Burkholderia (*Mallei and Pseudomallei*) Related Zoonosis Drastic Zoonotic and Biological Warfare Potential

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ABSTRACT

The Burkholderia genus consists of more than 20 species. The important pathogens in this group are B. Mallei and B. Pseudomallei. Burkholderia mallei and pseudomallei are rod-shaped, aerobic, non-spore-forming cocco-bacilli bacteria, which are involved in highly contagious diseases of equids, Glander and Melioidosis respectively. These pathogens are one of the main cause of financial and performance losses in developing nations. Their incubation period varies from days to weeks, or even months in some cases. They are extremely important pathogens if seen as bio-terror due to their highly zoonotic nature. B. Mallei has already been used as a potential biological terror agent in WW-II on both sides, leading to mass killing of horses, mules and donkeys employed in war as well as humans due to zoonoses. They both show typical respiratory and cutaneous signs making equids difficult to ride, or to be used for draught purposes. The diagnosis is based upon the signs and symptoms as well as ELISA, PCR and culture analysis. The Farcy Act was issued in 1899 to deal with Glanders effected equids, states that affected animals should be killed and disposed of properly. For prevention, identification of positive animals and culling is extremely important. Live vaccines for Glanders are available but have no satisfactory results and no vaccine is available for Melioidosis, so all prevention and control rely only on preventive measures. Treatment is quite tough as both bacteria are resistant to a number of antibiotics, making them more important as zoonotic agents, and is possible by a number of antibiotics, including Ceftazidime, Carbapenems, Amoxicillin-Clavulanic acid, Trimethoprim-Sulphadiazine, Danofloxacin, Norfloxacin, and Chloramphenicol and doxycycline.

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1. INTRODUCTION

Glanders is a highly contagious zoonotic disease, mainly present in horses, donkeys, and mules (Rahimabadi et al. 2023). It can cause infection in other animals like cats, dogs, and goats, and also cause disease in humans as well. Glanders is caused by Burkholderia mallei, a gram-negative bacterium (Elshafie and Camele 2021) and is a highly adapted pathogen to harsh environmental conditions. This bacterium can survive within the host cells and also replicate in it. There are different factors that help to survive the bacterium within cell, one being capsule formation, which prevents immune cells to kill the pathogenic bacterium. The route of transmission is primarily through direct contact with the animal and its secretions (Norris et al. 2018). The bacterium can enter the body also by some other routes like ingestion, inhalation, and abrasion of the skin. Mainly it is confined to the respiratory area, but later it can spread to the other parts of the body (Pinho et al. 2023). It is an ancient disease known for centuries. Hippocrates considers it as serious disease of equines.

In 350 BC it was given name 'melis'. In 1822, its etiology was described by isolating it from horse liver and spleen (Schadewaldt 1975).

After the discovery it is classified as Pfeifferella mallei, Loefflerella mallei, Actinobacillus mallei Malleomyces mallei, Mycobacterium mallei, Corynebacterium mallei, Bacillus mallei and Pseudomonas mallei. Now genus is classified as Burkholderia due to 16S rRNA gene typing, cellular lipid and fatty acid composition, DNA-DNA homology values and phenotypic characteristics (Whitlock et al. 2007). Glanders in Asia, America, Europe and Africa causes major morbidity and mortality in 19th and 20th century.

2. CHARACTERISTICS OF *B. MALLEI*

Genus Burkholderia comprises of more than 20 species mostly isolated from water or soil. The important pathogens in this group are *B. mallei* and *B. pseudomallei*. *Burkholderia mallei* is an anaerobic, non-spore-forming, gram-negative coccobacillus. *B. mallei* are non-motile (due to the absence of polar flagella present in other Burkholderia species). *B. mallei* are a non-fermenting bacterium and grow readily on MacConkey agar media (Gilligan et al. 2003). The specimen collection and transport methods, being used normally, are sufficient for recovering burkholderia species due to their capability to survive in hostile environments. Isolation is easier in the field due to survivability in hostile environments and can be isolated by culture or antigen-antibody basis (basis of ELISA in Glanders). *B. mallei*, isolated in 1944 from human postmortem, sequencing revealed two circular chromosomes (Nierman et al. 2004). More than 5,000 protein-encoding open reading frames (ORFs) have been recognized in its DNA.*B. mallei* sis capable to survives in 30% Normal Human Saline (NHS), and serum-sensitive strains lack Lipopolysaccharides. The capacity of *B. mallei* to grow in 30% NHS was assessed, at 2, 4, 8, and 18 hours, with a serum bactericidal assay. The bacterium survived in the presence of 30% NHS for 18 hours (DeShazer 2004). *B. mallei* shares lots of genes with *B. pseudomallei*, and both bacteria have almost same allelic profile (Godoy et al. 2003).

3. PATHOPHYSIOLOGY IN HOST

Pathophysiology of glanders in the host is shown in Fig. 1.

4. ROUTES OF INFECTION

The bacterial invasion of the oral, conjunctival, and nasal mucosa occurs by direct contact or through abrasions, deep lung deposits, and inhalation. The neck, arms, face, and head are the areas of exposed skin most frequently affected by the occupational exposures previously stated. Although penetrations or

