of the body most often exposed are the inner nose, mouth, conjunctiva, and skin is believed to be unlikely, patients may insist their skin was intact at the time of exposure. Among 105 chronic human cases associated with equid exposure described by Robins, only 40 (38%) reported a wound present.3 In 27 cases (17%) the absence of a wound was specifically noted.3

Laboratory infections have followed procedures that involved washing and aeration of cultures. Air samples and swabs from equipment, tables, and benches failed to detect residual contamination in laboratories after the six US laboratory-acquired events that occurred between 1944 and 1945. Seven of the eight Fort Detrick laboratory-acquired infections also occurred at a time when mouth-pipetting was common practice. The first six patients acknowledged using this technique to clear blocked pipettes and to blow contents out of pipettes that were calibrated to the tip. The eighth case patient involved a microbiologist who had 2 years of experience of working with B mallei in BSL-3 containment but did not always wear latex gloves.11,56 Based on the clinical manifestation of unilateral axillary lymphadenopathy, transmission in this case was believed to be percutaneous, yet a break in the skin or a specific exposure-associated laboratory incident was not recalled. This is not surprising as most laboratory-acquired infections are not associated with injury, or a recollection of injury.27 This patient had a 13-year history of diabetes, however, and collected blood via finger-stick morning and evening. It is possible that a recent finger-stick site may have been a potential entry point. Bacterial surveys of the laboratory found no contamination, and all engineering controls were validated as functional.

Human-to-human transmission is rare, but it has been reported. The majority of documented events were in medical practice, at autopsy, in the diagnostic laboratory, and in patient care settings before clearer understanding of universal precautions existed.1,3,11 Transmission also occurred in home settings. Close contact while caring for glanders-infected individuals at home led to infecting other family members.3 At least one entire family became infected. In this case, two children and the wife of a chronically infected stable hand contracted glanders. The wife was presumably infected sexually; the 4-year-old child was likely infected by close contact with a 2-year-old sibling who was presumably infected by one of the parents. Robins found that among the 156 chronic infections he studied, 10% were directly caused by another human case.3

Human infection by ingestion has not been definitively reported. Stomach contents were found to inactivate B mallei experimentally in 30 minutes.57 In his detailed 1886 report on the etiology of glanders, Loeffler describes several accounts of feeding meat...
from glanderalous horses to humans without causing disease.\textsuperscript{1} In one account, more than 100 glanderalous horses were slaughtered and fed to soldiers without incident. Although not clear in his report, it is most likely that in these cases the meat was cooked just as was customary for a military mess at that time. In another case, consumption of raw glanderalous meat by a veterinarian seeking to answer the ingestion question did not produce disease. An 1866 veterinary journal report, however, describes two persons who contracted glanders after consuming milk from a glanderalous mare. Because these individuals were also exposed to the mare, infection by ingestion could not be determined.\textsuperscript{1}

Monogastric animals, including the lion, tiger, domestic cat, dog, and bear, became infected with \textit{B mallei} after ingesting raw meat.\textsuperscript{1} Regarding wild animals, Loeffler posited that crunching bones might cause enough oral trauma to introduce the organism through defects in the oral mucosa rather than by entry through the healthy digestive tract.\textsuperscript{1} This explanation, however, does not explain infections in dogs, domestic cats, and captive wildlife that were fed only boneless meat from glanderalous horses. In 2010, four lions and one tiger at a zoo in Tehran died from glanders.\textsuperscript{28} Although not definitively proven, this outbreak was attributed to ingestion of soliped meat that had not been screened for \textit{B mallei}. Based on this limited collection of testimonies and the current understanding of glanders pathogenesis, one may infer that ingestion of the live organism by humans is unlikely to cause disease.

These features of transmission exemplify the requirement for BSL-3 containment and safety practices when working with \textit{B mallei}. Adherence to safety procedures and universal barrier precautions is also prudent. In the presence of potentially infected equids, transmission risk is also reduced by universal precautions as well as procedures that reduce inhalation risk of potentially contaminated aerosols. Advances in medicine, infection control, and therapeutics make it less likely today than 100 years ago for human-to-human transmission to occur even in the event of a human outbreak, whether related to bioterrorism or not. It is also highly unlikely that an equid reservoir would become established. Acute disease is expected to manifest in a significant proportion of exposed equids, which would allow emergency response, quarantine, traceback, and eradication procedures. Long-term exposure to asymptomatic chronically infected equids that evade detection and are handled without precautions could become a sporadic but perilous risk to humans, and less caution may be used around them.\textsuperscript{3}

Among equids, transmission is primarily by oro-nasal mucous membrane exposure, inhalation, and mastication (possibly ingestion) of skin exudates and respiratory secretions of infected animals, including those with latent and subclinical infection. The sharing of feed and water troughs facilitates this, as do common equid behaviors that include grooming and snorting.\textsuperscript{40,48,49} Since equids cannot breathe through their mouths, simple exhalation and snorting to clear nasal passages serve to finely aerosolize infectious nasal efflux from an infected equid, which poses a transmission risk to susceptible hosts (including humans) in the vicinity.

Transmission through ocular mucous membranes and abrasions in the skin is also possible. Vertical transmission from mare to foal has occurred naturally in horses. In utero transmission from sow guinea pig to pup has also occurred in housed laboratory animals.\textsuperscript{1} Sexual transmission from stallion or jack to mare or jenny has also been observed. The breeding of asymptomatic stallions was responsible for some glanders spread near the turn of the 20th century.\textsuperscript{4}

Carnivores can become infected after eating contaminated carcasses and meat.\textsuperscript{29,58} Reported outbreaks in captive wild felids suggest that they appear to be more susceptible than canids.\textsuperscript{40,48,58,59} Glanders has also been transmitted to goats housed with infected horses.\textsuperscript{1} Laboratory animals are also susceptible, including mice, hamsters, guinea pigs, rabbits, and monkeys.\textsuperscript{1} Cattle, swine, and chickens appear to be resistant, even after experimental infection.\textsuperscript{2,59,60} Pigeons were infected experimentally.\textsuperscript{1} A review of experiments on glanders in animals led Loeffler to suggest that the field mouse, donkey, mule, horse, goat, cat, and guinea pig were more susceptible to glanders infection and clinical disease than humans.\textsuperscript{1} Among other susceptible host species, the rabbit and dog appeared to be less susceptible to disease than humans. Recent reports have described the use of invertebrate species including wax moth larvae, cockroaches, and nematodes as viable experimental models of glanders that are primarily useful for identifying virulence factors.\textsuperscript{61-63}

**Pathogenesis**

**Overview of Pathogenesis**

Although glanders has been largely eradicated in humans, and for the most part in animal populations, \textit{B mallei} is considered a significant potential biothreat agent.\textsuperscript{64} Both the acute and chronic forms of glanders were described in detail long before effective treatments were available and when the disease was still prevalent. In the 1906 review of 156 chronic human glanders cases, Robins stated that distinguishing between chronic and acute disease was difficult because chronic disease was often interrupted with
acute symptoms and acute onset disease may run a chronic course. For convenience purposes he defined chronic cases as those lasting longer than 6 months. Most historical literature attempting to differentiate the two classifies a more fulminant and rapidly fatal clinical course (within 2 to 4 weeks) as an acute form of glanders. An acute course is found more often with untreated acute pneumatic and frank septicemic infection, whether primary or recurrent. Chronic infections are most common in horses where they comprise the majority of cases, whereas acute disease is more common in humans and donkeys.

*B mallei* most often enters the human body through abrasions or openings in the skin, particularly where occupationally exposed on the hands and forearms, face, and neck. An abrasion is not always present, however, at least grossly. Normal, intact skin resists penetration of the organism; however, in several human infections, the affected persons insisted no wound or penetration occurred during the likely exposure interval. Thus, a patient history in which there is no recollection of exposure to horses or of abrasion should not preclude glanders as a differential diagnosis. Organisms may also enter through oral, nasal, and ocular mucous membranes, as well as via inhalation. The latter has occurred in several laboratory-acquired infections; however, at least one laboratory-acquired case most likely occurred through cutaneous exposure. When present, the most characteristic feature of the disease is glanders nodes—small papular to egg-sized abscesses—that are very slow to heal if they open.

The incubation period for glanders is variable, ranging from less than a day to several weeks. Cutaneous and mucous membrane exposure generally leads to symptoms in 3 to 5 days; without direct inoculation of the organism, however, the duration may be longer. Inhalational exposure may incur a slightly longer range of about 7 to 21 days.

**Figure 8-2.** Interactions of *Burkholderia mallei* with host cells. (a) Proposed model of the intracellular lifestyle of *B mallei* in phagocytic cells. Following entry in host cells, *B mallei* rapidly escapes from the phagosome and enters into the cytosol where it can grow, polymerize host cell actin (red), spread cell to cell, and induce host cell fusion resulting in the formation of multinucleated giant cells. *B mallei* interacts with various pattern recognition receptors and evades host cell autophagy. Genes, proteins, or systems that are known to be important at various points are indicated in blue text. VirAG senses a signal within the phagosome that activates T6SS-1 gene expression; T3SS, is required for escape from the phagosome; BmA is necessary for actin-based motility and actin tail formation; T6SS-1 is critical for multinucleated giant cell formation. (b) Fluorescent micrograph of *B mallei* infected RAW 264.7 murine macrophages. *B mallei* was added to RAW 264.7 cells at a multiplicity of infection of 10; at 12 hours postinfection cells were fixed and stained for actin and nuclei. *B mallei* expressing green fluorescent protein is shown in green; actin stained with Alexa Fluor™ phalloidin is shown in red; nuclei stained with DRAQ5 are shown in blue. MNGC: multinucleated giant cell
Photograph: Courtesy of Mary N Burtnick, University of South Alabama, Mobile, Alabama.
Medical Aspects of Biological Warfare

**Intracellular Lifestyle**

*B. mallei* is a host-adapted deletion derivative of *B. pseudomallei* and is genetically more uniform and less diverse than the latter. The severity, clinical course, and frequent chronicity of glanders are likely related to the capacity of *B. mallei* to survive and persist within host cells and thus evade destruction by the immune system. As an intracellular pathogen, survival of *B. mallei* involves binding to and invading eukaryotic cells, successfully escaping phagosomal compartments, and growing in the cytosol. Relatively little is known concerning how the organism binds to cells or the surface receptor involved. *B. mallei* adheres poorly to and does not invade A549 and LA-4 respiratory epithelial cell lines, but readily invades phagocytic cell lines such as J774.2 and RAW 264.7 murine macrophages. As shown in Figure 8-2 and as discussed below, *B. mallei* uses a type III secretion system to escape from the phagosome into the cytosol, where it can multiply and use actin-based motility to move about the cell. In addition, *B. mallei* can induce cell-to-cell fusion resulting in the formation of multinucleated giant cells (MNGCs), which are thought to provide a protective niche and metabolic resources for the bacteria, but little is known about the bacterial or host factors that are required for MNGC formation. Recent evidence points to expression of a type VI secretion system as a requirement for efficient intra- and intercellular spread and host cell fusion. *B. mallei* can also evade innate host immune responses when present extracellularly due in part to its surface structures, which will be described below. However, little is known about the specific molecular mechanisms of *B. mallei* virulence, and more study is urgently needed.

**Animal Models**

Research on pathogenicity requires the availability of relevant, well-characterized animal models. Various animal models of glanders have been reported for use in studies on pathogenesis and countermeasure evaluation, including guinea pigs, mice, hamsters, nonhuman primates (NHPs), and several invertebrates. Major models will be described and are summarized in Table 8-1; several recent reviews are available that provide further details of the development of in vivo models for glanders.

**Guinea Pigs and Hamsters**

Guinea pigs and hamsters are the laboratory animals exhibiting the greatest susceptibility to *B. mallei*, and guinea pigs were initially used most extensively. However, these animals varied in their individual susceptibility to infection. Syrian hamsters proved to be uniformly susceptible to infection and have been used more extensively in recent studies on *B. mallei*. In 1999, Fritz et al characterized disease in these animals bacteriologically and pathologically to include gross, histological, immunologic, and electron microscopic pathology. The hamster was shown to be much more susceptible to *B. mallei* than is presumed for humans, with an LD$_{50}$ of less than 10 colony-forming units (cfu) by the intraperitoneal (IP) route, with mortality/morbidity monitored for 5 days. Nevertheless, the course of disease and extensive development of glanders-associated pathology in a broad range of organs, especially in reticuloendothelial organs (such as spleen, lymph nodes, liver, and bone marrow) but also in other organs such as lung and brain, are similar to those in humans. The changes observed consist of infiltrates with an equal mixture of macrophage and polymorphonuclear leukocyte inflammatory cells; these can become organized into discrete, often bacteria-filled, nodules referred to pyogranulomas. More extensive information on the pathology of glanders in these susceptible species is described elsewhere.

