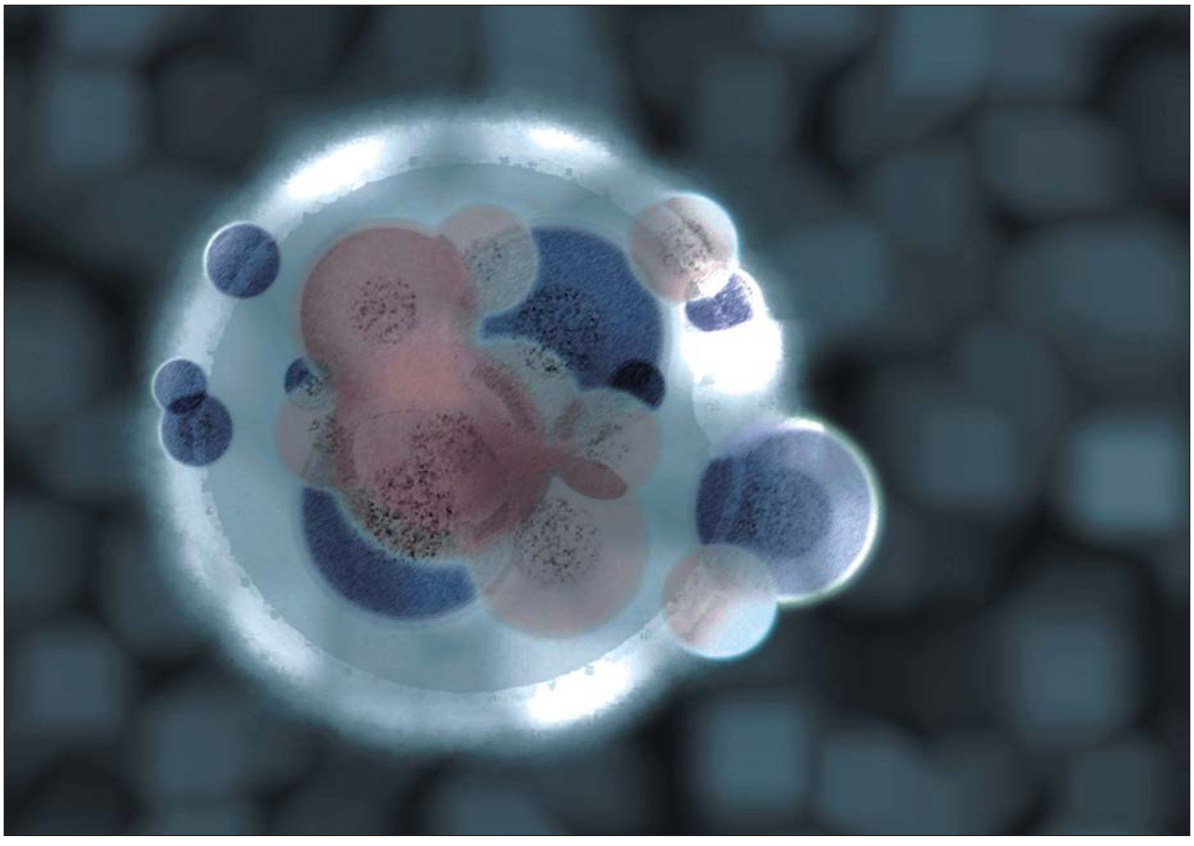

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Glanders-A Re-emerging Zoonotic Disease: A Review

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Abstract: Glanders is a contagious and highly fatal zoonotic disease affecting horses, donkeys and mules as well as man leading to formation of nodules and ulcerations in the upper respiratory tract and lungs. This is a notifiable disease under Glanders and Farcy Act, 1899. The disease is caused by *Burkholderia mallei*, a gram negative bacteria, non-spore forming, non-motile rod bacterium and is a facultative intracellular pathogen. The disease has been eradicated from many countries by testing and destruction diseased horses and restriction of import of animals. However, the disease is endemic in Africa, Asia, Mongolia, Middle East, Central and South America. In India, major glanders outbreaks were reported between 1976 to 1982 from different parts of the country. Later, sporadic cases were reported in 1988, 1990 and 1998. India was remained free of glanders for 8 years until recent re-emerging outbreaks started from 2006 to 2011. The occurrence of the disease leads to international trade restrictions. Glanders is primarily a disease of equines which causes chronic disease in horses and acute disease in donkeys and mules. Human is accidental host and the disease usually results from occupational exposure. Though the organism is susceptible to various antibiotics *in vitro* treatment is difficult and needs longer course with combination of antibiotics upon early diagnosis. It can be used as a biological weapon and has been classified by the Centers for Disease Control and Prevention (CDC) as a category B bio-threat agent and at present no vaccine is available for this bacterium either in humans or animals. This review describes this important disease covering its etiology, epidemiology, transmission, clinical signs, post-mortem lesions, public health significance, diagnosis, treatment and prevention and control strategies to be adapted to combat this deadly zoonotic pathogen.

