Burkholderia mallei: ‘GLANDERS’
Phylum B X VII Spriocacteria
Class II Betaproteobactera/Family
Burkholderiaceae/Genus 1 Burkholderia: Gram-negative aerobic rods and cocci

Definition/Overview
Glanders is an ancient, highly fatal, and usually chronic respiratory disease of solipeds caused by Burkholderia mallei (formerly Pseudomonas mallei) with humans being accidental hosts. The diagnosis is based on the presence of characteristic stellate scars in the nasal septum and a positive reaction to the mallein test, combined with a positive culture of B. mallei. Human infections are often fatal if untreated.

Aetiology
B. mallei is a facultative rod-shaped Gram-negative nonmotile, intracellular pathogen that can invade, survive, and replicate in epithelial and phagocytic cell lines (Ribot & Ulrich 2006). It is an obligate animal pathogen whose natural hosts are horses, donkeys, and mules, but infections can also occur in felines, camels, and goats. Virulence in B. mallei is multifactorial and several virulence determinants have been identified and characterized (Schell et al. 2007). Seventeen distinct ribotypes were identified from human and equine infections (Harvey & Minter 2005).

Epidemiology
Glanders is endemic in Africa, Asia, the Middle East, and Central and South America. Carriers that have made an apparent recovery from the disease are the most important source of infection, as the pathogen does not survive for more than 6 weeks outside the host (Lehavi et al. 2002).

Pathophysiology
Equines are generally infected orally (Schell et al. 2007). Following penetration of the mucosa, the pathogen is spread via the lymphatic tissues.

Incubation period
The incubation period varies from 1 to 2 days following intratracheal deposition, with rectal temperatures increased to above 40°C (Lopez et al. 2003).

Clinical presentation
Clinical signs include febrile episodes, cough, blood-encrusted material on nostrils, inflammatory nodules and ulcers developed in the nasal passages with a sticky yellow discharge, characteristic stellate scars in the nasal septum, purulent nasal discharge, enlargement of submaxillary lymph nodes, chronic lymphangitis, skin abscessation, progressive debility, orchitis, and dyspnæa associated with interstitial pneumonia. Furthermore, apparent neurological degeneration is seen in acute glanders (Lopez et al. 2003). Life expectancy was judged likely to have been less than 12 hours in B. mallei inoculated horses due to subsequent pulmonary oedema (Lopez et al. 2003).

Differential diagnosis
The differential diagnosis includes various causes of fever and dyspnæa (see p. 262).

Diagnosis
The presence of stellate scars in the nasal septum is regarded as pathognomonic. B. mallei can be cultured easily from purulent nasal discharge and the complement fixation test can be used for serology. Furthermore, the mallein test can also be used to identify infected horses; purulent exudate in the eye associated with blepharospasm of a glandered animal 24–48 hours following subconjunctival inoculation is regarded as a positive test result. Alternatively, the intracutaneous mallein test can be used with an increase in rectal temperature and a swelling at the point of injection regarded as a positive test result (Arum et al. 1999). When tested comparatively with Dutch PPD mallein as standard, trichloroacetic acid-precipitated proteins were comparable to Dutch PPD mallein in potency and innocuity, whereas ammonium sulphate-precipitated proteins elicited nonspecific reactions (Verma et al. 1994).

Competitive enzyme-linked immunosorbent assay (cELISA) test specificity for B. mallei was 99%. Concordance and kappa value between the complement fixation (CF) and the cELISA procedures for the serodiagnosis of B. mallei infection in experimentally exposed horses were 70% and 0.44, respectively (Katz et al. 2000). The cELISA offers the possibility for automatization, can be applied to noncomplement fixing sera, and used for various host species although the complement fixation test (CFT) is internationally mandatory for testing of equine sera for the absence of glanders to date (Sprague et al. 2009).

