Chapter 6

GLANDERS

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INTRODUCTION

Glanders, a highly contagious and often fatal zoonotic disease of solipeds, including horses, mules, and donkeys, is caused by infection with the bacterium *Burkholderia mallei*. Glanders is characterized by ulcerating granulomatous lesions of the skin and mucous membranes. Disease progression and pathology in humans and horses are similar, although the clinical presentation of any two cases in the same species—even if related by direct transmission—may vary significantly.[1-4] Generalized symptoms include fever, myalgia, headache, fatigue, diarrhea, and weight loss. After infection, the organism generally travels through lymph channels, first to regional lymph nodes often causing irritation (lymphangitis, lymphadenitis) en route. Unchecked, organisms may enter the bloodstream and travel throughout the body. Without proper treatment, the disease course may range from acute and rapidly fatal to slow and protracted with alternating remissions and exacerbations.

Glanders, an old disease that was described toward the beginning of recorded history, is less commonly known by other names, including equinia, malleus, droes, and farcy. Farcy is an ancient term for a particular cutaneous manifestation of glanders that before 1882 was believed to be a separate disease of horses. With this cutaneous manifestation of glanders, nodular abscesses (farcy buds) become ulcerated, and regional cutaneous lymphatic pathways become thickened and indurated (farcy pipes) and ooze a glanders-typical yellow-green gelatinous pus (farcy oil).[5] Pure farcy without ulceration of the mucous membranes was rare—if not just a temporary stage of glanders infection—as was vice versa.[3] Humans, goats, dogs, cats, rabbits, and carnivorous predators living close to infected equids or carcasses have been naturally infected.[2,6] Camels have also been infected and are associated with human disease.[6] Naturally occurring glanders has been eradicated in most countries, but is still found in parts of Africa, the Middle East, South America, and Eastern Europe. *B mallei* 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 launched 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 shipped from the United States to the Allies were inoculated with cultures of *B mallei*.[7] In 1914 Anton Dilger, a member of the German army and an American-educated surgeon, was sent home to live with his parents in Virginia after a nervous breakdown. He brought strains of anthrax and glanders and, with his brother's help, set up a laboratory 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.

Also, 4,500 mules in Mesopotamia were infected with glanders by German agents; 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, which successfully impaired artillery movement and troop and supply convoys. Concurrent with this increase in animal cases during and after the war, human cases increased in Russia. Attempts to contaminate animal feeds in the United States were also made. A report by the Monterey Institute of International Studies states that between 1932 and 1945 Japan developed *B mallei* as a biowarfare agent, infecting 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.[9] The former Soviet Union was alleged to have used weaponized *B mallei* against opposition forces in Afghanistan between 1982 and 1984.[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. Glanders 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 is the largest reported human case series in US medical literature.[1] The seventh case has not been previously published. 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]

In 1972 the United States signed the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin

Weapons and on Their Destruction, which banned development, production, stockpiling, acquisition, and retention of biological agents, toxins, and the weapons to deliver them.[8] All offensive biological warfare work at Fort Detrick had ceased by that time; any remaining biological weapons were destroyed by 1973. Research aimed at the biodefense of *B mallei* warfare is currently being conducted in the United States. There are no known current attempts for acquisition and use by terrorists.[12]

B mallei was considered a potential threat agent in 1947 because of its high infectivity, high degree of incapacitation among those infected, and agent availability.[13] It poses a more significant threat if weaponized. As exemplified by past clusters of laboratory-acquired infections, *B mallei* is infectious by the respiratory route, but it is not contagious among humans. A determined bioterrorist could likely gain access to the agent, whether from an infected animal, laboratory culture, or commercial culture. Because glanders is relatively unknown in the West and its clinical symptoms are protean and nonspecific, diagnosis and treatment may be delayed postattack, even in regions with the most advanced medical facilities. Delayed diagnosis and treatment could lead to significant morbidity and mortality. Treatment may be complicated by the relative scarcity of knowledge and experience in therapy. Because equids and some other animals are susceptible, further spread from animals to humans could occur long after an attack. Glanders is curable, and postexposure prophylaxis may be an option after an attack. As with other agents, genetic engineering could produce unpredictable virulence and atypical antibiotic resistance. If glanders were cultivated, concentrated, and delivered as a wet or dry bacterial aerosol, significant casualties could result.[14]

HISTORY

Aristotle first described glanders in horses in 330 BCE, and named it malleus, meaning hammer or mallet. Glanders was associated with various horse populations around the globe, particularly army horses and mules. The association of glanders with domesticated equids was so familiar that "horses and their glanders" commonly appeared together in early literature. Glanders was not studied systematically until the 19th century. In 1882 the causative agent now called *B mallei* was isolated from a glanderous horse's liver and spleen.[2] The first account of the disease in humans was published in 1821,[3] yet the medical community recognized it earlier as a syndrome. The first veterinary school was established in Lyon, France, in the mid-1700s to study rinderpest and glanders. Many researchers at the school became infected and died of glanders.[15]

Horses and mules were the primary modes of transportation in all developing economies until the Industrial Revolution. Particularly in urban locations, glanders passed from the infected to the uninfected animals housed in crowded conditions. Horses and mules were in high demand during the American Civil War. Thousands of animals passed through remount stations where glanders existed in epidemic proportions. The problem was exacerbated after the war, when glanders was spread to communities as infected military stock was sold to civilians. 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 meticulously disinfecting 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 cases increased countrywide. The notion of a carrier-state began to be accepted. Despite epidemic disease in equine populations, no simultaneous epidemics occurred in the human population.

Vaccines and therapeutic agents were developed but were unsuccessful in reducing the glanders incidence. By 1890 the mallein diagnostic skin test was developed. Control and eradication programs soon incorporated the testing of all contact equids, followed by quarantine and a recommendation for slaughter of all skin-test–positive animals. These programs failed in some locales at first because of lax enforcement and lack of incentive to owners for killing their nonclinically ill animals. Some horse owners hid contact animals to avoid testing, or they sold contact and asymptomatic test-positive animals to unsuspecting individuals to minimize their economic loss.[4] Inexpensive steam transportation aided disease spread when glanders carriers were shipped to other regions and countries. The United States was blamed for the import of glanders-infected horses to Cuba in 1872[3] and for the great increase of glanders cases in Canada, where tens of thousands of US horses were shipped annually, near the turn of the 20th century.[3,4]

Once control programs offered indemnity to test-positive and contact animals and people 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

