Glanders & Melioidosis: a zoonosis and a sapronosis

"Same same, but different"

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Abstract

(Use introduction)

Keywords

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Introduction

Glanders, caused by infection with *Burkholderia mallei,* primarily causes infection in equines, but may be transmitted to humans, and thus qualifies as a true zoonosis. Melioidosis is caused by *B. pseudomallei*, genetically very similar to *B. mallei*, but which is an environmental saprophyte capable of infecting humans and a wide range of other animals. Good evidence of animal-to-human, or even human-to-human, transmission of melioidosis is lacking, and so it most appropriately referred to as a

sapronosis, or at most a sapro-zoonosis. Although rare in Western countries, both micro-organisms have recently gained much interest because of their potential use as bioterrorism agents and widening geographic footprint. The increasing recognition of melioidosis in humans and recent outbreaks of glanders in animals have led to their description as emerging or re-emerging diseases, and melioidosis as a neglected tropical disease. Laboratory-associated infections with both organisms have also occurred, resulting in their categorisation as Hazard Group 3 pathogens. In this chapter we review the epidemiology of animal and human cases of glanders and melioidosis, the evidence for different modes of transmission, pathogenesis and clinical features, diagnosis and treatment, as well as public health and disease control issues.

Glanders

History & Epidemiology.

Symptoms of glanders in equines were reported as early as 425 BC by Hippocrates, however it was Aristotle who first described it as "μηλις" (malis in Latin, from which *B. mallei* takes its name) in approximately 350 BC.(Sharrer, 1995) *B. mallei* was first isolated from the liver of an infected horse in 1882.(Boerner, 1882) Infection resulted in significant morbidity and mortality in equines worldwide, and was occasionally transmitted to humans in prolonged close contact with horses, such as grooms, coachmen, veterinarians and butchers, or to other animals through direct or indirect contact.

Glanders has since been eradicated from Western Europe, USA and Canada due mainly to the reduction in the use of horses in everyday life, but also to improved animal husbandry and hygiene and strict programmes enforcing statutory testing and slaughter of infected animals,(Blancou, 1994, Derbyshire, 2002) however the disease persists in the Middle-East, parts of Asia, and South America. A possible re-introduction occurred in Germany in 2014, which was identified on routine pre-export serological tests despite a lack of epidemiological evidence of contact with *B. mallei* infected horses.(Kettle and Wernery, 2016) This possibly represented latent disease, which was not detected by standard diagnostics.(Kettle and Wernery, 2016) Within the last 20 years, increasing numbers of equine cases have been reported from countries including Pakistan, India, Bangladesh, Brazil, Turkey, Iran, Iraq, Afghanistan, Kuwait, Bahrain, UAE, Lebanon, Latvia, Belarus, Mongolia and China.(Rahman et al., 2018, Health, 2013, Khan et al., 2013) These are usually sporadic involving single or small numbers of animals, although occasionally larger outbreaks have occurred, such as that in India between 2006 and 2010 involving eight states and 164 equines.(Malik et al., 2012) Sporadic human cases have also been reported from Cameroon, Curaçao, Sri Lanka, and Turkey.(Office International des Épizooties 2011) Laboratory-associated human cases, such as that which occurred in a military research microbiologist in USA in 2000,(Srinivasan et al., 2001) the first case in the USA for over 50 years, have also been reported occasionally.

Due to its high fatality rates and transmissibility of the disease in animals and humans, glanders has long been considered as a potential biological weapon. When horses were widely used for military purposes, devastating natural outbreaks occurred, for example during the American Civil War.(Sharrer, 1995) *B. mallei* was actually used by the Germans in sabotage attempts during World War I,(Christopher et al., 1997) and in human experiments by the notorious Japanese Unit 731 in Manchuria in the period leading up to World War II.(Darling, 2004) It is reported to have been

weaponized by the Soviet Union, and used against the Mujaheddin in Afghanistan in the 1980s.(Alibek, 1999) With resurgent bioterrorism concerns, *B. mallei*, listed as a category B bioterrorism agent and Tier 1 Select Agent by the Centers for Disease Control and Prevention and the US Department of Agriculture, is now being studied in many laboratories throughout the world.

Modes of Transmission

It was initially thought that glanders was transmitted through the air, however in the early 18th century it was proposed that transmission took place through direct contact with infected horses, or indirectly through contaminated harnesses and water troughs.(Khan et al., 2013, Kinsley, 1911) Inoculation or ingestion of infected clinical material was demonstrated to cause infection in horses and other animals in experiments conducted in the late 19th and early 20th centuries, which also confirmed that 'glanders' and 'farcy' were different manifestations of the same disease.(O'Leary, 1908, Schutz, 1898) Later, nasal discharge and skin exudate from infected animals was shown to contain large numbers of bacteria that could be readily cultured, and it was shown that viable bacteria could survive for at least four weeks suspended in water.(Miller et al., 1948)

Once it has contaminated harnesses, grooming tools, hoof trimming equipment, water troughs or hands, *B. mallei* may transmit to new hosts through skin abrasions, mucous membranes, ingestion of water containing infective material, or inhalation of dried infected particles.(Carr Gregory, 2007) The disease spreads quickly in overcrowded, poorly hygienic, and humid environments.(Khan et al., 2013) Occasional cases have been reported in carnivores fed on infected meat.(Alibasoglu et al., 1986, Khaki et al., 2012) Vertical transmission has occurred naturally from mare to foal, and from laboratory infected guinea pig sows to their offspring.(Loeffler, 1886, Rutherford, 1906)

Zoonotic transmission to humans appears to be relatively uncommon. During World War II, human infections were rare despite a 30 % prevalence in horses in China.(Darling, 2004) Disease has occurred almost exclusively in individuals whose occupation involves close and prolonged contact with horses, but there is often no history or clinical evidence of inoculation or path of infection.(Bernstein and Carling, 1909) Lethal human glanders has also been documented to occur following a bite by an infected horse.(Pilcher, 1907) As is the case for melioidosis, diabetes may place humans at greater risk of infection after exposure, although reports of this are remarkably rare, perhaps because of the rarity of human glanders since diabetes became readily treatable.(Srinivasan et al., 2001) Human infection by ingestion has not been definitively reported, although it has been seen in carnivores (Alibasoglu et al., 1986, Khaki et al., 2012). In fact even where there is known to have been consumption of glanderous meat, human infection has not occurred.(Loeffler, 1886) Human-to-human transmission is also rare, but has been reported, and has usually involved close contact with the infected individual either as a family member, a carer, during medical procedures or post-mortem examination.(Loeffler, 1886, Robins, 1906) In a review of 156 reported cases at the turn of the last century, around 10 % were believed to have been acquired from human contact.(Robins, 1906) In the present day, improved living conditions, universal precautions, disinfection and available treatment make human-to-human transmission much less likely to occur. Recently, a study examining 538 in-contact individuals, including equine handlers, veterinarians/field assistants and laboratory workers processing *B. mallei* samples, found no seropositive individuals by complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA).(Singha et al., 2020a)

In developed countries, laboratory exposure seems to be a greater threat than animal contact, and anecdotal observations suggest that *B. mallei* may be more infectious in this setting than *B. pseudomallei*.(Howe and Miller, 1947) Some cases have occurred following obvious aerosol exposures during spillage of culture material, or direct inoculation injuries,(Howe and Miller, 1947) but many did not recall a particular exposure.(Srinivasan et al., 2001) It is suspected that most laboratory-acquired cases are a result of inhalation.(Carr Gregory, 2007)

Although outbreaks continue to occur in the endemic regions listed above, little is known about the ecology and population dynamics of *B. mallei*. A recent study investigated the molecular epidemiology of glanders in Pakistan. Isolates from 15 glanderous horses in the Punjab between 1999 and 2007 underwent variable number of tandem repeat (VNTR) analysis, phylogenetic analysis and comparison to 10 whole-genome-sequenced strains of *B. mallei*. The results confirmed the Punjab strains to be genetically distinct from the sequenced strains, and to form three distinct clades, with the majority belonging to a single clade temporally and geographically spread, suggesting that this is ecologically established in the Punjab region.(Hornstra et al., 2009) Together with additional epidemiological data, the authors concluded that human movement of equines contributed to the dispersal of *B. mallei* genotypes and that strains could persist for at least 1.5 years. Similarly, glanders infection in a dromedary in Bahrain was shown to be genetically similar to the Dubai 7 strain which caused an outbreak in horses in United Arab Emirates in 2004,(Wernery et al., 2011) and it was suggested that the strain was introduced from that region through international horse trade. The first molecular characterisation of a Brazilian *B. mallei* strain isolated from a mule in 2016 found that it was most closely related to an Indian strain isolated from a horse in 1932.(Laroucau et al., 2018) Using 45 *B. mallei* isolates, this study identified three distinct lineages; L1- included only two isolates from Turkey and United Kingdom , L2- isolates mainly from India, China and Burma, with some from Hungary, Iran, Pakistan and UAE, and L3- mainly Turkish isolates along with some from Brazil, Hungary, India, Iran, Russia and USA. Caution is advised due to the paucity of available *B. mallei* genome data, meaning there is likely to be bias in the geographic clustering seen so far.(Laroucau et al., 2018) In India, the state of Uttar Pradesh (UP) has become a glanders hotspot. Between 2013-2016, 10 isolates from horses and mules identified a cluster of five that were linked to UP.(Singha et al., 2021) However, further VNTR analysis identified considerable genotypic variability of *B. mallei* isolates from India. These were closely linked to isolates from Pakistan, followed by Turkey; which points to an ancestral clone which disseminated to geographically linked countries via equine movement over time.(Singha et al., 2021)

Microbiology

B. mallei is a facultative intracellular, aerobic, non-motile Gram-negative bacillus. The results of DNA-DNA hybridisation,(Rogul et al., 1970) multi-locus sequence typing (MLST),(Godoy et al., 2003) and whole genome sequencing(Nierman et al., 2004) have demonstrated unequivocally that *B. mallei* is actually a clone of *B. pseudomallei* which has undergone a substantial reduction in the size of its genome during the process of adaptation as an equine pathogen,(Nierman et al., 2004) differing at only a single nucleotide site in one of seven housekeeping genes studied. Based on these data it should not taxonomically be considered a separate species, however it retains species status due to its specific clinical and epidemiological behaviour.

