

# Glanders and melioidosis – diseases caused by *Burkholderia* species

*Burkholderia* spp. are ubiquitous in the environment; however, transmission to humans does occur and can result in serious, life-threatening disease. Here, Sally J Cutler and Ronald R Cutler provide an overview of these uncommon microbiological agents.

*Burkholderia mallei* and *B. pseudomallei* are the aetiological agents of glanders and melioidosis, respectively. Although glanders is primarily a disease of solipeds – animals with single hooves (eg horses and asses) – it can also infect humans. It is rarely reported but endemic pockets of infection persist in parts of Africa, the Middle East, South America, south and south-east Asia, and Turkey, and a recent cluster of cases at a racing stable in the United Arab Emirates serves to alert us against complacency.<sup>1</sup> The organism causing melioidosis is caused by an environmental saprophyte associated with mud or water in both tropical and sub-tropical regions. Melioidosis is considered endemic in south and south-east Asia and in northern Australia.

In man, both agents can cause severe and rapidly fatal disease, and this, coupled with aerosol transmission, a broad host range and resistance to many antimicrobial agents, has led to their inclusion on the category B list of critical biological agents by the CDC. Glanders was used as a biological weapon during the two major wars of the first half of the 20th century, when it was used against horses, civilians and prisoners of war. Later, it was also used against horses by the former Soviet Union during its war with Afghanistan.

Increasing globalisation and the frequent movement of animals through trade and of humans through tourism now provide ideal

routes for dissemination. This, coupled with global warming, which has extended the compatible climatic ranges for the saprophytic *B. pseudomallei* and increased susceptible human populations through improved supportive medical care of immunocompromised patients, provides opportunities for these infections to be classed as emerging pathogens.

This article examines current thinking on the *B. mallei*/*B. pseudomallei* complex, and identifies some gaps in current knowledge.

## FROM DISCOVERY TO TODAY

Early descriptions of glanders date back to Greek and Roman times when accounts of the infection were recorded by writers such as Aristotle, Apsyrus and Vegetius. The aetiological agent was first isolated simultaneously in 1882 by German and French microbiologists. Epidemics followed large-scale movements of horses, and typically were associated with early wars (Fig 1).

**Table 1.** History of glanders.

3 BC	Description by Aristotle
1664	Contagious nature recognised
1830	Zoonotic nature suspected
1891	Mallein test developed
1900	Control programmes implemented



**Fig 1.** Movement of large numbers of horses during the First World War.

A delayed hypersensitivity reaction, similar to that seen with the tuberculin test, following subcutaneous or intradermal inoculation of the crude cell extract mallein provided a diagnostic tool in 1890. Use of this test and the subsequent slaughter of all animal reactors, whether or not clinical signs were present, was instrumental in the eradication of the disease.<sup>2</sup> Glanders was a widespread disease during the 19th century, but it was finally eradicated from the UK by 1928.

Melioidosis was described initially by Alfred Whitmore and his colleague C S Krishnaswami as a glanders-like illness. This was first seen among morphine addicts from Rangoon, Burma, in 1911.<sup>3</sup> Although it caused a clinically similar picture in man, the causative agent of melioidosis could be distinguished by its motility and more luxuriant growth *in vitro*.

The more recent application of typing methods has substantiated the resemblance of both aetiological agents, which supports the hypothesis that *B. mallei*, the causative agent of glanders, is a clone of the more diverse *B. pseudomallei*, the agent of melioidosis.<sup>4</sup> Genomic sequencing has now provided confirmation of this.

## THE MICROBE

The organisms responsible for glanders and melioidosis belong in the genus *Burkholderia*, which contains over 30 species; however, only *B. mallei*, *B. pseudomallei* and *B. cepacia* are pathogens in man, the last being seen predominantly in cystic fibrosis patients.

These microbes are aerobic, non-spore-forming bacilli. Although most members of the genus are motile, *B. mallei* is the notable exception. Motility has been used for discrimination since *B. mallei* was first described. Molecular studies of the genomes have revealed that although *B. mallei* carries flagellin genes, one component, *fliP*, is non-functional because of the presence of a large DNA insertion.<sup>5</sup>

Phylogenetic studies show the remarkable similarity between these species, and *B. mallei* is now considered a 'pathovar' of *B. pseudomallei*.<sup>4</sup> Both organisms show a genome split between two large chromosomes, with the larger (4.07 Mb for *B. pseudomallei* [strain K96243] and 3.5 Mb for *B. mallei* [strain 23344]) carrying genes for cell metabolism and growth, and the smaller (3.17 Mb for *B. pseudomallei* [strain K96243] and 2.3 Mb for *B. mallei* [strain 23344]) carrying accessory genes required for survival and virulence factors.