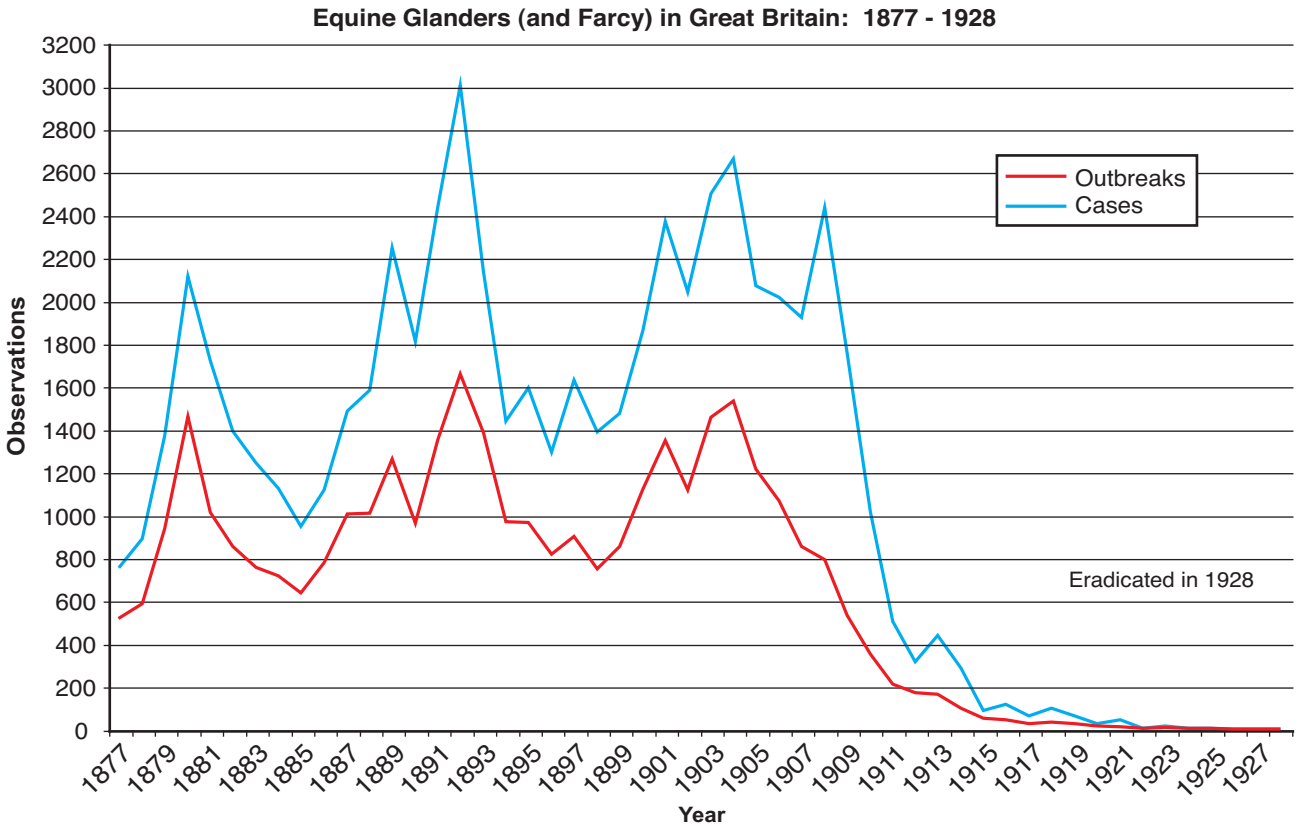

Fig. 6-1. Glanders cases and outbreaks reported to the Department for Environment, Food, and Rural Affairs in Great Britain, 1877–1928. Glanders was eradicated in Great Britain in 1928.
Data source: Available at: http://www.defra.gov.uk/animalh/diseases/notifiable/glanders/index.htm.

experience with the rise and fall of glanders outbreaks in equids[16] typifies many countries as shown in Figure 6-1. Great Britain eradicated glanders by 1928, about 30 years after eradication programs were initiated. The United States eradicated glanders by 1942.[17] 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 (OIE) in accordance with the International Animal Health Code.[18] Several countries still have eradication programs. In over 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. Between 1996 and 2003, glanders in livestock was reported in Bolivia, Belarus, Brazil, Eritrea, Ethiopia, Iran, Latvia, Mongolia, Myanmar, Pakistan, and Turkey. During the same time frame, glanders in humans was reported in Cameroon, Curaçao, Sri Lanka, Turkey, and the United States (laboratory-acquired).[17] Exhibit 6-1 depicts the year equine glanders was last reported to the OIE among countries and territories without glanders activity (by OIE report) since 1996. Bioterrorism should be considered as a possible source if confirmed glanders is found in the countries and territories listed in Exhibit 6-1.

INFECTIOUS AGENT

Glanders is caused by *B mallei*, a gram-negative bacillus that is a close relative to *B pseudomallei* (causative agent for melioidosis). *B mallei* is an obligate animal pathogen[19] and has not been found free-living in the environment; however, *B pseudomallei* can be isolated from tropical soil. The lack of motility is a primary means of differentiating *B mallei* from *B pseudomallei*. Growth requirements are not complex; *B mallei* can be cultivated on basic nutrient medium, and glycerol can be added to the medium to enhance growth. When stained, the cells typically exhibit bipolar staining.

EXHIBIT 6-1

YEAR EQUINE GLANDERS WAS LAST REPORTED TO OIE BEFORE 1996*

Country or Territory	Year	Country or Territory	Year
Australia	1891	Moldavia	1957
Austria	1952	Nambia	1925
Bulgaria	1954	Netherlands	1957
Canada	1938	Norway	1889
Croatia	1959	Poland	1957
Denmark	1928	Portugal	1952
Egypt	1928	Romania	1960
Estonia	1945	Serbia and Montenegro	1959
Finland	1943	Slovakia	1954
Yug Rep of Macedonia (former)	1957	South Africa	1945
France	1965	Spain	1956
Georgia	1960	Sudan	1989
Germany	1955	Sweden	1943
Greece	1965	Switzerland	1937
Hungary	1956	Taipei China	1950
India	1988	Great Britain	1928
Ireland	1920	Northern Ireland	1910
Israel	1951	United States of America	1942
Japan	1935	Zimbabwe	1911

*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 2003. (Data are available only for the listed countries and territories.)
OIE: Office International des Epizooties

B mallei is well-traveled taxonomically. Since its discovery, this microorganism has been placed in several genera, including *Bacillus*, *Corynebacterium*, *Mycobacterium*, *Loefflerella*, *Pfeifferella*, *Malleomyces*, *Actinobacillus*, and *Pseudomonas*,[20] and was finally assigned to the genus *Burkholderia* in 1992.[21] Not particularly hardy in the environment,[20] *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 for 1 month.[2,16,22] Experimentally and under the most favorable temperature and moisture conditions, Loeffler extended the viability of *B mallei* to 100 days. In nature, the organism's viability 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. Because of its high infectivity by aerosol, laboratory studies on this Category B pathogen[23] are performed at biosafety level 3 (BSL-3). Varying degrees of virulence among strains have been shown in the laboratory and in nature.[1,4,6] The infectious dose is low, depending on the route of infection, susceptibility, and strain virulence. One to 10 organisms of some strains by aerosol are lethal to hamsters.[1,24] Inhaling only a very few organisms may cause disease in humans, equids, and other susceptible species.

DISEASE

Epidemiology

Naturally acquired cases of glanders in humans or equids are sporadic and rare; most countries have eradicated the disease. Glanders is still infrequently reported in northern Africa, the Middle East, South America, and Eastern Europe.[17] Serologic cross-reactivity with *B pseudomallei* precludes the accurate distribution and prevalence of *B mallei* by serologic means alone. Although human outbreaks have been reported in Austria and Turkey, no human epidemic has been recorded.[25]

In nature, the horse is the reservoir of *B mallei* and may also be the amplifying host. A disease primarily of

solipeds, donkeys are considered most prone to develop acute forms of glanders, and horses are more prone to develop chronic and latent disease. Mules, a crossbred animal resulting from a horse and donkey, are susceptible to both acute and chronic disease as well as latent infections.[20,26,27] 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.[24] 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. Veterinarians and veterinary students, farriers, flayers (hide workers), transport workers, soldiers, slaughterhouse personnel, farmers, horse fanciers and caretakers, and stable hands have been naturally infected. Subclinical or inapparent infections in horses and mules pose a hidden risk to humans. Human-to-human transmission is rare. Infection by ingesting contaminated food and water has occurred; however, it does not appear to be a significant route of entry for human infections.[2,6,28] 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.[1]

Transmission

Glanders is transmitted directly by bacterial invasion of the nasal, oral, and conjunctival mucous membranes by inhalation into the lungs and by invasion of abraded or lacerated skin. The arms, hands, and face are most often exposed. Considering the affinity for warm and moist conditions,[2] *B mallei* may survive longest in stable bedding, manure, feed and water troughs (particularly if heated), wastewater, and enclosed equine transporters. Transmission from handling contaminated fomites, such as grooming tools, hoof-trimming equipment, harnesses, tack, feeding and husbandry equipment, bedding, and veterinary equipment, has occurred. Such equipment stored away from any contact with equids for at least 3 months—even without disinfection—is not likely to be a source of infection.