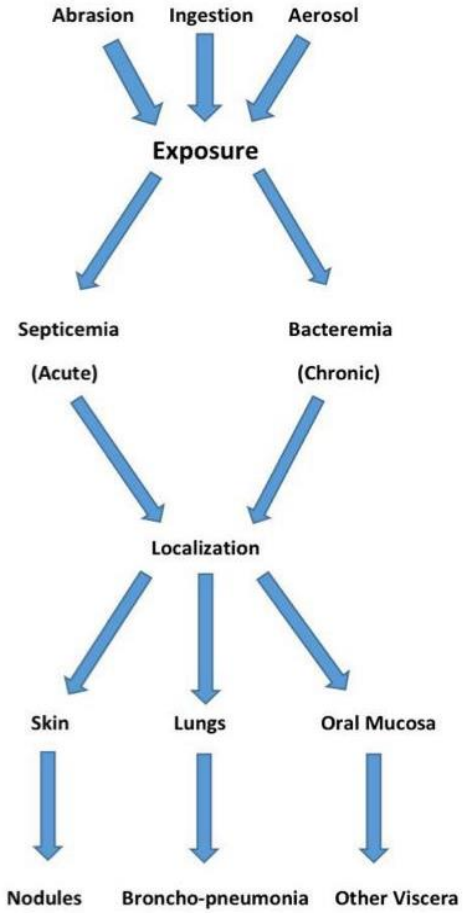

Fig. 1: Pathophysiology of Glanders In Host.

wounds during the anticipated exposure were not discovered, it is considered that *B. mallei* cannot enter normal intact skin (Pal et al. 2022). In fact, the major illnesses picked up in laboratories are not linked to injuries or memories of damage.

5. INCUBATION PERIOD

The incubation period for the acute form of the disease is typically 1–14 days, whereas the incubation period for the chronic form of the disease can last up to 12 weeks or more. Within one to five days of contact, a localized infection usually develops and may be distinguished by swelling of the afflicted area and weeping discharge. Before symptoms manifest, acute lung infection may occur between 10-14 days of incubation. Septicemia may occur right after exposure or up to fourteen days after the exposure. When left untreated, pneumonia develops quickly and is virtually always fatal between 10 and 30 days (Fhogartaigh et al. 2015).

6. SIGNS AND SYMPTOMS

Animals with acute Glanders typically exhibit the following symptoms (Fig. 2) after an incubation period of three to twenty days and death occurs within a few days (Howe et al. 1947).
Numerous Glanders types, such as chronic, disseminated, pulmonary, and septicemia, have been reported (Van Zandt et al. 2013).

7. MUCOSAL INVOLVEMENT

Photophobia and profuse lacrimation are symptoms of B. mallei infection of the eye and conjunctiva. Nasal involvement is characterized by swelling and inflammation of the nose and sinusitis followed by nasal discharge. Local lymph nodes may become irritated, and the face may enlarge. Bronchitis is accompanied by coughing and mucopurulent discharge if the infection spreads to the lower respiratory tract. The first few days after infection are usually characterized by moderate-to-low fever, excruciating headaches, and chills with or without rigors, in noon or evening. However, these symptoms could be very severe and continue through treatment. (Pal et al. 2023).

8. CUTANEOUS INVOLVEMENT

Papular lesions can appear anywhere on the body if the infection progresses chronically. An inflammatory reaction involving pain and swelling usually occurs after B. mallei penetration through an abrasion. In such circumstances, a Glanders node may initially appear as a blister, evolving into an ulcer, which would bleed profusely (Rathish et al. 2022). A localized infection, with a discharge, usually appears at the entering point. Inflammation can travel along local lymphatic, resulting in lymphangitis and several foci of suppuration. Escalating irritation and inflammation of the lymphatic smooth muscles are effects of endotoxins of B. mallei (Pal et al. 2022).

9. PULMONARY INVOLVEMENT

Pleural effusion, pleuritis, pulmonary abscess, and pneumonia are the typical symptoms of a lung infection. Dyspnea, cough, chest pain, and mucus in the sputum are all indications of lung infection. Respiratory infections are frequently accompanied by nonspecific signs and symptoms such as chills, dyspnea, discharge, myalgia, fever (typically above 102°F), headache, lymphangitis, fatigue, pleuritic chest discomfort, cough, tachypnea, sore throat, and gastrointestinal indications (Estes 2010). The onset of various symptoms can take up to two or three weeks. Rigors, weight loss, myalgia, mucosal eruptions, night sweats, dizziness, severe headaches, tachycardia, and nausea are examples of nonspecific symptoms that are frequently present and may point to widespread infection. (Khan et al. 2013).

10. DISSEMINATION OF INFECTION

Localized mucosal or cutaneous infection causes septicemia and the colonization of internal organs such as the liver, spleen, lungs, and the emergence of abscess. Septic shock and high mortality are frequently linked to these diseases.

11. ZOONOTIC ASPECTS OF BURKHOLDERIA MALLEI

B. mallei is an agent which requires a host animal to survive. Members of the family Equidae (donkeys, horses and mules) are the primary natural reservoir for B. mallei. Acute form of the infection usually occurs in mules and donkeys with respiratory problems and fever, whereas horses typically exhibit a more chronic course, especially in endemic areas, and may survive for years. Interestingly, the term Glanders refers to lymphangitis and lymphadenopathy in horses. The condition is known as Farcy when it has cutaneous symptoms. Humans, sometimes felids, wolves, dogs, bears, and camels are susceptible to illnesses, usually to less extent. Other carnivores may get the disease by ingesting the infected meat, but pigs and cattle are immune (Khan et al. 2013).