**Mice**

Mice vary by strain in their susceptibility to *B. mallei*, a finding that mirrors that observed in studies with *B. pseudomallei*. However, mice are all considered to be moderately resistant to infection, similar to humans. Depending on the strain, route, and dose of infection selected, they have been used to model a range of disease manifestations and states ranging from latent to acute or chronic. Since genetic constructs and reagents specific to mice are widely available, they are common tools for studies on pathogenesis and protection. In most studies, the C57Bl/6 strain was more resistant than BALB/c strain mice by the pulmonary route. Studies by Goodyear et al have shown that when *B. mallei* was administered intranasally to C57Bl/6 mice, high dose inocula (5,000 cfu) resulted in acute infections that were lethal within 3 to 4 days, whereas low dose inocula (500 cfu) resulted in chronic infections. In recent as well as previous studies, the relatively more sensitive BALB/c strain has been used most often. In acute infection studies with BALB/c mice, a lethal IP dose often results in splenomegaly with multiple splenic white foci consisting of pyogranulomatous inflammation. This pathology occurs also in other reticuloendothelial organs, as typically described for hamsters; but mice usually do not exhibit this pathology in other organs, such as the lung, as occurs later in infection of hamsters.
TABLE 8-1
EXPERIMENTAL ANIMAL MODELS OF GLANDERS

<table>
<thead>
<tr>
<th>Model</th>
<th>Route of Infection</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horses</td>
<td>IT</td>
<td>Physiologically relevant model that mimics the natural development of chronic glanders in its reservoir host. Horses typically exhibit a long disease course with periods of improvement and relapse.</td>
</tr>
<tr>
<td>Rhesus monkeys</td>
<td>SC and aerosol</td>
<td>Development of SC lesions that healed after 3 weeks. No evidence of chronic or acute infection. Evidence of potential nonlethal chronic infection after aerosol exposure.</td>
</tr>
<tr>
<td>African green monkey</td>
<td>Aerosol</td>
<td>Development of acute glanders resembling the human infection.</td>
</tr>
<tr>
<td>Syrian golden hamsters</td>
<td>IP</td>
<td>Extremely susceptible infection model (LD$_{50}$ &lt;10 cfu) with development of acute infection resulting in a rapid disease course. Bacteria are transported to the mediastinal lymph nodes and seeded to other tissues, forming lesions in the spleen as early as 1 day postinoculation and death occurring around 6 days later.</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td>SC and IP</td>
<td>Development of acute glanders. Bacteria are transported to inguinal and axillary lymph nodes (SC), or mediastinal and mesenteric lymph nodes (IP), at early time points postinoculation.</td>
</tr>
<tr>
<td>BALB/c mice</td>
<td>Aerosol, IN, and IP</td>
<td>Good model for acute and potentially chronic glanders. Commonly used model due to low cost, susceptibility to B mallei infection, and a well-documented disease pathology. Mice are more resistant to B mallei when delivered IP.</td>
</tr>
<tr>
<td>C57Bl/6 mice</td>
<td>Aerosol, IN, and IP</td>
<td>More resistant to B mallei infection than BALB/c mice by pulmonary routes. High doses delivered IN resulted in acute disease, whereas low doses produced chronic infections.</td>
</tr>
<tr>
<td>Wax moth larvae</td>
<td>Injection into hemocoe</td>
<td>Used to screen putative virulence mutants; between 3 to 200 cfu of wild type B mallei leads to &gt;90% killing within 6 days.</td>
</tr>
<tr>
<td>Madagascar hissing cockroaches</td>
<td>Injection into dorsal abdomen</td>
<td>Used to screen putative virulence mutants; highly susceptible infection model (LD$_{50}$ &lt;10 cfu) resulting in death within 5 days.</td>
</tr>
<tr>
<td>Nematodes</td>
<td>Feeding</td>
<td>Used to screen putative virulence mutants; limited sensitivity resulting from high infectious dose.</td>
</tr>
</tbody>
</table>

cfu: colony forming unit  
IN: intranasal  
IP: intraperitoneal  
IT: intratracheal  
LD: lethal dose  
SC: subcutaneous

Aerosol exposure to a lethal dose of B mallei produces gross and microscopic pathologic changes similar to those after IP injection, except that there is more lung involvement, that is, focal inflammation and necrosis early in infection develop into extensive consolidation with chronic inflammatory cell infiltration (David L Fritz, David DeShazer, David M Waag, USAMRIID, Fort Detrick, MD, unpublished data, 2013). It has been observed that aerosol-exposed BALB/c mice develop acute inflammation of the nasal passages, which later extends to the nasal sinuses and ultimately into the brain, especially the olfactory lobe (David M Waag, USAMRIID, Fort Detrick, MD, unpublished data, 2013). Unlike humans, mice are obligate nose breathers, an anatomical difference that may explain these findings. Recent models using an intratracheal (IT) route of infection address this potentially confounding issue. However, the brain infection in mice might serve as a potential protected site for bacterial survival and provide a model for studying recrudescence of disease. Acute infection virulence data obtained with murine models often used laboratory strains such as the type strain B mallei ATCC 23344 (aka China 7) or the most...
recent human clinical isolate of *B mallei*, referred to as the FMH 23344 strain of *B mallei* ATCC 23344. The 50% lethal doses (LD_{50}s) for BALB/c mice have varied by route, and the mice were more susceptible by pulmonary routes than by the IP route. The range of LD_{50}s, as determined 10 to 35 days postchallenge (most 21 or 28 days), is exemplified as follows for BALB/c mice: IP (7 x 10^5 cfu), intranasal (IN; 680 cfu), IT (818 cfu), whole-body aerosol (913 cfu), and nose-only aerosol (1,859 cfu). Recently, the virulence of *B mallei* strain FMH 23344 was reevaluated in C57Bl/6 and BALB/c mice challenged by the whole-body aerosol route. As expected, C57Bl/6 mice were significantly more resistant than BALB/c mice; the respective LD_{50}s (after 21 days) were 7,665 cfu and 395 cfu, respectively (David M Waag, USAMRIID, Fort Detrick, MD, unpublished data, 2013).

No well-defined murine models for long-term chronic or latent glanders exist. However, *B mallei* clearly establishes protracted persistent infections in mice as it does in horses and humans, as shown especially in BALB/c mice. In recent aerosol and IP challenge studies, spleen cultures revealed the presence of long-term residual infection with *B mallei* in surviving mice. Such infections were more consistently detected in BALB/c than in C57Bl/6 survivors, that is, BALB/c mice surviving greater than or equal to 60 days after aerosol challenge with the FMH derivative of strain *B mallei* ATCC 23344 had positive spleens; whereas C57Bl/6 survivors had usually cleared the infection (Christopher K Cote and David M Waag, USAMRIID, Fort Detrick, MD, unpublished data, 2012). Fritz et al conducted a natural history study of *B mallei* ATCC 23344 in mice infected lethally and sublethally by the IP route. Pyogranulomatous inflammation was observed histologically in multiple reticuloendothelial organs, and the incidence and severity of the changes did not decrease in the sublethally infected mice. Barnes et al treated mice that had been infected by aerosol with *B mallei* with a postexposure prophylactic regimen of trimethoprim-sulfamethoxazole that effectively prevented acute infection; two-thirds of the mice survived to study end at day 74. However, the treatment did not eradicate the bacteria and a clinical relapse of infection occurred by day 30 postchallenge. *B mallei* was detected in the organs of all surviving mice. Similar data have been reported, and the results overall suggest that BALB/c mice could serve as a useful model for both acute and chronic glanders in a relatively resistant host such as humans.

Nonhuman Primates

Although NHPs are phylogenetically the closest animal to humans, few studies have described their use as laboratory models. In one study, rhesus monkeys were given different doses of a virulent strain by the subcutaneous route, and the monkey receiving the highest dose developed a cutaneous abscess that resolved completely after 3 weeks. Russian investigators reported studies with baboons but few details are available. Different species of NHPs appear to vary significantly in susceptibility but overall there is a lack of substantial data characterizing experimental infection in these animals. The challenge is to develop a model(s) that best mimics the human acute and chronic responses to *B mallei*. Recent studies have shown that rhesus macaques are resistant to acute infection (John J Yeager and M Louise Pitt, USAMRIID, Fort Detrick, MD, unpublished data, 2012); however, Yingst et al showed that rhesus monkeys given subclinical doses of *B mallei* exhibited clinical presentations and pathological lesions that correlated well with those described for human cases of glanders. They suggested that the rhesus macaque is a potentially viable model for human disease albeit not acute lethal illness. A longer-term study is being done to include LD_{50} determinations with extensive clinical, pathological, and laboratory evaluations to further analyze three NHPs as models of human glanders (Patricia L Worsham, David M Waag, and Taylor B Chance, USAMRIID, Fort Detrick, MD, unpublished data, 2012). African Green monkeys (*Chlorocebus aethiops*) were much more sensitive to infection than cynomolgus macaques or rhesus macaques and appear to be a potential model for acute infection (Patricia L Worsham, David M Waag, and Taylor B Chance, USAMRIID, Fort Detrick, MD, unpublished data, 2013).

Invertebrate Models

Several nonmammalian surrogate models have recently been used to study virulence mechanisms and host-pathogen interactions of the human pathogenic species of *Burkholderia*, including insect larvae, cockroaches, a phagocytic amoeba, and soil-dwelling nematode. For *B mallei*, models using wax moth larvae (*Galleria mellonella*) and the Madagascar hissing (MH) cockroach have been reported. Insect models are useful mammalian surrogates for several reasons. Significant similarities exist between the innate immune systems. Both hosts harbor Toll receptors (insects) or Toll-like receptors (TLRs, mammals) that recognize pathogen markers and produce protective responses such as antimicrobial peptides; also insects possess...
phagocytic hemocytes that can take up and kill microbes, in a manner similar to that of neutrophils. Other advantages of using insects as models involve ready availability, reduced costs and facile housing/maintenance, and exemption from regulatory oversight and expense. *B mallei* was shown to be as virulent for the larvae as it was for hamsters and mice, whereas *Burkholderia* that are nonpathogenic in mammals were not pathogenic for the insects. Notably, in tests with mutants of *B mallei* harboring known virulence-associated gene defects (eg, in capsule production or in the type three secretion system [T3SS]$_{AD}$), lethality for wax moth larvae corresponded to the extent of reduced virulence of the mutants in hamsters and mice.65 Fisher et al found the MH cockroach to be easier to handle than wax moth larvae and their ability to grow at 37°C made them more amenable than other insects to mutant analysis with *B mallei*, a mammalian host-adapted pathogen.45 Thus, MH cockroaches appear to be a valid surrogate and alternative to mammals as a model for virulence mechanisms of *B mallei* important in host interactions.

**Virulence Mechanisms**

**Surface Polysaccharides.** Since *B mallei* appears to be genetically derived from the environmental saprophyte *B pseudomallei*, it shares many virulence factors with the latter.68,69 However, some of the *B pseudomallei* factors required for its independent lifestyle appear to have been lost in *B mallei* as a consequence to its adaptation to the equine hosts. In addition, differences in the presence or role of some virulence factors in *B mallei* compared to *B pseudomallei* have been described (possibly related to the increased presence in *B mallei* of insertion sequence elements and genetic rearrangements), as will be illustrated. The factors and activities identified as being essential for *B mallei* virulence and host persistence include the following:

- a capsular polysaccharide (CPS);
- lipopolysaccharide (LPS);
- animal pathogen-like T3SS (T3SS$_{AD}$);
- the cluster 1 T6SS (T6SS-1); and
- the VirAG two-component regulatory system.38,72,74,77,93

Other putative virulence factors, including various autotransporter proteins, adhesins, quorum sensing, and iron-binding compounds, have been identified but their roles in virulence are unconfirmed (as described below).

Two major polysaccharide (PS) antigens that are present on the surface of *B mallei*, a CPS and LPS, play important roles in the pathogenesis of glanders and in host responses to the infection. The presence of a CPS on the surface of *B mallei* was shown by immuno-electron microscopy.77 The structure of the CPS antigen has recently been characterized and shown to be identical to the CPS expressed by *B pseudomallei*, which is a homopolymer of -3)-2-O-acetyl-6-deoxy-β-D-manno-heptopyranose-(1-7,74 Consistent with this finding, anti-CPS monoclonal antibodies (mAbs) that have been characterized recognize the CPS of both pathogens.95–97 The surface-expressed nature of the LPS is evidenced by its availability to the host immune system and its ability to activate TLR4 complexes.97–99 The structure of *B mallei* O-polysaccharide (OPS) has been determined to be a repeating unit of -3)-β-D-glucopyranose-(1,3)-6-deoxy-α-L-talopyranose-(1- where the talose residues contain 2-O-methyl or 2-O-acetyl side groups.93,100 In comparison to *B pseudomallei*, *B mallei* OPS lacks 4-O-acetyl modifications on the talose residues.93,100,101 This structural difference explains why mAbs specific for either *B mallei* LPS or *B pseudomallei* can be isolated.93,102 The virulence roles of these PS antigens were demonstrated by the construction of mutant strains lacking either CPS or OPS, which proved to be avirulent in animal models.77,93,103 The precise functions of CPS and LPS in pathogenesis are not fully characterized; however, the CPS may contribute to survival in serum by inhibiting complement deposition, opsonization, and phagocytosis, as well as possibly conferring resistance to the harsh environment of the phagosome until the bacteria are able to escape.75 OPS is known to be critical for serum resistance since *B mallei* strains lacking OPS moieties are rapidly killed by 30% normal human serum.93 mAbs to both *B mallei* CPS and LPS have been identified that are either bactericidal for the organism or have strong opsonic activities.97 The roles of these two PS moieties in pathogenesis were further confirmed by demonstrating that passive administration of LPS- or CPS-specific mAbs effectively protected mice against lethal pulmonary challenge.97,104

**Secretion Systems and Secreted Proteins.** *B mallei*, a highly successful facultative intracellular pathogen that can survive in many eukaryotic cell lines, possesses a variety of mechanisms to adapt to and alter the host environment.68,69,71 The organism harbors an array of specialized secretory systems that are essential to this process. Little is known about these systems or the specific roles of their components, although a number of the genes identified appear to be homologous to the more extensively studied species *B pseudomallei.* *B mallei*-specific studies have focused primarily on characterization of T3SS$_{AD}$ and T6SS-1.68,72,74,77,93,100 Genes encoding other secretion systems, including the type II and type V systems, are also present in *B mallei.*75 The effector proteins delivered by these systems are
predicted to disable or modulate critical host proteins and pathways involved in cell signaling, cytoskeleton and ubiquitin function, and cell death pathways, thus facilitating pathogen survival and propagation in the host.70,75,107-109 In addition, both B mallei and B pseudomallei exhibit actin-based motility and the distinct ability to induce formation of MNGCs in tissue culture models, potential mechanisms that allow the pathogen to spread in the host via direct cell-to-cell passage (Figure 8-2).73,75,110

After B mallei is phagocytosed by the host cell, it escapes from the endocytic vacuoles into the host cell cytoplasm where it uses actin-based motility to spread intra- and intercellularly. Virulence-associated T3SS_Ap and T6SS-1, as well as other secreted proteins, appear to be essential for these various functions.66,69,71-73,103,111,112

Many gram-negative bacterial pathogens harbor such secretion systems and use them to synchronize the secretion and delivery of effector proteins directly into target host cells via needle-like injection apparatuses. B mallei, T3SS_Ap is required for virulence in animal models of infection, as well as for phagosomal escape and survival in macrophage tissue culture models of infection.69,72,75 Once free in the cytosol, the microbe activates processes for evading host cell killing and for polymerization of host cell actin.75 BimA, a type V secreted (T5S) protein, plays a major role in facilitating actin-based motility in B mallei.113,114