Reports of the circumstances surrounding zoonotic transmission are diverse. A few reports include equids snorting in the vicinity of humans or human food, and humans wiping equine nasal exudate off their arm with a blade of grass (local infection occurred at wipe site), sleeping in the same barn or stall as apparently healthy equids, accidentally puncturing themselves with contaminated equipment, wiping an eye or nostril after contact with an equid, being licked by a glandered horse, and cleaning stalls without any direct equine contact.[3,29,30] Horse handling requires physical work that often produces skin abrasions under normal circumstances. Although absorption through intact skin is probably unlikely, patients may insist their skin was intact when exposed. Among 105 people with chronic glanders associated with equid exposure described by Robins,[3] only 40 (38%) reported a wound present. In 27 cases (17%) the absence of a wound was specifically noted.

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 when mouth pipetting was a common practice. The first six patients acknowledged using this technique to clear blocked pipettes and blow contents out of pipettes that were calibrated to the tip. The eighth case involved a microbiologist who had worked with *B mallei* in BSL-3 containment for 2 years, but did not always wear latex gloves.[11] 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. Most laboratory-acquired infections are not associated with injury or a recollection of injury.[31] This patient had diabetes for 13 years, however, and collected blood via finger-stick morning and evening. 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 has occurred. The majority of reported events occurred in medical practice, at autopsy, in the diagnostic laboratory, and in patient care settings before a clearer understanding of universal precautions existed.[2,3,11] Transmission also occurred in home settings, where close contact during care of glanders-infected individuals led to infection of other family members.[3] At least one entire family became infected: the two children and wife of a chronically infected stable hand contracted glanders. The wife was presumably infected sexually;

the 4-year-old 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.

Human infection by ingestion has not been definitively reported. Stomach contents can inactivate *B mallei* experimentally in 30 minutes.[25] In his detailed 1886 report on the etiology of glanders, Loeffler describes several accounts of humans eating meat from glanderous horses without contracting disease. In one account, over 100 glanderous 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 setting at that time. In another case, a veterinarian ate raw glanderous meat to answer the ingestion question, but did not contract disease. An 1886 veterinary journal report, however, describes two persons who contracted glanders after consuming milk from a glanderous mare. Because these individuals were also exposed to the mare, infection by ingestion could not be determined.[2] Monogastric animals, including lions, tigers, domestic cats, dogs, and bears, have died from *B mallei* infection from ingesting raw meat.[2] 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 through the healthy digestive tract. This explanation, however, does not explain infections in dogs, domestic cats, and captive wildlife that were fed only boneless meat from glanderous horses. From this limited collection of testimonies and understanding of glanders pathogenesis, it appears that human ingestion of the live organism is unlikely to cause disease.

These features of transmission exemplify the requirement for BSL-3 containment and safety practices when working with *B mallei*. Laboratory workers should adhere to safety procedures and universal barrier precautions. In the presence of potentially infected equids, transmission risk is also reduced by universal precautions and procedures that reduce inhalation risk of potentially contaminated aerosols. The advances in medicine, infection control, and therapeutics make it less likely now than 100 years ago for human-to-human transmission to occur, even in a human outbreak, whether related to bioterrorism or not. It is also highly unlikely that an equid reservoir will become established. Acute disease is expected to manifest in a significant proportion of exposed equids, which would necessitate emergency response, quarantine, trace-back, 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.[3]

Among equids, transmission is primarily by oronasal mucous membrane exposure, inhalation, and mastication (possibly ingestion) of skin exudates and respiratory secretions of infected animals, including those with latent and subclinical infection. Sharing feed and water troughs facilitates this transmission,[20,26,27] as well as common equid behaviors that include grooming and snorting. Because equids are unable to breathe through their mouths, simple exhalation—and in particular, snorting to clear nasal passages—can finely aerosolize infectious nasal efflux from an infected equid. This snorting poses an absolute 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.[2] Sexual transmission from stallion or jack to mare or jenny has also occurred. The breeding of asymptomatic stallions resulted in the spread of glanders near the turn of the 20th century.[4]

Carnivores can become infected after eating contaminated carcasses and meat.[32] Reported outbreaks in captive wild felids suggest that they appear to be more susceptible than canids.[20,26,32,33] Glanders has also been transmitted to goats housed with infected horses.[2] Laboratory animals including mice, hamsters, guinea pigs, rabbits, and monkeys are also susceptible.[2,34] Cattle, swine, and chickens appear to be resistant to glanders, even after experimental injection.[2,33] Pigeons have been infected experimentally.[2] Loeffler suggested that field mice, donkeys, mules, horses, goats, cats, and guinea pigs were more susceptible to glanders infection and clinical disease than humans. Among other susceptible host species, rabbits and dogs appeared to be less susceptible to disease than humans.

Pathogenesis

The clinical course and potential chronicity of glanders show *B mallei* to be a hardy and persistent organism in situ that can evade attack from the immune system. The cytoplasmic membrane and cell wall consist of three layers.[24] In experimentally injected guinea pigs, *B mallei* produces a tenacious capsule that may protect it from phagocytosis.[35] The structure of the capsule described in this study is unknown. However, more recent genetic analysis has shown that the coding sequence of the *B mallei* capsule is 99% identical to the carbohydrate capsule encoded by *B pseudomallei*, which is a homopolymer of

-3)-2-*O*-acetyl-6-deoxy-ß-D-*manno*-heptopyranose-(1-.[36,37] Furthermore, a mutant strain without the capsule is avirulent in mice and hamsters.[36] The capsule does not stain with typical capsule stains. The organism has an affinity for the lymphatics and can be found within and outside the host cell. Where there are glanderous nodes of infection even deep within the musculature, ulceration and drainage to the outside of the body generally occur; internal organs are an exception. Some strains of *B mallei* produce an endotoxin that affects smooth muscle cells of various organs.[6] Tissue reactions, including lymphangitis and mucous membrane erosions, and the slow healing nature of local infections are clinical symptoms that support this local effect.

Acute and chronic glanders infections were described long before a viable treatment was available and before most countries had eradicated the disease. In his 1906 review of 156 chronic human glanders cases, Robins stated that distinguishing chronic and acute disease was difficult because chronic disease was often interrupted with acute symptoms and acute-onset disease may run a chronic course.[3] Robins defined chronic cases as those lasting longer than 6 months. Most historical literature attempting to distinguish between the two in humans and equids classifies a more fulminant and rapidly fatal clinical course (within 2–4 weeks) as an acute form of glanders. An acute course is found more often with untreated acute pneumonic and frank septicemic infection, whether primary or recurrent.[1,25,38] Chronic infections are most common in horses, where they comprise the majority of cases.[6] An acute disease course is more common in donkeys and humans.

B mallei most often enters the human body through abrasions or openings in the skin, particularly on the hands and forearms, face, and neck, where occupational exposure occurs. 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 there was no wound or penetration during the likely exposure interval. 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, which has occurred in several laboratory-acquired infections. However, at least one laboratory-acquired case most likely occurred through cutaneous exposure. When they are present, the most characteristic feature of the disease is glanders nodes, small papular to egg-sized abscesses, which are slow to heal if they open.

The incubation period 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; although without direct inoculation of the organism, the duration may be longer.[3] Inhalational exposure may incur a slightly longer range of about 7 to 21 days.[1,3]

Clinical Disease in Animals

B mallei naturally infects horses, donkeys, and mules,[20,39] although other species have occasionally become infected.[32,40] 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 varies from a few days to many months, most often falling between 2 and 6 weeks. The infectious process, disease progression, and pathology in equids are similar to those in humans. Donkeys are most likely to die from acute disease within a week to 10 days.[2,4] 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 increases temperature, such as infectious disease, roundup, transport, overwork, poor diet, exercise, vaccination, and even mallein testing.[2,4,41] Changes in season from winter to spring, and from summer to fall, have also been associated with recurrent disease.[4]

The primary route of infection in the natural host is oral, by chewing or contacting contaminated food and water, feeding and husbandry equipment, as well as by direct close contact with infected animals.[42] Tooth eruption, irregular tooth wear, coarse feeds, and bridling contribute to oral trauma, a common finding that leaves the mucosa and mucocutaneous junctions more vulnerable to infection. Equids are also very gregarious, preferring 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. Tack such as a harness can cause skin irritation that, if the tack is contaminated, may allow easy entry of the organism. Despite the oral route of infection, significant pathology is usually seen in the airways and lungs.[19]

With early infection or recurrence, constitutional signs are often the first to manifest including thirst, fever (low-grade to high), shivering, drooping of the head, tachycardia, tachypnea, weight loss, rough hair coat, indolence, prostration, and reluctance to move.[43] Limbs and joints may swell. 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.[2] In some cases (or at various disease stages), 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 characterized at least 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 signs. However, laryngeal, tracheal, and lower respiratory tract pathology is often present, 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.[3,30]

With clinical expression of upper respiratory infection, a highly infectious, sticky, yellow-gray to greenish viscous unilateral or bilateral nasal exudate is produced. The glottis may be edematous and the thickness of nasal discharge may 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 from 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, which 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 the aid of endoscopy and are near-hallmark signs of prior infection. Visible or palpable regional lymphadenopathy (particularly submandibular) and lymphangitis are usually present.