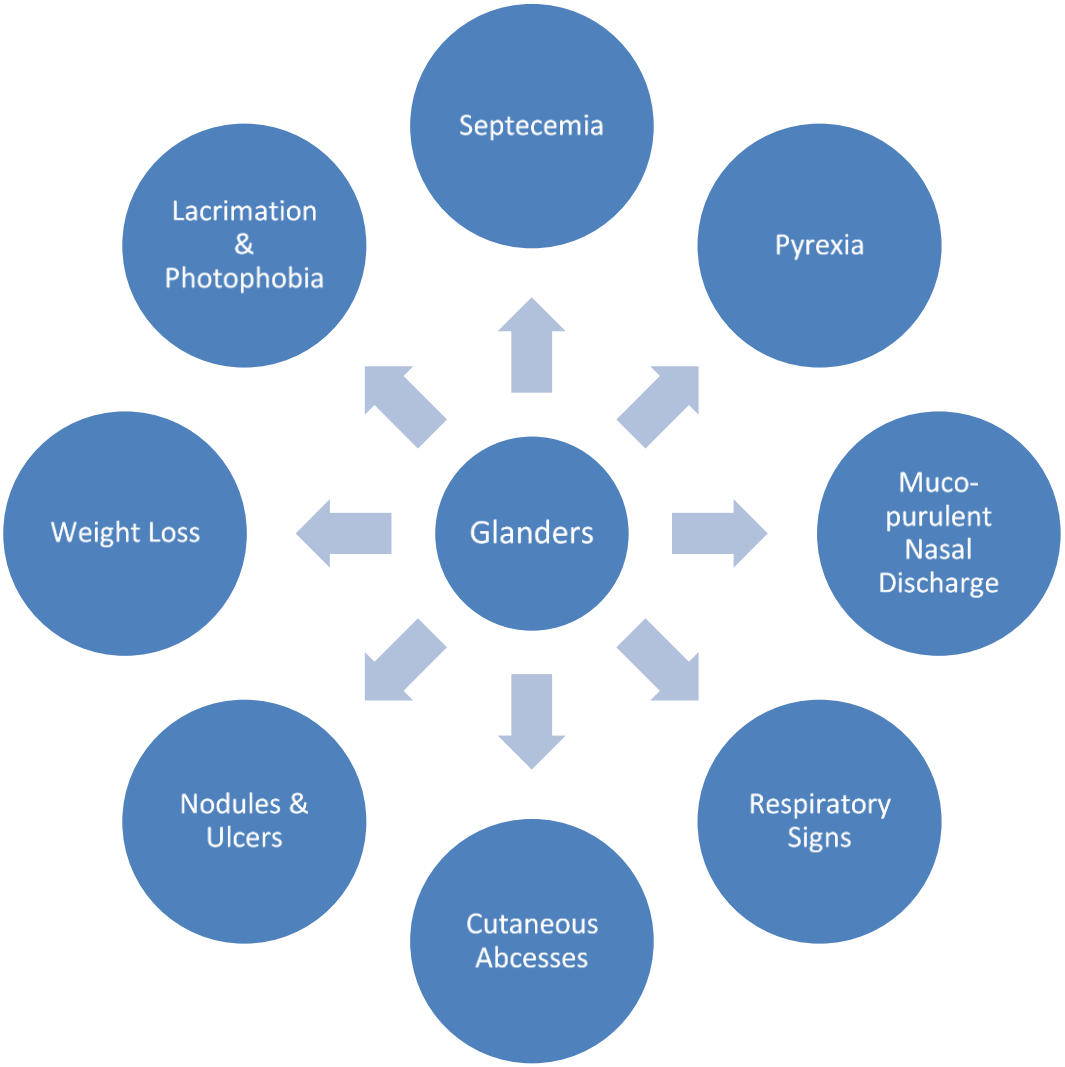

Fig. 2: Mostly observed signs and symptoms in humans in response of zoonotic infection.

The integument, gastrointestinal system, and mucous membranes are all routes for Burkholderia mallei to infect its host (Wernery et al. 2011). The frequency of infections in solipeds and other animals, such as zoo predators (tigers and lions) and camels, has gradually risen over the past two decades, and glanders can be considered a re-emerging illness. B. mallei is thought to be a potential bioterrorism agent due to the lethal and invasive property of the infection in humans (Wittig et al. 2006). The animals most likely to have acute Glanders are mules and donkeys. It can be fatal within days to weeks (Mota et al. 2010). In contrast to horses and donkeys, mules may be somewhat less vulnerable to Glanders, and the illness can have both an acute and chronic course (Khan et al. 2013). Anorexia, sadness, and weakness are the first symptoms of acute Glanders. Clinical symptoms include coughing and fluid flow from one or both nostrils and rapid growth of nodules and ulcerations in nasal sputum with thick, sticky, yellowish-white muco-purulent to blood discharge as these lesions progress, resulting in dyspnea. The sub-maxillary lymph nodes expand bilaterally and often become indurated. They commonly protrude from the jawbones and may even rupture. Septicemia and respiratory failure (bronchopneumonia) cause death within a few days to weeks (Radostits et al 2007).

12. POSSIBLE OUTCOMES OF GLANDERS IN MAN

Regardless of the low frequency of animal to human transmission, the vulnerability at work is still major risk for farriers (those who care for horses' hooves), students, flayers (those who work with leather), stable hands, transport workers, farmers, soldiers, veterinarians, slaughterhouse staff, and horse riders (Pal et al. 2022).

12.1. GENERAL SYMPTOMS

Numerous Glanders types, like chronic, pulmonary, and septicemia, have been stated. The many infection paths account for a considerable portion of the variability of infections. Localized infections are typically restricted to a specific geographic area. The pustules might bleed and ulcerate for a very long time. Localized infections can spread and cause septicemia, multi-tissue, or lung diseases. Furthermore; the following signs most often: moderate-to-low fever in the evening or noon, malaise, fatigue, headache, and myalgia such as backache, lymphadenopathy, and pain in the chest (Khan et al. 2013). After the first wave of disease signs, roughly fifty percent of the patients not only looked well but also became medically better. Within a few days to two months the patients began showing signs and symptoms. (Khan et al. 2013).