Very few recent clinical isolates are available for study, so knowledge of the characteristics of *B. mallei* is based on historical descriptions and archived strains, which may be laboratory-adapted to

varying degrees. The organism often has an irregularly stained appearance on Gram's stain due to the presence of 'lipoid granules'.(Worley and Young, 1945) Miller noted that when the organism was stained in clinical specimens, there was an impression of a capsule, however this is not apparent using common capsule stains.(Miller et al., 1948) It is nutritionally versatile, being able to use a wide range of organic compounds as a carbon source, and can oxidise glucose and usually mannitol. It is able to grow on most laboratory media, but requires glycerol for optimum growth,(Miller et al., 1948) initially forming shiny and translucent colonies which tend to become mucoid with age. Selective agars have been developed and may be useful when clinical specimens from non-sterile sites are collected.(Kinoshita et al., 2019) Most strains are oxidase positive. The optimal temperature for growth is 37°C; many strains grow poorly below 25°C but all will grow at 41°C. *B. mallei* is unable to survive in dried pus for longer than a few days, or for 24 hours when exposed to sunlight, although it can survive in tap water for at least four weeks(Miller et al., 1948, Howe and Miller, 1947) and for three to five weeks in wet, humid, or dark environmental conditions. Following inoculation onto non-porous laboratory materials (e.g. rubber gloves, glass and stainless-steel), *B. mallei* China 7 viability decreased to nondetectable levels within 4-7 days. However, it was inactivated on exposure to vapour phase hydrogen peroxide.(Rogers et al., 2016)

It grows less luxuriantly on laboratory media than *B. pseudomallei*, from which it may be distinguished by its susceptibility to aminoglycosides and lack of motility. Like *B. pseudomallei*, it is intrinsically resistant to colistin and polymyxin B. *B. mallei* is non-flagellated, despite retaining flagellar genes that are not expressed,(Song et al., 2010) whereas *B. pseudomallei* has 2-4 polar flagella per cell.

Pathogenesis

B. mallei has the ability to invade and replicate inside epithelial and phagocytic cells and evade host immune mechanisms, resulting in an acute and fatal course, or a more chronic persistent infection state. *In vivo* animal models of glanders, in particular hamster and mice models, have provided important data on various pathogenic mechanisms. Non-human primate models highlight striking differences in pathological features observed between melioidosis and glanders.(Nelson et al., 2014) With *B. pseudomallei* infection, most pathological features were hepato-splenic; multi-focal suppurative lesions with areas of variable necrosis, whereas *B. mallei* lesions, although multi-focal, were non-necrotic, and more severe in the lungs. All *B. mallei*-challenged animals had multi-focal necrotising pneumonia, but only one-third of *B. pseudomallei*-challenged animals exhibited pneumonia.(Nelson et al., 2014)

B. mallei ATCC 23344 genome contains at least two *luxI* and four *luxR* homologues, which are quorum sensing (cell-density) based regulators of virulence factor expression. When inactivated, reduced bacterial virulence was observed in mice.(Ulrich et al., 2004) The genome also encodes a *virAG* two-component regulatory system that is required for virulence in hamsters(Nierman et al., 2004) and overexpression results in transcription of approximately 60 genes.(Schell et al., 2007)

Like many pathogenic Gram-negative bacteria, *Burkholderia* spp. use a Type III secretion system (T3SS) to interact with and invade host cells. This system involves secretion of a protein onto the membrane of a host cell, to which the bacteria can subsequently bind, form a pore, and insert effector proteins directly into the host cell cytosol. *B. mallei* contains two T3SS, one of which is the animal pathogen-like Bsa T3SS (T3SSAP) which is required for rupture of endocytic vacuoles, escape

into the host cell cytoplasm (Ribot and Ulrich, 2006) and actin-based motility to promote spread within and between cells.(Ulrich and DeShazer, 2004) A type VI secretion system , T6SS-1, part of the *VirAG* regulon, is essential for *B. mallei* virulence in the hamster model of glanders,(Schell et al., 2007) and has an important role in growth and actin-based motility following uptake of *B. mallei* by murine macrophages.(Burtnick et al., 2010) *B. mallei* also exhibits Bim-A dependent intracellular actin-based motility, similar to that discussed later for *B. pseudomallei*. Multinucleated giant cell (MNGC) formation is characteristic of both organisms and is believed to be involved in the establishing persistent infection by facilitating intercellular spread and immune evasion.(Duval and White, 1907, Burtnick et al., 2011)

In laboratory infected guinea pigs, *B. mallei* has been shown to produce a thick carbohydrate capsule,(Popov et al., 1991) the coding sequence of which is 99% identical to that of the *B. pseudomallei* capsule.(DeShazer et al., 2001) This enables *B. mallei* to resist macrophage and complement-mediated killing, promoting survival in serum.(Burtnick et al., 2002) Furthermore, mutated strains lacking a capsule appear non-pathogenic in mice and hamsters.(DeShazer et al., 2001) Lipopolysaccharide has also been shown to be a potent activator of Toll-like receptor-4 *in vitro*.(Brett et al., 2007)

Through the various modes of transmission outlined previously, using these pathogenic mechanisms, *B. mallei* is able to penetrate mucosae, invade lymphatics, and spread haematogenously. Post-mortem examinations of glanderous animals have revealed nodules and ulcers of the nasal passages, larynx, lip and other tissues (Figure 1); sero-sanguinous fluid in the nasal cavity, paranasal sinuses and trachea; sub-pleural lung nodules; diffuse, miliary granulomatous nodules with caseo-necrotic centres; pulmonary oedema or severe bronchopneumonia; and less frequently, muscle abscesses.(Khan et al., 2013) Some ulcerating lesions are believed to be endotoxin mediated.(Carr Gregory, 2007)

Clinical presentation in animals

In addition to the normal equine hosts, glanders has been confirmed in camels, bears, wolves, dogs and felines(Health, 2013, Wernery et al., 2011) and in laboratory experiments guinea-pigs and hamsters appear to be susceptible, whereas cattle, fowl, rats and pigs appear to be more resistant.(Hu, 1958, Minnet, 1959) Donkeys are particularly susceptible and develop an acute fatal form of infection, whereas horses tend to develop a chronic, more insidious, yet eventually fatal illness. The clinical presentation of equine glanders may be acute or chronic, and it typically manifests as a respiratory illness with pulmonary and nasal involvement (“glanders”; Figure 2) or with cutaneous nodules and lymphangitis (“farcy”; Figure 3.1 and 3.2), although these forms often coexist, and pulmonary involvement is almost invariably found at post-mortem. The incubation period varies from a few days to several months.(Health, 2013) The clinical presentation in other susceptible animals appears similar to that in equines. General clinical signs noted may include fever, rough hair coat, breathing difficulty, joint and limb swelling, inappetence and gradual emaciation.(Singha et al., 2020b)

Acute infection in donkeys begins with fever, anorexia, loss of stamina, and respiratory symptoms such as nasal discharge and cough. This is shortly followed by swelling of the nostrils, nodules and ulceration of the nasal septum, mucopurulent nasal discharge, submaxillary lymphadenopathy

(often with suppuration), and increasing shortness of breath.(Health, 2013, Wernery et al., 2011, Minnet, 1959) Death occurs within a few days to weeks as a result of respiratory failure and sepsis.

In horses, glanders generally follows a more chronic course with episodic exacerbations followed by improvement in symptoms. The animal may have intermittent, low-grade fever, and mild respiratory symptoms, however the disease may remain latent for months to years without significant symptoms or signs. As disease progresses, cough, weakness and signs of wasting develop, and nasal and cutaneous forms ensue with inflammatory nodules and ulceration of the nasal cavity and upper respiratory tract (see Figure 1), yellow-green purulent nasal discharge (see Figure 2), and lymphangitis or nodular lymphadenitis particularly affecting the limbs (Figure 3.2). The skin nodules may also ulcerate, and deep lesions are often associated with joint swelling and oedema of the hind quarters resulting in lameness. Shortness of breath progresses as lung nodules and abscesses develop, and nodules are often found in the liver and spleen. Neurological involvement has been noted but is rare.(Dobberstein, 1935) Although chronic cases may survive for many years, the animal will usually become increasingly debilitated and eventually die.(Health, 2013, Khan et al., 2013, Minnet, 1959, Saqib et al., 2012) Chronic and subclinical cases are potential sources of transmission to other animals or humans through shedding of bacteria in respiratory secretions and skin exudate.(Wittig et al., 2006) Increased incidence of glanders has been recorded during the seasonal transition from spring to summer followed by the humid rainy season, with working stress also playing an important role.(Singha et al., 2020b, Fonseca-Rodriguez et al., 2019, Ghori et al., 2017)

Clinical presentation in humans

Knowledge of the clinical features of glanders in humans is based on a relatively narrow window in the literature of just over 100 years between the early 19th and the early 20th centuries, and a few more recently published laboratory-acquired cases.(Srinivasan et al., 2001, Robins, 1906, Howe and Miller, 1947) The clinical manifestations appear to relate to the route of infection, and whether the disease remains localised or disseminates, which probably accounts for the relative frequency of involvement of the head, neck and upper limbs. Typically, it results in pneumonia, septicaemia and chronic suppurative skin infection. Average incubation is 1-14 days.(Nelson et al., 2014) Localised infection typically produces pus-forming, ulcerating nodules and abscesses of the skin, subcutaneous tissues or mucous membranes, with associated lymphangitis or regional lymphadenopathy. Depending on the site affected, there may be swelling and increased discharge from nasal, ocular, or respiratory mucous membranes. Fever, malaise, headache, myalgia and gastrointestinal upset are common accompanying features.