Although the T3SS_Ap is important for phagosomal escape and survival within host cells, the exact roles of specific effector proteins delivered by this system have yet to be clearly identified. Many proteins are predicted to be part of the B mallei T3SSs by in silico annotation; some have been partially characterized, and the potential roles of a few in virulence have been studied.73,92 These proteins include BopA and BopE; Bip B, C, and D; and BapB, as described in detail in previously published reports.70,75,92 In B mallei a mutation in the T3SS-encoded BopA effector protein resulted in a slower growth rate in macrophages and an apparent reduced ability to escape the cells. This mutation also attenuated infection by B mallei in BALB/c mice, suggesting BopA may contribute to survival within—and possibly escape from—host alveolar macrophages.70,115

In B pseudomallei, BopA appears to enhance survival by helping the microbe evade autophagy-induced phagocytic vacuole degradation.116 Bip B, C, and D proteins appear to be structural components of the T3SS injector apparatus that are involved in contact of the tip with host cells.75

T6SS-1, which is important during the intracellular lifestyle of the B mallei, is essential for B mallei virulence in a hamster model of glanders.83,73 In a RAW 264.7 macrophage model, T6SS-1 expression was shown to occur following internalization of B mallei, but before escape of the organism from the phagosomal environment.73 Once in the cytosol of host cells, T6SS-1 mutants displayed defects in actin polymerization and an inability to induce MNGC formation.73,108 T6SSs are proposed to resemble inverted bacteriophage tail-like structures involved in delivering effector molecules directly into target cells. These systems are tightly regulated at the genetic level so that they are only expressed at the appropriate time and place. Two key components of T6SS-1 are the Hcp1 and VgrG1 proteins, which are both secreted and structural components of the T6SS apparatus and are considered reliable indicators of T6SS function.74,112,117,118 Various components of T6SS-1 are being characterized because these proteins may represent potential diagnostic, therapeutic, or vaccine targets.73,74,105,111,112

The VirAG two-component system is an important regulator of virulence gene expression in B mallei and is required for virulence in hamsters.36 Approximately 60 genes are under the regulatory control of VirAG, including the T6SS-1 gene cluster and genes involved in actin-based intracellular motility (bimBCADE).74 When the VirAG system is overexpressed in vitro, Hcp1 and VgrG1 are secreted into culture supernatant by T6SS-1. VirAG also controls the expression of tssM, which encodes a putative ubiquitin-specific protease (ie, deubiquitinase) that is expressed shortly after intracellular uptake and provides the bacteria with an enzymatic tool that can potentially regulate multiple eukaryotic cell processes.74,108 Shanks et al characterized the expression and regulation of B mallei TssM, and demonstrated that it was a potent ubiquitin-specific protease.108 Ubiquitin is a host protein that attaches to other proteins so as to direct their intracellular fate. Bacterial deubiquitinases remove the ubiquitin residues, disrupting this process and promoting bacterial evasion of host immune responses and survival. Although the TssM protease may provide B mallei a selective advantage within the cell during infection, a role in virulence for hamsters was not shown. Interestingly, even though tssM is coregulated with and physically linked to the T6SS-1 gene cluster, TssM is not secreted by either T6SS-1 or TSS_Ap.108 More research is needed to determine the signal sensed by VirAG in vivo that ultimately results in the expression of this important regulon.

**Autotransported and ATP-binding Cassette Proteins.** Several autotransporter (AT) and ATP-binding cassette (ABC) transporters that may have roles in the infectious process have been described for B mallei. ATs are large families of outer membrane proteins in gram-negative bacteria that are secreted by the T5S pathway, and they include virulence-associated
invasins, adhesins, proteases, and actin-nucleating factors.\textsuperscript{119,120} AT proteins have three common features: (1) an N-terminal signal sequence for periplasmic translocation, (2) a central functional domain(s), and (3) an outer membrane channel-forming C-terminus needed for surface export of the central domain.\textsuperscript{119} Eight of the 11 AT analogs of \textit{B pseudomallei} are shared by \textit{B mallei} and include BimA, an AT involved in actin tail formation and actin-based motility, and BoaA, an AT with a potential role in bacterial adhesion to epithelial cells.\textsuperscript{74,76,114,121} BimA is expressed by both \textit{B pseudomallei} and \textit{B mallei}, although their sequences vary. In both species BimA is required for actin-based motility and MNHC formation in infected tissue culture monolayers.\textsuperscript{74,76,114} Interestingly, in \textit{B mallei}, the bimBCADE genes were found to be dispensable for virulence in hamsters.\textsuperscript{14}

A group of immunodominant \textit{Burkholderia} antigens, designated Hep-Hag autotransporter (BuHA) proteins, shares structural similarities with hemagglutinins and invasins.\textsuperscript{123} These proteins were present in 53\% of a \textit{B mallei} expression library examined and only 3\% of a \textit{B pseudomallei} library. They appear to function as surface proteins that modulate interactions of the bacterial cell with the host and environment; homologs in other bacteria have significant roles in virulence, but their possible roles in \textit{B mallei} virulence and immune modulation require further study. Finally, several ABC protein systems with established roles in the virulence and pathogenicity of various gram-negative pathogens have been identified in the \textit{Burkholderia}.\textsuperscript{124,127} Although their contribution to \textit{B mallei} pathogenicity has not been evaluated, some components (eg, the ABC transporter protein LolC) have been shown to be immunogenic and to elicit significant partial protection against both \textit{B mallei} and \textit{B pseudomallei}. These proteins deserve further analyses as both putative virulence factors and vaccine targets.

\textbf{Quorum Sensing Systems.} Quorum sensing (QS) permits bacteria to monitor their population density and modify gene transcription at critical population levels.\textsuperscript{126} Many host-associated bacteria use small amphipathic acyl-homoserine lactone signals for QS; and Duerkop et al identified octanoyl-homoserine lactone as the predominant BmaI1 synthase-produced acyl-homoserine lactone signal and activator for the \textit{B mallei} LuxR QS system.\textsuperscript{126} Nevertheless, numerous animal pathogens lack such systems, yet are virulent. Using mutants in bmaI genes of the \textit{B mallei} QS systems, Ulrich et al showed that QS was critical for virulence of \textit{B mallei} in aerosol-exposed BALB/c mice.\textsuperscript{127} However, using constructs with similar mutations in bmaI, Majerczyk et al recently determined that QS was not required for lethal infection of mice exposed by aerosol to \textit{B mallei}.\textsuperscript{128} These studies do not exclude a role for QS in glanders in other animal models or the natural equine host.

\textbf{Other Potential Virulence Factors.} A role for pilin/fimbriae structures in the pathogenesis of glanders is poorly defined.\textsuperscript{75,129} For instance, type IV pili are required for virulence of \textit{B pseudomallei}, and although they are expressed by \textit{B mallei} in vivo and are highly immunogenic, a role for them in adherence and virulence of \textit{B mallei} has yet to be shown. Neither active nor passive immunization with pilin or anti-pilin antibodies protected mice against subcutaneous or aerosol challenge. Its protective role in a natural host or incidentally infected human or after exposure by different means remains to be determined.\textsuperscript{129} Several other virulence mechanisms are being investigated, and innovative techniques and combination approaches (in silico, in vitro, and in vivo) are being used to identify putative novel virulence factors with possible roles in pathogenesis of \textit{B mallei} or in protective immunity.\textsuperscript{62,92} One of these virulence mechanisms is the carboxy-terminal processing protease of \textit{B mallei} and compounds potentially involved in iron acquisition, such as the siderophore malleobactin.\textsuperscript{130–132}

\textbf{Clinical Disease in Animals}

\textit{B mallei} naturally infects horses, donkeys, and mules, but other species have occasionally become infected.\textsuperscript{41,45,58,133} If glanders is suspected as a differential diagnosis, local and regional animal and public health authorities must be immediately notified. The incubation period for glanders in equids ranges from a few days to many months, with most between 2 to 6 weeks. The infectious process, disease progression, and pathology in equids are similar to those in humans. Donkeys are most likely to succumb to acute disease and die in a week to 10 days.\textsuperscript{1,4} Horses are more likely to incur a slowly progressive, chronic disease. Recurring clinical disease and even death in horses may manifest months to years after dormancy, particularly after any stress that causes a rise in temperature such as infectious disease, roundup, transport, overwork, poor diet, exercise, immunization, and even mallein testing.\textsuperscript{1,4,134} Changes in season, from winter to spring and from summer to fall, have also been associated with recurrent disease.\textsuperscript{4}

The primary route of infection in the natural host is oral, by chewing or contacting contaminated food and water, feeding and husbandry equipment, and by direct close contact with infected animals.\textsuperscript{135} Tooth eruption, irregular tooth wear, coarse feeds, and bridling contribute to oral trauma, a common finding that leaves the mucosa and mucocutaneous junctions more

\textbf{Glanders}
vulnerable to infection. Equids are also gregarious and prefer to be in close contact with at least one other. Grooming and nibbling behavior also exacerbate the potential for exposure from direct contact. Contaminated aerosols, such as those produced by snorting or coughing, may also easily find their way into the eyes, mouth, or skin abrasions of other equids in the vicinity. Tack, such as a harness, can cause skin irritation that—if contaminated—may allow easy entry of the organism. Despite the oral route of infection, significant pathology is usually seen in the airways and lungs.60

With early infection or resurgence, constitutional signs are often the first to manifest. These signs may include thirst, fever (low grade to high), shivering, head drooping, tachycardia, tachypnea, weight loss, rough hair coat, indolence, prostration, and a reluctance to move.136 Swelling of the limbs and joints may be seen. The lungs, mucosa of the respiratory tract, and lymphatic system are most frequently involved wherever the infection originates. Horses experimentally infected by cutaneous flank injection of infectious material developed a respiratory tract infection within a few weeks.1 In some cases (or at various stages of disease) the lungs may appear to be the only organ involved. Regional or diffuse pneumonia and pleuritis are common. The lungs and upper respiratory tract are also the organs and tissues that show the oldest signs of chronic disease. Lung pathology is typically more marked and extensive in donkeys than in horses.

The nasal form of glanders classically described in equids is a somewhat local infection of the nasal cavity at least characterized by yellowish-green unilateral or bilateral nasal discharge, with or without nodules or ulcers on the nasal mucosa. Regional lymphadenopathy and lymphangitis most often accompany nasal pathology. Laryngeal, tracheal, and lower respiratory tract pathology is often present, however, even if microscopically supporting the concept that a local infection is more likely just early infection, or rare. Nasal signs are common with recurrence of chronic infection. Although the nasal form has been associated with equids, similar pathology has been described in humans.3,54

With clinical expression of upper respiratory infection, a highly infectious, sticky, yellow-gray to greenish viscid unilateral or bilateral nasal exudate is produced. The glottis may be edematous and nasal discharge may be so thick as to obstruct nasal passages. The margins of the external nares are often swollen and crusted. The exudate may be periodically blood-tinged. The muzzle and distal forelimbs may be covered with this exudate; the latter is due to wiping the nose. The nasal mucosa may be nodular and ulcerous with ulcers often rapidly spreading. Ulcers may be deep and coalesce forming larger ulcers. Mucosal abscesses of the septum and nasal conchae may have swollen edges and display small yellow and gray nodules. These abscesses may invade the turbinates and cartilaginous structures, leading to perforation and erosion of the nasal septum. Particularly where the larger ulcers heal, white stellate or radial scars are left on the mucosa. These scars may be seen with an endoscopy, and they are near-hallmark signs of prior infection. Visible or palpable regional lymphadenopathy (particularly submandibular) and lymphangitis are present.

The equid will frequently snort to clear nasal passages, effectively showering the immediate area with the infectious exudate. The animal may cough, or a cough may be easily elicited by placing pressure on the throat over the larynx when there is laryngeal involvement. The air-exchange produced by a cough may exacerbate nasal discharge as equids breathe through their nose and not their mouths. Dyspnea, particularly inspiratory, may result from swelling in the nasal cavity or larynx. Expiratory dyspnea is also not uncommon, particularly with chronic involvement of the upper and lower respiratory tract.50 Auscultation and diagnostic imaging findings may support localized or diffuse lung disease and pleurisy. Clinical signs may be mild and transient, or severe and progressive. Death may occur within a few days.

At necropsy, glanders nodes will likely be found in the lungs, even if incidentally. Their consistency may be caseous to calcified depending on lesion age. These nodes may be of any size and occur as just a few or as hundreds in a diffuse miliary pattern. Pleuritis may also be found at necropsy. The microorganism is relatively abundant in the affected tissues. Animals may die within 3 to 4 weeks from bronchopneumonia and septicemia.

The progression of cutaneous and mucous membrane infection in the equid is similar to infections in humans. An entry wound may not be found. Lymphatic involvement may be more visible, however. Subsequent to cutaneous or mucosal infection, regional lymphangitis develops within 7 to 10 days. Typically, the lymphatics undergo a visible or palpable “string of pearls” stage within 10 days and then turn to more solid, fingerlike cords that can be traced to regional lymph nodes. Nodules along the lymphatic vessels may erupt, exuding gelatinous pus. Lymph nodes may be enlarged and indurated, and less frequently they may rupture and suppurate. With disease progression, more eruptions, enlargement of eruptions, and coalescence of lesions are expected. As a rule these are very slow to heal. Thick crusts of wound secretions, hair, bedding, and dirt may mat around the lesions. With ocular involvement, photophobia, excessive lacrimation, mucopurulent ocular discharge, conjunctivitis, and apparent partial
blindness may occur, and this may result in behavioral changes such as avoidance or fear. With disseminated disease, cutaneous and mucous membrane lesions may appear anywhere, particularly the respiratory tract, as previously mentioned, and the limbs. The hind limb is more commonly affected than the forelimb.\textsuperscript{52, 60}

Acute septicemia may occur at any stage of infection. A septicemic course is typically progressive with signs leading to multiple organ failure including watery diarrhea, colic, marked dyspnea, prostration, cardiovascular collapse, and death. Donkeys are particularly susceptible to \textit{B mallei} septicemia; this form manifests in most of those naturally and experimentally infected. Disseminated disease in horses is typically more protracted, however. Clinical signs vary widely and may include any of those previously mentioned. Horses may be asymptomatic. They may appear slightly thin, unthrifty, or have an occasional or persistent nasal discharge. There may be a transient mild to moderate fever. Mucous membrane and cutaneous lesions as well as lymphadenopathy and lymphangitis may also be transient or chronic. Visceral abscess is common, and the spleen and the liver are frequently involved. Intact males may have orchitis, which may not be evident without a reproductive examination.\textsuperscript{40, 137} Remission is unlikely with disseminated disease particularly if it involves visceral organs.