The equid frequently snorts 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 because equids breathe through their nose, not their mouths. Dyspnea, particularly inspiratory, may result from swelling in the nasal cavity or larynx. Expiratory dyspnea is also common, particularly with chronic involvement of the upper and lower respiratory tract.[29] 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. Animals may die within a few days, or within 3 to 4 weeks from bronchopneumonia and septicemia.

At necropsy, glanders nodes are likely found in the lungs, even if incidentally. Their consistency may be caseous to calcified depending on lesion age. These nodes may be 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.

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 into more solid, fingerlike cords that can be traced to regional lymph nodes. Nodules along the lymphatic pathways 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. The lesions are 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, which may result in behavioral changes such as avoidance or fear. With disseminated disease, cutaneous and mucous membrane lesions may appear anywhere, particularly in the respiratory tract as previously mentioned, and on the limbs. The hind limb is more commonly affected than the forelimb.[22,26]

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 *B mallei* septicemia; this form manifests in most donkeys that are naturally and experimentally infected. In horses, however, disseminated disease is typically more protracted. Clinical signs are widely variable and may include any of those previously mentioned. Horses may be asymptomatic, or 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 male donkeys may have orchitis, which may not be evident without a reproductive examination.[20,44] 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 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 eradication (by test-and-slaughter) of the disease.

Chronically infected horses may display cycles of worsening disease followed by apparent recovery when few symptoms are displayed. Clinical signs include intermittent cough; lethargy; and lesions in the nasal region, lungs, and skin, just as with acute disease.[43] Lungs may develop lesions similar to tubercles. Nodules may appear in the submucosa of the nasal cavity, particularly in 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.[45] Attempts to isolate *B mallei* from chronically infected animals are usually unsuccessful. Thromboses can be found in the large venous vessels of nasal mucous membranes.[46] Nodules in the skin along lymphatics may be seen as they thicken in chronically infected animals. Nodules may ulcerate and rupture, spewing a thick exudate that can 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 be variable. At least six forms of infection have been described, including nasal, localized (the nasal form is also a localized form), pulmonary, septicemic, disseminated, and chronic infection; none are exclusive. The most important distinction is whether the infection is truly localized, which is unusual except early in the infectious process. The variety of forms is largely explained by various routes 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. Clinical courses will be discussed in detail below because they are associated with route of entry and disease spread.

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 pathways that drain the entry or infection site. Mucus production from affected ocular, nasal, and respiratory mucosa is often increased. Localized infections typically disseminate, leading to pulmonary, septicemic, or disseminated infection.

Constitutional signs and symptoms typically occur early in the course of disease, and some may persist through treatment and be severe, leaving the patient exhausted. 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 (including night sweats); altered mental status; and fatigue. Other nonspecific signs, any of which may be present, include tender lymph nodes, sore throat, chest pain, blurred vision, splenomegaly, abdominal pain, photophobia, and marked lacrimation.

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, although 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.[6,29] Localized infection with a mucopurulent discharge develops at the entry site. Inflammation may extend along regional lymphatics and cause lymphangitis with numerous foci of sup-

puration along their course. This irritation is caused by endotoxins present in some *B mallei* strains affecting the smooth muscle of the lymphatics. Lymphatic pathways 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 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 studies have described glanders nodes anywhere, including the face, neck, shoulders, lumbosacral region, arms, and legs. When the legs are affected, glanderous nodes occur more often on the medial aspect than the lateral. At first these glanderous nodes may be hard and painful, but eventually they ulcerate and slough. The 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 mucous 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 infected, swell, and weep a serosanguineous 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 (affecting the 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 there may be copious nasal discharge. Infection may invade the nasal septum and bony tissues, causing fistulae and tissue destruction. The face may swell, and regional lymph glands may inflame and suppurate. Infection may also extend lower in the respiratory tract, resulting in tracheitis and bronchitis that may be accompanied by cough and mucopurulent sputum production. If mucous membrane involvement is extensive, constitutional signs, such as fever, severe headache, fatigue, prostration, earache, and various neurologic signs are also usually severe.

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, such as fatigue, fever, chills, headache, myalgias, and gastrointestinal signs, often accompany respiratory infections. Pulmonary abscess and pleuritis are common sequelae. Symptoms, which may take up to 2 to 3 weeks to develop, include tender cervical lymph nodes, fatigue, lymphangitis, sore throat, pleuritic chest pain, cough, fever (often exceeding 102°F), chills, tachypnea, dyspnea, and mucopurulent discharge. Nonspecific signs, such as night sweats, rigors, myalgia, severe headache, tachycardia, nausea, weight loss, dizziness, and mucosal eruptions, are also usually present. 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 necrotizing nodules, or a localized (lobar to bilateral) bronchopneumonia are other potential radiographic signs. Developing abscesses may be well circumscribed and circular, later becoming cavitated with evidence of central necrosis. Pleural irritation may also be visible on imaging studies. Untreated acute bronchopulmonic or pneumonic disease has a rapid onset of symptoms and was once said to be almost uniformly fatal within 10 to 30 days.[1] Most laboratory-acquired infections have been caused by inhalational exposure resulting in pulmonary infection.

Clinical features of eight laboratory-acquired infections from Camp (later Fort) Detrick are summarized in Table 6-1. 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.[11] 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 (see Table 6-1). 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

TABLE 6-1

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

Signs and Symptoms*	Patient 1† November 1944	Patient 2† November 1944	Patient 3† February 1945	Patient 4† April 1945	Patient 5† August 1945	Patient 6† August 1945	Patient 7† July 1953	Patient 8† March 2000
Fever, PM rise††	99.0–99.4	99.0–101.2	101.0–103.4	99.0–103.8	99.0–102.8	-	99.0–101.4	99–104.5
Rigors, chills			+	+				+
Night sweats				+			+	+
Pain in chest	+				+	+	+	
Myalgia	+	+						
Malaise	+		+	+	+	+	+	+
Headache		+	+	+	+	+		
Backache			+	+	+			
Stiff or sore neck			+					
Dehydration	+		+					
Earache			+					
Cough		+			-		+	
Mucopurulent sputum		+						
Oro-pharyngeal	Postnasal drip	Blister under tongue; nasal obstruction				Sore throat		
Pharynx injected	+	+			+			
Lymphadenopathy	Cervical		Cervical	-	Cervical			L axilla
Neurologic signs			Stupor	Carpo-pedal spasm				
Drowsy			+	+				
Apprehension			+				+	
Dizziness				+				
Fatigue	+	+	+		+		+	+
Weight loss	+						+	+
Anorexia				+			+	
Blurred vision				+				
Lacrimation				+				
Photophobia			+	+				
Abdominal signs			-	Pain L-upper quadrant; spasm		Diarrhea	Indigestion, flatulence, belching	Epigastric tenderness
Nausea, vomiting				+				
Enlarged spleen				+				+
Chest radiographs	R-upper; ~Abscess	R-lower; ~Abscess	R-upper, ~Abscess	Clear	L-middle, ~Abscess	L-lower, pneumo-nitis	L-hilum ~Abscess	Clear

(**Table 6-1** *continues*)

Table 6-1 *continued*

WBC	Normal-low; neutropenia	Normal	High; neutrophilia	High to normal to low; Neutrophils	Normal	Normal to high-normal; Neutrophils	Normal, L-shift; atyp mono, lymph	Normal late in disease
Primary site	Pulmonary	Pulmonary	Pulmonary	Unknown	Pulmonary	Pulmonary	Pulmonary	Cutaneous
Disseminated			Possible	Likely spleen	Possible			+
Secondary sites				Unknown				Liver, spleen
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 72	-	-	NA
Successful treatment	Sulfadiazine 10 days	Sulfadiazine 10 days	Sulfadiazine 36 days	Sulfadiazine 20 days	Sulfadiazine 20 days	Sulfadiazine 20 days	Aureomycin 28 days	Doxycycline 6.5 mos
Onset of antibiotic[¶]	Day 60	Day 60	Day 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 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.
Patient 8: Initial blood culture was negative; data from Srinivasan A, Kraus CN, DeShazer D, et al. Glanders in a military research microbiologist. *N Engl J Med*. 2001;345:256–258.