Cutaneous inoculation or entry of *B. mallei* via mucous membranes typically results in a localised infection at the site of entry within 1-5 days. Although involvement of the nasal or oral mucosa has been well described, this is by no means invariable and certainly not as prominent as it is in horses, but pustular lesions around the face appear to be common. If untreated, lymphatic or haematogenous spread takes place after one to four weeks, resulting in pulmonary, septicaemic or disseminated infection with abscesses in many organs, but particularly the spleen, liver and lungs.(Carr Gregory, 2007) Multiple, painful skin and soft tissue nodules and abscesses may be a particularly prominent feature, and these often contain a characteristic oily pus (”farcy oil”). Pulmonary involvement, secondary to aerosol inhalation or as part of disseminated infection, may present with cough, purulent sputum, shortness of breath and chest pain as a result of pneumonia, lung abscess, or pleural effusion. Pneumonia, abscesses with cavitation and miliary nodules have

been seen on chest radiographs.(Carr Gregory, 2007) Septicaemia may develop immediately or up to two weeks after initial exposure or recurrence, and has a poor prognosis. The most recent reported case of human glanders occurred in Brazil in 2020, when an 11-year-old child, who was known to be in constant close contact with families who owned horses, presented with fever, chest pain and breathing difficulty, going on to develop septic shock. Chest x-rays identified pericardial and pleural effusions with pneumonia. *B. mallei* was subsequently cultured from abscesses which appeared on his trunk. An abrasion on his left knee was presumed to be the portal of infection.(Santos Junior et al., 2020)

Although human glanders was generally fatal over days to weeks before antibiotics were available, a more protracted course of disseminated infection interrupted by latent periods has also been described,(Bartlett, 1988) as well as cases of localised abscesses which responded to incision and drainage only.(Bernstein, 1909) Recent naturally occurring and laboratory-acquired cases have survived with antibiotic treatment similar to that used for melioidosis despite delays in making the diagnosis.

Diagnosis

A definitive diagnosis of glanders, in animals or humans, generally requires isolation and identification of *B. mallei* from clinical samples, although seroconversion following known exposure would also be highly suggestive of infection. Specimens from suspected or confirmed cases should be handled with appropriate laboratory containment. All suspected cases should have blood and urine culture, together with sputum, pus, exudate from superficial lesions and other samples as available or appropriate. Guidelines for culture and identification of *B. mallei* have been developed.(Microbiology, 2008) Gram's stain of clinical samples may demonstrate the irregularly stained Gram-negative bacilli. The organisms are difficult to demonstrate in tissue sections where they may have a beaded or encapsulated appearance.(Miller et al., 1948) Isolation from non-sterile sites may be optimised by using a selective medium such as *Burkholderia cepacia* agar, although selective media containing aminoglycosides designed for *B. pseudomallei*, such as Ashdown's agar, are inhibitory to the aminoglycoside-susceptible *B. mallei*.(Glass et al., 2009) *B. mallei* is often not correctly identified by API 20NE (Amornchai et al., 2007) and other commercial identification systems.(Glass and Popovic, 2005) Well-resourced laboratories are now using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry for bacterial identification. However, accurate results require good sample preparation and a well-developed database. To ensure highly pathogenic organisms are nonviable and safe for handling, the American Society for Microbiology document "Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases" recommends that laboratories use the tube extraction method followed by filtration through a $\leq 2\mu m$ pore size filter for suspected biothreat agents.(Rudrik et al., 2017) Caution is advised with a genus-level identification of *Burkholderia* species by MALDI-TOF MS. All suspected *B. mallei* isolates should be referred to the relevant national reference laboratory for molecular confirmation.

Molecular techniques have been developed to identify *B. mallei* in laboratory culture(Lee et al., 2005, Thibault et al., 2004b, U'Ren et al., 2005) and although their use is currently restricted to research and reference laboratories, they could potentially be used to detect the organism in clinical specimens. PCR targeting the flagellin gene of *B. mallei* (fli-P) was used successfully to detect the organism in clinical samples taken during a glanders outbreak in horses.(Scholz et al., 2006)

In suspected cases of glanders in animals, the mallein skin test was historically used for diagnostic purposes. The test is based on a hypersensitivity reaction to a protein fraction (mallein) of *B. mallei* following intrapalpebral or subcutaneous injection or administration in eyedrops, leading to marked eyelid swelling, a painful raised lesion, or conjunctivitis respectively after one to two days, often accompanied by fever.(Health, 2013) Mallein testing can, however, lead to subsequent false positive results in other serological tests,(Hagebock et al., 1993) and may be falsely negative in animals with acute glanders or in the late stages of chronic disease.(Neubauer et al., 2005) It is no longer recommended due to animal welfare concerns, but may be used in remote endemic regions where storage and transport of samples for serological testing is problematic.(Health, 2013)

Numerous serological tests for the diagnosis of glanders in horses exist including complement fixation test (CFT), various enzyme-linked immunosorbent assay (ELISA), immunoblot (IB), Rose Bengal Test (RBT), indirect haemagglutination assay (IHA), agar-gel immunodiffusion (AGID), indirect fluorescent assay test (IFAT), counter immunoelectrophoresis (CIE) and dot ELISA. Many of these methods are not widely validated, cross react with other *Burkholderia* species and, like the mallein test, may give false negative results in acute cases or very debilitated animals. CFT represents the current method of choice for the diagnosis of glanders and is required before international horse trade by the World Organisation for Animal Health (OIE).(Health, 2013) It is 90-98% sensitive, becoming positive within one week of infection, and remaining positive in chronic cases and exacerbations of latent cases.(Health, 2013) A cELISA has been developed using an uncharacterised anti-lipopolysaccharide monoclonal antibody, and shown to have similar performance characteristics to the CFT.(Katz et al., 2000) However, both methods have suboptimal specificity,(Neubauer et al., 2005) particularly when testing serum samples from animals in glanders-endemic areas.(Khan et al., 2012) A recently optimised BimA protein-based indirect enzyme-linked immunosorbent assay (iELISA) exhibited 96% sensitivity and 91% specificity, with 100% repeatability and minimal decrease in diagnostic efficacy after storage of ELISA plates at room temperature or 37°C for 90 days.(Singh et al., 2018) ELISAs based on recombinant antigens (TssA, TssB and Hcp1) and semi-purified fractions of *B. mallei* (IDVet) have been shown to have significantly higher specificity, offering a suitable alternative for serological testing of equids.(Elschner et al., 2019, Elschner et al., 2021) CFT performance is also significantly affected by the availability of quality reagents and specifically by the *B. mallei* antigen applied. False positive tests can have serious consequences in terms of animal slaughter and financial losses. Performing an immunoblot as a confirmatory test for all positive CFT results has been suggested as a means of overcoming the sensitivity issues.(Khan et al., 2012) The serodiagnosis of glanders in animals should only be undertaken and interpreted by specialists with relevant expertise. Ideally, the diagnosis should be confirmed by culture if possible. The European Union Reference Laboratory (EURL) for glanders, nominated in 2008, found good intra-laboratory repeatability of CFT testing, however, a risk of inter-laboratory inconsistency was highlighted which may misclassify positive samples with low CFT titres.(Laroucau et al., 2016) A field deployable recombinase polymerase amplification-lateral flow (RPA-LF) assay, which is highly sensitive (down to 10fg of *B. mallei* genomic DNA) and specific, shows promise as a tool for use in endemic areas with limited laboratory resources.(Saxena et al., 2019)

No validated serological test is currently available for the diagnosis of human glanders, although numerous melioidosis serodiagnostic tests are in use around the world and, given the serological cross-reactivity between *B. mallei* and *B. pseudomallei*, it is likely that these would become positive

in many cases of human glanders. Recent molecular and immunological research is leading to the identification of more specific and immunogenic *B. mallei* antigens to optimise serological diagnosis.

Treatment

B. mallei is intrinsically resistant to a range of antimicrobial agents, including early beta-lactams, but unlike *B. pseudomallei*, *B. mallei* remains susceptible to aminoglycosides and macrolides.(Kenny et al., 1999, Thibault et al., 2004a) Most strains are susceptible to carbapenems, ceftazidime, amoxycillin–clavulanic acid, piperacillin, doxycycline and trimethoprim-sulfamethoxazole.(Thibault et al., 2004a, Kenny et al., 1999, Al-Izzi and Al-Bassam, 1989, Heine et al., 2001) Despite low mean inhibitory concentrations *in vitro*, certain antimicrobials, including aminoglycosides, may not be effective *in vivo* due to the intracellular nature of the infection. Doxycycline and fluoroquinolones, such as ciprofloxacin and ofloxacin, with good intracellular and tissue penetration, have demonstrated efficacy when used to treat experimental infection in animals,(Batmanov, 1991, Iliukhin et al., 1994, Russell et al., 2000) however in one study relapse occurred in some doxycycline treated animals.(Russell et al., 2000) Doxycycline has also been shown to have some efficacy as post-exposure prophylaxis following aerosol and intraperitoneal challenge in animals.(Russell et al., 2000, Iliukhin et al., 1994)

Trials to determine optimal treatment for animal and human glanders are lacking. Until recently, glanderous animals, including those that are asymptomatic with positive serological tests, have been euthanized according to strict veterinary public health policies to prevent spread to other domestic animals or humans. In the case of high value animals, such as those in equestrian sports, an expensive treatment regimen may be justified. During an outbreak of culture confirmed glanders in 23 horses at the Lahore Polo Club in Pakistan, a combination of intravenous enrofloxacin and trimethoprim-sulphadiazine for three weeks, followed by oral doxycycline for a total of 12 weeks, successfully treated all infections.(Saqib et al., 2012)

Recommendations for treatment of human glanders adopt the same antimicrobial regimens as those validated for melioidosis, which are based on clinical trial evidence. This consists of an intensive phase of intravenous antimicrobial therapy (ceftazidime or a carbapenem) for a minimum of 10-14 days, followed by an eradication phase of oral antimicrobial (trimethoprim-sulfamethoxazole) for 12-20 weeks, or longer if there is widespread visceral disease.(Lipsitz et al., 2012) Without the latter phase, there is likely to be a high risk of relapse, particularly in those with disseminated infection. Drainage of abscesses where possible is an important adjunct to antimicrobial therapy.