In the event an equid presents with clinical or necropsy signs consistent with glanders, the premises should be immediately quarantined and local and regional animal health authorities should be notified. Treatment should not be attempted. Although a clinical prognosis for various forms of glanders infection may be surmised, it is less relevant now because of the global interest in eradicating (by test-and-slaughter) the disease.

Chronically infected horses may display cycles of worsening disease followed by apparent recovery where few symptoms are displayed. Clinical signs include intermittent cough, lethargy, and lesions in the nasal region, lungs, and skin, just as with acute disease.\textsuperscript{136} Lungs may develop lesions similar to tubercles. Nodules may appear in the submucosa of the nasal cavity, particularly the nasal septum and turbinates. Nodules found in the liver and spleen may be up to 1 cm in diameter and have a purulent center surrounded by epithelioid and giant cells.\textsuperscript{138} Attempts to isolate \textit{B mallei} from chronically infected animals are usually unsuccessful. Thrombosis can be found in the large venous vessels of nasal mucous membranes.\textsuperscript{25} Nodules in the skin along lymphatics may be seen in chronically infected animals as they thicken. Nodules may ulcerate and rupture, spewing a thick exudate that may be a source of infection.

Clinical Disease in Humans

Even during its peak near the turn of the 20th century, human glanders was uncommon but well documented. The clinical course of glanders is based on reports of hundreds of cases published before antibiotics were developed and from a small series of cases that occurred in the United States since the discovery of sulfonamides. The earlier reports describe a nearly always fatal disease of short (a few days to weeks) to long (months to years) duration that was usually acquired from close contact with infected equids. The most recent cases were laboratory acquired, and all patients survived.

Glanders manifestations can vary. At least five forms of infection have been described, including localized, pulmonary, septicemic, disseminated, and the aforementioned chronic infection, but none is exclusive. The most important distinction is whether the infection is localized, which is unusual except early in the infectious process. The variety of forms is largely explained by route of infection, regional lymphatic inflammation and drainage, and loci of dissemination and embolism via hematogenous or lymphatic spread. With disease progression and chronicity, all forms may manifest.

Localized infections are regionally confined and typically characterized by pus-forming nodules and abscesses that ulcerate and drain for long periods of time. Lymphangitis or regional lymphadenopathy may develop in the lymphatic vessels that drain the entry or infection site. Increased mucus production from affected oronasal, nasal, and respiratory mucosa is often present. Localized infections typically disseminate, leading to pulmonary, septicemic, or disseminated infection.

Constitutional signs and symptoms typically occur early in the disease and some may persist through treatment. These signs and symptoms may be severe, leaving the patient extremely prostrate. Common signs and symptoms include fever or low grade fever in the afternoon to evening, chills with or without rigors, severe headache, malaise, generalized myalgias (particularly of the limbs, joints, neck, and back), dizziness, nausea, vomiting, diarrhea, tachypnea, diaphoresis (includes night sweats), altered mental status, and fatigue. Other nonspecific signs may be tender lymph nodes, sore throat, chest pain, blurred vision, splenomegaly, abdominal pain, photophobia, and marked lacrimation. Any or many of these signs may be present. Following constitutional signs, clinical courses are discussed in greater detail as they are associated with route of entry and disease spread.

Cutaneous manifestations include multiple papular or pustular lesions that may erupt anywhere on the body. Cutaneous or mucosal infections may spread,
leading to disseminated infections. Dissemination to internal organs produces abscesses in virtually any organ, most commonly the spleen, liver, and lungs. Disseminated infections are associated with septic shock and high mortality, yet they may also produce a more chronic, indolent course of infection.

With cutaneous entry through an abrasion, an inflammatory response of varying degrees (virulence dependent) occurs with accompanying pain and swelling. A glanders node may appear usually as a single blister, gradually developing into an ulcer that may be hemorrhagic. \(^7,55\) Localized infection develops at the entry site with a mucopurulent discharge. Inflammation may extend along regional lymphatics and cause lymphangitis perhaps with numerous foci of suppuration along their course. This irritation is caused by endotoxins present in some \(B\) \(mallei\) strains affecting the smooth muscle of the lymphatics. Lymphatic vessels may be easily palpable as firm, ropy cords. Regional lymph nodes become involved and similarly inflamed. Infection may remain localized, but more often spreads, particularly without adequate treatment. Further spread occurs via the lymphatics and through hematogenous dissemination as thrombi and emboli are formed. Local endothelial tissue inflammation and suppuration can occur at any place along the route of spread, producing abscesses that may drain through the skin. Superficially, these abscesses may appear as papules or diffuse abscesses in inflamed skin, or larger (egg-sized) swellings deeper in the subcutaneous tissue and superficial musculature. Published case descriptions have described glanders nodes anywhere including the face, neck, shoulders, lumbosacral region, arms, and legs. \(^7,55\) When the legs are affected, glanders nodes occur more often on the lateral aspect than the medial. At first these glanders nodes may be hard and painful, but eventually they ulcerate and slough. These nodes may exude relatively tenacious pus that varies in consistency from mucopurulent to gelatinous to oily, depending somewhat on chronicity. The nodes heal slowly and recur without adequate treatment. At any time the patient may become acutely ill and septicemic. Other organs and tissues may also be showered with infectious emboli.

The infectious process through the oral, nasal, or ocular mucus membrane is similar to the cutaneous process. Weakened or abraded membranes are more vulnerable to entry than are intact membranes. Potential entry may be associated with contaminated hands, fingers, objects, and aerosols contacting the eye, nose, and mouth. A localized infection typically follows. Within 1 to 5 days the affected membranes become injected, swell, and weep a serosanguinous to mucopurulent discharge. Papular and ulcerative lesions similar in character to those in the skin may appear. Single or multiple oral blisters and sores may also appear. Hyperemia may be diffuse (entire pharynx, conjunctiva, etc) or localized. With ocular involvement, excessive lacrimation and photophobia are common. With nasal involvement, the nose may become greatly swollen and inflamed and copious nasal discharge may occur. Infection may invade the nasal septum and bony tissues, causing fistulae and tissue destruction. The entire face can become swollen, and regional lymph glands may inflame and suppurate. Infection may also extend lower in the respiratory tract resulting in tracheitis and bronchitis, which can be accompanied by cough and the production of mucopurulent sputum. If mucous membrane involvement is extensive, constitutional signs are also usually severe including high fever, severe headache, fatigue, prostration, earache, and various neurologic signs.

Infection of the respiratory tract may be anticipated after aerosol exposure or secondarily as a consequence of disseminated infection. A pulmonary infection typically produces pneumonia, pulmonary abscess, pleuritis, and pleural effusion, with associated signs and symptoms such as cough, dyspnea, chest pain, and mucopurulent sputum. Nasal exudate and cervical lymphadenopathy may also be present if the upper respiratory tract is involved. Nonspecific signs and symptoms often accompany respiratory infections, such as fatigue, fever, chills, headache, myalgias, and gastrointestinal signs. Pulmonary abscess and pleuritis are common sequelae. Symptoms including tender cervical lymph nodes, fatigue, lymphangitis, sore throat, pleuritic chest pain, cough, fever (often exceeding 102°F), chills, tachypnea, dyspnea, and mucopurulent discharge may take 2 to 3 weeks to develop. Nonspecific signs are also usually present including night sweats, rigors, myalgia, severe headache, tachycardia, nausea, weight loss, dizziness, and mucosal eruptions. Some of the latter symptoms may indicate disseminated infection. Imaging studies may show diffuse or localized infiltration depending on the stage of infection. Miliary to nodules or a localized (lobar to bilateral) bronchopneumonia are other potential radiographic signs. Developing abscesses may be well circumscribed and circular, later becoming cavitary with evidence of central necrosis. Pleural irritation may also be visible on imaging studies. Acute bronchopulmonic or pneumonic disease untreated tends to have a rapid onset of symptoms and was once said to be almost uniformly fatal within 10 to 30 days. \(^5\) Most laboratory-acquired infections have resulted from inhalational exposure resulting in pulmonary infection.

Clinical features of eight laboratory-acquired infections from Camp (Fort) Detrick are summarized in Table 8-2. These infections include the six-case series.
published by Howe and Miller in 1945, a previously unpublished case that occurred in 1953, and the 2000 case first presented by the Centers for Disease Control and Prevention. The most common symptoms experienced by at least four of the eight include—in order of most common occurrence—afternoon to evening low-grade fever, malaise, fatigue, headache, myalgias including backache, lymphadenopathy, and chest pain (Table 8-2). Shaded elements in the table represent the first signs and symptoms according to the medical records of the first seven patients, and according to the published case description of the eighth patient. An important clinical feature that is not reflected in the table is that at least half of the patients not only “felt better” but also were clinically better for a time after the first wave of disease symptoms. This period lasted from a few days for patient 7, to 2 months for patient 2. Inhalation is suspected as the route of exposure for the first seven patients, whereas percutaneous exposure probably led to the eighth case.

Septicemic glanders results from the seeding of B mallei into the bloodstream, whether as a primary event, secondary to a local or pulmonary infection, or as a relapse to chronic or latent infection. Septicemia may be passing and lead to protracted disseminated infection or be fulminant and rapidly fatal. Septicemic glanders may produce numerous signs consistent with a highly pathogenic bacterial septicemia. Without aggressive treatment, B mallei septicemia runs an acute course and may lead to death in 7 to 10 days. The thromboembolic process of glanders was well described by the early 1900s. B mallei causes damage and subsequent death of the endothelial cells lining the vessels. As the cells detach, the endothelial lining is predisposed to thrombosis. Thrombi serve as an excellent culture medium and seed the bloodstream with bacteria. The embolic process may be realized by the patient as sharp stinging pain in the receiving part or tissue of the body. Robins describes one protracted chronic infection in which the patient was always aware of pain before multiple impending dissemination sites. Bacteremia is transient; however, the more acute or sudden the onset of a septicemic course, the more likely B mallei may be isolated from the blood. Bacteremia is also more likely shortly before and during the appearance of multiple eruptions and pustules, if they occur.

Century-old accounts of acute septicemic glanders suggest that virulent organisms and toxins may be so rapidly absorbed that systemic disease is actually primary, preceding the more patent ulcerative and lymphoglandular manifestations. Death may occur before these develop, however. Clinical signs and symptoms of the septicemic process may develop immediately or up to 2 weeks after initial infection or resurgence. These signs and symptoms include any severe constitutional sign and any of the cutaneous, mucous membrane, nervous, and respiratory signs previously discussed. Multiple organs may be involved. Erythroderma, jaundice, severe gastrointestinal distress, abdominal spasm, and severe respiratory signs may develop. Tachycardia, blurred vision, photophobia, excessive lacrimation, altered mental status, hepatomegaly, splenomegaly, granulomatous or necrotizing lesions, and lymphadenopathy may also be present. Death usually occurs in 7 to 30 days without adequate treatment. The prognosis for acute B mallei septicemia is guarded regardless of treatment.

Dissemination can also occur in a more benign process resulting in a chronic course, which may be interrupted with latent periods of up to 10 years. Dissemination typically occurs without adequate treatment 1 to 4 weeks after B mallei infection of the lymph nodes. The organs most often involved in disseminated infection are the spleen, liver, and lungs, although any can be affected. Other sites include the skeleton, brain, meninges, musculature, and any cutaneous or mucous membrane locations. The kidneys are rarely affected, however. Clinical signs may be absent, limited simply to weight loss, or be highly severe and variable and include any of the aforementioned. Cutaneous eruptions may appear anywhere on the body and often originate from deep pockets of infection in the musculature. The extremities are often affected. Generalized lymphadenopathy with induration, enlargement, and nodularity of regional lymphatic vessels may be found on the extremities and in other affected areas. Miliary abscesses of organs and tissues may resemble tuberculosis. Robins described several cases of disseminated chronic infections in which no clinical symptoms were apparent, yet at autopsy patients were riddled with abscesses, including in the lungs. Robins chronicles a patient with the longest known infection (15 years, only 5 of which were latent) who finally died of disseminated disease. Symptoms of this particular disseminated infection included nasal and aural discharge, submaxillary adenitis, phlegmon of the nose, perforation of the nasal septum, jaundice, diarrhea, and amyloid disease.
**TABLE 8-2**

**CLINICAL FEATURES OF EIGHT US LABORATORY-ACQUIRED *BURKHOLDERIA MALLEI* INFECTIONS**

<table>
<thead>
<tr>
<th>Signs and Symptoms*</th>
<th>Patient 1† November 1944</th>
<th>Patient 2‡ November 1944</th>
<th>Patient 3‡ February 1945</th>
<th>Patient 4‡ April 1945</th>
<th>Patient 5‡ August 1945</th>
<th>Patient 6‡ August 1945</th>
<th>Patient 7‡ July 1953</th>
<th>Patient 8‡ March 2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever, pm rise‡</td>
<td>99.0–99.4</td>
<td>99.0–101.2</td>
<td>101.0–103.4</td>
<td>99.0–102.8</td>
<td>99.0–101.4</td>
<td>99–104.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rigors, chills</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Night sweats</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain in chest</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Myalgia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Malaise</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Headache</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Backache</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stiff or sore neck</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dehydration</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Earache</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cough</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mucopurulent sputum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oropharyngeal</td>
<td>Postnasal drip</td>
<td>Blister under tongue;</td>
<td>Nasal obstruction</td>
<td>Sore throat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pharynx injected</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>Cervical</td>
<td>Cervical</td>
<td>-</td>
<td>Cervical</td>
<td>L axilla</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurologic signs</td>
<td>Stupor</td>
<td>Carpopedal spasm</td>
<td>+</td>
<td>Drowsy</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Apprehension</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Weight loss</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anorexia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blurred vision</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lacrimation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photophobia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal signs</td>
<td>-</td>
<td>Pain L-upper quadrant;</td>
<td>Spasm</td>
<td>Diarrhea</td>
<td>Indigestion, flatulence, belching</td>
<td>Epigastric tenderness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea, vomiting</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Enlarged spleen</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chest radiographs</td>
<td>R-upper; ~Abscess</td>
<td>R-lower; ~Abscess</td>
<td>R-upper, ~Abscess</td>
<td>Clear</td>
<td>L-middle, ~Abscess</td>
<td>L-lower, pneumonitis</td>
<td>L-hilum ~Abscess</td>
<td>Clear</td>
</tr>
</tbody>
</table>