[††] 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
NA: not applicable or not done
WBC: white blood cell

for patient 2. Inhalation is suspected as the route of exposure for the first seven patients, and 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 in chronic or latent infection. Septicemia may be passing and lead to protracted disseminated infection or be fulminant and rapidly fatal. Without aggressive treatment, *B mallei* septicemia runs an acute course and may lead to death in 7 to 10 days. Septicemic glanders may produce numerous signs consistent with a highly pathogenic bacterial septicemia. The thromboembolic process of glanders was

well described by the early 1900s.[2,3] *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 patient may recognize the embolic process 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.[3] 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. However, death may occur before these manifestations develop. Clinical signs and symptoms of the septicemic process may develop immediately or up to 2 weeks after initial infection or recurrence. 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. Patients die within 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.[5] Dissemination typically occurs without adequate treatment 1 to 4 weeks after *B mallei* infection of the lymph nodes. The organs most 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 or limited to weight loss, or they may be severe, variable, and include any of those mentioned earlier. Cutaneous eruptions may appear 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 pathways are 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 had abscesses in the lungs and on the body. Robins chronicles a patient with the longest known infection (15 years, only five of which were latent) who finally died of disseminated disease. Symptoms of this particular disseminated infection included nasal and aural discharge, submaxillary adenitis, nose phlegmon, nasal septum perforation, jaundice, diarrhea, and amyloid disease.[47]

The amount of infection and pathology in a surviving patient can be particularly alarming when compared to a usually more rapidly fulminant disease such as septicemic anthrax. Protracted disseminated infections are associated with septic shock and a guarded prognosis. Diagnostic imaging studies are indicated to identify potential locations of infection. Before antibiotics, disseminated infection was ultimately fatal either by recurrence of acute disease or from chronic wasting. Based on the few cases treated with antibiotics, survival is likely if early and long-term effective therapy is instituted. Even with treatment, clinical symptoms may continue for several months before complete resolution, particularly if treatment is delayed.

Complete blood count and chemistry studies for glanders patients vary depending on the disease's location 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 (see Table 6-1). Neutropenia or neutrophilia, with or without a left shift, may be transient findings. Leucopenia with mild to moderate relative lymphocytosis was seen in three of the six laboratory-acquired infections reported by Howe and Miller,[1] 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 that the mortality rate for localized disease is 20% when treated, and the overall mortality rate is 40%.[38] 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 patients (37%) experiencing a delay in effective treatment of 2 months. Even 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 patients who received delayed treatment still recovered with only 10 days of sulfadiazine, although 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.

Diagnosis

Definitive diagnosis of glanders is by isolation and positive identification of the organism. Physical findings that support the differential diagnosis of glanders may be linked to the potential route of infection. With pulmonary involvement—likely from aerosol exposure—suspect clinical signs and symptoms include oropharyngeal injection, headache, chest pain, fever, rigors, night sweats, fatigue, cough, nasal discharge, and diagnostic imaging studies that support localized or lobar pneumonia, bronchopneumonia, miliary nodules, lobar infiltrative pneumonia, and consolidation (early) or cavitating (later) pulmonary lesions (see Table 6-1). Neurologic signs may also be present, with or without obvious pulmonary signs. With cutaneous involvement and regional lymphadenopathy likely from percutaneous exposure to infected equids or contaminated fomites, clinical signs and symptoms include lymphadenopathy with or without ulceration and single or multiple cutaneous eruptions that may heal slowly, particularly along lymphatic pathways (see Patient 8, Table 6-1). For presentation at autopsy, suspect findings include disseminated nodular and ulcerative disease, particularly involving the spleen, lungs, and liver. Cultures of nodules in septicemic cases usually establish the presence of *B mallei*. These presentations support glanders as a differential diagnosis and prompt further testing to rule out *B mallei* infection.

The development of adequate diagnostic tests that could identify infected animals, particularly those that were asymptomatic, finally allowed glanders control through test and slaughter programs. Until this breakthrough, isolating the agent, particularly from chronically infected animals, was difficult. A potential glanders clinical presentation in a human patient should prompt immediate notification of local animal health authorities to explore potential cases of glanders in livestock, particularly equids. The converse is also true; glanders as a potential differential diagnosis in livestock warrants immediate notification of local regulatory animal and public health authorities. Cutaneous ulcerative disease outbreaks in sheep, goats, and swine accompanying suspected human cases would be more consistent with a *B pseudomallei* (melioidosis) outbreak than with *B mallei*. Because of the rarity of natural glanders infection, bioterrorism should also be immediately suspected, particularly in regions where glanders has been eradicated. Human glanders without animal exposure or more than one human case is presumptive evidence of a biowarfare attack. With this suspicion, regional public health authorities can initiate an appropriate emergency public health response for disease prevention, environmental decontamination, epidemiological investigation, and criminal investigation.[23,48]

Because *B mallei* has a high potential for aerosol or droplet production and laboratory-acquired infection, BSL-3 personnel and primary containment precautions are indicated for activities attempting to rule out *B mallei* infection. Aseptically collected exudates from abscesses, cutaneous and mucous membrane lesions, sputum, and blood as well as aspirates from preerupting nodules and abscesses are excellent culture sources. Blood cultures are often not productive unless disease stage is near terminal.[49] Bacteremia is more likely during febrile peaks (and acute disease), thus sampling during such peaks may enhance chances for a productive culture. Among the eight US laboratory-acquired infections, blood cultures were attempted at least once within several weeks of initial presentation. In at least the first seven cases, special media were used to enhance growth of *B mallei*. All were negative (see Table 6-1). In the eighth case, a positive blood culture was obtained 2 months after initial presentation during an acute septicemic relapse in which the patient was in a guarded condition.[50]

Growth and Morphology

In endemic regions, biochemical assays and observation of colony and cell morphology may still be a practical means to definitively diagnose glanders. These methods may take 2 to 7 days to confirm a diagnosis.[51] Gram stains of pus from lesions may be productive, but microorganisms are generally difficult to find, even in acute abscesses.[49] *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, proflavine, penicillin) or *B mallei*-selective media may be useful.[52,53] Optimum growth temperature is approximately 37°C.[47] Growth is typically slow on nutrient agar, but is rapid (2 days) when

enhanced with 1% to 5% glucose and/or glycerol, and on most meat infusion nutrient media.[52,54] *B mallei* colonies typically are about 1 mm in width, white (turning yellow with age), and semitranslucent and viscid on Loeffler's serum agar and blood agar. Colonies have a clear honey-like layer by day three, later darkening to brown or reddish-brown when grown on glycerin-potato medium. Selective inhibition of *B pseudomallei* and *Pseudomonas aeruginosa* growth may be enhanced by noting the following: *B mallei* does not grow at 42°C; *B pseudomallei* and *P aeruginosa* do. Nor does *B mallei* grow at 21°C; *P aeruginosa* does. Furthermore, *B mallei* does not grow in 2% sodium chloride solution, nor on MacConkey agar; both *B pseudomallei* and *P aeruginosa* do.[6]

