



## ***Burkholderia mallei* and *Burkholderia pseudomallei* as Bioterrorism Agents: National Aspects of Emergency Preparedness**

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*Burkholderia mallei* and *Burkholderia pseudomallei* are the causative organisms of glanders and melioidosis, respectively. Although now rare in western countries, both organisms have recently gained much interest because of their unique potential as bioterrorism agents [1]. Despite being unique organisms, *B. mallei* and *B. pseudomallei* share many similarities and may be considered together in the context of a deliberate-release event.

These pathogens are less familiar to medical and laboratory personnel than other select bioterrorism bacterial agents such as *Bacillus anthracis*, *Yersinia pestis* and *Francisella tularensis* and, therefore, a review of glanders and melioidosis is crucial in order to guide emergency preparedness and response to a deliberate-release event. The aim of this paper is to review the unique characteristics of these organisms in the context of bioterrorism, with special emphasis on national aspects of preparedness in Israel.

### **What makes *B. mallei* and *B. pseudomallei* “attractive” bioweapons?**

These pathogens have many characteristic features that make them nearly “perfect” agents for biological terrorism. Some of these characteristics are shared by the two bacteria, while others are unique or more prominent in one of them. It should be stressed that despite the abundant literature relating to *B. pseudomallei*, much of the knowledge of *B. mallei* relies on indirect evidence or expert opinion. Relevant key features are summarized in Table 1 and are elaborated below.

### **Epidemiology**

Melioidosis is endemic in several parts of Southeast Asia. During the 20th century, sporadic cases also occurred in western countries [2] but involved mainly returning travelers or military veterans, or reactivation disease. The most notable endemic foci today are in northern Australia and Thailand [3], and to a lesser extent in Singapore, Vietnam, Malaysia and Burma. Sporadic cases have been documented worldwide, notably in the Americas, the Caribbean region, the Pacific region, and Africa [4]. Confirmed sporadic cases in the Middle East have been reported

in Iran, and suspected cases in Egypt, the Gulf Emirates and Saudi Arabia, but not Israel.

Glanders primarily affects animals and can be transmitted both from animal to animal and animal to human, while human-to-human transmission is rare, at least in nature. Most human cases during the 20th century were occupational infections among laboratory workers, horse handlers, butchers and veterinarians [5].

**Table 1.** Key features contributing to the bioterrorism potential of *B. mallei* and *B. pseudomallei*

Aspect	Key features
Epidemiology	<ul style="list-style-type: none"> <li>• Rare diseases in western countries</li> <li>• Melioidosis endemic in Southeast Asia and Oceania, glanders rare and mostly sporadic</li> <li>• Not a reportable disease in Israel</li> </ul>
Pathogenesis	<ul style="list-style-type: none"> <li>• Environmental persistence (weeks for <i>B. mallei</i>, years for <i>B. pseudomallei</i>)</li> <li>• Infection through inhalation, ingestion or percutaneous inoculation</li> <li>• Low infective doses</li> <li>• Highly variable incubation period</li> </ul>
Clinical features	<ul style="list-style-type: none"> <li>• Acute, subacute or chronic disease; possibility of relapse and late reactivation</li> <li>• Wide spectrum of manifestations – “great imitators”</li> <li>• High prevalence of severe sepsis and septic shock</li> <li>• Significant mortality</li> </ul>
Diagnosis	<ul style="list-style-type: none"> <li>• Lack of experience among both clinicians and laboratory personnel</li> <li>• Identification by routine laboratory methods difficult to impossible</li> <li>• Need for specialized laboratory reagents, equipment and expertise</li> <li>• Unusual biosafety requirements</li> </ul>
Therapy	<ul style="list-style-type: none"> <li>• Complex protocols utilizing intravenous antimicrobials</li> <li>• Long duration of therapy (months)</li> <li>• Frequent need for surgical interventions and supportive critical care</li> <li>• Antimicrobial resistant isolates not uncommon</li> </ul>
National preparedness	<ul style="list-style-type: none"> <li>• Species highly accessible</li> <li>• No national reference laboratory</li> <li>• Difficulty in stockpiling relevant antimicrobials</li> <li>• Antimicrobial resistance</li> <li>• No available vaccine</li> <li>• No evidence regarding post-exposure prophylaxis</li> <li>• Lack of awareness in Israeli medical community</li> </ul>