*(Table 8-2 continues)*
Glanders

Survival is likely if early and long-term effective therapy is instituted. Even with treatment, clinical symptoms may continue several months before complete resolution, particularly if treatment is delayed. Radiographic imaging is useful to monitor pulmonary infection. Early radiographic signs are typically infiltrative or support early abscess formation. Segmental or lobar infiltrates are common. With time, pulmonary abscesses tend to undergo central degeneration and necrosis, which radiographically resembles cavitation, and these may be single or multiple. Unilateral or bilateral bronchopneumonia may be seen, as well as a smattering of miliary nodules. Because of the potential for disseminated disease, survival is likely if early and long-term effective therapy is instituted. Even with treatment, clinical symptoms may continue several months before complete resolution, particularly if treatment is delayed. Radiographic imaging is useful to monitor pulmonary infection. Early radiographic signs are typically infiltrative or support early abscess formation. Segmental or lobar infiltrates are common. With time, pulmonary abscesses tend to undergo central degeneration and necrosis, which radiographically resembles cavitation, and these may be single or multiple. Unilateral or bilateral bronchopneumonia may be seen, as well as a smattering of miliary nodules. Because of the potential for disseminated disease,

<table>
<thead>
<tr>
<th>WBC</th>
<th>Normal-low; neutropenia</th>
<th>Normal</th>
<th>High; neutrophilia</th>
<th>High to normal to low; Neutrophils</th>
<th>Normal to high-normal; Neutrophils</th>
<th>Normal, L-shift; atyp mono, lymph</th>
<th>Normal late in disease</th>
</tr>
</thead>
</table>

**Primary site** | Pulmonary | Pulmonary | Pulmonary | Unknown | Pulmonary | Pulmonary | Pulmonary | Cutaneous |
**Disseminated** | **Secondary sites** | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown |

**Likely route of entry** | Inhalation | Inhalation | Inhalation | Inhalation | Inhalation | Inhalation | Inhalation | Percutaneous |
**Sputum/throat culture** | - | - | - | - | - | - | + | NA |
**Blood culture** | - | - | - | - | - | - | - | + at 2 mos |
**Isolation of organism** | - | - | - | - | - | - | + | + |
**CFT positive** | Day 50 | Day 50 | Day 12 | Day 40 | - | - | - | NA |
**Agglutinin positive** | Day 50 | Day 50 | Day 5 | Day 23 | Day 22 | Day 23 | Day 19 | NA |
**Mallein test positive** | Day 58 | Day 58 | Day 21 | Day 18 | Day 18 | Day 72 | - | NA |
**Successful treatment** | Sulfadiazine 10 days | Sulfadiazine 10 days | Sulfadiazine 36 days | Sulfadiazine 20 days | Sulfadiazine 20 days | Sulfadiazine 20 days | Sulfadiazine 20 days | Aureomycin 28 days | Doxycycline 6.5 mos |
**Onset of antibiotic** | Day 60 | Day 60 | Days 2, 15, 115 | Day 18 | Day 16 | Day 9 | Day 21 | ~ 5 wks |
**Recovery time post trx** | 21 days | Immediate | 188 days | 12 days | 15 days | Immediate | Immediate | > 6.5 mos |

---

*Shaded elements in the table represent the first signs and symptoms according to the medical records of the first seven patients and according to the eighth patient's published case description.

†Patients 1 through 7: Data from original case files. WBC deviations involved only neutrophils. Absolute lymphocyte counts were all normal. Patients 1 and 2: Glanders as a differential diagnosis was delayed. CFTs positive > 10 months, agglutinin titers positive > 10 months, mallein positive > 16 months.

Patient 3: First sulfadiazine treatment was halted because of falling sedimentation rate; two more treatments followed at onset days indicated.

Patient 4: See “Patients 1 through 7” note above.

Patient 5: Eleven normal complete blood counts except occasional slight relative lymphocytosis; lymphadenopathy also at axillary, epitrochlear, and inguinal.

Patient 6: Patient did not take temperature but felt feverish. Agglutinin test considered positive due to titers rising from zero to 1:320.

Patient 7: Previously unpublished case. Early WBC cytology showed transient atypical monocytes and lymphocytes (atyp mono, lymph).


‡Temperature ranges represent the span of recordings that exceeded normal.

§CFTs were considered positive if >/= 1:20.

¥Agglutinin titers were positive if >/= 1:640 because of background titers in healthy patients of up to 1:320.

¶Onset of antibiotic refers to the day of disease that the successful antibiotics were started; Patient 8 received two prior unsuccessful courses.

+ positive or present
- negative or not present
[blank]: not reported or no mention
CFT: complement fixation test
mos: months

WBC: White blood cell
post trx: Posttreatment
posttreatment: Posttreatment
wks: Weeks
computed tomography scan is useful for monitoring deep tissues and visceral organs.

Complete blood count and chemistry studies for glanders patients vary depending on the disease's location of infection and duration, and the degree of dissemination or septicemia. Complete blood count may be normal early and throughout the pretreatment disease course. Based on the laboratory-acquired cases, deviations in the white blood cell count typically involve only the absolute neutrophil count rather than other cell lines (Table 8-2). Neutropenia or neutrophilia, with or without a left shift, may be transient findings. Leukopenia with mild to moderate relative lymphocytosis was seen in three of the six laboratory-acquired infections reported by Howe and Miller, which may be attributed to a low absolute neutrophil count. Absolute lymphocyte counts were consistently within normal limits.

Historically, mortality rates have been reported to be 95% without treatment and up to 50% with treatment. A more recent analysis estimates the mortality rate for localized disease is 20% when treated, and the overall mortality rate is 40%. Since the near eradication of glanders and the development of effective antibiotics, even these may be high estimates. Successful cure was achieved in 100% of the eight US laboratory-acquired cases, despite three of the eight cases (37%) experiencing a delay in effective treatment of 2 months. A brief period of “apparent recovery” is a common clinical feature that can easily lead to delayed treatment and complications. Four of the eight patients were successfully treated with sulfadiazine for at least 20 days. The first two who received delayed treatment still recovered with only 10 days of sulfadiazine, yet recovery was protracted. The most recent patient (patient 8) had disseminated disease, which included abscesses of the spleen and liver, and required ventilatory assistance before improving on a prolonged course of several antibiotics. These recent cases imply that prognoses range from good with localized infection and prompt treatment to guarded with septicemic infection.

**Laboratory Diagnosis**

**Morphology and Growth Characteristics**

A definitive glanders diagnosis in humans occurs when the organism is isolated in culture and correctly identified. In endemic regions, phenotypic characteristics such as colony and cell morphology in combination with biochemical assays may still be a practical means to definitively diagnose glanders. These methods may take 2 to 7 days to confirm a diagnosis. Gram stains alone of pus from lesions may be productive, but microorganisms are generally difficult to find or isolate. *B mallei* can be cultured and identified with standard bacteriological media. In potentially contaminated samples, supplements to inhibit the growth of gram-positive organisms (eg, crystal violet, bacitracin, penicillin) and some gram-negatives (eg, polymyxin B) and facilitate selective isolation of *B mallei* can be useful. The optimum growth temperature is approximately 37°C. Growth is typically slow on nutrient agar, but is more rapid (2 days) when enhanced with 1% to 5% glucose and/or glycerol, and on most meat infusion nutrient media. *B mallei* colonies typically are smooth and about 1 mm in width, white (turning yellow with age), semitranslucent and viscid on Loeffler’s serum agar and blood agar. After incubating for 3 days on sterile potato slices, growth appears as a shiny, moist, yellowish transparent film. Selective differentiation from the related organisms *B pseudomallei* and *Pseudomonas aeruginosa* may be achieved by examining the following phenotypes. Whereas *B mallei* does not grow at 42°C or at 21°C or in the presence of 2% sodium chloride, *B pseudomallei* and *P aeruginosa* do. Also, it has been reported that *B mallei* does not grow on MacConkey agar, whereas both *B pseudomallei* and *P aeruginosa* grow. However, others found that *B mallei* strains grew on this agar as nonlactose fermenting colonies.

*B mallei* is a small, nonsporulating, aerobic gram-negative bacillus approximately 2 to 4 µm long and 0.5 to 1 µm wide. It is nonmotile, a characteristic that differentiates it from related organisms such as *B pseudomallei*. The presence of a thick polysaccharide capsule can be demonstrated on the surface by immuno-electron microscopy. In the presence of nitrogen, the organism can grow as aerobic and facultative anaerobe. Size may vary by strain and by environmental factors including temperature, growth medium, and age of culture. Organisms from young cultures and fresh exudate or tissue samples typically stain in a bipolar fashion with Wright stain and methylene blue. Organisms from older cultures may be pleomorphic. In vivo *B mallei* is found most often to be extracellular. Since the disease is rare, samples should be designated as “glanders suspect” until confirmed by more extensive testing. Sample security to include appropriate chain-of-custody documentation is also prudent for all samples.

**Isolation**

The isolation of *B mallei* in culture is the gold standard for a glanders diagnosis. However, it can be difficult to obtain clinical specimens harboring viable bacteria; and invasive techniques, such as aspiration
and biopsy, may be required. Even then, *B mallei* bacteria are often difficult to find, even in acute abscesses. Although isolation from blood has sometimes been successful in acute human cases, blood cultures are frequently negative until the disease’s terminal stages and do not generally appear to be a reliable indicator of infection, at least in animals such as NHP (Patricia L Worsham, David M Waag, and Taylor B Chance, USAMRIID, Fort Detrick, MD, unpublished data, 2013; Samuel L Yingst and Mark J Wolcott, USAMRIID, Fort Detrick, MD, unpublished data, 2013).24,44,56 To amplify the presence of low numbers of bacteria in normally sterile sites, animal inoculation methods were often used previously. However, such studies are impractical and inadvisable now for several reasons:

- the time required for disease to manifest;
- logistical requirement for special containment facilities;
- stringent current animal regulatory requirements; and
- adverse public reception of such animal work.

Isolation of the agent in nonendemic regions or from potentially contaminated samples may require use of selective media. Several media are commercially available for isolation of human *Burkholderia* pathogens, such as BCA (*Burkholderia cepacia* agar also referred to as PC [*Pseudomonas cepacia*] agar), OFPBL (Oxidative-Fermentative-Polymyxin B-Bacitracin-Lactose agar), and *Burkholderia Cepacia* Selective Agar. These and other media have been described previously.145–147 For example, BCA was originally developed as a selective medium to isolate *Burkholderia cepacia* complex from the sputum of individuals with cystic fibrosis. Commercial preparations typically contain crystal violet, bile salts, ticarcillin, and polymyxin B (Remel, Lenexa, KS). It was found to be sensitive and selective for both *B pseudomallei* and *B mallei*.146,147 OFPBL agar is another commercially available selective medium used to isolate *B cepacia* from cystic fibrosis patients. It permitted growth of 80% of strains tested, even though the colonies are very small and translucent; the growth often causes the agar to change from green to yellow due to lactose fermentation.146 OFPBL can be used in conjunction with (but not in place of) BCA agar for selective isolation of *B mallei* or *B pseudomallei*. Although both media are significantly discriminating for *B mallei* and *B pseudomallei*, they are not totally selective and strains of *B cepacia*, other *Burkholderia*, and some non-*Burkholderia* organisms can be expected to be isolated with them.146

**Identification**

**Biochemical Identification.** Automated biochemical kits are available commercially, such as API 20NE (bioMerieux, Durham, NC), RapID NF (Remel, Lenexa, KS), VITEK (bioMerieux, Durham, NC), and Biolog Inc phenotype microarray systems; however, they have often misidentified the *Burkholderia*. In 2012, USAMRIID Supervisory Research Microbiologist Mark Wolcott relayed in several written and oral communications that *B mallei* and *B pseudomallei* have been misidentified as nonpathogenic bacteria or other pathogens such as the *B cepacia* complex or *Pseudomonas*.24,56,148 Another drawback is that many of these methods require the organism to be cultured in vitro before testing, resulting in a delay in the diagnosis.

The MIDI Sherlock Microbial Identification System (Microbial Identification System, version 4; MIDI Inc, Newark, DE) can identify isolates of the *Burkholderia* by gas–liquid chromatography of cellular fatty acids. Inglis obtained good results using MIDI with *B pseudomallei*; and gas–liquid chromatography was used to correctly classify the *B mallei* from the most recent case of human glanders in the *Burkholderia*.36,150 However, the bacteria must first be cultured under specific standardized conditions, and sample preparation is laborious and time-consuming. It does not usually allow speciation of the *Burkholderia* because of, for example, the highly similar cellular fatty acid compositions.53 Yet specific fatty acids and derivatives, such as methyl esters, are being identified that appear to be *Burkholderia* species-specific.148,151

**Nucleic Acid-based Identification**

A major obstacle to isolating the pathogenic *Burkholderia* directly from samples is their low concentration in tissues and biological fluids of infected hosts. The development of methods for the reliable detection of glanders that does not rely on isolation of the organism is especially important for the diagnosis of chronic glanders. Therefore, many attempts have been made to develop indirect assays, such as nucleic acid analysis. The genomes of at least nine strains of *B mallei* have been sequenced,352 and the data are being used to enhance the ability to specifically identify this microorganism and increase understanding of how *B mallei* interacts with its host.35 Several nucleic acid-based diagnostic methods can confirm specific identification of *B mallei*,...
often within several hours. Whereas some of the DNA-based procedures reported could be performed directly on clinical samples, others required preliminary culturing to isolate the bacteria. Many of the methods include polymerase chain reaction (PCR)-based and DNA gene sequencing-based assays. The latter have included the 16S and 23S rRNA-encoding genes, S21 ribosomal protein gene loci sequences, and MLST procedures. For instance, Frickmann et al showed that the sequence comparison of a 120 base pair ribosomal protein S21 gene fragment was useful as a diagnostic procedure for the discrimination of *B mallei* and *B pseudomallei* from the nonpathogenic *B thailandensis* and several other environmental species of *Burkholderia*, but it did not differentiate between *B mallei* and *B pseudomallei*. Gene fragments and a single nucleotide polymorphism in the 16S rRNA gene have been reported to differentiate *B mallei* from *B pseudomallei*. Analysis of the 16S ribosomal RNA gene sequence analysis identified *B mallei* from other *Burkholderia* species in the 2000 US laboratory-acquired infection. However, it will be necessary to analyze many different species of *B mallei* and *B pseudomallei* (and other species) to establish the specificity of these and similar single-locus typing procedures. For discrimination of the closely related species *B mallei* and *B pseudomallei*, sequencing of the entire 16S rRNA gene, sequencing of the 16S-23S rRNA intergenic spacer, or the addition of a specific PCR or other genotyping method to a 16S rRNA gene fragment test has been recommended.