12.2. MUCOSAL INVOLVEMENT

Severe lacrimation and photophobia are signs of a B. mallei infection of conjunctiva and the eye. Following B. mallei inhalation, nasal involvement can be marked by inflammation of the nose. There may be lots of nasal discharge with this. Additionally, infection may enter the bone structures of the nasal septum, leading to fistulas and tissue damage. Local lymphatic nodes may enlarge and swell up in the face. Lower respiratory tract infections may also spread, leading to bronchitis, which may be accompanied by coughing up muco-purulent sputum. In the early days after infection, constitutional symptoms like a fever and chills are widespread. These symptoms may also be severe and continue after treatment. Fever during the middle of the day or night; shivers with or without rigors; and a severe headache are a few examples of common signs and symptoms (Pal et al. 2022).

12.3. CUTANEOUS INVOLVEMENT

Papular lesions, which can develop on the body anywhere and have a more extensive, indolent infection, are examples of cutaneous symptoms. After B. mallei's entrance through an abrasion, an inflammatory reaction with indicators swelling and pain is typical. In these situations, a Glanders Node may initially present as the only blister, evolving over time into an ulcer that can bleed rapidly (Waag and DeShazer 2005), at the point of entrance, a discharge-producing localized infection usually appears. Regional lymphatics may become inflamed and develop multiple foci of suppuration along their path, leading to lymphangitis. By producing more irritation and inflammation in the lymphatics, the endotoxins found in some strains of B. mallei have a damaging effect on the smooth muscle cells of the lymphatics (Liu et al. 2014).

12.4. PULMONARY INVOLVEMENT

Influenza, pulmonary abscess, pleuritis, and extensive fluid are common complications of a lung infection. Cough, breathing difficulties, chest discomfort and mucopurulent sputum are all signs of lung infection. Nonspecific signs and symptoms include fatigue, fever (typically above 102°F), a shiver, pain, myalgia, lymphangitis, and pain in the throat, pleuritic, chest discomfort, cough, breathlessness, nasal discharge, and

gastrointestinal indicators. Many symptoms can take up to two to three weeks to manifest. Non-specific symptoms include rigors, fatigue, vomiting, sweating during the night, severe migraines, dizziness, a rapid heartbeat, losing weight and mucosal eruptions, which may indicate a widespread infection. (Khan et al 2013).

12.5. WARFARE POTENTIAL

Burkholderia mallei is a potential bioterrorism pathogen because of its ability to cause severe illness in humans and animals. The bacteria can be spread by aerosolisation, making it a potential weapon for bioterrorism. In addition, B. mallei is resistant to many antibiotics, making it a challenge to treat once the disease has occurred. The use of B. mallei as a biological weapon has been reported previously. During World War I, the bacteria was used to infect military horses and mules. While no recent activities have been reported, it remains a concern for public health officials and military planners due to its potential to cause widespread illness and death (Pal and Gutama 2022).

12.6. DIAGNOSIS

- Clinical Picture of case/Signs and Symptoms
- Culture and Sensitivity (nasal and throat swabs)
- ELISA
- PCR
- Antigen Detection (Ab is produced against pathogen by immune system)
- NAATs (Nucleic Acid Amplification Tests)

12.7. DIAGNOSIS IN EQUIDS AND OTHER ANIMALS

For a clinical diagnosis of Glanders, nodules, ulceration, scarring, and weakened state may be sufficient. Specific diagnostic tests should be utilized as soon as feasible because these symptoms typically do not appear until the disease is well advanced. The diagnosis is confirmed by B. mallei cultured from lesions.

- Based on cutaneous nodules oozing a honey-like discharge or nasal discharge and ulcers on the nasal mucosa, it is suspected.
- Proven using ELISA, PCR, culture, and the complement fixation test (Saqib et al. 2012)

12.8. MALLEIN TEST

Mallein, a secreted glycoprotein of B. mallei when injected is detected in culture supernatant, into the palm, one can test for delayed hypersensitivity. Within 24-8 hours, purulent conjunctivitis and eyelid edema appear in infected hypersensitive horses (Dvorak et al. 2008). Due to reservations about using animals, neither trade testing nor general recommendations are made for the Mallein test. In addition, malleinized sero-conversion may occur and may subsequently exhibit false positive results in additional diagnostic techniques like CFTs. In endemic rural areas, however, the Mallein test may be helpful (Saqib et al. 2012).

13. COMPLEMENT FIXATION TEST (CFT)

Additionally, complement fixation is employed to check for infection. CFT has been used for monitoring, confirmation of outbreaks, and trade testing for decades; also, a required based recommendation from the World Organization for Animal Health (OIE). Studies have demonstrated that the CFT is quite sensitive, but regrettably, a sizable number of false-positive results are generated by this test, which subsequently cause

threats to cross-border trade. Because there are no accessible international standard standards, CFT also has technological drawbacks, being labor-intensive and challenging to standardize (Saqib et al. 2012).

13.1. ELISA

The term ELISA means "Enzyme-linked Immunosorbent Assay" It is an effective method for determining the concentrations of mg/ml to g/ml ordered materials in solutions, including sperm, serum, culture supernatant, and urine. Competitive ELISA has greater sensitivity than CFT and can detect an infection as early as three days after exposure. It is an antigen-antibody reaction. Alkaline phosphatase, Horse Radish Peroxidase, and beta-Galactosidase are just a few of the enzymes deployed in ELISA tests (Richard 2002). Each enzyme utilizes specific substrates for results. For example, Ortho-phenyl-diamine-dihydro-chloride is used for peroxidase, while Para-nitro-phenyl phosphate is utilized for alkaline phosphatase. These substrates react with the enzymes to produce colored end products. Antibodies or antigens present in serum are detected by antigen or antibodies coated on a solid surface by matching. Based on the arrangement of the binding sites for the antibodies and the antigens, three categories of ELISA may be made: indirect, direct, and sandwich (Saqib et al. 2012).