While *B. pseudomallei* contains genomic islands amounting to approximately 6% of its genome, suggestive of horizontal DNA acquisition, no such findings are apparent for *B. mallei*. The latter shows 'selection by gene deletion', with a notable absence of genes required for environmental survival.

## HUMAN INFECTION

Clinical presentation is very variable, but the spectrum is similar for both organisms. Human melioidosis has been encountered significantly more frequently than glanders. The only recent case of human *B. mallei* infection (glanders) was the laboratory-acquired infection of a microbiologist undertaking research on this organism.<sup>6</sup>

Presentation can vary according to the route of infection and any potential microbial factors (eg as yet unidentified virulence determinants residing on the ancillary genome).<sup>7</sup> The disease can manifest as either progressive or chronic remitting infection, making diagnosis on clinical grounds alone unreliable. If strains are used in deliberate biological attacks, however, it is worth remembering that they may have



**Fig 2.** The removed nasal septum of a horse showing different stages of lesions caused by glanders.

been modified in order to enhance or alter virulence.

Patients often present with septicaemia resulting from a primary focal infection at the site of introduction (lung, skin or soft tissue involvement). New metastatic foci can follow, particularly affecting the lungs, liver, spleen and kidneys, skin and soft tissues (cellulitis, pustules), bones and joints, lymph nodes and prostate. Some cases present as pyrexia of unknown origin, and these are associated with microbial shedding from a deep-seated abscess. If the infection is detected soon enough, mortality in septicaemic cases can be reduced from 100% to around 40% with appropriate therapeutic management.

The lung is most frequently affected in melioidosis, resulting in areas of consolidation, particularly in the upper lobes, that may be confused with pulmonary tuberculosis. Other frequent presentations are soft tissue infection, visceral abscesses, especially in the spleen, liver and kidney, lymphadenitis, osteomyelitis/septic arthritis, and brain abscess. In children, melioidosis often presents as a parotid abscess, with mortality among those with focal manifestation of approximately 5%.

Studies of melioidosis suggest that there are certain risk factors for human disease. These include underlying conditions such as diabetes mellitus (particularly type 2), thalassaemia, renal disease, splenectomy, chronic lung disease, or other infections (eg tuberculosis or dengue haemorrhagic fever). In general, males above the age of 45 years appear to be at greatest risk. These observations underscore the importance of the host factors in susceptibility to melioidosis.

Studies of host-microbial interactions demonstrate multiplication in phagocytic vacuoles and subsequent endosomal lysis. Direct cell-to-cell spread is believed to occur, sometimes associated with fusion to form multinucleated giant cells. Transposon mutagenesis and investigations into the significance of exotoxins in virulence for these microbes have failed to identify a significant role in pathogenesis. Type III secretion systems are believed to be essential for pathogenicity of these organisms, with approximately 18 genes associated with this identified to date.

The *Burkholderia* secretion apparatus (*bsa*) resembles the *ipa/mxi/spe* cluster described in *Shigella flexneri*<sup>8</sup> and endows the ability to invade and escape from phagosomes and spread to adjacent cells. Both capsular polysaccharide (CPS) and lipopolysaccharide (LPS) are immunogenic. To date, four CPS genes have been described (I–IV), with CPSI having a proven role in virulence, probably through inhibition of complement factor C3b binding to the microbial cell. In contrast, CPSII has a suggested role in environmental survival.

Analysis of LPS from multiple isolates reveals that this is largely conserved, with only 3% of isolates showing aberrant profiles. Surprisingly, there is a lack of immunological cross-reactivity between the 'typical' and 'atypical' LPS types. Homology between the LPS of *B. pseudomallei* and that of *B. thailandensis* has led to the suggestion that LPS is not important for virulence. A detailed review of the pathogenicity mechanisms employed by these microbes is outside the scope of this article, but these were reviewed recently by Wiersinga and others.<sup>8</sup>

There is no vaccine for glanders. People avoid its transmission from animals by eliminating it from the animal population. When and if human cases do occur, steps must be taken to prevent spread into healthcare settings.