Pathology
B. mallei infection results in pyogranulomatous and necrotic pulmonary nodules, and ulcerative nodular skin and respiratory mucosal lesions with characteristic white stellate scars in the nasal septum. Histologically, lung lesions comprise liquefactive necrosis including neutrophils and surrounding epithelioid macrophages and fibrosis. The dermal disease of ulcerations including lymphangitis is named ‘farcy’ (Jubb et al. 2007). Remarkably, Streptococcus equi subsp. zooepidemicus was isolated from the brain of all B. mallei inoculated horses (Lopez et al. 2003).

Management/Treatment
Horses with a tentative diagnosis of glanders should be isolated to prevent possible human exposure. Treatment should not be attempted as the pathogen has important public health significance and glanders is a reportable disease.

Hydrolysis probe-based real-time PCR using the uneven distribution of type III secretion system genes afforded considerable improvements in the specificity and rapidity of the diagnosis of B. pseudomallei, B. mallei, and B. thailandensis, and allows rapid discrimination from opportunistic pathogens such as members of the B. cepacia complex (13), that routine diagnostic laboratories are more likely to encounter (Thibault et al. 2004).

Pathogenic bacteria
Bacterial diseases
Bacterial diseases

Public health significance
Humans are accidental hosts of B. mallei and the majority of cases have been the result of occupational contact with infected horses. While equines are generally infected orally, the primary route of infection in humans is contamination of skin abrasions or mucous membranes with nasal discharge or skin lesion exudate from an infected animal (Schell et al. 2007). Person-to-person spread of B. mallei is extremely rare. In humans, glanders is characterized by initial onset of fever, rigors and malaise, culminating in a rapid onset of pneumonia, bacteraemia, pustules and abscesses, leading to death in 7–10 days without antibiotic treatment. The course of infection is dependent on the route of exposure. Direct contact with the skin can lead to a localized cutaneous infection. Inhalation of aerosol or dust containing B. mallei can lead to septicaemic, pulmonary, or chronic infections of the muscle, liver, and spleen. The disease has a 95% case fatality rate for untreated septicaemia infections and a 50% case fatality rate in antibiotic-treated individuals (Mandell et al. 1995). Burkholderia infections are difficult to treat with antibiotics and no vaccine exists (Whitlock et al. 2007).
**Burkholderia pseudomallei: MELIOIDOSIS**

Phylum: BXXVII Spirochaetes

Class II Betaproteobacteria/Family Burkholderiaceae/Genus I Burkholderia: Gram-negative aerobic rods and cocci

**Definition/Overview**

Melioidosis is a rare disease caused by *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*) characterized by an intracellular life cycle. Both humans and animals (including birds, crocodiles, and kangaroos) are susceptible to melioidosis with both latency and a wide range of clinical manifestations. Some species may develop melioidosis only if immunocompromised. Sheep, goats, and horses are particularly susceptible, but zoonotic transmission to humans is extremely unusual (Neubauer et al. 1997, Choy et al. 2000). Melioidosis has important public health significance and is a reportable disease.

**Aetiology**

*B. pseudomallei* is a Gram-negative, bipolar-staining, pleomorphic, motile bacillus, which is principally an environmental saprophyte responsible for melioidosis.

**Epidemiology**

This saprophyte inhabitant of telluric environments is mainly encountered in southeast Asia and northern Australia, but is sporadically isolated in subtropical and temperate countries (White 2003). Melioidosis has become an increasingly important disease in endemic areas such as northern Thailand and Australia (Currie et al. 2000a). In endemic areas, the positive rates of antibodies against *B. pseudomallei* in humans, horses, oxen, and pigs were 4–15%, 9–18%, 7–33%, and 35%, respectively (Li et al. 1994).