With quarantine and veterinary control, glanders had been eliminated from most parts of Western Europe and North America by 1939 [6]. Enzootic foci continue in South America, the Middle East, Africa and Asia. Currently, the disease is considered potentially endemic in several countries in which recent infections have been described, such as Brazil, Manchuria, Eritrea, Ethiopia, Turkey, Latvia and India (last report in 1988). In Israel the last case occurred in 1951 and involved one horse but not humans.

Both glanders and melioidosis are not considered reportable infectious diseases by the Israel Ministry of Health. Current Health Ministry protocols do require the immediate reporting of "dangerous" infectious agents that might be implicated in bioterrorism such as anthrax, plague, tularemia, botulism, viral hemorrhagic fevers and smallpox, but not melioidosis and glanders [7].

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*Emergency preparedness should focus on increasing the awareness of the medical community, improvement of diagnostic capabilities and preparation of national response protocols*

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### Pathogenesis

Melioidosis affects both humans and animals. The main reservoir for *B. pseudomallei* is the contaminated environment (e.g., in endemic regions), especially soil and water. Human-to-human as well as zoonotic transmission is extremely rare. It has a saprophytic nature and is capable of surviving in relatively hostile environments for years [8].

*B. pseudomallei* survival capabilities are also associated with its adaptation to various hosts; the organism produces a wide variety of virulence and pathogenicity factors and is resistant to various elements of the innate immune system, including phagocytes and the complement system [9-11]. Its survival is also enhanced by formation of a glycolcalyx biofilm and transformation into several phenotypic variants [12]. Underlying conditions associated with qualitative and/or quantitative neutrophil abnormalities are thus recognized risk factors for melioidosis (e.g., alcoholism, diabetes mellitus, congestive heart failure, malignancy, chronic renal failure).

Humans (and animals) acquire melioidosis through percutaneous inoculation, inhalation or ingestion, and more rarely sexual transmission. The percutaneous route is thought to be the predominant portal of entry, even for patients with pneumonic melioidosis; pneumonia in such cases therefore involves hematogenous dissemination after percutaneous inoculation. However, pulmonary infection may occur directly via inhalation [13]. Ingestion mostly involves contaminated unchlorinated water. While these data apply for naturally occurring melioidosis, the natural history of inhalational exposure to *B. pseudomallei* in a

deliberate-release scenario generating much higher inocula may be quite different.

The pathogenesis of *B. mallei* infection is far more enigmatic. Unlike *B. pseudomallei*, it is not an environmental pathogen and its main reservoir is animals. It has limited surviving capabilities in the environment and has been described to persist for up to 6 weeks in infected horse stables. It is primarily a disease of equids (including horses, donkeys and mules) but can also affect goats, sheep, dogs and cats. The mode of infection in glanders is not at all clear and probably several routes of infection are possible, including inhalation, percutaneous inoculation, and ingestion. The incubation period may range similar to that of melioidosis, from 1-5 days in inhalational infection to many months. Relapse and reactivation disease may also occur in both diseases. Reactivation of melioidosis more than 30 years after the initial infection has been described.