B mallei is a small, nonmotile, nonsporulating, nonencapsulating aerobic gram-negative bacillus approximately 2 to 4 μm long and 0.5 to 1 μm wide (Figure 6-2). *B mallei* is facultatively anaerobic in the presence of nitrate.[47,55] 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.[52] In vivo, *B mallei* is found most often to be extracellular. Samples should be designated as "glanders suspect" because of the rarity of disease. Sample security, including appropriate chain of custody documentation, is also prudent for all samples. Automated bacterial identification systems may misidentify the organism. In the eighth US laboratory-acquired infection, such an automated system identified the agent as *Pseudomonas fluorescens* or *P putida*.[50] *B mallei* may have a beaded appearance in histopathology sections, where organisms tend to be difficult to demonstrate.[34]

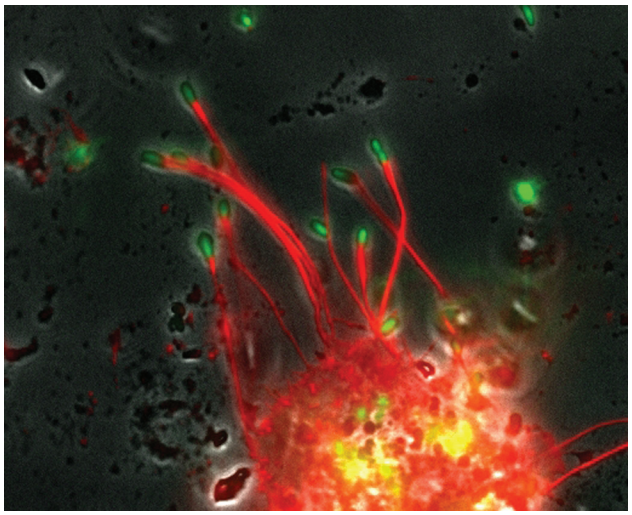

Fig. 6-2. The *B mallei* ATCC 23344 animal pathogen-like type 3 secretion system is involved in the induction of actin-based host cell membrane protrusions. J774.2 cells were infected with wild-type *B mallei* expressing green fluorescent protein at a multiplicity of infection of 10 bacteria to 1 macrophage. At 6 hours postexposure, cells were fixed and cellular actin was stained with Alexa Fluor^{568} phalloidin and viewed at a magnification x 630.
Photograph: Courtesy of Dr Ricky Ulrich, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

Isolation

Animal inoculation studies have been used to isolate the organism, but such studies may be impractical now for two reasons: (1) the time required for disease to manifest, and (2) logistical requirement for special containment facilities. Intraperitoneal inoculation of suspect *B mallei* exudate into intact male guinea pigs was once popular because they are nearly universally susceptible to infection and tend to produce a well-described localized peritonitis and associated orchitis. Loeffler first described this consistent experimental syndrome in 1886,[2] and it later was called the Strauss reaction.[22,54] Although this method of testing is sensitive, the clinical course runs nearly a month, which precludes rapid diagnosis.[2] Because *B mallei*, *B pseudomallei*, and *P aeruginosa* also produce identical clinical signs in intact male guinea pigs,[6] positive identification of the organism from the testes is still required to enhance sensitivity.

The field mouse (*Arvicola arvalis*) was also considered as a potential host for inoculation and isolation because of extremely high susceptibility to infection (even more so than the donkey) and predictable short disease course ending with sudden death in 3 to 4 days.[2] Upon necropsy, generalized subcutaneous infiltrate extending into superficial musculature, lymphangitis and lymphadenitis, enlarged spleen, liver infiltration, normal kidneys, and normal testicles are consistent findings in field mice. However, if exudates with mixed bacterial flora (which may be common with nasal exudates and sputum) are used in field mice, organisms causing other bacterial disease may competitively exclude expression of glanders disease.[2] In the seventh US laboratory-acquired infection, two mice injected with the patient's sputum died within 24 hours. From peritoneal washings taken from the mice, gram-positive cocci in pairs typed as pneumococci were readily observed, as were occasional gram-negative rods found to be "*Malleomyces mallei*" (name for *B mallei* at the time).

Organism Identification

The *B mallei* genome has been sequenced (see the Institute for Genomic Research Web site, www.tigr.org),[56] which results in an enhanced ability to specifically identify this microorganism and further demonstrate how *B mallei* interacts with its host. Several relatively new molecular-method diagnostic capabilities exist to reliably confirm specific identification of *B mallei* within several hours, including polymerase chain reaction-based assays and DNA gene sequencing.[57-59] The latter methods, as phenotypic testing and 16S ribosomal RNA gene-sequence analysis, identified *B mallei* from other *Burkholderia* species in the 2000 US laboratory-acquired infections.[50] A polymerase chain reaction procedure based on differences detected in ribosomal DNA sequences was also developed to distinguish *B mallei* from *B pseudomallei*.[57]

Polymerase chain reaction-based techniques and DNA gene sequencing are increasingly used in clinical settings and public health laboratories for bacterial identification.[60] Automation of sequencing and improved efficiencies of reagents have 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.[57] These methods are not yet widely available for *B mallei* identification; however, the current interest in biowarfare defense research is prompting a continued increased capability based on recent publications.[57-59,61,62]

Pulsed-field gel electrophoresis and ribotyping have been used to identify strains of *B pseudomallei* in outbreaks.[63] These methods have also been used to differentiate pathogenic *B pseudomallei* strains from less virulent strains.[64] Pulsed-field gel electrophoresis and ribotyping may be as useful for identification and virulence testing of *B mallei*, although these methods may be more labor intensive and time consuming than gene sequencing. Gas liquid chromatography of cellular fatty acids was used to help identify the organism as a *Burkholderia* genus in the laboratory-acquired infection in 2000.

Imaging Studies

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. Pulmonary abscesses, which may be single or multiple, undergo central degeneration and necrosis, which radiographically resemble cavitation. Unilateral or bilateral bronchopneumonia and a smattering of miliary nodules may be seen. Because of the potential for disseminated disease, computed tomography imaging is useful for monitoring deep tissues and visceral organs.

Serology and Mallein Testing

There are no specific serologic tests for human glanders diagnosis. The agglutinin test, complement fixation test (CFT), and mallein testing are not consistent in humans, nor are they particularly timely. The indirect hemagglutination and CFTs have been tried,[65,66] but the CFT may not detect chronic cases of glanders.[42] Serologic tests were instrumental, however, in diagnosing all seven US laboratory-acquired infections between 1944 and 1953 (see Table 6-1). Although sensitive, agglutinin tests may be difficult to interpret because of potentially high background titers of up to at least 1:320. Titers rising from 0 to 320 may be significant, however, as was the case with patient 6 (see Table 6-1). For at least four of the seven aforementioned cases, agglutinin titers developed in 3 weeks from disease onset (see Table 6-1). The CFT was initially used in the diagnosis of glanders in 1909[67] shortly after the mallein test was developed. The CFT is still used for glanders screening in animals in the United States; mallein testing is used only in animals positive for complement fixation antibodies.[39] The CFT is believed to be more specific than the agglutinin test; a positive titer is considered to be ≥ 1:20. In at least one patient (patient 6), however, the CFT was persistently negative. Patient 5 was also persistently negative but may not have been tested for a 70-day interval between the 17th and 87th day after disease onset; the agglutinin test was diagnostic by the 22nd day.

The US Army Medical Research Institute of Infectious Diseases has developed an enzyme-linked immunosorbent assay (ELISA) for human glanders. In laboratory testing, an ELISA could differentiate serum from a glanders patient from sera from patients with clinical cases of anthrax, brucellosis, tularemia, Q fever, and spotted fever.[68] However, an ELISA cannot distinguish glanders from melioidosis, caused by *B pseudomallei*, a closely related microorganism.