PCR-based techniques and DNA gene sequencing are increasingly used in clinical settings and public health laboratories for bacterial identification. Automation of sequencing and improved reagents have also reduced the cost per test and the time required for identification. Furthermore, because killed bacteria or their templates may be used, these techniques also have the advantage of reducing the risk of exposure and infection to laboratory personnel compared to conventional methods. The current interest in biowarfare defense research prompted an increased capability based on recent publications. Numerous PCR assays have been described, some of which were recently described in detail in a review on PCR methods. They target various genetic elements, such as specific insertion sequences or single nucleotide polymorphisms, secretion system genes, and flagellar biosynthetic genes. Most of these assays require evaluation using many more diverse strains of the pathogens *Burkholderia* and related/unrelated species and testing for diagnostic applicability in a controlled infection study, such as an animal natural history study, for full validation. For instance, a real-time PCR assay, BurkDiff, was designed to target a unique conserved region in the *B mallei* and *B pseudomallei* genomes containing a single nucleotide polymorphism that differentiates the two species. Assay sensitivity and specificity were assessed and confirmed by screening BurkDiff across 469 isolates of *B pseudomallei*, 49 isolates of *B mallei*, and 390 nontarget isolates. The agreement of results with traditional identification methods and lack of cross-reactivity prompted the suggestion that BurkDiff may be a robust and specific assay for the detection and differentiation of *B mallei* and *B pseudomallei*; however, test results may be difficult to interpret and the assay must be assessed for its diagnostic applicability in controlled models for *B mallei* infection.

Several PCR assays have targeted genes of the T3SS or other secretion systems; these multigenic systems have been shown to be important in the pathogenesis of *B mallei* and *B pseudomallei*. Two assays based on the bimA (*Burkholderia* intracellular motility A) gene of the type V secretion system were developed. It was demonstrated that the N-terminal nucleotide sequence of bimA contains a unique *B mallei* region not present in the *B pseudomallei* and *B thailandensis* bimA genes, as verified in tests with numerous strains of these three species. The value of the assays for early, rapid, and specific diagnosis of glanders in mice infected by the aerosol route was shown. However, it was reported later that the bimA of *B pseudomallei* strains from Australia contain an N-terminus identical to that in *B mallei* bimA and a *B thailandensis*-like strain was detected in a bimA-based PCR assay. Several multiplex PCR assays that target several and partially alleviate these issues have been developed. For example, Lee, Wang, and Yap described a sensitive and specific multiplex PCR using a short variable copy repetitive sequence, a metalloprotease gene fragment, and a sequence unique to *B thailandensis* that could distinguish *B mallei*, *B pseudomallei*, and *B thailandensis*. Koh et al separated the species *B mallei*, *B pseudomallei*, *B thailandensis*, and the *Burkholderia cepacia* complex by using four specific primers: (1) a putative sugar binding protein, (2) a hypothetical protein, (3) a putative outer membrane protein, and (4) 16S rDNA.

Other DNA-based techniques using pulsed-field gel electrophoresis and ribotyping have been used to identify strains of *B pseudomallei* and differentiate...
their virulence; these methods have not been tested with *B mallei* and would likely be more time and labor intensive than gene sequencing. More recently, improved in silico probe design based on short unique regions of the target genome has aided in the potential use of microarray procedures to selectively distinguish *B mallei* and *B pseudomallei* genetically; more microarray tests of these genetic targets with many bacterial strains are needed.

**Immunological Detection**

Immunoassays that detect the presence of specific microorganisms can be useful in disease diagnosis. No such tests specifically for *B mallei* are established despite advancements in immunodetection of *B pseudomallei*. Similar efforts to develop *B mallei*-specific immunodetection methods are important because antibiotics that are efficacious for one disease might not be effective for the other. mAbs elicited by antibiotics that are efficacious for one disease might not be effective for the other. mAbs elicited by *B mallei* whole cells and that targeted the LPS have been described. They appeared to be specific for *B mallei*, failing to recognize *B pseudomallei*, and might be useful reagents for a direct immunoassay for *B mallei*. In related studies, two groups developed large panels of mAbs to capsule polysaccharide and LPS that were specific for *B mallei*, *B pseudomallei*, or both and demonstrated strong binding to the bacteria. The study by Zou et al also revealed additional mAbs of possible diagnostic value, specifically, a pathogenicity-linked antigen epitope(s) on capsule-like polysaccharides found only in the pathogenic species of *Burkholderia* (both *B mallei* and *B pseudomallei*), and several *B mallei* LPS-specific mAbs. It is possible that by using a combination of mAbs from different antigen groups, different strains of *B mallei* and *B pseudomallei* can be effectively differentiated from each other and from nonpathogenic *Burkholderia* species.

**Serologic Diagnosis**

Although serological tests have been developed for diagnostic use in equines, no such tests exist to identify glanders specifically in humans. The mallein skin test has been primarily used to detect glanders in horses. A human version of the skin test was of little diagnostic value because of the multiweek delay to obtain a positive result. However, modified tests yielded somewhat improved results. In eight laboratory-acquired, confirmed cases of human glanders in the United States, the test was negative in two, not completed in one, and first positive in five on days 18 to 72 postinfection. Overall, it appears that this diagnostic test for human glanders is minimally useful.

In vitro tests to include the indirect hemagglutination assay (IHA) and complement fixation test (CFT) have been used for serologic glanders detection. The IHA, which is the most frequently used serological test for human melioidosis, can also be used to identify glanders cases. In melioidosis testing, the failure of the IHA to detect antibody responses despite culture-confirmed disease has been observed. The CFT is still used universally in veterinary medicine as a reasonably reliable and low-cost procedure for animal glanders diagnosis. However, the CFT can be nonspecific and may not detect all cases or stages of glanders. In addition to occasional false negative results, it has also produced frequent false positive results (low specificity) and been hampered by inhibitory effects on complement of sera. The major problem leading to the low sensitivity and specificity of the CFT and other in vitro procedures has been linked to the test antigens currently used, that is, crude preparations of whole cells. Use of such antigens has led to frequent false positive results resulting from cross-reactive antigens. To address these shortcomings, numerous new tests have been reported for animal diagnosis (described below); however, improved assays are also clearly needed for human glanders serodiagnosis.

Several assays and reagents have been described recently for the improved serodiagnosis of human glanders. Waag et al developed a whole cell enzyme-linked immunosorbent assay (ELISA) using irradiation-killed *B mallei*. The test identified patients that have melioidosis or glanders (and excluded other differential diagnosis candidates, ie, anthrax, brucellosis, tularemia, Q fever). Similarly, Parthasarathy et al developed a polysaccharide microarray using extracted CPS and LPS to facilitate specific detection of *B pseudomallei* and *B mallei* antibodies in animal and human sera. However, neither assay can discriminate between the two *Burkholderia* diseases because of serological cross-reactivities. By using bioinformatic or similar state-of-the-art approaches, others have identified *B mallei*-specific proteins that have potentially improved prospects for glanders-specific serodiagnosis. For example, Varga et al used pre-and postexposure sera from 2,000 cases of human glanders and a protein array platform made for studying melioidosis patients to characterize the human immunological response to *B mallei*. Significantly increased antibody responses to 17 of 156 peptides were detected and antibodies to only two (a pilus biosynthetic protein and 50S ribosomal protein) were shared between the two diseases, implying that the human antibody response to *B mallei* is markedly distinct from that to *B pseudomallei*. The results of these recent studies
suggest that these antigens may be useful for an improved glanders diagnosis; however, additional studies to include nonspecific and glanders-specific sera from human (and animal model) sources are needed.

Other Identification Methods

New methods to verify identification of isolated organisms as exemplified by phage-based identification have been described. Bacteriophages have been isolated which exhibit infectivity and high specificity for \( B \) mallei. For instance, \( \phi \)E125 and \( \phi \)1026b infect \( B \) mallei, but not \( B \) pseudomallei.\(^{196,197} \) Efforts to develop phage variants with species-specific receptors offer the potential for convenient phage-based diagnostics. Mass spectrometry methods, such as matrix-assisted laser desorption/ionization mass spectrometry and Raman spectroscopy, are being exploited for the identification of \( B \) mallei and \( B \) pseudomallei.\(^{198,199} \) For example, signatures specific to pathogens at the species level were developed to evaluate a Raman chemical imaging spectroscopy method for reagentless detection and identification.\(^{198} \) Raman chemical imaging spectroscopy combines Raman and fluorescence spectroscopy and digital imaging to allow detection of low levels of biothreat organisms in the presence of complex environmental backgrounds without prior amplification methods. Raman spectra for viable selective agents and toxins including \( B \) mallei and \( B \) pseudomallei were reported; however, most of the studies were conducted to distinguish \( Burkholderia \) from other pathogens (eg, \( B \) anthracis) and more efforts to distinguish near-neighbors of \( B \) mallei are needed. Other new assays for direct detection of the organism in culture and tissues and that use probes for surface or secreted antigens of the pathogenic \( Burkholderia \) are also being evaluated.\(^{133,200} \) One example is a fluorescence in situ hybridization assay that correctly identified all of the \( B \) mallei, \( B \) pseudomallei, and \( B \) thailandensis strains.\(^{193} \) However, the assay requires a relatively high bacterial concentration; thus, fluorescence in situ hybridization analysis may be more useful for evaluating tissue sections in pathogenesis studies than for diagnosis of direct clinical samples.

New combination methods are being developed. An assay coupling biothreat group-specific PCR with electrospray ionization mass spectrometry and using DNA extracts from killed bacteria correctly identified seven bacterial biothreat bacterial species (including \( B \) mallei and \( B \) pseudomallei) to the genus if not species level.\(^{201} \) A gas chromatography-mass spectrometry method was used to identify several cellular fatty acid methyl ester fragments that could differentiate the species \( B \) mallei, \( B \) pseudomallei, and \( B \) thailandensis.\(^{148} \)

Diagnosis in Equids

Several diagnostic tests have been developed and used extensively for glanders diagnosis in equines;\(^{24,53} \) Khan et al provides a comprehensive review and evaluation of diagnostic tests developed previously as well as an overview of recent assays aimed at improving the sensitivity and specificity of glanders diagnosis.\(^{24} \) Tests used currently consist primarily of serodiagnostic assays, that is, the CFT and the mallein skin test. Serum anti-\( B \) mallei antibodies are detected by the CFT within a week of infection, and the CFT can detect carriers and animals chronically infected with \( B \) mallei;\(^{24,189} \), it is the only mandatory serological test for international trade of equids, but it can be hampered by the problems detailed in the previous section.\(^{22,187} \) The mallein test is another well-known and established test used for glanders diagnosis in animals and involves injecting a purified protein derivative of the \( Burkholderia \) glycoprotein mallein intradermally. An immune cellular response as manifested by a delayed type hypersensitivity reaction is observed and considered diagnostic for glanders.\(^{24,177,182} \)

More recently developed assays include competitive and indirect ELISAs using \( B \) mallei-specific monoclonal Abs, ELISAs with purified recombinant proteins used as test antigens, and Western blot techniques.\(^{189,202,203} \) Using sera from horses in an endemic region of South East Asia, Elschner and Khan et al reported the significantly improved specificity and sensitivity of the CFT when it was combined with a complement independent immunoblot technique, an approach that increased both the detection rate and specificity of the test for glanders serodiagnosis,\(^{189,202} \) Kumar et al cloned a novel recombinant \( Burkholderia \) intracellular motility A (rBimA) protein and used it with many positive and potentially negative serum samples in an indirect ELISA to detect equine glanders.\(^{203} \) The results revealed 100% sensitivity and 98.9% specificity. Also, rBimA protein did not react with melioidosis patient and normal healthy human serum samples, and thus showed high specificity.

Treatment

Because human cases of glanders are rare, limited information exists regarding the use of modern antibiotic treatment for humans. \( B \) mallei infection responds to antibiotic therapy; however, recovery may be slow after a delayed diagnosis or with disseminated disease. Reports in the scientific literature indicate that most strains of \( B \) mallei are susceptible to the following antibiotics in vitro:
Glanders

- amikacin,
- netilmicin,
- gentamicin,
- streptomycin,
- tobramycin,
- azithromycin,
- novobiocin,
- piperacillin,
- imipenem,
- ceftazidime,
- tetracycline,
- oxytetracycline,
- minocycline,
- doxycycline,
- ciprofloxacin,
- norfloxacin,
- ofloxacin,
- enrofloxacin,
- erythromycin,
- sulfadiazine,
- trimethoprim/sulfadiazine,
- trimethoprim/sulfmethoxazole (co-trimoxazole), and
- amoxicillin-clavulanate (co-amoxiclav).

Aminoglycosides and other antibiotics incapable of penetrating host cells probably will not be useful in vivo because B mallei is a facultative intracellular pathogen.204,205,211 Susceptibility to streptomycin and chloramphenicol in vitro has been inconsistent, with some reporting sensitivity and others reporting resistance.7,205,209,212 B mallei is susceptible to the lytic action of human granulysin, a broad-spectrum antimicrobial peptide, and silver containing compounds.213,214

Most B mallei strains exhibit resistance to the following antibiotics:

- amoxicillin,
- ampicillin,
- penicillin G,
- bacitracin,
- chloromycetin,
- carbenicillin,
- oxacillin,
- cephalothin,
- cephalaxin,
- cefotetan,
- cefuroxime,
- cefazolin,
- ceftriaxone,
- metronidazole, and
- polymyxin B.7,11,47

In addition, a study focused on 41 isolates of B mallei obtained from various outbreaks of equine glanders occurring between 1999 and 2006 in Punjab, Pakistan, reported that less than 50% of the isolates were resistant to oxytetracycline, roxithromycin, and norfloxacin, and less than 40% were resistant to ciprofloxacin.212 Consistent with the published literature, all of the Pakistani isolates were susceptible to co-amoxiclav, chloramphenicol, doxycycline, gentamicin, and trimethoprim-sulfamethoxazole, and approximately 95% were susceptible to both enrofloxacin and ofloxacin.