Prevention & Control

Control and eradication of glanders has to date depended on the detection and elimination of infected animals to prevent onward transmission. A requirement for serological testing of animals prior to international transport in order to prevent the introduction of glanders into glanders-free regions, has been recommended by the World Organisation for Animal Health.(Health, 2003)

Attempts to develop vaccines against *B. mallei* have so far been experimental and no vaccine against glanders is yet available for either human or animal use. Intranasally vaccinated BALB/c mice using an iron-acquisition-deficient *B. mallei*$_{tonB}$ strain had 100% survival on subsequent challenge. However, necropsy and organ colony-forming units (CFU) enumeration showed splenomegaly and abscess formation with persistence of the attenuated *B. mallei,* which poses a significant safety

concern.(Hatcher et al., 2016) In contrast, a recombinant Parainfluenza virus 5 expressing BatA (autotransporter protein) resulted in 74% survival, with and complete bacterial clearance from the lungs and spleen in 78%.(Lafontaine et al., 2019) Interestingly, using a double mutant by deletion of tonB and hcp1 genes produced clearance from all organs by 21 days post inoculation, with unremarkable histopathology. Furthermore, serum from these mice was able to inhibit bacterial growth when co-cultured with *B. mallei*. Greatest protection was observed in mice with the highest total IgG titres and IgG2a/IgG1 ratios (markers of Th1-driven immune response and protection). Together, these studies demonstrate that live-attenuated vaccines can elicit a strong humoral response that contributes towards protection.(Hatcher et al., 2016) The addition of the Toll-like receptor 9 (TLR9) agonist CpG oligodeoxynucleotide (activating B and NK cells, antibody production and Th1 cell development) as an adjuvant may yet provide better protection and reduce the number of vaccine doses required.(Hatcher et al., 2016) More recent work is taking advantage of genome-wide bio- and immune-informatic analysis to predict highly immunogenic antigens. This led to the development of a nano-glycoconjugate vaccine (containing OmpW, OpcP and Haemagglutinin protein antigens alongside LPS) which offered complete protection in an inhalational glanders mouse model.(Tapia et al., 2020) Vaccine development is gaining momentum and much progress has also been made with melioidosis vaccines (*see below*), which may offer cross-protection.

If animal or human glanders is suspected, the case should be isolated, and personal protective equipment (PPE) worn by any person who must come into contact with the patient or samples. Local and national public health and veterinary authorities must be notified immediately and confirmed cases in animals reported to the World Organization for Animal Health. Any confirmed human glanders case occurring without equine exposure should prompt consideration of a deliberate release of the organism. In human cases, isolation and appropriate infection control precautions (according to the site of infection) should be taken until the patient is culture negative.

Confirmed animal cases and serologically positive animal contacts should be destroyed humanely, with the provision of adequate compensation to owners. Reasonable compensation schemes helped to eradicate glanders in Canada.(Derbyshire, 2002) In contrast, in some developing countries as little as $1.1 US dollars is paid in compensation for slaughter of a glanderous animal which may be the basis of the owner's livelihood, thus forcing them to sell the animal and risk onward transmission to other animals and regions.(Khan et al., 2013, Saqib et al., 2012) Premises and facilities of infected animals should be quarantined, cleaned and disinfected. Carcases as well as contaminated bedding, feed, manure and equipment in the vicinity should be buried or incinerated.

Prevention of laboratory-acquired human infection depends on a full risk assessment, appropriate containment and practices, the use of personal protective equipment, and the institution of appropriate guidelines in the event of accidental laboratory exposure.(Lipsitz et al., 2012)

Melioidosis

History and Epidemiology

Melioidosis was first described by Whitmore and Krishnaswami as a "glanders-like….pyaemic or septicaemic" illness occurring in morphia addicts in Rangoon in 1911 (Whitmore and Krishnaswami,

1912) and was documented in 5% of post-mortem examinations in Myanmar around this time.(Cheng and Currie, 2005) Fulminant presentations at autopsy were characterised by widespread caseous consolidation of the lungs and typically abscesses in the liver, spleen or other organs.(Whitmore, 1913a) The name originates from the Greek “μηλις” (distemper of asses) and “ειδος” (resemblance), and the name was suggested by Stanton and Fletcher in 1921,(Stanton and Fletcher, 1921) who went on to report a number of human and animal cases around Kuala Lumpur.(Stanton and Fletcher, 1932) It was later demonstrated that the causative bacterium, now known as *B. pseudomallei*, was saprophytic and could be cultured from soil and surface water in Vietnam,(Chambon, 1955) and subsequently from many other parts of southeast Asia and northern Australia. In Australia, *B. pseudomallei* was first identified in sheep in 1949,(Cottew, 1952) and the first human case occurred in a diabetic patient who died of septicaemic melioidosis in north Queensland in 1950.(Rimington, 1962) Using MLST (Currie et al., 2007), and more recently whole-genome sequencing(Chewapreecha et al., 2017), phylogenetic analysis has suggested that Australian isolates, which demonstrate greater genetic diversity, are ancestral to those found in southeast Asia.(Pearson et al., 2009) This supports the present hypothesis that Australia was the original reservoir for the current *B. pseudomallei* population, which expanded to southeast Asia, where the Mekong subregion has emerged as a hotspot for *B. pseudomallei* evolution.(Chewapreecha et al., 2017) Further dissemination to Africa and Central and South America is thought to have occurred between the 17th and 19th centuries.(Chewapreecha et al., 2017) Gee et al. used a typing scheme for length polymorphisms in the 16S-23S internal transcribed spacer (ITS) of *Burkholderia* spp. and identified ITS type G isolates (containing the *Yersinia*-like fimbrial (YLF) gene) as associated with the Western Hemisphere.(Gee et al., 2014) Analysis of single-nucleotide polymorphisms (SNPs) from whole genome sequencing is proving valuable in linking clinical isolates with geographic provenance. For example, a US military veteran who had spent time in Southeast Asia during World War II was initially reported as having the longest latency period (62 years) before developing melioidosis.(Ngauy et al., 2005) There was no history of travel to other known endemic regions. However, WGS found the isolate to belong to the Western Hemisphere clade and grouped with genomes from patient isolates with a travel history to Guatemala, Panama and Peru. This isolate also belongs to the ITS type G cluster, which suggests that his exposure to *B. pseudomallei* may not actually have occurred during his internment in World War II.(Gee et al., 2017) In the Darwin cohort periods of latency were thought to have occurred in 3% of melioidosis cases.(Currie et al., 2021)

Melioidosis is endemic in many tropical regions, mainly between latitudes 20°N and 20°S, although *B. pseudomallei* is unevenly distributed in the environment in these areas and the true distribution has not been accurately defined.(Dance, 2000a) The highest isolation rates have been found in rice paddies, rubber plantations and other cleared and cultivated areas(Nachiangmai et al., 1985, Strauss et al., 1969) but high rates have also been seen in urban sports fields in Singapore,(Thin et al., 1971) and grazing sites of animals with melioidosis in Australia.(Thomas et al., 1979) Factors that may influence environmental distribution include temperature, humidity, rainfall, ultra-violet exposure, soil composition, vegetation, fertilisers and soil disturbance such as excavation or ploughing.(Inglis et al., 2001) Recent modelling and epidemiological studies highlighted the underdiagnosis and under-reporting of melioidosis, which was estimated to have infected 165,000 people (95% credible interval 68,000–412,000) and caused 89,000 deaths (36,000–227,000) worldwide in 2015.(Limmathurotsakul et al., 2016) This equates to 4.6 million disability adjusted life-years (DALYs), a greater burden than those for dengue and leptospirosis, and suggests that melioidosis

should be formally categorised as a re-emerging neglected tropical disease.(Limmathurotsakul et al., 2016, Birnie et al., 2019, Savelkoel et al., 2021) India, Bangladesh, Vietnam, Nigeria and Indonesia are predicted to contribute almost three-quarters of the total global disease burden.(Birnie et al., 2019) Despite this, only ~1300 cases were reported annually worldwide in 2010, less than 1% of the estimated incidence.(Limmathurotsakul et al., 2016) Whether this reflects the inadequacy of current surveillance systems or the inaccuracy of the modelling remains to be determined.

The relatively small numbers of cases reported in endemic areas during the latter half of the 20th century probably reflects the limited culture facilities in many rural, high risk regions.(Dance, 1991) This is supported by the fact that western armed forces, with access to high quality laboratory diagnostics, reported at least 100 confirmed cases of melioidosis amongst French(Rubin et al., 1963) and American(Sanford, 1985) soldiers respectively during the conflicts in Vietnam in contrast to the numbers of cases identified among the indigenous population. In Thailand, very few cases were reported until the improvement of district microbiology laboratories and increased clinical awareness in the 1980s, which led to in around 800 case reports by 1986(Leelarasamee and Bovornkitti, 1989) and an average of nearly 1800 culture-positive cases annually between 2012 and 2015.(Hantrakun et al., 2019) Sri Lanka has become a case study for uncovering the hidden burden using enhanced surveillance, awareness and WHO's laboratory capacity building program.(Corea et al., 2016) Despite only a handful of cases identified since 1927, and at times even being considered non-endemic for melioidosis(Cheng and Currie, 2005), rising annual cases have been identified there since 2006, totalling 250 cases over 10 years in eight out of nine provinces.(Corea et al., 2018)

Warm climates favour the persistence of *B. pseudomallei* in the environment, however when introduced to a non-endemic area the organism may persist for several years in soil. This apparently occurred during a prolonged outbreak in France in the 1970s, which was thought to have followed the importation of an infected panda.(Mollaret, 1988) More recently, cases occurring in the USA have been linked to imported tropical fish (see below) and an aromatherapy spray.(Dawson et al., 2020, CDC, 2021) With increasing movement of humans, animals and goods around the world, new endemic foci may become established. Sporadic cases have been reported in the Americas, the Caribbean and sub-Saharan Africa,(Cheng and Currie, 2005, Dance, 1991) although the true incidence in these areas is unclear because of a lack of laboratory facilities and clinical awareness. Ongoing mapping of the distribution of *B. pseudomallei* and melioidosis is available at https://www.melioidosis.info/map.aspx.