### Clinical manifestations

The clinical manifestations of melioidosis are protean and this disease has been claimed to be one of the "great imitators," since primary infection and suppurative complications may involve virtually every body organ. A substantial percentage of melioidosis cases manifest with bacteremia (40-60%), septic shock is present in one-fifth of the patients, and mortality is highly significant (up to 60%) [13-15]. A substantial portion of patients thus require admission to the intensive care unit. Pneumonia is by far the most common syndrome associated with *B. pseudomallei* infection, occurring in half the cases. Lung infection can be acquired either by hematogenous dissemination or direct inhalation. In endemic areas, melioidosis may be the most common cause of community-acquired pneumonia [16]. Pneumonia in melioidosis may range in severity and may be acute, subacute or chronic, with the latter resembling tuberculosis [13]. A wide variety of other syndromes may occur that usually require surgery, e.g., prostatic abscesses, paralytic encephalomyelitis or intra-abdominal abscess formation [5,13]. Mortality rates among patients with bacteremia and multi-organ involvement or septic shock may be as high as 90% [17].

In animals, *B. mallei* causes glanders and "farcy," depending on the route of infection [6]. Glanders occurs predominantly through inhalation or ingestion whereas farcy is thought to occur through direct inoculation. In glanders, there is an acute or chronic lung infection and abscess formation in internal organs is common [6]. Farcy, on the other hand, appears as swelling in the skin and subcutaneous tissues that ulcerate. The surrounding lymphatic vessels, like the regional lymph nodes, become hard and enlarged (giving rise to "farcy pipes" and "farcy buds").

Human glanders, if acquired via the inhalational route, produces fever, ulcerative necrosis of the upper and lower respiratory tract with a typical purulent nasal discharge, extensive pneumonia, cervical or mediastinal lymphadenopathy, and pustular skin lesions (which may resemble smallpox). Prostration, disproportionate to the clinical signs, is a classic finding [6]. Septicemia invariably follows with involvement of various internal organs, as in melioidosis [18]. Without treatment, death occurs within

10 days. Chronic human glanders is associated with multiple subcutaneous and intramuscular abscesses, lymphadenopathy and lymphangitis and comprises half of naturally occurring infections. Visceral involvement is not rare in this disease variant and nasal involvement commonly occurs. This form of the disease may be active for month or years.

Contrary to naturally occurring glanders, inhalational glanders contracted through intentional aerosolization can be expected to produce a clinical syndrome similar to melioidosis [18], with acute fever, chills, myalgia, and symptoms of acute respiratory infection, especially cough and hemoptysis and various radiological forms of pneumonia. Unless a very low inoculum is involved, melioidosis and glanders in the context of deliberate release may thus be indistinguishable from one another [18].

## Diagnosis

The genus burkholderia contains more than 20 valid species, 3 of which are significant human pathogens – *B. mallei*, *B. pseudomallei* and *B. cepacia* complex. Owing to their ability to survive in hostile environments, standard specimen collection and transport principles are sufficient for recovering burkholderia species in clinical practice. Methods for isolating and identifying *Burkholderiaceae* may include culture-based, antibody/antigen-based and molecular-based techniques. *B. pseudomallei* grows well on standard culture media, but special media, such as the modified Ashdown agar, are needed for isolating it from non-sterile sites, especially sputum [19]. *B. mallei* is somewhat more fastidious and improved growth can be achieved with specialized media as well [20]. The processing of suspected clinical samples should be performed in a biosafety level 2 laboratory, while processing clinical isolates requires biosafety level 3 which is not available in most sentinel laboratories.

## Melioidosis and glanders constitute a major threat to Israel in the context of bioterrorism

*B. pseudomallei* has several basic phenotypic features that might raise suspicion, including colonial morphology, odor, motility and biochemical reactions, while *B. mallei* is non-distinct and thus may be extremely difficult to diagnose [21]. Identification of these two pathogens using commercial systems has been attempted, but significant rates of misidentification have been reported with both manual and automated platforms [22-24]. While *B. pseudomallei* is accurately identified to some extent by these systems, the identification of *B. mallei* is even more limited. Of note is that in the recent case of occupational glanders reported in a laboratory worker in the United States in 2000 [25], an automated system misidentified *B. mallei* as *Pseudomonas fluorescens* or *Pseudomonas putida*. Clues for laboratory diagnosis of these organisms are provided in Table 2.