**Pathophysiology**

Following ingestion via contaminated soil or faeces, a diverse assortment of virulence factors (quorum sensing, type III secretion system, lipopolysaccharide and other surface polysaccharides) allows *B. pseudomallei* to become an effective opportunistic pathogen; its intracellular life cycle also allows it to avoid or subvert the host immune system (Adler et al. 2009, Wiersinga & van der Poll 2009). The BoaA and BoaB genes specify adhesins that mediate adherence to epithelial cells of the human respiratory tract. The BoaA gene product is shared by *B. pseudomallei* and *B. mallei*, whereas BoaB appears to be a *B. pseudomallei*-specific adherence factor (Balder et al. 2010).

**Incubation period**

This is not established in the equine species yet. The incubation period in man from defined inoculating events was previously ascertained as 1–21 (mean 9) days (Currie et al. 2000b).

**Clinical presentation**

Clinical signs include fever, septicemia, oedema, colic, diarrhoea, and lymphangitis of the legs. A case of acute meningoencephalomyelitis caused by infection with *B. pseudomallei* has been described associated with inability to stand; opisthotonus, facial paralysis (14) and nyctagnus, rapidly progressing to violent struggling (Ladds et al. 1981).

**Differential diagnosis**

The differential diagnosis includes various causes of internal abscessation (without characteristic stellate scars in the nasal septum as seen in *B. mallei*) (see p. 262). Listerial abscess should be considered in a case of meningoencephalitis.

**Diagnosis**

*B. pseudomallei* can be cultured easily from purulent nasal discharge. The diagnosis is based on a positive reaction to the mallein test combined with a positive culture.

**Pathology**

Multiple abscesses in most organs are characteristic of the disease. The encapsulated nodules with caseous centres are composed of necrosis, neutrophils, lymphocytes, and epithelioid macrophages. In a case of acute meningoencephalomyelitis gross examination revealed malacia and haemorrhage in the medulla oblongata and adjacent spinal cord. Microscopically there were disseminated focal neutrophilic accumulations in affected areas, perivascular cuffing with mononuclear cells and lymphocytes, and marked oedema. Intracellular bacteria were identified in sections stained by the Giemsa method (Ladds et al. 1981).

**Management/Treatment**

Horses with a tentative diagnosis of melioidosis should be isolated to prevent possible human exposure. Treatment should not be attempted as the disease has important public health significance. Furthermore, the ubiquitous bacterium is characterized by remarkable insensitivity to antimicrobial drugs. For instance, *B. pseudomallei* is intrinsically resistant to aminoglycosides and macrolides, mostly due to AmrAB-OprA efflux pump expression (Trunck et al. 2009). Immunisation with heat-inactivated *B. pseudomallei* cells provided the highest levels of protection against either melioidosis or glanders, indicating longer-term potential for heat-inactivated bacteria to be developed as vaccines against melioidosis and glanders (Sarkar-Tyson et al. 2009).

**Public health significance**

Melioidosis has important public health significance and is a reportable disease. It is a life-threatening disease that is mainly acquired through skin inoculation or pulmonary contamination, although other routes have been documented (Neubauer et al. 1997). Primary skin melioidosis occurred in 12% of human patients. Secondary skin melioidosis (multiple pustules from haematogenous spread) was present in 2%. Patients with primary skin melioidosis were more likely to have chronic presentations (duration of a minimum of 2 months) [Gibney et al. 2008]. Severe septicemia secondary to melioidosis carries a high mortality. Although melioidosis can involve most tissues and organs, pericardial involvement is rare (De Keulenaer et al. 2008). Of human cases, 46% were bacteraemic and 19% died (Currie et al. 2000a).

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**Bordetella bronchiseptica**

Phylum BXXVII Spirochaetes
Class II Betaproteobacteria/Family III Alcanigenaceae/Genus III Bordetella: Gram-negative aerobic rods and cocci

**Definition/Overview**
The opportunistic bacterium *Bordetella bronchiseptica* is a rare cause of acute respiratory disease and abortion/infertility.