## ANIMAL INFECTION

Glanders or farcy, both caused by *B. mallei*, are well documented in solipeds. Typically, glanders produces ulcerative lesions of the nasal epithelium (Fig 2) or turbinate bones, with characteristic copious purulent nasal discharge accompanied by fever. Death can follow in just a matter of days.

In horses, infection can be more protracted, with infected animals surviving for some years. Such chronic and subclinical cases pose a significant risk of infection for new susceptible hosts. Dermatological presentation (farcy) manifests as ulcerative lesions of the skin and various body locations. Lymph nodes become enlarged and nodular abscesses develop, ulcerate and discharge purulent oily pus.

Although primarily affecting solipeds, infection has also been reported in members of the cat family (both in the wild and in zoological parks), in camels, bears, wolves and dogs. Although inhalation or inoculation through minor injuries are the major transmission routes, it is believed that infection in carnivores follows feasting on infected meat.

*B. pseudomallei* demonstrates a wider range of susceptible host species, including camels, horses, sheep, cattle, goats, pigs, kangaroos, koalas, alpacas, deer, cats, dogs and marine mammals.<sup>9–11</sup> As in man, melioidosis in animals can be highly variable in its clinical presentation, ranging from fulminant septicaemic infection with

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## ARTICLE

associated haematogenous spread and a high mortality, to focal disease or subclinical infection. Infection is associated with emaciation, weakness, oedema, lymphangitis of limbs, respiratory signs sometimes with nasal discharge and generalised signs of colic with or without diarrhoea.

Dogs and cats are also susceptible to infection with *B. pseudomallei*. Acute, subacute and chronic disease is described in dogs. As seen in other animals, the clinical signs range from acute septicaemia, pneumonia and diarrhoea, through to lymphadenitis and lymphangitis.<sup>10</sup>

### TREATMENT PROTOCOLS

Melioidosis has been managed successfully with one of the following options: ceftazidime, meropenem or imipenem/cilastatin). The duration of intravenous treatment required depends on the site of infection and the clinical response; however, a minimum course of 14 days' treatment should be given to all patients.

As human cases have been sporadic, clinical trials of efficacy have not been possible to evaluate different therapeutic regimens. Unlike *B. pseudomallei*, *B. mallei* is susceptible to aminoglycosides. In general, treatment protocols are often extended over 20 weeks, with an initial 10–14 days of intravenous therapy, usually with ceftazidime or carbapenem, followed by an oral therapeutic phase.<sup>8</sup> Prolonged clinical follow-up is advised as these organisms have the ability to persist in their host.

### EPIDEMIOLOGICAL PATHWAYS

#### Transmission routes

Inhalation rather than inoculation appears to be the primary route of infection. This is supported by the impact of climatic conditions on infection rates, such as that seen with high rainfall and pneumonia and septicaemia. Inoculation into small abrasions in contact with contaminated soil or water provides another important route for infection, and ingestion could provide a less frequent route of infection. Outbreaks among swine in northern Australia have been attributed to this route, with contaminated water supplies providing the likely vehicle for transmission. This was controlled effectively with chlorination.

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Fig 3. *B. pseudomallei* colonies on a blood agar plate.

#### Infectious dose

The infectious dose for both *B. mallei* and *B. pseudomallei* in humans is unknown, but will vary considerably according to host resistance and microbial virulence factors. Use of susceptible animals indicates the LD<sub>50</sub> by inoculation with *B. pseudomallei* to be less than 10 organisms. Even less is known about the infectious dose for *B. mallei* in humans, although the high incidence of infection among laboratory workers in the past implies that it may be even more infectious than *B. pseudomallei*.

#### Risks

Risk assessments undertaken for glanders conclude that the likelihood of importation to the UK is low. This is based on the few cases detected and reported in recent years. Diagnostic assays for glanders still require further improvement, as not all animals infected give sufficiently elevated titres. Also, there are specificity problems with cross-reactive antibodies from other sources, and a surprisingly low success rate for antigen detection by polymerase chain reaction (PCR) methods.

These factors, together with the potential for latency that could reactivate if the host is stressed, such as through transportation, could result in imported infection. This was illustrated by the introduction of glanders in a stable in Dubai, following transportation of horses from Syria.<sup>1</sup> Significant movement of racehorses between the UK and locations such as the United Arab Emirates is now normal practice.