13.2. PCR

It is possible to identify a specific organism using PCR based on 23S and 16S rRNA genes sequences. Burkholderia mallei was specifically identified in clinical samples and pure culture samples from outbreaks using a polymerase chain reaction (PCR) test that targets the flagellin P (fliP)-I S407A genomic region. While other closely related species failed to amplify the 989-bp fragment from each of the 20 B. mallei strains under investigation, primers derived from the known fliP-IS407A sequence of B. mallei American Type Culture Collection (ATCC) 23344T were successful. Horses with a widespread infection of B. mallei had their tissues amplified for B. mallei DNA as well. The created PCR assay can be utilized as an easy, quick approach for detecting B. mallei in clinical samples that is sensitive and specific (Saqib et al. 2012).

13.3. TREATMENT

Treatment is contraindicated due to the lack of availability of effective treatment and antibiotic resistance but some antibiotics are still effective against B. mallei. Isolates of B. mallei are susceptible to Doxycycline, Amoxicillin-clavulanic acid, Chloramphenicol, Gentamycin and Trimethoprim-sulphadiazine. Enrofloxacin is now not very much effective due to its vast use and developing resistance. Horses can be treated by giving the antibiotic course for 12 weeks in which Enrofloxacin (8mg/kg of body weight) and Trimethoprim-sulphadiazine (32mg/kg B/W) are given I.V. once a day for 1 week and their dose is reduced to half during 2nd and 3rd week. Doxycycline (6mg/kg B/W) orally twice a day from week 4 to 12 (Saqib et al. 2012).

In humans and laboratory animals Sulfonamides provide a satisfactory result. Autogenous vaccine (once) and Trimethoprim-sulphadiazine (20 mg/kg B/W P/O) for 1 month alter the disease course if given to infected horses (Al-Ani and Roberson 2007).

A study in mice showed that Finafloxacin controls B. mallei at the organ level and also controls the signs which are going to develop (Barnes et al. 2022).

Bacteria are sensitive to co-Trimoxazole, Danofloxacin, Norfloxacin, and Chloramphenicol. Treatment for 4 days can be given to animals with Ringer's-lactate-dextrose 500ml, 60-80 ml Dimethyl-sulfoxide I.V. and inj. Norfloxacin 5% 35-50 ml I.M. (Muhammad et al. 1998).

14. PREVENTION AND CONTROL

Glanders and Farcy Act of 1899 states that: affected animals should be killed and disposed of. For proper prevention, identify positive animals and eliminate them. According to WOAH, control measures include surveillance, identification, euthanasia, quarantine, cleaning, disinfection, and proper disposal by incineration.

Vaccination for proper prevention is not available. Live vaccines are available but have no satisfactory results. Affected animals should be separated from healthy animals and checked at intervals of three weeks until all animals are negative for Glanders. If the animal dies, don't perform a necropsy. Proper disinfection should be done. Various disinfectants can be used, such as 70% Ethanol (C2H5OH), Potassium Permanganate (KMnO4), household bleach, Iodine, and Mercuric Chloride in alcohol. UV rays and heat also kill the bacteria (Verma et al. 2013).

15. MELIOIDOSIS (BURKHOLDERIA PSEUDOMALLEI)

Melioidosis is infectious and zoonotic disease caused by gram negative bacteria named as Burkholderia pseudomallei (Norman and Chen 2023), also known as Pseudomonas pseudomallei (Somprasong et al. 2023). Genetic makeup is complex, contributing to its pathogenicity (Fang et al. 2016). Commonly found in water and soil, especially in South East Asia and Northern Australia. Main transmission route is contact with contaminated water and soil. Open wounds contribute to bacterial entry into the body. Transmission from person to person is rare (Mohapatra 2023). The symptoms of the disease vary widely, but it affects the liver, lungs, spleen, skin, and joints (Ignee et al. 2023). Diagnosis is confirmed through laboratory tests like sputum analysis and blood cultures (Noparatvarakorn et al. 2023).

Fig. 3: Melioidosis warfare potential (Samy et al. 2017).

15.1. HISTORY

The bacterium *B. pseudomallei* was discovered in Rangoon Burma, now Myanmar, by A. Whitmore and C. S. Krishan Swami in 1912 from the spleen of a man who died from an unknown illness. The pathogen was named Whitmori, and the disease was called Whitmore's disease at first. Again in 1913, the same pathogen was discovered in Malaysia and was named *Pseudomonas pseudomallei.* As the signs of the disease were almost similar to the Glanders, a condo caused by the bacterium mallei, the name was given as *pseudomallei*. A British medical officer cap. J. Simpson was the first to describe the symptoms officially in 1915 in Malaysia and named the disease Melioidosis after the pathogen's name. During World War II, the disease was highly prevalent among soldiers. This disease has gained quite an attention in recent years after the possible zoonotic and bioterrorism risk (Fig. 3) due to its possibility to cause high mortality, antibiotics resistance remains a sound issue yet (Foong et al. 2014). WHO and other authorities are working on it providing efficient medication and vaccines for the prevention and control.

16. AGENT CHARACTERISTICS OF *B. PSEUDOMALLEI*

It is a gram-negative, aerobic, non-sporulating saprophytic coccobacilli. Polar flagella make *B. pseudomallei* motile and on MacConkey agar it appears as a non-fermenter. *B. pseudomallei* shows a typical bipolar staining. Main reservoirs of this bacterium are contaminated soil and water (Wuthiekanun et al. 1995). Epizootic infections are possible and are caused by various animals that come in contact with the agent and act as reservoir hosts (Cheng and Currie 2005).

For isolation Ashdown agar medium is being used, specifically for non-sterile samples such as samples from throat, rectum or sputum (Ashdown 1979). Pathophysiology of melioidosis is shown in Fig. 4.