Molecular tools have demonstrated that environmental isolates are often identical to epidemiologically related human or animal strains, that there is considerable diversity among isolates persisting in a particular region, and that clonal outbreaks have occurred when the organism is introduced to a non-endemic region.(Gee et al., 2017, Cheng and Currie, 2005, Currie et al., 1994)

By the year 2000, melioidosis was regarded as an emerging infection due to increasing reports of confirmed cases in endemic regions, particularly Thailand, where it is estimated that more than 2,500 culture-positive cases of human melioidosis occur annually, increasing reports of cases from regions where the disease was not known to be endemic (e.g. the Americas and the Caribbean) (Cossaboom et al., 2020, Sanchez-Villamil and Torres, 2018), and concerns that it could be spread to non-endemic regions by infected animals.(Dance, 2000b) Much of the increase has been due to improved diagnostics and clinical awareness, but the increasing prevalence of predisposing medical

conditions such as diabetes in populations of endemic areas (Dance, 2000b) and possibly climate change and increasing travel and migration have also impacted on melioidosis epidemiology. Analysis of melioidosis amongst returned travellers in Europe identified Thailand as the main source of infection (53%), with one-fifth of patients being misdiagnosed.(Le Tohic et al., 2019) However, even in countries where notification is mandatory, such as the UK, many cases are still going unreported.(O'Connor et al., 2020)

Currently, the greatest burden of melioidosis is reported in Thailand (especially the northeast) and Northern Australia where annual incidence rates vary between 4 – 50/100,000/year.(Parameswaran et al., 2012, Hantrakun et al., 2018) Melioidosis is now the third most common cause of death from infectious disease in northeast Thailand,(Limmathurotsakul et al., 2010) although this has been under-recognised through routine surveillance systems (Hantrakun et al., 2019) and is the commonest cause of fatal community-acquired bacteraemic pneumonia in the Northern Territory of Australia.(Currie et al., 2000b) The disease is highly seasonal, with 75-85 % of cases presenting during the rainy season(Suputtamongkol et al., 1994, Currie et al., 2010) and incidence rates as high as 102.4/100,000 have been recorded in the indigenous Australian population during severe rains.(Parameswaran et al., 2012, Currie et al., 2004) Large case series have identified diabetes mellitus as by far the most important risk factor for infection, with occupational exposure to soil and water, male sex, Aboriginal Australians, alcoholism, chronic lung disease, chronic renal disease, thalassaemia, and kava and steroid use all additional risk factors for melioidosis.(Cheng and Currie, 2005, Suputtamongkol et al., 1994, Currie et al., 2010) The majority of cases have a predisposition, but in around 20 % none is identified.(Currie et al., 2010) Presence of a single risk factor increases the risk of death from melioidosis by 8.4 times (95% CI 2.7-26.0).(Currie et al., 2021)

With respect to animal infection*, B. pseudomallei* appears to affect a broader range of animal hosts than glanders, with infection in equines being relatively rare, although it may occasionally cause severe infections in horses. Species that have been infected include terrestrial and aquatic mammals, birds and fish. Goats, sheep, pigs and camels appear particularly susceptible, whereas dogs, cats and cattle appear more resistant, but these may develop disease if they become immunocompromised.(Choy et al., 2000) Sporadic cases or small outbreaks have been reported in various primates, marsupials, deer, buffalo, camels, llamas, zebras, horses, mules, rabbits, meerkats, rodents, iguanas, parrots, crocodiles, dolphins, and seals.(Elschner et al., 2014, Sprague and Neubauer, 2004) Animal cases have also been reported in other regions, such as southern and western Australia,(Currie et al., 1994, Ketterer et al., 1986) China,(Li et al., 1994) Iran,(Baharsefat and Amjadi, 1970) Saudi Arabia,(Barbour et al., 1997) United Arab Emirates,(Wernery et al., 1997) South Africa,(Van der Lugt and Henton, 1995) and the Americas.(Zehnder et al., 2014, Galimand and Dodin, 1982) Epizootics have been reported after importation of animals from areas of endemicity. This was believed to be the source of a cluster of infections in sheep, goats and pigs in Aruba in 1957,(Fournier, 1965) an outbreak in a Paris zoo which spread to other zoos and equestrian clubs in France in the 1970s,(Mollaret, 1988) and an outbreak in primates in the UK in the 1990s.(Dance et al., 1992) More detail on confirmed cases of melioidosis in different animal species worldwide can be found in a review by Sprague and Neubauer.(Sprague and Neubauer, 2004)

Modes of Transmission

Whitmore's early observations of melioidosis in guinea pigs led him to believe that the infection was transmitted by consumption of food and drink contaminated by urine, sputum, or other secretions

containing viable bacteria, from infected persons or animals.(Whitmore, 1913b) In the 1930s, Stanton & Fletcher also proposed that infection occurred by ingestion, although they believed that rodents were a zoonotic reservoir.(Stanton and Fletcher, 1932) It was subsequently observed that human infections commonly followed exposure to mud and water, and that *B. pseudomallei* could be isolated from mud and surface water,(Chambon, 1955) leading to the current knowledge that it is an environmental saprophyte. Well documented modes of transmission include inoculation and aspiration of water during near drowning (such as during the Asian tsunami in 2004), and laboratory-acquired infection (although only two such instances have been reported in the literature). Epidemiological evidence and animal studies also suggest a role for inhalation and ingestion, although it is often impossible to define precisely how and when infection occurred. Although sporadic cases have been anecdotally associated with infection in animals, there is limited evidence for zoonotic or person-to-person spread,(Dance, 2000a) and it is equally likely that both humans and animals have acquired infection from the same environmental source. The few suspected human-to-human *B. pseudomallei* transmissions have been in siblings with cystic fibrosis (Holland et al., 2002) and diabetes(Arauz et al., 2020), an American Vietnam veteran diagnosed with *B. pseudomallei*-associated prostatitis and his spouse,(McCormick et al., 1975) and cases of mother-to-child transmission via transplacental, breast or perinatal routes.(Aziz et al., 2020, Rodriguez et al., 2020, Ralph et al., 2004, Kunakorn et al., 1991) Recently, a case of transmission from a breastfeeding mother with mastitis was confirmed using WGS.(Aziz et al., 2020)

Inoculation of organisms through penetrating injuries or pre-existing skin lesions appears to be the major mode of acquisition, particularly in farmers who are continually exposed whilst working in the mud and surface water of paddy fields.(Suputtamongkol et al., 1994) Twenty five percent of patients in one case series recalled a previous inoculation injury, but often there is no such history.(Currie et al., 2000b) *B. pseudomallei* is most abundant in soil depths of >10cm, however during the rainy season it can rise and concentrate at the surface.(Limmathurotsakul et al., 2013a) Inoculation is the method most frequently used to induce infection in animal models, and natural infection in animals occurs in this way by entry of bacteria through minor skin trauma, bite wounds and scratch injuries. This was the likely mode of infection in a patient from Maryland, USA with no travel history who developed melioidosis in 2019. Isolates of *B. pseudomallei* that were indistinguishable by WGS and clustered with isolates from southeast Asia were obtained from both the patient and a freshwater aquarium in which all the fish had died. The patient recalled reaching into the aquarium with bare hands and arms a month prior to onset of illness.(Dawson et al., 2020) Nosocomial infections have also occasionally been reported, mainly through use of contaminated medical supplies and solutions(Merritt et al., 2016).

Infection after inhalation has been repeatedly demonstrated in laboratory animals,(Jeddeloh et al., 2003) and this may be an important and underestimated mode of acquisition in humans. In Australia, *B. pseudomallei* recovered from an air sample was linked to a clinical isolate from a patient with mediastinal melioidosis by WGS (Currie et al., 2015), and the north-easterly winds during the typhoon season in Taiwan were associated with detection of *B. pseudomallei*-specific DNA in aerosols and a hot spot of transmission.(Hsueh et al., 2018) It is now established that during periods of very heavy rainfall, increases in pneumonic cases of melioidosis occur, probably as a result of aerosolisation of the bacteria.(Currie and Jacups, 2003) Inhalation was previously thought to be

the primary mode of transmission due to the high incidence of melioidosis in U.S. military helicopter crews during and after the Vietnam war.

Ingestion has also been proposed as a mode of infection in both humans and animals. Contaminated water supplies have been implicated by PFGE as the point source of melioidosis outbreaks in Australia.(Currie et al., 2001, Inglis et al., 1999) Suppurative parotitis, a common presentation in children with melioidosis in southeast Asia, is believed to be due to the ingestion of contaminated water or soil, resulting in the ascent of bacteria from the mouth to the parotid gland.(Stoesser et al., 2012) Although not confirmed, an untreated river water supply was implicated in melioidosis outbreaks occurring in intensive piggeries.(Ketterer et al., 1986) In these outbreaks, an oral mode of transmission was suspected due to the common finding of infected gastro-hepatic nodes. Faeco-oral transmission was felt to be unlikely due to the fact that *B. pseudomallei* was infrequently isolated from faecal samples of infected pigs. However, faecal shedding has been detected from wallabies and wild stock, suggesting it may be a means of expanding the geographical distribution of *B. pseudomallei*.(Höger et al., 2016) Recent non-human primate models showed that ingestion of >6 x 10^6 cfu resulted in acute-febrile, lethal disease(Nelson et al., 2021). Enteritis was observed in fatal disease with the lungs being the first organ colonised outside of the gastro-intestinal tract. Severe pathological feature in the mesenteric lymph nodes suggests that lymphatic drainage maybe an important route of dissemination post ingestion.(Nelson et al., 2021)

Despite early theories, there is relatively little evidence for melioidosis being a true zoonosis. In addition to the aquarium-associated case described above, three anecdotal cases of possible zoonotic infection in Australia were described by Choy et al.(Choy et al., 2000) In one case, *B. pseudomallei* was cultured from a wrist lesion of a meat worker in Darwin; secondly, a vet in rural Queensland developed abscesses on the arm, but this does not appear to have been confirmed as melioidosis by culture; and similarly, a goat farmer had a lesion on his hand resembling a “milker’s lesion” for two months preceding a diagnosis of melioidosis, which again does not appear to have been culture-confirmed. In Malaysia, a case of suspected sheep-to-human-transmission was reported in a 10 year old boy.(Idris et al., 1998) The evidence for this was entirely circumstantial, and it is more likely that he contracted the illness from soil and water in the environment (from which *B. pseudomallei* was also isolated). Earlier anecdotal evidence of animal-to-human transmission of melioidosis was reported during “L’affaire du Jardin des Plantes”, an outbreak of melioidosis that started in a Paris zoo and spread to other zoos and equestrian clubs in France through transport of infected animals and contaminated manure. At least two fatal human cases were said to have occurred during this outbreak, although details were never published.(Dodin and Galimand, 1986) In none of these cases has there ever been genotypic evidence of the relationship between the human and animal isolates, and so the case for animal-to-human transmission remains unproven. Nonetheless, the potential for zoonotic transmission can lead to significant public health concerns and responses, such as those that occurred following the importation into the USA of a rescue dog from Thailand that was subsequently found to have *B. pseudomallei* urinary tract infection. Fortunately, there were no resultant human infections.(Ryan et al., 2018)

There have been concerns that goats, which appear to be particularly susceptible to melioidosis and often develop mastitis as a manifestation of the infection (Figure 4), could transmit the disease via infected milk. However, small studies of infected goats have found that the organism is only isolated from body fluids in a minority of cases.(Thomas et al., 1988a) Furthermore, a recent literature

review of bacterial infections following animal bites world-wide did not identify any cases of melioidosis, supporting the fact that transmission from body fluids is unlikely.(Abrahamian and Goldstein, 2011) However, clearly it makes sense in public health terms to avoid drinking milk or eating meat from infected animals.