**Aetiology**
Pasturellaceae are Gram-negative bacteria with an important role as primary or opportunistic, mainly respiratory, pathogens in domestic and wild animals. Some species of Pasteurellaceae cause severe diseases with high economic losses in commercial animal husbandry and are of great diagnostic concern (Dousse et al., 2008). Sixteen distinct ribotypes were identified in *B. bronchiseptica* strains (Regster et al., 1997). Four main types of variation of the *B. bronchiseptica* lipopolysaccharide (LPS) are apparent: (1) heterogeneity of the core, (2) presence or absence of O-chains, (3) differences at the level of the hinge region between the O-chain and the core, and (4) differences in the association with other cell surface constituents. Isolates from different animal species did not show significant differences in their patterns of reactivity with monoclonal antibodies (LeBlay et al., 1997).

**Epidemiology**
Glucose nonfermenting Gram-negative bacilli have been recognized as opportunistic pathogens of humans. The most common veterinary glucose nonfermenting Gram-negative bacilli were *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus*, *B. bronchiseptica*, and *Pseudomonas pseudocaligines*. Of all clinical veterinary specimens submitted for cultures, 10% contained nonfermenters (Mathewson & Simpson, 1982). *B. bronchiseptica* was isolated from bronchial lavage specimens in distal respiratory tract disease (nasal discharge, cough, pneumonia) in 13% of foals (1–8 months old) (Hoffman et al., 1993).

**Pathophysiology**
Either *B. bronchiseptica* does not persist inside animals or susceptible animals possess specific receptors for smooth-type LPSs, in contrast to man (Le Blay et al., 1997).

**Incubation period**
Not established in the equine species yet.

**Clinical presentation**
Clinical presentation includes respiratory disease in foals (17) (Koehne et al., 1981), coughing in Thoroughbred racehorses (Christley et al., 2001), bronchopneumonia (Saxegaard et al., 1971), abortion (Mohan & Obwolo, 1991), and infertility (Mather et al., 1973). *B. parapertussis* did not grow in tracheobronchial washing from a horse (Porter & Wardlaw, 1994).

**Differential diagnosis**
The differential diagnosis includes various causes of fever and dyspnoea (see p. 262).

**Diagnosis**
Diagnosis primarily depends on culture of the bacterium from tracheobronchial washing samples combined with clinical signs. Analysis of tracheobronchial washing samples for known *Bordetella* nutrients revealed concentrations of amino acids and nicotinic acid averaging 0.35 mM and 0.56 μg/ml, respectively (Porter & Wardlaw, 1994).

**Pathology**
Common lesions caused by *B. bronchiseptica* include a catarrhal to suppurative bronchopneumonia and a (sero)fibrinous pleuropneumonia. These are usually opportunistic secondary infections preceded by viral infections in juvenile animals.

**Management/Treatment**
Treatment of diseased animals is supportive and specific treatment should be based on *in-vitro* antimicrobial susceptibility testing.

**Public health significance**
The absence of smooth-type LPSs appears to be rather frequent in human isolates, since long-chain LPSs were detectable in only 52% of human isolates, whereas 94% of animal isolates contained molecules of that type (Le Blay et al., 1997). *B. bronchiseptica* might have some public health significance and its zoonotic risk should be minimized.
**Taylorella equigenitalis: Contagious Equine Metritis**

**Phylum** Bacteria

**Class** Betaproteobacteria

**Family** XI Alcanigenaceae

**Genus** Taylorella

**Description**

*T. equigenitalis* is a Gram-negative, microaerophilic, fastidious slow-growing cocccobacillus with streptomycin-sensitive and -resistant biotypes (Timoney 1996). Isolates of *T. equigenitalis* obtained from European horses analysed by pulse-field gel electrophoresis (PFGE) were classified into 18 genotypes (Kagawa et al. 2001). High sequence similarity (99.5% or more) was observed throughout isolates from Japan, Australia, and France, except from nucleotide positions 138 to 501 where substitutions and deletions were noted (Matsuda et al. 2006). A phylogenetic analysis revealed a position of *T. equigenitalis* in the beta subclass of the class Proteobacteria apart from the position of *Haemophilus influenzae*, which belongs in the gamma subclass of Proteobacteria. A close phylogenetic relationship among *T. equigenitalis*, *Alcaligenes xylosoxidans*, and *Bordetella bronchiseptica* was detected (Bleumink-Plumy et al. 1993). Lipopolysaccharide O-Ps could be a specific marker for identification and differentiation of *T. equigenitalis* and *T. asinigenitalis*, and provide the basis for the development of specific detection assays for *T. equigenitalis* (Brooks et al. 2010).