Smooth colonies of *B. pseudomallei* are formed within 24-48 hours with a putrid odor, mostly yellow to orange-colored, which after a few days, form wrinkled and dry colonies like *Pseudomonas stutzeri*. Earthy and musty odor is produced during growing phase.

B. cepacia medium and *B. pseudomallei* Selective Agar (BPSA) are newly introduced growth media. Growth of mucoid *B. pseudomallei* colonies on BPSA medium is more than compared to the Ashdown medium (Howard and Inglis 2003).

17. CLINICAL SIGNS AND SYMPTOMS

Due to range of signs and symptoms it is frequently misdiagnosed with other diseases (Fong et al. 2015). From mild to severe disease, patients may exhibit a range of clinical symptoms such as headaches, fever, muscle discomfort, abscesses, labored pneumonia and cough. (Karunarathna et al. 2018). Site of the infection/inoculation may have an impact on the clinical appearance. The time that clinical symptoms first appear, or the incubation period, varies greatly for Melioidosis. It could last anywhere from 1 to 21 days or for many years (Chakravorty and Heath 2019).

18. FORMS OF MELIOIDOSIS

According to (Alwarthan et al. 2018), Melioidosis can manifest clinically in a number of ways (Fig. 5):

18. SUBCLINICAL FORM

Subclinical form is caused by the seroconversion the agent in population living in an endemic area. It is considered that all new cases have a recent history of infection and that there is no sufficient evidence to justify the emergence of clinical cases of seroconversion (Currie 2014).

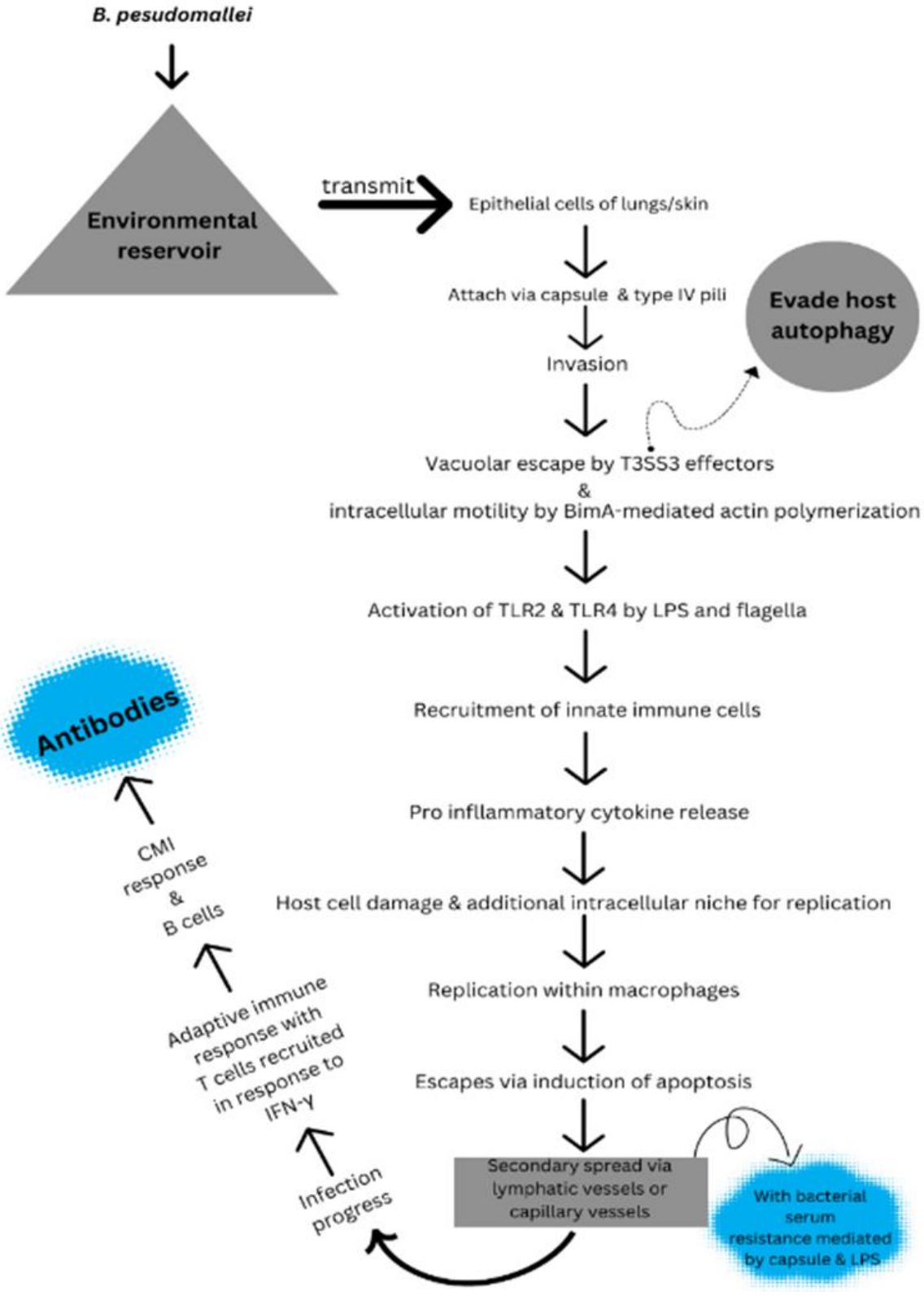

Fig. 4: Pathophysiology of Melioidosis (Adler et al. 2009).