For *B. pseudomallei*, various techniques have been used to

**Table 2.** Key phenotypic features that should raise the suspicion for *B. mallei* or *B. pseudomallei* in the sentinel laboratory

Key feature	<i>B. mallei</i>	<i>B. pseudomallei</i>
Gram-stain morphology	Gram-negative coccobacilli	Bipolar Gram-negative bacilli
Growth on medium	Growth on blood agar within 24-48 hours; delayed or no growth on MacConkey agar	Growth on blood/MacConkey agar within 24-48 hours
Morphology of colonies	Grey, translucent, smooth, no pigment	White, smooth, yellow/no pigment, aging colonies become wrinkled
Odor	Odorless	Earthy and musty
Motility	Non-motile	Motile
Cytochrome oxidase activity	Variable	Positive
Indole production	Negative	Negative
Polymyxin B susceptibility	Resistant	Resistant
Nitrate reduction to gas	Negative	Positive
Sugar utilization	Non-fermenter	Non-fermenter

detect specific antibodies. Antibody detection is important mainly for epidemiological purposes and serological surveys since *B. pseudomallei* recovery rates are high during clinical illness. The most used method is indirect hemagglutination or enzyme-linked immunosorbent assay but this technique has many limitations, especially in endemic areas [26]. Conversely, during acute infection, antibodies are usually not detected until seroconversion occurs at a later stage. Of note is the marked cross-reactivity that was found between *B. mallei* and *B. pseudomallei* and, therefore, serological testing cannot be used to differentiate between melioidosis and glanders [27]. An alternative in the form of direct antigen testing using immunofluorescence or agglutination has also been utilized. One-step immunofluorescence has been shown to be highly specific for detecting *B. pseudomallei* in clinical samples (> 99%). However, sensitivity has been disappointing (66%) [28].

In recent years, molecular methods for identifying these pathogens in clinical samples have gained much interest because of difficulties in identifying these organisms, especially in non-endemic areas [22,23,29]. Molecular tools may be applied directly to clinical specimens for diagnosing (or ruling out) the presence of *B. mallei* or *B. pseudomallei*, or alternatively, for final identification of isolates recovered from clinical specimens. Many studies have used such molecular tools, which are mostly experimental and only a few have clinical applications in the routine laboratory work flow. These assays include 16S rRNA analysis using real-time polymerase chain reaction [30] as well as methods relying on 23S rDNA [29], real-time PCR of type III secretion system genes [31] and TaqMan allelic-discrimination assay [32]. A promising multiplex real-time PCR with molecular beacons for diagnosing anthrax, tularemia, plague and glanders was recently described [33]. None of these molecular assays has yet become 'point of care' in sentinel laboratories.

PCR = polymerase chain reaction

Evaluation of antimicrobial susceptibility for *B. mallei* and *B. pseudomallei* is also challenging. Although susceptibility breakpoints for these organisms have recently been introduced [34], resistance determination requires broth dilution methods that are not routinely performed in sentinel laboratories. Although the E-test may prove to be a reasonable alternative to broth-based methods, susceptibility testing of *B. mallei* and *B. pseudomallei* requires biosafety level 3 conditions and is thus beyond the capability of the average sentinel laboratory.

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*Appropriate measures to ensure national emergency preparedness to the deliberate release of B. mallei and B. pseudomallei are crucial*

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### Treatment

As a general rule, bactericidal agents are preferred for the initial treatment phase of melioidosis (also termed the intensive phase), while bacteriostatic agents may be used for maintenance therapy in the so-called eradication phase. Nine randomized controlled trials have been carried out to date in melioidosis patients, six for the intensive phase and three for the eradication phase. These trials were recently summarized in a Cochrane Collaboration review [35]. Trials in the intensive phase included 619 patients with moderate-to-severe disease for whom outcome data were reported. All studies included an arm of ceftazidime alone or in combination with trimethoprim-sulfamethoxazole. For comparison the drugs mostly used were imipenem, amoxicillin-clavulanate and cefoperazone-sulbactam. Ceftazidime showed decreased mortality as compared to older drugs [36,37] and similar outcome to that of the newer drugs [38,39]. For the eradication phase, trials included various combined regimens of oral trimethoprim-sulfamethoxazole, amoxicillin-clavulanate, chloramphenicol and doxycycline.