Microbiology

B. pseudomallei is an irregularly staining, oxidase-positive, motile Gram-negative bacillus which sometimes exhibits marked bipolarity microscopically. It can be distinguished from *B. mallei* by its motility and usually its resistance to aminoglycosides. It grows readily on most routine culture media, initially forming smooth creamy, non-haemolytic colonies (Day 2) which may become dry and wrinkled (Day 4) with a metallic sheen on prolonged incubation (Figure 5), sometimes with a zone of haemolysis surrounding confluent growth. Considerable variability in colonial morphology may be seen between, and even within, strains. It is often dismissed as a contaminant or misidentified (e.g. as *Acinetobacter* spp. *Pseudomonas* spp. or *Bacillus* spp.), especially on non-selective agars, where it may be outgrown by other microbial flora. Ideally, selective media (e.g. Ashdown's agar) should be used, particularly where laboratory staff are unfamiliar with its characteristics or when polymicrobial growth is expected, with daily examination of plates for up to 4 days in suspected cases. In respiratory samples from low-incidence settings, this approach improved sensitivity (87.5%) and allowed for quicker identification than routine media (50%)(Subakir et al., 2020). Important characteristics include arginine dihydrolase and gelatinase activity, the inability to assimilate arabinose (distinguishing *B. pseudomallei* from the closely related avirulent *B. thailandensis*), and growth at 42°C. Its intrinsic resistance to aminoglycosides (although clonal isolates susceptible to gentamicin are common in Sarawak, Malaysia)(Podin et al., 2014), polymyxins and the early beta-lactams, but susceptibility to co-amoxiclav, is particularly characteristic, and any oxidase positive Gram-negative bacillus with these characteristics should be assumed to be *B. pseudomallei* until proved otherwise.(Trinh et al., 2018) The species is antigenically homogeneous, but a number of molecular techniques, most usefully MLST and WGS, can distinguish between isolates.

Pathogenesis

A range of bacterial factors have been associated with virulence, but the relative contributions of individual virulence factors to the disease process have not been fully characterized. A variety of adhesins, in particular type 4 pili, appear to be involved in attachment of bacteria to different eukaryotic cell types, and expression is regulated by the *pilA* gene.(Allwood et al., 2011) Capsular polysaccharides also act to inhibit opsonophagocytosis and complement-mediated killing (Egan and Gordon, 1996). Like *B. mallei, B. pseudomallei* utilizes up to three T3SS, including Bsa T3SS. *In vitro* experiments have demonstrated the importance of this system, and its individual components, in host cell invasion, escape from endosomes and intracytoplasmic survival.(Stevens et al., 2002) Mutations in components of the T3SS in *B. pseudomallei* have reduced ability to cause disease in animal models.(Stevens et al., 2004) Cell-to-cell spread takes place by actin-based motility which is dependent on the BimA protein(Stevens et al., 2005) (mutations of which have been linked to central nervous system diseases, especially in Australia) and the cluster 1 type VI secretion system (T6SS-1) which mediates endocyte escape and membrane fusion during intracellular spread via VgrG5 spike protein(Toesca et al., 2014). The antiphagocytic polysaccharide capsule, quorum sensing mechanisms, and bacterial components such as lipopolysaccharide, flagella, secreted products (protease, lipase, lecithinase, various toxins) and a siderophore ('malleobactin') also have important

roles in environmental protection and adaptation, and host immune system evasion.(Wiersinga et al., 2018, Cheng and Currie, 2005) The ability of the organism to survive and grow intracellularly or become metabolically inactive within granulomas probably contributes to the persistent nature of the infection and the risk of relapse.

The clinical outcome after exposure to *B. pseudomallei* in the environment varies from person to person, ranging from asymptomatic seroconversion (the commonest outcome) or localized infection to fulminant sepsis and death, and is dependent on the size and route of the inoculum, the virulence of the infecting strain, and host immune factors. On the host side, innate immune mechanisms, macrophage and neutrophil function, and both cellular and humoral responses all play a role in defense against the organism, hence the strong associations with immune-suppressing conditions such as diabetes (12-fold increased risk compared with the normal population)(Limmathurotsakul et al., 2010), thalassaemia, renal impairment (associated with disease severity in India)(Shaw et al., 2019) and alcohol excess.(Cheng and Currie, 2005, Currie et al., 2004) In a prospective study of over 1000 patients with melioidosis, a third of whom had diabetes; there was a statistically significant survival advantage in diabetics compared with non-diabetics. However, this was confined only to patients taking glyburide (a second generation sulfonylurea which acts as K_{ATP}-channel blocker and broad-spectrum ATP-binding cassette (ABC) transporter inhibitor used in treating type 2 diabetes mellitus).(Koh et al., 2011) Subsequent diabetic mice models demonstrated an anti-inflammatory effect of glyburide by reducing IL-1b, diminished cellular influx and reduced bacterial dissemination to distant organs.(Koh et al., 2013)

Interestingly, HIV does not appear to be a risk factor despite murine studies showing a role for the adaptive immune system in control of infection, with increased survival correlated to CD8+ T cell responses in humans.(Jenjaroen et al., 2015) An exaggerated host response with high levels of pro-inflammatory cytokines such as TNF-alpha may also have a pathogenic role,(Nuntayanuwat et al., 1999) whereas hypofunctional TLR5 has been associated with decreased organ failure, improved survival and functional cytokine response(Chantratita et al., 2014, West et al., 2014). Antigens and epitopes (e.g. BopE – Type III secreted protein, AhpC – alkyl hydroperoxide reductase, PilO – Type IV pilus biosynthesis protein), immunodominant in survivors, have been identified as immune correlates of protection.(Dunachie et al., 2017) Diabetic patients were noted for their impaired response to GroEL proteins (chaperonins that assist in protein folding) during acute infection.(Dunachie et al., 2017)

Clinical Presentation in Humans

The majority of infections appear to be subclinical with 60-70% of populations in endemic areas acquiring antibodies to *B. pseudomallei* by the age of 4 years without clinically apparent disease.(Wuthiekanun et al., 2006) When disease manifests, it may be localised or disseminated with septicaemia. The incubation period varies depending on the mode of acquisition and infecting dose, with most cases occurring within 3 weeks after an inoculation injury (median 4 days, IQR 3-7 days),(Currie et al., 2021) and as soon as 24 hours after a near-drowning event.(Currie et al., 2000b, Suputtamongkol et al., 1994) The median age at presentation with melioidosis is 50 years, with 4-10% of patients under 16 years of age.(Currie et al., 2010, McLeod et al., 2015) Pneumonia is the commonest presentation, and is evident in around half of all cases.(Currie et al., 2010) Cavitation

may occur in the upper zones mimicking tuberculosis. Localised abscesses may occur in any other organ including the skin and soft tissues, lymph nodes, liver, spleen, genitourinary tract (especially the prostate gland in males), parotid gland, bone or joint, and nervous system. Localised disease without bacteraemia generally has a good outcome and low mortality. However, in 50-75% of cases the patient is bacteraemic, although this is lower in paediatric populations,(Turner et al., 2016, Currie et al., 2021) and just over one fifth of these are in septic shock at presentation, which has a mortality approaching 50% despite optimal treatment.(Birnie et al., 2019, Currie et al., 2010, Suputtamongkol et al., 1994) There appear to be geographical variations in manifestations, with hepatosplenic abscesses more common in Asian populations and suppurative parotitis in Asian children, and higher rates of prostatic and neurological melioidosis seen in Australia,(Cheng and Currie, 2005) although this could be biased by better access to imaging. Recrudescent melioidosis after treatment occurs in up to 5% of cases.(Currie et al., 2021) This is due to reactivation of the original strain (relapse) in approximately 75% of cases, which is usually associated with a failure to sterilise deep-seated foci of infection in disseminated disease, but may also be associated with poor adherence to therapy or an insufficient duration of eradication therapy.(Currie et al., 2000a) Reinfection with a different strain accounts for ~25% of recurrent infections.(Currie et al., 2021)

Clinical Presentation in Animals

As outlined above, a wide range of animal species may be affected by melioidosis, with a range of clinical manifestations and severity. In fact, the disease in animals is usually similar to that in humans, with subclinical infections common and abscesses occurring in any organ, particularly lungs, liver, spleen, and associated lymphatics. The acute form presents as fulminant sepsis with haematogenous dissemination and high mortality, often associated with respiratory distress and diarrhoea, and tends to occur in younger animals of susceptible species. The chronic form presents as a more non-specific illness in older animals, with low grade fever, anorexia, cough, progressive emaciation and lameness.(Choy et al., 2000) In sheep, goats, and horses, nasal and ocular discharge (similar to that seen in glanders) is common, and central nervous system involvement may contribute to paralysis, convulsions, nystagmus and blindness.(Sprague and Neubauer, 2004) Mastitis appears to be a particular feature in goats (Figure 4), orchitis has been described in rams and boars, and skin lesions, limb oedema, lymphangitis and meningoencephalitis in horses.(Sprague and Neubauer, 2004) Monkeys are affected in a similar way to horses, but neurological involvement is more unusual.(Sprague and Neubauer, 2004)

Diagnosis

Melioidosis should be considered in any person or animal who has visited or migrated from an endemic area presenting with septicaemia and/or abscesses, especially if they have a predisposing condition such as diabetes. Confirmation of the diagnosis relies on culture of the organism from blood, sputum, pus, or other body fluid indicated by the clinical presentation. Liaison with the microbiology laboratory is of utmost importance if melioidosis is suspected. Firstly, the organism is a hazard group 3 pathogen and must be handled in appropriate laboratory containment in case of transmission to laboratory staff. Secondly, selective media such as Ashdown's or *B. cepacia* media may be used to optimise the isolation of the organism from sites with a normal flora. And thirdly, if not aware of the clinical context, growth in cultures may be dismissed as a contaminant by the unwary.