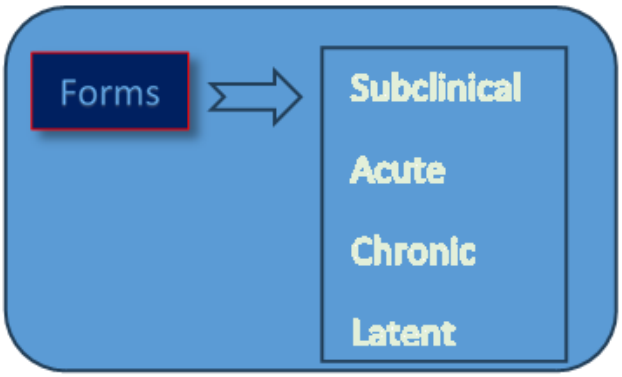

Fig. 5: forms of melioidosis

18.1. ACUTE FORM

A severe case which results in septicemia, shock and death. Exposure through inhalation/aspiration leads to acute form. Lungs are mainly affected (Mahendra et al. 2022). More than half of patients exhibit bacteremia in which 20% suffers septic shock (Chakravorty and Heath 2019). Lungs are more affected in adult cases. Children experience skin infections more frequently. Bacteria enters bloodstream, colonize in organs (liver, spleen, kidney, genitalia and brain) and then cause inflammation and development of visceral abscesses. Central nervous system is less impacted (Currie 2014).

18.2. CHRONIC FORM

Symptoms persist for longer than two months. Chronically infected people make up around 11% of all instances and can resemble the clinical symptoms of cancer, tuberculosis, or fungal infections, such as weight loss, fever and cough that may be bloody (Alwarthan et al. 2018).

18.3. LATENT FORM

Bacteria might occasionally become active or relapse after months or years. It is regarded as being in latent form in that case (Wiersinga et al. 2012).

19. ZOONOTIC ASPECT OF *B. PSEUDOMALLEI*

It is endemic in the north of Australia and Southeast Asia. During the last twenty-five years, it caused significant illness and death in this region (Limmathurotsakul and Peacock 2011). Pneumonia is most common than skin and soft tissue infection, parotitis and prostatitis (Cheng and Currie 2005).
Diabetes mellitus is the risk factor, as evidenced by research in Australia and Thailand, where as much as sixty percent of Melioidosis sufferers are diabetic, primarily type 2 (Cheng and Currie 2005). *B. pseudomallei* have a diverse host range in addition to humans such as cattle, goats, and swine are the most commonly recorded domestic animals (Ouadah et al 2007). The occasional instances or small outbreaks of the disease have been observed in apes, gibbons, orangutan species like cows, zebras, deer, kangaroos, cattle, camels, sheep, wallabies, koalas, pets such as horses, dogs, cats, shoes, parrots, rabbits, rat, guinea pigs, squirrels, dolphins, seals (Sprague and Neubauer 2004). Recently in California *B. pseudomallei* disease in two domestic iguanas was reported (Zehnder et al. 2014). Melioidosis is present in both acute and chronic forms in animals. Anorexia, pyrexia, wheezing, skin dehydration, and lesions are common symptoms in animals (Galyov et al. 2010).

20. POSSIBLE OUTCOMES OF MELIOIDOSIS IN HUMAN

B. pseudomallei can infect humans. This organism is mostly acquired from the environment; however, a few zoonotic examples have been described.

20.1. CLINICAL SIGNS

B. pseudomallei can induce a wide range of clinical symptoms in humans. While many infections appear insignificant, others cause acute lung illness, septicemia, or localized long-term suppurative disorders. The prevalence of various disorders varies by geography (Mariappan et al. 2017). Parotid abscesses, for example, are prevalent in children in Thailand but uncommon in Australia. If the organisms move to other locations, one sickness can evolve into another (Benoit et al. 2015).

20.2. ACUTE LOCALIZED INFECTIONS

Acute localized infections can happen at the point of injection. Localized skin disease was observed to be a prevalent type of Melioidosis among children in Australia. Scars in the skin often manifest as grey or white, hard nodules and ulcers that are frequently but not usually single. Caseating nodules are frequently surrounded by inflammation. Regional lymph nodes and lymphangitis may accompany them. Suppurative parotitis/parotid abscesses, damaging corneal ulcers seen following ocular trauma, and illnesses that simulate necrotizing fasciitis are all examples of acute localized infections. Prostatic abscesses are a common symptom of the genitourinary tract. Although localized infection can spread, systemic illness is not necessarily preceded by localized infections. The skin and subcutaneous tissues can be affected through the hemorrhagic transfer of microorganisms from other sites (Cheng et al. 2015).

20.3. PULMONARY DISEASE

People commonly suffer from lung illness. It can occur as a separate condition or as a part of septicemia, and it can emerge quickly or gradually following a nonspecific prodromal sickness. The severity of pulmonary Melioidosis ranges from moderate acute or chronic pneumonia to respiratory difficulty with severe septic shock. Fever, wheezing, pleuritic chest pain, and, in certain situations, hemoptysis are common symptoms. Individuals with pneumonia as part of septicemia may have a cough or pleuritic pain while being febrile and very unwell. Chronic lung Melioidosis may increase and decrease, and symptoms include a decrease in weight, fevers, sweating during the night, and a productive cough with blood-tinged sputum. Nasal ulcers and nodules are occasionally observed, and the septum can perforate. Pneumothorax, empyema, and pericarditis are all possible consequences. Severe instances can develop into septicemia (Dance 2014).

20.4. SEPTICEMIA

The most severe form of Melioidosis is septicemia. It is common in those who already have conditions like diabetes, cancer, or kidney failure. The onset is frequently abrupt, with fever, rigors, and other characteristic sepsis symptoms. However, it may appear gradually, with a variable fever and substantial weight loss. Fever, severe headache, anxiety, pharyngitis, upper abdomen discomfort, stools, jaundice, and considerable muscle tenderness are common signs of septicemic Melioidosis. Pulmonary symptoms, such as dyspnea, are prevalent, and arthritis or hepatitis may be present. A diffused pustular redness with

regional lymphadenopathy, cellulitis, or lymphangitis is seen in many cases. Septic shock is a common and potentially fatal condition (Limmathurotsakul and Peacock 2011).