Therefore, intravenous therapy for acute melioidosis should at least include ceftazidime or imipenem (or meropenem) as first-line agents, or a beta-lactam/beta-lactamase inhibitor combination as second-line (amoxicillin-clavulanate). Initial-phase therapy should last at least 14 days, while 6 weeks of intravenous therapy is indicated for suppurative complications (e.g., intra-abdominal abscesses or septic arthritis). For the eradication phase, oral therapy should include a combination of trimethoprim-sulfamethoxazole and doxycycline with or without chloramphenicol [35] for a duration of at least 3 to 6 months. Oral monotherapy with trimethoprim-sulfamethoxazole, or amoxicillin-clavulanate (in pregnant women or children) is also acceptable.

The antibiotic susceptibility pattern of *B. mallei* is generally similar to that of *B. pseudomallei*. A recent survey of 15 *B. mallei* isolates showed 100% susceptibility to cefotaxime, ceftazidime, amoxicillin-clavulanate, piperacillin-tazobactam, imipenem, chloramphenicol, trimethoprim-sulfamethoxazole and tetracyclines [40]. There are no specific data for human glanders infection. It is generally thought that empiric therapy for melioidosis is also

appropriate for glanders [18], but no evidence-based regimens are available. Moreover, there are no data, clear-cut recommendations or sufficient evidence regarding post-exposure antibiotic prophylaxis in melioidosis or glanders.

### Emergency preparedness

Glanders was implicated in the first modern attempt of biological warfare when used by the Germans against cavalry horses of the United States, Argentina, Spain, Norway and Romania during the Great War. Weaponization of *B. mallei* was also used by Japan in World War II [18] and to some extent by the United States and the Soviet Union.

An occupational *B. mallei* infection occurred recently (in 2000) in the United States and involved a microbiology laboratory worker [25]. The patient developed fever and lymphadenopathy, followed by diabetic ketoacidosis and intra-abdominal abscess formation that was eventually successfully treated with antibiotics. Interesting points emanating from this case are: a) the acquisition of *B. mallei* infection due to inadequate safety precautions while working with the organism, b) the difficulty in diagnosing clinical *B. mallei* infection, and c) the misidentification of the clinical isolate by routine laboratory methods.

*B. mallei* and *B. pseudomallei* are now included in formal emergency preparedness plans and guidelines issued by various authorities in the United States and Europe. The actual risk for deliberate release of either of these agents is unknown, at least publicly, and therefore most efforts are concentrated on the efficiency and safety of laboratory-based diagnosis of melioidosis and glanders. Recommendations regarding post-exposure prophylaxis and antibiotic stockpiling are lacking and no vaccine is available.

Melioidosis and glanders constitute a bioterrorism threat to Israel until proven otherwise. Currently, efforts should target several main issues at the national levels:

- Recognition of the threat posed by *B. mallei* and *B. pseudomallei* by all relevant official health and national authorities and development of appropriate national emergency preparedness and response protocols. Defining melioidosis and glanders as reportable infectious diseases by the Ministry of Health is mandatory.
- Increasing the awareness of the medical community to the clinical features and principles of diagnosis and treatment of glanders and melioidosis, among first responders, hospital and community physicians, laboratory personnel and public health practitioners.
- Development of diagnostic capabilities at the level of sentinel and reference laboratories in order to allow accurate diagnosis in a timely fashion.

In conclusion, melioidosis and glanders are increasingly recognized worldwide as potential biological weapons. Emergency preparedness to a deliberate-release event involving either melioidosis or glanders should be an integral part of national biodefense efforts, with particular focus on increasing the awareness of the medical community, improvement of diagnostic capabilities and preparation of national response protocols.

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