Culture may take several days, and meanwhile microscopy of pus, sputum or urine may reveal bipolar or unevenly staining Gram-negative rods, although this appearance is not specific. Immunofluorescent staining of such samples is a useful rapid diagnostic tool but is not widely available.(Wuthiekanun et al., 2005) Once cultured, commercial identification kits such as the API 20NE usually identify the organism correctly but may give misleading results,(Amornchai et al., 2007) so presumptive isolates should be sent to a Reference Laboratory if in doubt as to the identity. Well-equipped laboratories are increasingly using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry for bacterial identification, provided an appropriate database including hazardous pathogens is available as discussed above.(Lasch et al., 2016) A latex agglutination test using a monoclonal antibody to the 200 kDa extracellular polysaccharide is also useful for screening suspect colonies or positive blood culture fluid, with 95 % sensitivity and 99.7 % specificity.(Anuntagool et al., 2000) In low-resource or -incidence settings, selective media followed by real-time PCR has been shown to improve diagnosis of melioidosis at a reasonable cost.(Subakir et al., 2020)

Direct detection of *B. pseudomallei* antigens in clinical samples is another approach that might be particularly useful in areas where culture is not available. A lateral flow test using a monoclonal antibody specific for capsular polysaccharide (CPS), with a limit of detection of ~0.2ng/ml, the Active Melioidosis Detect Lateral Flow Immunoassay (AMD LFI; InBios, USA), is not yet on the market but has undergone evaluation in several settings. Testing against a large panel of *B. pseudomallei* isolates showed an analytical reactivity of 98.7%, with cross-reactivity in only 2.8% of near neighbour species.(Houghton et al., 2014) The AMD-LFI was 99% sensitive and 100% specific on turbid blood culture bottles. The high specificity was maintained across all sample types, with relatively high sensitivity in pus and sputum samples but poor sensitivity on serum. In this cohort from Laos, urine samples had a positive predictive value of 94% for diagnosing melioidosis; a potential game changer for diagnostics in resource limited settings.(Woods et al., 2018) Similar findings were reproduced from prospective cohorts in India, although highest discordance was demonstrated from serum – 34.1%. This was corroborated in Thailand where sensitivity in serum was 31.3%.(Wongsuvan et al., 2018)

There is no standard serological test for melioidosis. An indirect haemagglutination (IHA) test, using a crude mixture of poorly characterized antigens, is most widely used, but it lacks sensitivity and specificity in humans, particularly in endemic areas where background seropositivity rates are high (e.g. 29%, 38% and 12.8% in studies in India, Thailand and Australia respectively).(Vandana et al., 2016, Chaichana et al., 2018, Smith et al., 2018) A rapid immunochromatographic test for IgG appeared to be more sensitive and specific than IHA in populations of endemic areas but is no longer commercially available.(Wuthiekanun et al., 2004) Recently, ELISAs employing better characterised antigens such as purified O-polysaccharide (OPS) and haemolysin co-regulated protein (Hcp-1) have been shown to have better performance than the IHA for serodiagnosis of melioidosis in endemic areas (Pumpuang et al., 2017), with IgG antibody levels for both antigens raised from an early stage.(Pumpuang et al., 2019) A rapid immunochromatography test (ICT) using Hcp-1 was evaluated in cohorts of melioidosis patients, healthy controls and patients with other infections, demonstrating an overall sensitivity of 88.3%.(Phokrai et al., 2018) However, until kits using more refined, standardised antigens are available, the utility of serological tests is largely limited to non-endemic regions. Despite the limitations, serology continues to be used in veterinary medicine, and a two-step method by screening with IHA followed by confirmation with a complement fixation test was

shown to be sensitive and specific in caprine melioidosis.(Thomas et al., 1988b) Molecular methods have also been developed but are not yet used for routine diagnostic purposes.

Radiology is an important adjunct to microbiological diagnosis, and may demonstrate diffuse nodular infiltrates, abscess, or cavitating pneumonia on chest radiograph. Liver, splenic, prostatic or other intra-abdominal abscesses on ultrasound or CT may be suggestive of the diagnosis.(Huson et al., 2020)

Treatment

B. pseudomallei is intrinsically resistant to many classes of antibiotics, including some third generation cephalosporins, early penicillins, aminoglycosides, colistin and polymyxin, and exhibits relative resistance to quinolones and macrolides.(Cheng and Currie, 2005) Acquired resistance may occur but rarely compromises the choice of antibiotic treatment,(Wuthiekanun et al., 2011, Fen et al., 2021) although one recent paper from China reported nearly 13% of isolates as resistant to ceftazidime.(Rao et al., 2019)

The treatment of melioidosis may be classified into acute and eradication phases. In the acute phase the aim is to kill bacteria in the circulation and prevent patients dying of overwhelming sepsis, and in the eradication phase the aim is to kill any residual bacteria in abscesses or tissues and prevent relapse of infection. Currently, ceftazidime or a carbapenem for 2 weeks is the treatment of choice for the acute phase, and co-trimoxazole for 12-20 weeks for eradication.(Anunnatsiri et al., 2020) Recent updates to Australian guidelines recommend a minimum of 3 weeks intravenous therapy for multi-lobar pneumonia without bacteraemia, 4 weeks if bacteraemic, and minimum 3 weeks for those with only single lobar pneumonia with concomitant bacteraemia. Even longer courses of treatment (up to 8 weeks intravenously and 6 months orally) are recommended for those with deep-seated foci of infection, including bone and joint, central nervous system and intravascular involvement.(Sullivan et al., 2020)

In a trial of 161 patients in Thailand (65 with confirmed melioidosis, 54 of these septicaemic), ceftazidime (120mg/kg/day) in the acute phase reduced mortality from 74% to 37%, compared with the conventional combination regimen of chloramphenicol, doxycycline and co-trimoxazole.(White et al., 1989) Other cephalosporins, such as cefotaxime and ceftriaxone, were associated with significantly greater mortality compared with ceftazidime in retrospective analyses.(Chaowagul et al., 1999) Subsequent trials assessed ceftazidime with and without the addition of co-trimoxazole in the acute phase of melioidosis, and failed to demonstrate any difference in mortality between the monotherapy and combination groups.(Chierakul et al., 2005) Median time to defervescence of fever was 9 days.(Simpson et al., 1999b) In the Darwin cohort, extending the acute intensive phase to 4 weeks, resulted in a relapse rate of only 1.2%.(Pitman et al., 2015)

Carbapenems are the most active drugs *in vitro* against *B. pseudomallei*, and are more rapidly bactericidal.(Smith et al., 1996) A randomised trial comparing ceftazidime (120 mg/kg/day) with imipenem/cilastatin (50 mg/kg/day) for a minimum of 10 days, was unfortunately terminated early and therefore underpowered. It showed no difference in mortality between the two groups, but higher rates of treatment failure in the ceftazidime group (41.3 % versus 20.3 %).(Simpson et al., 1999a) Co-amoxiclav is considered second line therapy for the acute phase. One study from Malaysia has suggested veterinary cases, especially those involving novel ST 1130 isolates show significantly

higher likelihood of resistance to meropenem.(Sadiq et al., 2018) *B. pseudomallei* isolates carrying the carbapenemase blaOXA-57 have also been identified, although, >90% of blaOXA-57 carrying isolates were phenotypically susceptible to imipenem.(Amladi et al., 2019) The concern is however that IS (insertion sequences) family transposases (carried by these isolates), which facilitate mobilisation of extended-spectrum -lactamase (ESBL) and carbapenemase genes, would have been missed had hybrid genome assembly not been performed.(Amladi et al., 2019) This highlights the wider threat of AMR and virulence gene acquisition and need for robust surveillance systems globally.

The conventional combination of chloramphenicol, doxycycline and co-trimoxazole for eradication therapy was extremely poorly tolerated leading to reduced compliance and increased rates of relapse. Omitting chloramphenicol was shown to be beneficial in terms of side effect profile, with no adverse treatment outcomes.(Chaowagul et al., 2005) Years of clinical experience in Australia,(Currie et al., 2021) however, suggest that co-trimoxazole monotherapy for 12-20 weeks is probably adequate to prevent relapse. The MERTH trial conducted in Thailand, also supported the use of co-trimoxazole monotherapy on the basis of efficacy, safety and tolerance by patients.(Chetchotisakd et al., 2014) Another recent RCT ,which did not meet the primary end point (culture-confirmed recurrent melioidosis), found that all-cause mortality was significantly lower with a 12-week regimen (0.3%) compared to 20-weeks (3%), meeting the criteria for non-inferiority for the secondary composite end-point (overall recurrent melioidosis and mortality).(Anunnatsiri et al., 2020) In the rare cases of co-trimoxazole resistance (determined by MIC), and where co-trimoxazole is contraindicated, co-amoxiclav is the preferred choice for eradication therapy, although this is associated with increased rates of relapse.(Rajchanuvong et al., 1995) In a cohort of >3000 patient isolates, only 0.33% were resistant to co-trimoxazole. Encouragingly, all resistant isolates were susceptible to co-amoxiclav, but only 91% to doxycycline.(Saiprom et al., 2015) As mentioned previously, poor compliance also contributes to relapse, and so it is crucial that each patient is counselled in the importance of completing the full treatment course regardless of symptomatic improvement.