Antibiotics have been tested against glanders in hamsters, mice, equids, guinea pigs, and monkeys.207,208,211,215–217 Sodium sulfadiazine—but not penicillin or streptomycin—was effective for treating acute glanders in hamsters.208 Doxycycline and ciprofloxacin were also examined in the hamster model of glanders.211 Doxycycline therapy was superior to ciprofloxacin therapy, but relapse did occur in some of the treated animals 4 to 5 weeks after challenge. Hamsters were also infected subcutaneously or by aerosol with B mallei and were treated with ofloxacin, biseptol, doxycycline, and minocycline.215 All of the antibiotics exhibited some activity in animals challenged subcutaneously, but ofloxacin was superior. None of the antimicrobials demonstrated appreciable activity against a high dose of B mallei delivered by aerosol, but doxycycline provided 70% protection against a low dose delivered by this route.214

Ceftazidime and levofloxacin were examined as treatments for glanders in BALB/c mice infected intranasally with B mallei ATCC 23344.218 Despite good in vitro activity against B mallei, intraperitoneal delivery of the antibiotics failed to eradicate the organism and resulted in the development of nonlethal, chronic glanders. More recent studies using a BALB/c model of inhalational glanders reported that oral administration of co-trimoxazole twice daily for 14 days prevented the development of acute disease, but was not able to completely eliminate B mallei and ultimately resulted in the establishment of a chronic infection.89 Co-trimoxazole is recommended for postexposure prophylaxis in humans; however, an extended course of therapy of up to 21 days is likely to be indicated.89,219,220

In most countries, strict regulations mandate that animals testing positive for glanders are destroyed rather than treated. Given the difficulties in implementation of such regulations in some countries along with the high monetary value of horses in equestrian sport and breeding, the usefulness of modern antibiotics for treatment or postexposure prophylaxis of horses has recently been reevaluated. In a 2012 report Saqib et al described the effectiveness of antibacterial therapy on 23 culture positive horses involved in a
confined glanders outbreak at the Lahore Polo Club in Pakistan.\textsuperscript{37} A treatment protocol was implemented consisting of once daily parenteral administration of enrofloxacin and trimethoprim/sulfadiazine followed by twice daily oral administration of doxycycline for 12 weeks (84 days). All horses showed a marked improvement during the initial week of treatment. Abatement of fever, renewal of appetite, and healing of ulcerated nasal septa were reported by the end of week 1. Nodules and ulcers were cleared in most horses by the end of week 3. No clinical signs were observed in any horse following week 12. To confirm the absence of disease, immunosuppression was induced (by daily corticosteroid injection for 10 days) in six randomly selected horses starting on day 90 following completion of therapy. No recrudescence of disease as determined by a lack of clinical signs was observed. By 8 months posttreatment, the IHA titers of all of the horses were considered to be negative (≤1:320). At the time of the report (September 2012), nine of the horses in the study were still at the Lahore Polo Club and remained healthy; the status of the remaining eight was unknown. Whereas the findings of the Saqib et al are promising, the widespread treatment of glanderous horses is unlikely to replace the practice of “testing and culling” because the latter is a more cost-effective and efficient means of containing the disease spread. This study does, however, provide important information that may be applicable in certain situations where treatment is desirable.

The majority of human glanders cases occurred before the antibiotic era, and more than 90% of cases resulted in death.\textsuperscript{221} Several human glanders cases have been recorded since the 1940s, primarily in laboratory workers, and these have been successfully treated with antibiotics.\textsuperscript{5,56,222,223} Sulfadiazine was used successfully in the first six US laboratory-acquired infections.\textsuperscript{5} The seventh was successfully treated with the tetracycline compound, aureomycin. Two additional cases were successfully treated with sulfadiazine in 1949 and 1950.\textsuperscript{222} Disseminated glanders in a stablehand who had only indirect contact with horses was also treated successfully with aureomycin in Austria in 1951.\textsuperscript{35} Streptomycin was used to treat a patient infected with \textit{B. mallei} and \textit{Mycobacterium tuberculosis}.\textsuperscript{222} Treatment with streptomycin reportedly cured the glanders, but had little effect on the bone’s tuberculosis in this patient. In a recent case of laboratory-acquired glanders, the patient received imipenem and doxycycline intravenously for 1 month followed by oral azithromycin and doxycycline for 6 months.\textsuperscript{66} Susceptibility testing of the \textit{B. mallei} isolate in this case demonstrated sensitivity to the former two drugs, although retrospective susceptibility testing found that the organism was resistant to azithromycin.\textsuperscript{51} Diagnostic imaging of the patient’s splenic and hepatic abscesses through the 6-month course showed their near complete resolution.

No Food and Drug Administration-approved therapy for glanders exists. Recommendations for antibiotic therapy depend on the infection site and severity. Localized disease should be treated with at least a 2-month—and preferably a 6-month—course of antibiotics based on sensitivity. Without susceptibility test results and for mild disease, oral doxycycline (100 mg twice/day) plus trimethoprim-sulfamethoxazole (4 mg/kg/day in two divided doses) for 20 weeks is recommended.\textsuperscript{224} Historically, oral chloramphenicol has been added to this regimen for the first 8 weeks; however, based on recent recommendations for melioidosis treatments, this may no longer be necessary. Amoxicillin/clavulanate is the recommended alternative for pregnant women and children or individuals that cannot tolerate trimethoprim-sulfamethoxazole. For severe disease ceftazidime at 40 mg/kg intravenously (IV) every 8 hours or imipenem IV at 15 mg/kg every 6 hours (maximum 4 g/day) or meropenem at 25 mg/kg IV every 8 hours (maximum 6 g/day) plus trimethoprim-sulfamethoxazole at 8 mg trimethoprim/kg per day IV in four divided doses is recommended. Intravenous therapy should be continued for at least 14 days and until the patient is clinically improved. Oral maintenance therapy as for mild disease can be continued from that point.\textsuperscript{224} Combined therapy for at least the first month should be considered for patients even with the mildest of systemic symptoms. For visceral and severe disease, prolonged treatment up to a year is recommended. Abscesses may be surgically drained, depending on their location.\textsuperscript{68} Because of the intractable nature of glanders, long-term follow-up and possibly prolonged, tailored therapy is indicated for infections that are slow to clear. Patients should be followed at regular intervals for at least 5 years after recovery. Diagnostic imaging is useful to follow the reduction and resurgence of abscesses, serology may help to monitor the clearing of antibody, and inflammatory markers may also suggest resurgence of a latent infection. Patients should be advised of the lifelong risk of relapse and to alert their healthcare providers of their previous history, particularly if they develop a febrile illness. This situation becomes even more important when potentially dealing with a genetically engineered strain of \textit{B. mallei}. Current postexposure prophylaxis recommendations for laboratory workers consist of oral trimethoprim-sulfamethoxazole for 21 days.\textsuperscript{219,220,224} If the patient is allergic to or intolerant of this, or the organism is known to be resistant to the first choice, the second-line choice is oral amoxicillin/clavulanic acid (co-amoxiclav) for 21 days.\textsuperscript{219}
Prophylaxis

Host Immunity

*B. mallei* can establish chronic or acute infections in multiple species, which suggests that native host immune responses are unable to eradicate wild type organisms upon initial challenge. In addition, no evidence indicates previous infection provides immunity against glanders. Infections in horses that appeared to symptomatically recover from glanders would recrudesce when the animals were challenged with *B. mallei*. Inoculation of *B. mallei* into chronically infected horses produced at least local infections most of the time, and occasionally a manifestation of classic glanders. Similar to equines, protective immunity in humans after recovery from glanders is not believed to occur. In an 1869 human case report from Poland as told by Loeffler, one attempt at autoinoculation with the fluid from a pustule produced more pustules. Thus, patients who recover may still be susceptible, making reuse of the agent in biowarfare necessary to consider.

Immune responses to glanders appear to be complex requiring both humoral and cell-mediated immune (CMI) responses. The role of antibodies in immune protection has been investigated experimentally in animals as well as retrospectively in a recent human case. Experiments on horses regarding protective immunity have given ambiguous results. Passive immunity experimentation using equine sera has also failed. Conversely, passive immunization of BALB/c mice using *B. mallei* OPS- or CPS-specific mAbs conferred nonsterilizing protection against a lethal bacterial challenge delivered either intranasal or by aerosol. Consistent with a role for antibodies in protection against glanders, investigations by Whitlock et al involving the depletion of B cells in BALB/c mice demonstrated a significantly decreased survival time following *B. mallei* infection compared to control mice. In contrast, Rowland et al reported that when B cell knockout mice (μMT C57Bl/6 mice) were infected with *B. mallei*, survival of B cell deficient animals did not differ from wild type mice. The reason for these contrasting results is unclear, but it is possible that these differences may be a consequence of the manner in which the B cells were depleted (ie, antibody depletion versus genetic deletion) or the specific mouse models used.

Analysis of humoral immune responses from a laboratory-acquired case of glanders in 2000 indicated that *B. mallei* specific immunoglobulin A, immunoglobulin G, and immunoglobulin M levels were highly elevated (8-, 16- and 4-fold, respectively) by 2 to 4 months postinfection and then began to decline. By 14 months postinfection antibody titers returned to near baseline levels. More extensive characterization of the antibody responses in this case demonstrated reactivity of highly increased (≥2-fold compared to pre-exposure serum) antibodies with a variety of *B. mallei* and/or *B. pseudomallei* proteins including T3SS and T6SS components, type IV pili, outer membrane proteins, chaperones (eg, GroEL and GroES), and hypothetical proteins. Screening of equine glanders serum against a bacteriophage expression library revealed the presence of antibodies to a *Burkholderia Hep_Hag* autotransporter (BuHA) proteins, a family of immunodominant antigens predicted to be hemagglutinins and invasins. Such proteins represent potential candidate antigens to develop glanders diagnostics, therapeutics, and vaccines.

Since *B. mallei* is a facultative intracellular pathogen, CMI mechanisms as well as cytokine and chemokine expression, significantly contribute to the clearance of the organism from infected hosts. *B. mallei* is capable of infecting and surviving in many cell types including professional phagocytes. In a BALB/c model of glanders, neutrophils and macrophages infiltrated the spleen 5 hours postinfection and an increase in activated macrophages, neutrophils, and T cells occurred by 24 hours postinfection. When neutrophils were depleted, mice became acutely susceptible to *B. mallei* infection and succumbed within 5 days. In contrast, if mice were depleted of both CD4+ and CD8+ T cells, they did not succumb until 14 days postinfection. In wild type C57Bl/6 mice, macrophages have been shown to be important for reducing the susceptibility of the animals to pulmonary *B. mallei* infection. Monocyte chemoattractant protein-1 (MCP-1), a chemokine involved in the chemotraction of macrophages to sites of infection, plays an important role in protective immunity to *B. mallei* infection. Mice lacking either MCP-1 or the MCP-1 receptor were more susceptible to disease following IN challenge and exhibited higher bacterial burdens in organs at 3 days postinfection compared to wild type mice. Monocyte and inflammatory dendritic cell recruitment was defective in the MCP-1 knockout mice and increased numbers of neutrophils were observed in the lungs. These data support a critical role for phagocytic cells (neutrophils, monocytes and macrophages, and dendritic cells) in controlling the early, innate responses to *B. mallei* infection while T cells appear to be important later in infection.

Interferon-gamma (IFN-γ) plays an important role in macrophage activation and clearance of intracellular organisms. Examination of cytokine responses 24 hours following IP injection of female BALB/c mice with *B. mallei* exhibited a strong IFN-γ response and
elevated levels of IL-18, IL-12, IL-27, IL-6, and MCP-1.229 IFN-γ knockout mice infected with B mallei died within 2 to 3 days after infection, and uncontrolled bacterial replication in several organs confirmed a critical role for this cytokine during innate immune responses to B mallei. Similar findings were reported when specific antibodies were used to deplete IFN-γ in mice.228 The proinflammatory cytokines IL-12 and IL-18 were shown to be critical for IFN-γ production at early time points postinfection.229 Neutralization of IL-12 in vivo led to increased susceptibility of mice to lethal infection, possibly from its role in promoting IFN-γ production by natural killer cells and T cells.230 Consistent with this notion, in vitro assays have confirmed that natural killer cells and CD8+ T cells were the main cellular sources of IFN-γ generated in response to B mallei.228 Furthermore, MyD88 knockout mice (which cannot produce IFN-γ) were also highly susceptible to pulmonary challenge with B mallei compared to wild type mice.228 Treatment of MyD88 knockout mice with exogenous recombinant IFN-γ helped to restore effective immunity and significantly increased survival.228,231,232 Thus, B mallei appears to be susceptible to CMI responses promoting the expression of type 1 cytokines (eg, IFN-γ and IL-12). Although the initial burst of IFN-γ was able to control bacterial replication, clearance was not achieved.229 These studies highlight the importance of cytokine and chemokine production during B mallei infection and suggest that strategies targeting the production of IFN-γ should be considered when attempting to achieve effective vaccine induced immunity.

LPS and various microbial products can activate macrophages through TLRs and other pattern-recognition receptors. Pattern-recognition receptors signaling leads to transcription of genes encoding cytokines, chemokines, and enzymes, such as inducible nitric oxide synthase that aids in the clearance of intracellular bacteria. Purified B mallei LPS is a strong activator of human TLR4 complexes and stimulates production of tumor necrosis factor-alpha (TNF-α), IL-6, and regulated upon activation normal T-cell expressed, and secreted (RANTES) protein in human macrophages and dendritic cells.98 The expression of these cytokines and chemokines by antigen presenting cells reflects signaling through both MyD88-dependent (TNF-α and IL-6) and -independent (RANTES) pathways. Recent studies using LPS-activated macrophages and iNOS-2 knockout mice suggest that iNOS activity and the production of reactive nitric oxide species contribute to the killing of intracellular B mallei.96,227 These studies illustrate the importance of TLR4 signaling and reactive nitric oxide species in macrophage activation and clearance of intracellular B mallei. The role of other pattern-recognition receptors (eg, TLR2 and NOD-like receptors) in B mallei infection remains to be investigated.