**Aetiology**

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**Incubation period**

Horses challenged with *T. equigenitalis* showed seroconversion from day 11 post-inoculation (Katz & Geer 2001). Clinical presentation

CME can be the cause of short-term infertility sometimes associated with mucopurulent discharge and, very rarely, abortion in mares (Fontijn et al. 1989). Unlike the mare, stallions exposed to *T. equigenitalis* and *T. asinigenitalis*, and provide the basis for the development of specific detection assays for *T. equigenitalis* (Timoney 1996). It has been concluded that *T. equigenitalis* is of limited significance in horse breeding (Parlevliet et al. 1997).

**Differential diagnosis**

Atypical (donkey-origin) *Taylorella* spp. infections should be considered as a differential diagnosis of equine infertility in mares (Katz et al. 2000). *T. asinigenitalis*, resembling *T. equigenitalis*, was recently isolated from the urethral fossa, urethra, and penile sheath of a 3-year-old stallion of the Ardennes breed when it was routinely tested for CEM. However, the colony appearance, the slow growth rate, and the results in the API ZYM test differed slightly from those of *T. equigenitalis*. Sequence analysis of 165 rRNA genes was shown to be a reliable tool for differentiation of donkey-related *T. asinigenitalis* from *T. equigenitalis*, as well as for identification of these species. The *T. asinigenitalis* strain had a low minimum inhibitory concentration (MIC) of gentamicin (≤1 μg/ml) but a high MIC of streptomycin (>16 μg/ml) (Bäverud et al. 2006).

**Diagnosis**

Diagnosis is based primarily on culture of the bacterium from its predilection sites in the reproductive tract of the mare and the stallion (18, 19) (Timoney 1996). However, the rate of *T. equigenitalis* detection was higher with PCR than with the classic bacteriological examination. PCR is especially valuable in cases of intensive bacterial and fungal contamination of swabs where the isolation of *T. equigenitalis* usually fails (Zdovc et al. 2005). A direct-PCR assay was developed for the rapid detection of *T. equigenitalis* in equine genital swabs without need for a preliminary step of DNA extraction or bacterial isolation (Duquesne et al. 2007). The assay is also able to discriminate between *T. equigenitalis* and *T. asinigenitalis* (Wakeley et al. 2006).

In chronically infected mares, the organism was detectable in the clitoral swabs of nearly 93%, but in the cervical swabs of only 31%. In contrast, in acutely infected mares, the organism was detectable in the clitoral swabs of nearly 69%, but in the cervical swabs of 84% (Wood et al. 2005). There was close agreement between CFT and ELISA methodologies during the post-exposure time period used to detect CEM serodiagnostics in regulatory animal health testing programmes. Unlike the CFT, which requires an overnight incubation step, the ELISAs are more convenient and can be completed in 3 hours (Katz & Geer 2001).

**Pathology**

Macroscopically no vaginal lesions are apparent; the endometrial mucosa may be swollen and corrugated with a scant mucopurulent exudate. Histology of uterine biopsies might reveal a mild endometritis, characterized by interstitial mucosal oedema and a mild inflammatory infiltrate composed of neutrophils; later plasma cells may be more evident (Jubb et al. 2007).

**Management/Treatment**

Aggressive systemic antibiotic therapy accompanied by routine topical therapy might be required to treat CEM-positive stallions (Kristula & Smith 2004).

**Public health significance**

Not convincing yet.