Novel agents such as cefiderocol, a siderophore cephalosporin which inhibits peptidoglycan synthesis and is described as universally stable against β-lactamases (providing greater efficacy than carbapenems, cephalosporins and other inhibitor combinations), shows promise as it is highly active in vitro against *B. pseudomallei*.(Burnard et al., 2021)

Apart from appropriate antibiotic therapy, the management of melioidosis must also incorporate optimal supportive treatment for sepsis, including maintenance of blood pressure, adequate glycaemic control, and management of respiratory and acute renal failure. Around one quarter of cases require admission to intensive care.(Currie et al., 2021) The drainage of abscesses should also take place where possible. Adjunctive granulocyte colony stimulating factor (G-CSF) has been used to boost host neutrophils in an attempt to control infection, but despite promising outcomes in a retrospective study, G-CSF did not significantly reduce mortality in a randomised controlled trial.(Cheng et al., 2004, Cheng et al., 2007)

Even with appropriate antimicrobial and supportive therapy, mortality remains high for septicaemic cases. Poor prognostic factors include shock, absence of fever, leucopenia, abnormal liver function, renal impairment, high level or persistent bacteraemia, hypoglycaemia and acidosis.(Currie et al.,

2021, Limmathurotsakul et al., 2011) However, over the past 5 years, a combination of optimal sepsis management and antibiotic therapy has reduced the overall mortality from melioidosis in Darwin, Australia, to only 6%.(Currie et al., 2021)

The efficacy of post-exposure prophylaxis (PEP) in protection against developing melioidosis remains unknown. Animal models show that PEP simply delays the onset of disease, rather than preventing it.(Dance et al., 2017) In over 70-years of combined experience in diagnostic laboratories from endemic regions which handle thousands of *B. pseudomallei* samples at containment levels less stringent than U.S. biosafety level 3, leaders in the melioidosis field have not once been consulted about a case of laboratory-acquired infection.(Dance et al., 2017) Exposure of 30 healthcare workers (HCWs) in South Korea, including 5 high risk exposures to pus/blood through non-intact skin and 25 low risk contacts with blood through intact skin along with two laboratory staff who opened the lid of an agar plate growing *B. pseudomallei* outside a biological safety cabinet, resulted in no seroconversion or symptoms of melioidosis. The only two well described laboratory-acquired cases to date involved sonication outside a safety cabinet and cleaning up a spillage of *B. pseudomallei* culture with bare hands whilst having an ulcerative lesion on a finger.(Jun et al., 2017) A recent study in Queensland (Australia) identified no infections or seroconversions following 1,267 instances when *B. pseudomallei* was handled outside a safety cabinet.(Gassiep et al., 2021) Using bioaerosol sampling and *B. thailandensis* as a bioequivalent surrogate in handling experiments, the authors found no evidence of environmental contamination. This suggests the risk of laboratory-acquired melioidosis may be lower than that for glanders or some other hazard group 3 agents. In the rare occasion where a high-risk exposure or low risk exposure in a lab worker with underlying risk factors has occurred, PEP with co-trimoxazole may be considered following a careful discussion of risks and benefits.(Peacock et al., 2008) As a caution, of two lab technicians who were exposed to aerosolised *B. pseudomallei* whilst manipulating cultures outside a biosafety cabinet, neither of whom developed melioidosis or seroconverted, one had to take time off work due to adverse drug reaction (fever, cough and rash) to co-trimoxazole, so the decision to offer PEP should not be taken lightly.(Mitchell et al., 2017)

As is the case for glanders, the long duration of treatment of melioidosis in animals can be expensive and ineffective. In cases where treatment is deemed necessary, such as in animals of economic value, treatment regimens are as for human cases.

Prevention and Control

A cost-benefit analysis has suggested that immunisation against melioidosis would be worthwhile if used in high-risk populations, even when only partial protection is assumed.(Luangasanatip et al., 2019) A Steering Group on Melioidosis Vaccine Development (SGMVD) was created to advise the scientific community in 2015.(Limmathurotsakul et al., 2015) A key recommendation was that vaccine candidates should also be tested in diabetic animal models. The Defense Threat Reduction Agency (DTRA) recently funded the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) to conduct head-to-head comparisons of promising vaccine candidates in 2020. As recommended by SGMVD, this study should provide concrete evidence for antibody- and cytokine-mediated responses that confer protection and preferably sterilising immunity.(Khakhum et al., 2020) However, although potential candidates are under investigation, as yet there is no licensed human or animal vaccine for melioidosis.

Due to the complex pathogenicity of *B. pseudomallei* and strain heterogeneity, it has become evident that a multicomponent vaccine using a combination of protective antigens will be required for complete protection. Thankfully, intense research has resulted in several multivalent platforms such as glycoconjugates, multivalent subunit preparations, live-attenuated bacteria, nanoparticle platforms and outer membrane vesicles (OMV), that have proven highly effective in experimental animal models of melioidosis, conferring 40-100% efficacy.(Morici et al., 2019) OMVs hold particular promise as they lack any observable toxicity, are self-adjuvanting (driving dendritic cell maturation) and can contain intracellular stage-specific proteins such as T3SS-3 or T6SS-1.(Baker et al., 2021) A recent study using purified *B. pseudomallei* CPS covalently linked to recombinant CRM197 (a non-toxic mutant of diphtheria toxin) produced opsonizing antibody responses with high IgG titres. Mice vaccinated with a combination of CPS-CRM197 and recombinant Hcp1 showed 100% survival in a lethal inhalational challenge model, with 70% sterilising immunity.(Burtnick et al., 2018) This candidate is planned to be the first melioidosis vaccine used in a human phase 1 clinical trial in diabetic and non-diabetic volunteers.

In the absence of a licensed vaccine, preventive measures must therefore focus on avoidance of contact with *B. pseudomallei* in the environment. A matched case-control study carried out in northeast Thailand found that working in rice fields, walking barefoot, bathing in pond water, exposure to rain, water inhalation, and having an open wound all significantly increased the odds of acquiring melioidosis.(Limmathurotsakul et al., 2013b) A lower risk of melioidosis was associated with wearing protective clothing such as long trousers and rubber boots, and washing with clean water after working in the fields. The authors, therefore, recommended avoidance of direct contact with soil and environmental water whenever possible, but wearing protective clothing and washing after exposure if this is unavoidable. Wounds should be kept covered until they have completely healed, and the application of herbal remedies to wounds should be avoided, as this was also associated with an increased risk of melioidosis. Since there was a small but significant risk observed with drinking untreated water, and since *B. pseudomallei* was found in water drunk by 7% of cases and 3% of controls, including borehole, wells and piped supplies, it was also recommended that only treated water should be drunk in endemic areas.(Limmathurotsakul et al., 2013b) It has also been recommended that goat's milk be pasteurised to avoid potential zoonotic transmission by ingestion,(Choy et al., 2000) although this has never been reported, but this makes sense in general public health terms.

Very recently, the results of PREMEL, a stepped-wedge cluster-RCT on the effectiveness of a multifaceted prevention programme for melioidosis in diabetics from 116 primary care units in northeast Thailand, have been published.(Suntornsut et al., 2021) Although rates of culture-confirmed melioidosis were not decreased in participants who had received an intervention in the form of a behavioural support group session, they had a lower incidence of hospital admissions involving infectious diseases and of all-cause mortality. Proposals for modification/addition of behavioural techniques and need for more frequent intervention have been suggested.(Suntornsut et al., 2021)

Due to the low but theoretical risk of person-to-person transmission, human cases should be nursed in isolation with contact precautions and care taken when handling any body fluids. People with strongly associated predisposing conditions, such as diabetes, should be informed of their increased

risk of melioidosis, and advised to avoid the above high-risk activities. Unfortunately, in rural areas, and during heavy rain and winds, exposure may be unavoidable for many.

It has been recommended that animals be removed from contaminated sources, such as soil or water in endemic regions, to prevent melioidosis outbreaks in herds,(Choy et al., 2000) however infections have still occurred when pigs were reared on artificial, hard surfaces such as concrete.(Thomas et al., 1981) Chlorination of water has been shown to eliminate *B. pseudomallei*,(Howard and Inglis, 2005) but only if pH and concentrations of organic substrates are carefully controlled, and this could prove difficult in water troughs which may become highly contaminated.(Choy et al., 2000) When an animal becomes infected in an endemic area, it has been suggested that strict maintenance of a hygienic environment may prevent a larger outbreak, although supporting evidence is lacking. Regular disinfection with potassium hypochlorite and cresol (to include all surfaces and the lower limbs of the animal), removal of infected excrement several times per day, and the avoidance of large quantities of water were used in an effort to curtail the outbreak in Paris zoos and equestrian clubs in the 1970s.(Sprague and Neubauer, 2004) Infected carcasses of animals must be condemned and destroyed. Guidelines for handling and disposal are available in the Manual for Meat Inspection in Developing Countries (http://www.fao.org/docrep/003/t0756e/T0756E05.htm#ch4.2.9). There are no mandatory requirements for serological screening for melioidosis in animals that are transported internationally, although it is possible that such animals might react in serological or skin tests to the closely related *B. mallei*. Serological testing of imported primates for melioidosis was used following an outbreak amongst Cynomolgus monkeys in the United Kingdom in the 1990s but has never been used routinely.(Dance et al., 1992)

Conclusion

Both glanders and melioidosis may be regarded as re-emerging infections with the ability to infect both animals and humans, although only glanders is a true zoonosis. Glanders has been eradicated from many countries, whereas melioidosis is widespread across the tropics, particularly South and Southeast Asia and tropical North Australia. Various factors have contributed to their emergence such as increasing awareness and diagnostic capability, increasing prevalence of underlying predisposing conditions, increased transport of animals (and associated contaminated waste and equipment) and other products internationally, human migration patterns, and adventure travel to tropical regions. Sub-clinically infected human and animal carriers risk further transmission of infection in the case of glanders, as well as persistence in a new environment under the right physical conditions. Global warming may extend the current geographic limitations of melioidosis and place a greater population at risk of exposure. This is particularly of concern as the prevalence of diabetes and other immunosuppressive states increases in many developing countries. Finally, we must be alert to the possible use of these agents in bioterrorism. Increased awareness of these pathogens is important so that early recognition, treatment, and public health action occurs, and so that organisms are handled at the appropriate level of containment to prevent laboratory-associated cases. Further research is required to develop effective vaccines and optimise prevention strategies.

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