20.5. CHRONIC CASES

Chronic cases can result in infections and suppurative lesions in multiple organs. While the spleen, liver, skeletal muscle, and prostate gland are frequently affected, lesions can also be seen in the skin, lung, kidney, heart, bone, joints, lymph nodes, and testes. There are also mycotic aneurysms. Melioidosis can cause brain infections, encephalomyelitis (with multiple symptoms, including flaccid paralysis), or meningitis in rare cases. In situations of encephalitis, there may be significant residual abnormalities (Loveleena and Dhawan 2004).

20.6. DIAGNOSIS

1. Clinical signs of Melioidosis make a clinical diagnosis particularly challenging.
2. Travel history to an endemic region.
3. Cultural analysis, biochemical tests and gram staining.

21. ASHDOWN SELECTIVE AGAR (ASA)

ASA is selective medium in endemic areas, which exhibits huge, round, wrinkled purplish colonies. However, it has recently been found that some bacterial strains are unable to grow on this medium. *Burkholderia pseudomallei* Selective Agar (BPSA) was created for better isolation (enrich gentamicin susceptible strains). Smooth colonies are formed on chocolate agar, horse blood agar and MacConkey agar. The microbe should be cultured for at least 4 days with daily examination because it grows slowly. While there are various commercial kits available for bio typing (Walk-Away, VITEK-2, and 1156576 and 1156577). Their sensitivity and specificity remain debatable. It may be mistaken for contaminant or *Pseudomonas* (Pal et al. 2022).

22. ANTIBIOTIC DISC DIFFUSION TEST

It may be employed as a presumptive test. It is based on the observation that the majority of isolates are susceptible to Colistin but not to Co-amoxiclav (Pal et al. 2022).

22.1. SERUM TESTS

The sensitivity and specificity of serological diagnostic methods such immunofluorescence, complement fixation tests and indirect haem agglutination have been reported to be low. Due to cross-reactions and high background antibody in endemic places and antibody-based tests are prone to producing false positive results. As a result, only the bacterial culture approach may be used for confirmatory assays (Pal et al. 2022).

22.2. LATERAL FLOW IMMUNOASSAY (LFI)

Another diagnostic technique that makes use of monoclonal antibodies (mAb 3C5) is the LFI, created by In BiOS (Active Melioidosis Detect), to identify the capsular polysaccharide. A promising technique for detecting *B. pseudomallei* may be AMD LFI. It is equipment-free diagnostic test, user-friendly, inexpensive, quick and reliable but it has low sensitivity for samples that are not blood.

Many molecular methods for identifying species have been developed, with various advantages and disadvantages. These methods include particular PCRs, rtPCR and 16S rDNA sequencing. Quick and affordable detection method is matrix-assisted laser desorption/ionization of time-of-flight mass spectrometry (MALDI-TOF MS). Additionally, imaging tests like CT, chest radiography or ultrasound are frequently very helpful for figuring out how severe the illness is and for looking for subclinical abscesses (Pal et al. 2022).

22.3. TREATMENT

Prolonged antibiotic treatment is required (Laws et al. 2019). Antimicrobials should be selected by culture and sensitivity tests. *B. pseudomallei* is resistant to Macrolides, Gentamicin, Ampicillin, Polymyxin, 1st and 2nd generation Cephalosporin, Tobramycin, and Penicillin (Currie 2015).
Two phase treatment is given, the acute phase and the eradication phase. In the acute phase, drugs including Ceftazidime (50mg/kg TID), Carbapenems, Amoxicillin-Clavulanic acid, and Trimethoprim-Sulphadiazine are given by parenteral route for 10-14 days, then orally Trimethoprim-Sulphadiazine or amoxicillin-clavulanic acid to prevent relapse for 12 weeks (Dance 2014).
The minimum inhibitory concentration of Imipenem and Meropenem is low. They are more bactericidal than Ceftazidime. Drug of choice for acute phase is Ceftazidime while Trimethoprim-Sulphadiazine for the eradication phase (Wiersinga et al. 2018). Abscesses may be surgically drained.

23. PREVENTION AND CONTROL

To control Melioidosis effectively, its risk factors should be controlled, such as diabetes, alcohol, smoking, immunosuppression, environmental exposure, and chronic diseases of the kidney, heart, liver, and lungs (Mohapatra and Mishra 2022).
After handling aquariums, snails and fish wash hands with soap. Don't allow less than 5y children to clean the aquarium (Dawson et al. 2021).
According to CDC:

1. Avoid contact with standing water and soil
2. Wear boots specially for agriculture workers
3. Healthcare workers use standard precautions

No vaccine is available, so all prevention and control rely only on preventive measures (Norman and Chen 2023). Drink only treated or boiled water. If an animal outbreak occurs, euthanize infected animals and disinfect the environment. Avoid rain and dust (Pal et al. 2022).

24. CONCLUSION

Glanders and Melioidosis are two main diseases caused by the genus Burkhulderia. The diseases are of primary importance in terms of performance, economics, trade, work force and, above all, zoonosis. Signs and symptoms of both diseases are quite similar and often it becomes important to diagnose the one differently for better treatment and management of infected equines. Due to the zoonotic aspect, both have major health concerns for workers like farriers, grooms, game players, volunteers and veterinarians working closely with them. Moreover, in developing countries, a large number of the population is directly or indirectly involved with horses, mules, and donkeys to meet their financial needs. It is important to devise a regulatory framework to screen and separate the affected ones from healthy ones to minimise the risks. There is also need to develop screening tests which are more specific and easier to perform,

while cost effectiveness remains primary concern. Treatment due to AMR resistance and quarantine of effected equines remains major concern too.

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