As a significant disease in animals, melioidosis has been overshadowed by glanders from the livestock perspective. It is now considered to be an emerging disease, however, with isolates from Spanish horses, an outbreak associated with a zoological park in Paris, and introduction in the UK following the importation of infected monkeys.

### ENVIRONMENTAL SURVIVAL

*B. pseudomallei* is resilient to hostile environmental conditions such as those associated with nutritional deficiency. It can persist not only when deprived of nutrients but also in antiseptic and detergent solutions

(ie in acidic environments [pH 4.5] for up to 70 days), at temperatures of 24–32 °C; and with little water (ie soil water content <10%).<sup>3</sup> The organism is also reported to have survived for several years in soil in France during an outbreak in the 1970s.<sup>10</sup>

Survival appears to vary with soil type, with clay layers able to yield the organism from depths of 25–120 cm. Certainly, many infectious episodes relate to environmental events such as agricultural or construction practices that involved disturbance of the soil.

Although *B. mallei* is generally less robust than *B. pseudomallei*, it is reported to survive for up to four weeks in tap water, and is likely to survive under suitable environmental conditions for a couple of months. Both organisms are susceptible to killing by a variety of disinfectants (eg sodium hypochlorite at 500 ppm chlorine for five minutes, 5% phenol for 10–15 minutes), although little recent work has been done on this subject.

Soil decontamination was attempted using hypochlorite during the French outbreak, but it is unclear what significance this played in the ultimate disappearance of the organism from contaminated areas.

### DIAGNOSTIC DILEMMAS

As diagnosis is difficult on clinical grounds alone, laboratory confirmation is essential. Direct immunofluorescence microscopy on pus or secretions can provide rapid laboratory confirmation and plays a significant role in endemic areas, but currently it is not available in the UK. Thus, a specific diagnosis usually depends on culture and identification of *Burkholderia* spp. from clinical material.

The organisms can be cultivated from many sample types, but typically these include blood cultures, sputum, pus or swabs and urine. Serology can be useful later in the disease, particularly against LPS and capsular antigens. Diagnostic samples include blood cultures, sputum or nasal discharges, pus from suppurative lesions, urine and acute and convalescent serum for serological investigations. These materials should be processed accordingly for containment level 3 pathogens.

Cultivation of *B. pseudomallei* is not difficult (Fig 3); however, isolation of *B. mallei* presents more of a challenge. Numbers of organisms in ulcerative lesions or environmental samples may be low, so concentration and enrichment can improve yield significantly. Antimicrobial resistance of *B. pseudomallei* has been used to develop selective media such as Ashdown's medium, which incorporates gentamicin (4 mg/L) and crystal violet,<sup>12</sup> and more recently with the addition of colistin. Comparison of this with *B. cepacia* medium revealed comparable recovery, but the latter proved less selective.<sup>13</sup>

Ideally, a non-selective medium should be included in parallel. This is especially important as the crystal violet used in Ashdown's medium may inhibit mucoid phenotypes or those 'damaged' by

## ARTICLE

These once largely neglected pathogens have received a resurgence of interest through their inclusion on the CDC list of agents that could be used as biological weapons. Application of molecular genetics to analyse genomic sequencing data has disclosed some of the underlying mechanisms used by these organisms to survive in and subvert host cells. Further interrogation of these data, together with the additional genomic sequences nearing completion, will help to unravel the host–pathogen interactions employed by these microbes.

Analysis of the proteome and secretome, coupled with the host immunological interactions, will provide further insights into the pathogenicity mechanisms of these organisms. The significance of lateral gene transfer in the acquisition of further virulence determinants has yet to be established.

Clinically, it remains unclear why childhood incidence is low and parotitis predominates, in contrast to the manifestations seen later in life. What is the pathological basis for the associated risk factors observed in clinical studies? Furthermore, it is yet to be determined how, where and why some hosts become latent carriers. How can diagnostic ability to detect these cases be improved? Therapeutic management could be further refined, particularly in the case of glanders.

Much of this research has focused on vaccine development. What is becoming apparent, however, is the need to stimulate cell-mediated and humoral immunity, but which vaccine candidate to use, under what circumstances and in which populations are questions that have yet to be resolved. ■

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