Development of a human mallein skin test was attempted, but delay of up to several weeks postinfection for positive result rendered it of little diagnostic value.[69] Modified equine mallein tests have infrequently been used in humans, however.[1,3] At the station hospital at Camp Detrick, 0.1 mL of 1:10,000 diluted commercial mallein was injected intradermally into the forearm, and the test was read at 24 and 48 hours. Five of the first seven patients tested positive as early as the 18th

day of disease. In one patient (patient 4), the modified mallein test was the first of the three tests to show positive results (see Table 6-1). In contrast, patient 5 did not test positive until the 72nd day postdisease onset, whereas agglutinin was positive by day 22. The CFT, agglutinin titer, and mallein tests remained positive for no less than 10 months in the two patients (patient 1 and patient 2) whose diagnoses were delayed and who received the shortest course of antibiotics. Both responded quickly to treatment, however. Patient 3 also had persistently positive serology and a protracted illness. Serology may be useful to monitor cure post-treatment, if not for initial diagnosis.

Diagnosis in Equids

Whether naturally occurring or related to bioterrorism, a suspected case of human glanders warrants the investigation of potential contact equids or fomites. Physical findings in equids that support the differential diagnosis of glanders include fever; white-to-greenish viscous unilateral or bilateral nasal exudate that dries, forming thin yellowish crusts along the external nares; irregularly shaped abscesses on the nasal septum; regional lymphadenopathy; boil-like lesions with thick, ropy lymphatic pathways tracking from them; swelling of the limbs; dull hair coat; cough; weakness; and emaciation. Universal precautions are warranted when handling animals or fomites suspected or known to be infected. Because glanders may be latent or clinically inapparent, potential contacts to a human (or livestock) case should undergo systematic testing to help identify a potential outbreak.[18,20,26,44]

In the United States glanders has been considered a foreign animal disease (FAD) since its eradication in 1942. US Department of Agriculture (USDA) veterinarians are trained to recognize and control FADs—including glanders—and help mitigate the shortfall created by the unfamiliarity with glanders in human patient care settings. In the United States the USDA and the Department of Homeland Security have elements of regulatory authority for unintentional FAD outbreaks. When a FAD or other federally regulated disease is suspected in the United States, an emergency response system is activated. Where intentional transmission is suspected, the Federal Bureau of Investigation should be contacted immediately, and it will take the lead in the investigation. Many other countries have a corresponding FAD (includes glanders) emergency response system. Therefore, human patient care and public health systems around the globe should partner with local and regional animal health authorities when there is any suspicion of zoonotic disease.

The OIE provides technical support to member countries that request assistance with animal disease control and eradication operations, including zoonoses. The OIE also publishes the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, a compilation of diagnostic procedures and a useful reference for any diagnostic laboratory, to coordinate methods for the surveillance and control of the most important zoonotic and animal diseases, including glanders.[54] The manual includes standards for the most current laboratory and diagnostic tests, and the production and control of biological products for veterinary use around the world.

For any case in which glanders must be ruled out in livestock, regionally assigned veterinarians respond after notification to quickly identify, contain, diagnose, and eradicate glanders from livestock in accordance with local or regional animal and public health authorities. The veterinarians also work with regional veterinary reference laboratories to ensure diagnostic samples are harvested and submitted accordingly.

Aseptic collection of specimens and laboratory-handling procedures are similar to those described for humans. *B mallei* may be isolated from fresh cutaneous lesions, blood (when pyrexic), nasal exudate, and various lesions at necropsy. Several tests are available for regulatory veterinarians to help diagnose glanders in equids. The CFT, indirect hemagglutination test, and ELISA are among the most highly sensitive tests for glanders in equids. The CFT is reported to be 90% to 95% sensitive with the ability to detect positive sera as soon as 1 week after infection. In chronic cases, sera are typically positive for a long time.[20] A limitation to the CFT is that a large percentage of donkey, mule, and pregnant mare sera are anticomplementary and cannot be effectively tested.[54] Counter immunoelectrophoresis[70] and immunofluorescence tests as well as agglutination and precipitin tests are available, although the latter two are unreliable for horses with chronic glanders and animals in poor condition. An immunoblot method has also been developed.[71]

A recently developed dot ELISA that rivals other tests economically was found to be the most sensitive compared to CFTs, indirect hemagglutination, and counter immunoelectrophoresis tests, and it is faster and easier to administer. The dot ELISA is not influenced by potential anticomplementary activity of some sera or other spurious activity that can be associated with the CFT.[72] The test is named for the positive reaction that is indicated by the appearance of a clearly visible brown dot in the antigen-coated area. Mallein testing within 6 weeks interferes with test results, however. Thus, dot ELISA subsequent to mallein testing must be delayed. Low antibody levels of ≤ 1:100 can be demonstrated in the normal equine population. Natural infection and

sensitized equids (eg, from mallein) have dot ELISA titers that range from 1:400 to 1:25,600.[72] Positive dot ELISA titers may be seen 4 days postinfection, are present by 6 days, and persist for at least 7 weeks. All serologic tests for glanders in equids cross-react with those for *B pseudomallei*, which causes melioidosis. Thus, where melioidosis is endemic, serologic testing may result in false positive results.[22]

The mallein test was the first diagnostic test for glanders and has been the bastion of field diagnosis and eradication programs since the 1890s. Russian military veterinarians Gelman and Kalning first developed the test in 1891,[4,6] and the United States and Canada began using it as a diagnostic tool in 1905.[20] Originally cultured for 4 to 8 months, mallein is a heat-treated lysate of *B mallei* containing both endotoxins and exotoxins produced by the organism. The test works similarly to tuberculin testing. Glanders-infected animals become hypersensitive to mallein, exhibiting local pain and swelling, as well as a systemic reaction including a marked temperature increase, after inoculation. After confirmation of normal body temperature, mallein is injected intradermally either into the lower palpebrum (intradermo-palpebral test) or subcutaneously in the neck region (subcutaneous test). A third and slightly less reliable procedure is to instill a few drops of mallein onto the eye near the medial canthus (ophthalmic test). The intradermo-palpebral test is preferred.[73] Subsequent monitoring of the animal and interpretation of positive results depend on the method of administration and should be done by the animal health authorities who administered the test. In advanced clinical disease in horses and acute infection in donkeys and mules, however, mallein testing may give inconclusive results.[74] Also, testing of chronically infected or debilitated equids may give negative or inconclusive results. In either case additional testing methods are required. Mallein testing (inoculation) may trigger a humoral response and subsequent serologic reaction to the CFT, particularly when administered subcutaneously. Although thought to be transient, this seroconversion may become permanent after repeated mallein testing, which is an important consideration for equids that may be exported to regions that depend on the CFT.

Treatment

Because human glanders cases are rare, limited information exists regarding antibiotic treatment for humans. *B mallei* infection responds to antibiotic therapy; however, recovery may be slow after a delayed diagnosis or with disseminated disease. The scientific literature reports that *B mallei* is susceptible to the following antibiotics in vitro:

- amikacin,
- netilmicin,
- gentamicin,
- streptomycin,
- tobramycin,
- azithromycin,
- novobiocin,
- piperacillin,
- imipenem,
- ceftazidime,
- tetracycline,
- oxytetracycline,
- minocycline,
- doxycycline,
- ciprofloxacin,
- norfloxacin,
- ofloxacin,
- erythromycin,
- sulfadiazine, and
- amoxicillin-clavulanate.[75-82]

Aminoglycosides and other antibiotics incapable of penetrating host cells are probably not useful in vivo because *B mallei* is a facultative intracellular pathogen.[79,80,82] Susceptibility to streptomycin and chloramphenicol in vitro has been inconsistent, with some researchers reporting sensitivity and others reporting resistance.[6,78,80]

Most *B mallei* strains exhibit resistance to the following antibiotics:

- amoxicillin,
- ampicillin,
- penicillin G,
- bacitracin,
- chloromycetin,
- carbenicillin,
- oxacillin,
- cephalothin,
- cephalexin,
- cefotetan,
- cefuroxime,
- cefazolin,
- ceftriaxone,
- metronidazole, and
- polymyxin B.[6,11,25]

Antibiotics have been tested against glanders in equids, hamsters, guinea pigs, and monkeys.[77,81-85] Sodium sulfadiazine—but not penicillin or streptomycin—was effective for treating acute glanders in hamsters.[81] Doxycycline and ciprofloxacin were also examined in the hamster model of glanders.[82] Doxycycline therapy was superior to ciprofloxacin therapy, but some of the

treated animals relapsed in 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.[83] Although all of the antibiotics exhibited some activity in animals challenged subcutaneously, 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.[83]