Relatively little is understood regarding the role of T-cell subsets in controlling B mallei infections. Recent studies suggest that strong T-cell helper (Th1) polarized responses will likely be required for protection against B mallei infection.231,232 More research is needed in this area. Maximizing the effectiveness of CMI responses is predicted to limit the duration of B mallei infections and to reduce disease pathology, and understanding how innate and adaptive immune responses fail to result in complete bacterial clearance may provide clues for the design of effective vaccination strategies.

**Vaccine Candidates**

No human or veterinary glanders vaccine exists. From 1895 to 1928 numerous attempts to vaccinate horses and laboratory animals against glanders were unsuccessful. Vaccines were initially prepared by treating bacterial cells with urea or glycerin or by drying the glanders bacilli.225 For most chronically infected horses, experimental vaccination did not change the course of their illness. Experiments on protective immunity in horses have given ambiguous results.1,7 Mendelson reported guarded postvaccination success in a youth with severe ocular and oro-nasal involvement.54 Although attempts (with limited success) to develop a glanders vaccine were initiated more than 100 years ago, using modern approaches to identify virulence factors and studying the ways putative vaccines modulate the immune system may aid in developing an efficacious glanders vaccine.

Interest in glanders vaccine development has increased in recent years, mostly resulting from B mallei’s biothreat potential. Several up-to-date reviews provide further details of glanders vaccine development.233–235 The most desirable glanders vaccine will be a recombinant protein or a biochemically purified preparation that gives long-term sterile immunity when administered. As previously mentioned, since B mallei is an intracellular pathogen it is likely that both CMI and humoral responses will be critical in developing protective immunity. Activating both arms of adaptive immunity and ultimately achieving sterile immunity will be significant challenges in B mallei vaccine design. A better understanding of the correlates of vaccine-induced immunity is needed.

**Killed Whole Cells**

The initial attempts to protect mice against an aerosol-acquired infection with B mallei using either an irradiation-killed B mallei or heat-killed B pseudomallei
cellular vaccine resulted in an increased time to death, compared to controls, but spleens of survivors were not sterile.236 A nonviable B mallei cellular vaccine failed to protect mice from a parenteral live challenge.237 This vaccine stimulated a weak mixed Th1- and Th2-like immune response. This study suggested that nonviable B mallei cell preparations may not protect mice because of the failure inducing a strong Th1-like immune response. In a subsequent study, adding IL-12 to an irradiated B mallei vaccine preparation stimulated a Th1-like antibody response and induced an increase in splenocyte proliferation and IFN-γ production in comparison to mice vaccinated with killed B mallei alone.238 Following an IP challenge with B mallei, increased survival was observed in mice vaccinated with both IL-12 and killed bacteria in comparison to control mice. Sterile immunity was not achieved, and the spleens of the vaccinated survivors were enlarged and heavily infected with B mallei. More recent studies by Sarkar-Tyson et al and Whitlock et al using heat killed B mallei and/or B pseudomallei are consistent with the findings of Amemiya et al, and indicate that TNF-α, IFN-γ, and B cells are necessary for an effective immune response against the organism.239,240

**Live Attenuated Vaccines**

Three attenuated strains of B mallei, a CPS (wcbB) mutant, a branched-chain amino acid auxotroph (ilvI) mutant, and a carboxy-terminal protease (ctpA) mutant have been evaluated as vaccines in a BALB/c mouse model of glanders.77,90,131 The CPS and ilvI vaccine strains were delivered aerogenically, and mice were subsequently exposed to wild type B mallei via whole-body aerosols. Immunization with the CPS mutant resulted in a Th2-like antibody response that failed to protect mice (all animals died within 5 days of infection). In contrast, immunization with the ilvI mutant resulted in a Th1-biased immune response that conferred significant protection against lethal aerosol challenges. At 1 month postchallenge, 25% and 50% of the mice had survived high- and low-dose aerosol challenges, respectively. Analysis of bacterial loads in the organs of the surviving mice revealed high numbers (>10⁵ cfu) of B mallei in the spleens of all of the ilvI mutant vaccinated mice. B mallei was also present in the livers and lungs of most of the surviving animals, suggesting the development of a chronic infection. A B mallei ctpA mutant, which was evaluated as a live attenuated vaccine in CD1 mice, provided partial protection against an IP challenge of wild type B mallei.131 Whereas 75% of the ctpA mutant-vaccinated mice survived the 15-day postchallenge, all survivors displayed splenomegaly and high splenic loads of B mallei. A mixed Th1/Th2-like antibody response was noted. Based on these studies, it appears that live attenuated strains promoting Th1-like antibody responses may be useful as glanders vaccine candidates.

**Protein Subunit Vaccines**

Two studies by Whitlock et al describe the identification and testing of purified B mallei protein antigens as potential vaccine candidates.125,239 Immunogenic antigens were identified from genomic screens of B mallei expressed proteins. In initial studies, the candidate protein antigens included LolC (ABC transporter protein), BimA (autotransporter protein), BopA (T3SS effector protein), and Hcp1 (T6SS-1 component).239 Immunization with purified recombinant LolC, BimA, BopA, and Hcp1 alone or as a quadrivalent mixture, administered with CpG 2395 and ISCOM adjuvants, provided BALB/c mice significant protection against an IN challenge of B mallei. While the recombinant proteins protected the mice from the initial acute infection, sterile immunity was not achieved. In a subsequent study, recombinant forms of five additional B mallei proteins and GroEL were evaluated for their protective capacity in BALB/c mice.125,134 Three of these proteins and GroEL provided partial protein against an IN challenge of B mallei. Several of the highly immunogenic proteins (eg, LolC and Hcp1) evaluated in these studies are also considered promising components for melioidosis vaccine development.105,124,239

**Polysaccharide-based Subunit Vaccines**

B mallei isolates appear to be capable of expressing only a limited repertoire of structurally diverse CPS and LPS antigens.36,93,97 The protective efficacy of LPS and CPS as vaccines against B pseudomallei infection was tested in BALB/c mice and partial protection was observed.240,241 Active immunization studies using CPS or LPS for protection against B mallei have not been reported; however, it has been shown that CPS- and LPS-specific mAbs passively protect animals from challenge with B mallei.97,104 In addition, the bacterial or opsonophagocytic activities of various anti-LPS or anti-CPS mAbs appeared to correlate with their ability to passively protect mice.97,104 Such findings confirm the protective capacity of these surface exposed antigens and support the rationale for developing CPS and OPS-based glanders vaccines. However, since carbohydrates such as CPS and OPS are T-independent antigens that would be poorly immunogenic if administered in purified form, methodologies for the preparation of CPS- and OPS-protein conjugates have been developed.242-245 Immunization of mice with CPS- or OPS-based glycoconjugates resulted in the generation of high titer carbohydrate-specific antibody responses.
Depending on the adjuvant system used, Th1- or Th2-polarized antibody responses could be achieved. The protective capacity of these glycoconjugates in animal models of glanders has not been reported. Further development of these antigenically defined CPS- and OPS-based vaccine candidates is an active area of research.

**Immunotherapies**

In addition to passive immunization studies with PS-specific antibodies, activators of innate immune responses have been experimentally evaluated as potential immunotherapies for pre- or postexposure prophylaxis. BALB/c mice pre-treated with CpG-containing oligodeoxynucleotides (CpG 7909) that signal through TLR9 exhibited elevated levels of IL-6, IL-12, and IFN-γ following an aerosol challenge with _B mallei_, resulting in lower numbers of bacteria in lungs and spleen, and prolonged survival.232 Similarly, mucosal administration of cationic liposome DNA complexes (CLDC), potent activators of innate immunity, to BALB/c mice before or shortly after bacterial challenge generated nearly complete protection from inhalational challenge with 100% lethal doses of _B mallei_.86 Substantially reduced acute organ pathology was observed in CLDC-treated mice in comparison to controls. Protection was dependent on CLDC-mediated induction of IFN-γ responses in lung tissues and was partially dependent on the activation of natural killer cells. These findings suggest that preexposure or timely postexposure therapy with CpG or CLDC may help to protect individuals exposed to aerosolized _B mallei_.

**Control and Decontamination**

Historically, no vaccines were successful in protecting animals from glanders. Control and eradication of the disease has been dependent on eliminating infected horses and preventing infected horses from entering glanders-free stables. The greatest risk for glanders exposure to humans—outside of a biowarfare attack—is infected equids, particularly the asymptomatic horse. When glanders infection is considered as a differential diagnosis in countries with ongoing or completed eradication programs, local and state public health and veterinary authorities should be contacted immediately. Where human infection has occurred, potential exposure to infected equids should be investigated by a team approach involving patient care personnel, public health officials, and local veterinarians. Equids suspected as a possible human exposure source should be tested and, if positive, humanely destroyed in accordance with the local regulatory animal health authority. Facilities and transporters traced back to positive equine cases should be quarantined and disinfected in accordance with the local animal health authority. Stall bedding, feed, and manure in the vicinity of infected livestock should be burned.

In the event of deliberate release of _B mallei_, emergency response personnel entering a potentially heavily contaminated area should wear personal protective equipment, which includes Tyvek coveralls (DuPont USA, Wilmington, DE), gloves, and powered air purifying respirators. Decontamination procedures for the patient include the removal and containment of outer clothing. Such clothing should be regarded as contaminated or high risk and handled in accordance with local protocol. All waste should be managed in accordance with BSL-3 containment protocols. Patient showers are indicated, preferably in a facility for which decontamination and containment can be managed. The risk of acquiring infection from contaminated persons and their clothing is low.26 Personal protective equipment may prevent infection in those potentially exposed, including emergency responders.

Environmental contamination will decline over time as a result of sunlight exposure and drying. Monitoring highly contaminated areas is indicated, however, and the advice of foreign animal disease experts should be sought. _B mallei_ can remain viable in tap water for at least 1 month.86 _B mallei_ can be destroyed by heating to at least 55°C for 10 minutes, and by ultraviolet irradiation. It is susceptible to several disinfectants including 1% sodium hypochlorite, chlorine dioxide, 5% calcium hypochlorite, 70% ethanol, 5% Micro-Chem Plus (National Chemical Laboratories Inc, Philadelphia, PA), 2% glutaraldehyde, 1% formaldehyde, iodine, benzalkonium chloride, 1% potassium permanganate, 3% solution of alkali, and 3% sulfur-carbolic solution. Phenolic and mercuric chloride disinfectants are not recommended.7,42,247,248

Because human-to-human transmission has occurred nosocomially and with close personal contact, standard precautions are recommended. These precautions include use of disposable gloves, face shields, surgical masks, and — when appropriate — surgical gowns to protect mucous membranes and skin. Personnel, microbiological, and containment procedures for BSL-3 are advised in the laboratory. Appropriate barriers to direct skin contact with the organisms are mandatory at all times.249,250 Family contacts should be advised of blood and body fluid precautions for patients recovering at home. Barriers protecting mucus membranes, cuts and sores, and potential skin abrasions from genital, oral, nasal, and other body fluids are recommended.
Many countries have import restrictions for equids. Veterinary health authorities may require testing within a few weeks of shipment and again at the place of disembarkation, as well as documentation of the animal’s location in the exporting country for the 6 months before shipment. Restrictions vary by country and glanders-free status under the International Animal Health Code. The most current information regarding import and export should be sought from the regional animal health authority.

SUMMARY

*B. mallei* is designated as a Tier 1 select agent by the Centers for Disease Control and Prevention, since the organism is considered to present a high risk of deliberate misuse and could pose a severe threat to public health and safety. *B. mallei* also is believed to be moderately easy to disseminate, and enhancements to current diagnostic capabilities and disease surveillance are required to diagnose the disease rapidly and accurately. Given the biothreat potential associated with *B. mallei*, raising the clinical index of suspicion for glanders in humans is crucial. The rarity of recent human cases may make glanders a difficult diagnosis even in regions with exceptional medical facilities. As with many rare diseases, final diagnosis and appropriate treatment is often delayed, with sometimes disastrous results. Without a higher index of suspicion, diagnosis is warranted. Considerable research on treatments for *B. mallei* infections, the disease remains difficult to treat. Moreover, due in part to the development of chronic infections, the disease remains difficult to treat. More research on treatments for *B. mallei* is warranted. Considering an aerosol threat from a virulent strain, studies to distinguish the effectiveness of therapeutic agents for treating septicemic and pulmonary infections are indicated. The potential for prophylactic treatment regimens should be further investigated.

Significant progress has been made toward a better understanding of host immunity to *B. mallei* infection. Effective innate immune responses are essential for controlling the early phase of the infection, and monocytes and macrophages are crucial for limiting dissemination of the organism. The role of adaptive immune responses in controlling *B. mallei* infection requires more investigation, although it is evident that T cell responses will be important for vaccine-induced immunity. Efforts aimed at developing effective vaccine candidates for prevention of glanders are critical, and research is active in this area. Overcoming chronic infections and achieving the ultimate goal of sterile immunity will be challenging. Investigations focusing on determining correlates of vaccine-induced protection in both acute and chronic infection are needed.

Aerosol dissemination of *B. mallei* would likely cause disease in humans, equids, goats, and possibly cats in the vicinity. Unintentional infection may first manifest in equids or humans. Thus, public health workers should team with animal health officials in a suspected outbreak to expedite identification and control of an event. Although a formal surveillance system for glanders does not exist in the United States, local and state veterinary and public health authorities would be among the first to recognize a potential outbreak regardless of intent. These agencies would then work with the US Department of Agriculture, the Centers for Disease Control and Prevention, the Department of Health and Human Services, and the Department of Defense to control and eradicate the disease.

REFERENCES


Medical Aspects of Biological Warfare

4. Rutherford JG. Special Report on Glanders (from the Veterinary Director-General and Livestock Commissioner). Ottawa, Canada: Canada Department of Agriculture, Health of Animals Branch; 1906.


Medical Aspects of Biological Warfare


Medical Aspects of Biological Warfare


Glanders


Medical Aspects of Biological Warfare


Medical Aspects of Biological Warfare


Medical Aspects of Biological Warfare