The majority of human glanders cases occurred before antibiotics, and over 90% of these people died.[86] Several human glanders cases have been recorded since the 1940s—primarily in laboratory workers—and these have been successfully treated with antibiotics.[1,50,87,88] Sulfadiazine was used successfully in the first six US laboratory-acquired infections.[1] The seventh case was successfully treated with the tetracycline compound aureomycin. Two additional cases were successfully treated with sulfadiazine in 1949 and 1950.[87] Disseminated glanders in a stable hand who had only indirect contact with horses was also successfully treated with aureomycin in Austria in 1951.[29] Streptomycin was used to treat a patient infected with *B mallei* and *Mycobacterium tuberculosis*.[88] Treatment with streptomycin reportedly cured the glanders, but had little effect on the tuberculosis of this patient's bone. 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.[50] Susceptibility testing of the *B mallei* isolate in this case demonstrated sensitivity to the former two drugs.[50] A 6-month course of doxycycline and azithromycin followed, although retrospective susceptibility testing found that the organism was resistant to azithromycin. Diagnostic imaging of the patient's splenic and hepatic abscesses through the 6-month course showed their near complete resolution.

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 and trimethoprim-sulfamethoxazole are recommended for at least 20 weeks plus oral chloramphenicol for the first 8 weeks.[24] For severe disease, either ceftazidime at 40 mg/kg intravenously (IV) every 8 hours, or imipenem IV at 15 mg/kg every 6 hours (maximum 6 g/day), or meropenem at 25 mg/kg IV every 8 hours (maximum 6 g/day) and trimethoprim-sulfamethoxazole at 8 mg trimethoprim/kg per day IV in four divided doses is recommended. IV therapy should be continued for at least 14 days and until the patient is clinically improved. Oral maintenance therapy for mild disease can be continued from that point.[24] Patients with the mildest of systemic symptoms should consider combined therapy for at least the first month. For visceral and severe disease, prolonged treatment for up to a year is recommended. Abscesses may be surgically drained, depending on their location.[38] For infections that are slow to clear, long-term follow-up and possibly prolonged tailored therapy is recommended because of the intractable nature of glanders. Patients should be followed at regular intervals for at least 5 years after recovery. Diagnostic imaging is useful to follow the reduction and recurrence of abscesses, serology may help to monitor the clearing of antibody, and inflammatory markers may also suggest recurrence of a latent infection. Patients should be informed of the lifelong risk of relapse and advised to alert their healthcare providers of their previous history, particularly if they develop a febrile illness. These actions are especially important if the patient might have been infected with a genetically engineered strain of *B mallei*.

Prophylaxis

There is no evidence that previous infection or vaccination provides immunity against glanders.[6,89] Infections in horses that seemed to symptomatically recover from glanders have recrudesced when the animals were challenged with *B mallei*. Inoculating *B mallei* into chronically infected horses generally produced at least local infections and occasionally a manifestation of classic glanders. Numerous attempts to vaccinate horses and laboratory animals against glanders were unsuccessful between 1895 and 1928. For most chronically infected horses, experimental vaccination did not change the course of their illness. Vaccines were made by treating bacterial cells with urea or glycerin[6] or by drying the glanders bacilli.[89] Experiments on protective immunity in horses have given ambiguous results.[2,6] Passive immunity experimentation using equine sera has also failed.[6] A nonviable *B mallei* cellular vaccine failed to protect mice from a parenteral live challenge.[90] This vaccine stimulated a mixed T-cell helper (Th)1- and Th2-like immune response. This study suggested that nonviable *B mallei* cell preparations may not protect mice because of the failure to induce a strong Th1-like immune response. Because no vaccines protected animals from disease, control and eradication of glanders were dependent on eliminating infected horses and preventing them from entering glanders-free stables.

Protective immunity in humans after infection 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. Mendelson reported guarded postvaccination success in a young person with severe ocular and oro-nasal involvement.[30] Thus, patients who recover

may still be susceptible, which makes reuse of the agent in biowarfare necessary to consider.

Although unsuccessful attempts to develop a glanders vaccine were initiated over 100 years ago, using modern approaches to identify virulence factors and studying the ways putative vaccines modulate the immune system could possibly result in the development of a vaccine to induce sterile immunity. The initial attempts to protect mice against an aerosol-acquired infection using an irradiation-killed *B mallei* cellular vaccine resulted in an increased time to death, compared to controls, but spleens of survivors were not sterile.[91] The most desirable glanders vaccine would be a recombinant protein or a biochemically purified preparation that provides long-term sterile immunity.

Antibiotics may offer some protection, however, against a *B mallei* strategic attack. Prophylaxis with doxycycline and ciprofloxacin given before and coincident with intraperitoneal inoculation in rodents caused the minimum lethal dose to rise several thousand-fold, but did not completely protect against infection.[82] This approach is limited by the possibility that the biological agent may be engineered to resist the anticipated antibiotic regimen (as is true for other types of biowarfare).

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, patient care personnel, public health officials, and local veterinarians should investigate any potential exposure to infected equids. 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 case of deliberate release of *B mallei*, emergency response personnel entering a potentially heavily contaminated area should wear protective gear, including a mask with a biological filter. 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 according to local protocol. All waste should be managed according to 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 probably low.[48] Prophylactic treatment with ciprofloxacin or doxycycline may help to prevent infection in those potentially exposed, including emergency responders.

Environmental contamination declines after sunlight exposure and drying. Monitoring highly contaminated areas is indicated, however, and seeking the advice of FAD experts is recommended. *B mallei* can remain viable in tap water for at least 1 month[20] and 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, at least 5% calcium hypochlorite, 70% ethanol, 2% glutaraldehyde, iodine, benzalkonium chloride, at least 1% potassium permanganate, at least 3% solution of alkali, and 3% sulfur-carbolic solution. Phenolic and mercuric chloride disinfectants are not recommended.[6,22]

Because human-to-human transmission has occurred nosocomially and with close personal contact, standard precautions are recommended, including 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 should be used in the laboratory. Appropriate barriers to direct skin contact with the organisms are mandatory.[92,93] Family contacts should be advised of blood and body fluid precautions for patients recovering at home. Barriers protecting mucous 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.[18] 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

Glanders is a Category B disease of concern for bioterrorism by the Centers for Disease Control and Prevention because the agent is believed to be moderately easy to disseminate. Dissemination would result in moderate morbidity and low mortality, and enhancements to current diagnostic capabilities and disease surveillance would be required to rapidly and accurately diagnose the disease.

Because *B mallei* is a contender for use as a biological warfare or terrorism agent, the clinical index of suspicion should increase for glanders disease in humans. The rarity of recent human cases may make glanders a difficult diagnosis even in regions with exceptional medical facilities. As is the case with many rare diseases, final diagnosis and appropriate treatment are often delayed, sometimes with disastrous results. Without a higher index of suspicion, diagnostic laboratories might not conduct tests appropriate to detect *B mallei*, which happened in 2000 in the eighth US laboratory-acquired infection case.[50]

Further studies are needed to fully assess the usefulness of 16S rRNA sequencing in epidemiological investigations and the potential of using the subtle variations in the 16S rRNA gene sequence as a subtyping method for virulence and toxin production.

The genetic homology between *B mallei* and *B pseudomallei* may cause confusion in identifying the infectious agent, especially in areas endemic for *B pseudomallei*, which presents another challenge and invites further research. The capability to distinguish virulent strains from nonvirulent naturally occurring strains would also be useful. Finally, more research on antibiotic susceptibilities to *B mallei* is also warranted. Specifically, studies to consider an aerosol threat from a virulent strain and to distinguish the effectiveness of therapeutic agents for treating septicemic and pulmonary infections are indicated. The potential for prophylactic treatment regimens should also be investigated.

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. Therefore, 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 USDA, the Centers for Disease Control and Prevention, and the Department of Health and Human Services to control and eradicate the disease.

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