Key words: Glanders, *Burkholderia mallei*, horses, epidemiology, clinical signs, diagnosis, treatment, public health, vaccine, bioterrorism

INTRODUCTION

Zoonotic diseases are diseases that are transmitted between man and animal under natural conditions. These diseases are in focus due to difficulties faced in prevention of their transmission and spread. Due to factors like increasing population, change in climate and global warming, various diseases are emerging and re-emerging including vector borne and zoonoses viz., tuberculosis, anthrax, brucellosis, salmonellosis, campylobacteriosis, rabies, dengue, Japanese encephalitis, chikungunya, bird flu, swine flu, West Nile virus, Hendra virus infections etc. (Taylor *et al*., 2001; Rogers and Randolph, 2006; Verma *et al*., 2007, 2008; Jones *et al*., 2008; Bhatia and Narain, 2010; Kumar *et al*., 2009, 2012; Myers and Patz, 2009; Mahima *et al*., 2012a; Dhama *et al*., 2010a, b, 2011, 2012a, 2013a, b, c). These are adversely affecting animal and human health and posing serious socio-economic threats and huge sufferings (Bhatia and Narain, 2010; Cascio *et al*., 2011).

Glanders is a highly contagious and fatal disease of horses, donkeys and mules (solipeds) caused by *Burkholderia mallei* (formerly called as *Pseudomonas mallei* or *Actinobacillus mallei*), characterized by nodules and ulcerations in the upper respiratory tract and lungs (Whitlock *et al*., 2007; OIE, 2008; Larsen and Johnson, 2009; Malik *et al*., 2010; Burtnick *et al*., 2012; Saqib *et al*., 2012; Khan *et al*., 2013). Its skin affection or subcutaneous form is known as

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'farcy' (Lehavi *et al*., 2002). The disease is of high public health significance. Glanders has been eradicated from the developed countries. Due to its ability to infect via inhalation route, *B. mallei* can be used as a biological weapon and thus has biodefense concern so it should be considered more important from practical point of view (Wheelis, 1998; Lehavi *et al*., 2002; Horn, 2003; Bojic *et al*., 2007; Whitlock *et al*., 2007; Gilad, 2007; Bondi and Goldberg, 2008; Estes *et al*., 2010; Ricketti *et al*., 2011; Anderson and Bokor, 2012; Burtnick *et al*., 2012). During World War I (20th century) *B. mallei* has been implicated for use as a biological warfare agent.

HISTORY

The disease was first identified by Hippocrates in the 4th century BC (Colahan *et al*., 1999). Later Aristotle named the disease as *malleus* which is a Latin word means depicting a malignant disease. In 16th century, William Shakespeare written about glanders in his comedy book called 'The Taming of the Shrew' and in 17th century Alexandre Dumas also mentioned about glanders in his novel, 'The Three Musketeers' (Wilkinson, 1981). Throughout history glanders has been known by various names including equinia, malleus, droes, morve, pacin, carn and farcy. In 1664, Sollysel in France first recognized the contagious nature of the disease (Howe, 1949; Derbyshire, 2002). Its zoonotic potential was not reported until the beginning of the 19th century. In 1882, Loeffler and Schutz in Germany first time isolated the causative agent of the glanders, *Burkholderia mallei* (Colahan *et al*., 1999). In 1890 on the pattern of Koch's tuberculin, Helman from Estonia, Kalning from Latvia and Pearson from USA prepared mallien from cultures of *B. mallei* which was used for specific diagnostic test (Verma, 1975; Wilkinson, 1981).

Etiology: *Burkholderia mallei* is a gram negative bacteria, straight or slightly bent (2-5 μ long and 0.3-0.8 μ wide), non-spore forming and facultative intracellular, rod shaped bacterium (Gilad, 2007; Galyov *et al*., 2010; Estes *et al*., 2010; Malik *et al*., 2010). The bacterium is an obligate aerobic (except in media containing nitrate) (DeShazer, 2004). The bacteria grow aerobically and prefer media that contain glycerol as enrichment agent (Evans, 1966). On Glycerol Dextrose Agar (GDA), there was a confluent, slightly cream-coloured growth that was smooth, moist and viscid after 24 h of incubation. With continued incubation, the growth thickened and became darker and tough (Malik *et al*., 2009). The capsule-like coat has been demonstrated by electron microscopy. The capsule is consisting of neutral carbohydrates and it protects the cell from unfavourable environmental factors. The organisms are closely relative to *Burkholderia pseudomallei* but *B. mallei* have no flagellae and are nonmotile (Krieg and Holt, 1984; Sprague and Neubauer, 2004). The organisms are difficult to demonstrate in tissue sections and it has beaded appearance (Miller *et al*., 1948). In culture media, they vary in appearance depending on the age of the culture and type of medium. The organisms showed much pleomorphism in older cultures. Branching filaments are seen on the surface of broth cultures (Neubauer *et al*., 2005). *B. mallei* is sensitive to the external environment and destroyed by exposure to direct sunlight within 24 h and is killed by most of the common disinfectants such as phenol, potassium permanganate, copper sulphate, formalin and chlorine (Howe, 1949; Van der Lugt and Bishop, 2004). The organism could remain viable for 3 to 5 weeks in damp media and decomposing material, may survive for up to 4 weeks in clean water and for about six weeks in contaminated stables (Silva and Dow, 2013).

Susceptible host: Susceptible host species are horses, mules and donkeys, but carnivores like lion may be infected by eating meat. Sheep and goat may also get infection. The natural reservoirs of *B. mallei* are the Solipeds. Usually the disease is chronic in horses, while it occurs in acute form and often fatal in donkeys and mules often fatal (Wittig *et al*., 2006; Van Zandt *et al*., 2013). Laboratory animals are also susceptible to glanders including, hamsters, mice and guinea pigs. This susceptibility makes the basis of the Strauss reaction in the diagnosis of glanders (Fritz *et al*., 1999, 2000; Lever *et al*., 2003). Veterinarians, farriers and animal workers are susceptible to this important occupational disease (Howe and Miller, 1947; Georgiades and Fishman, 2001; Srinivasan *et al*., 2001). Pigs, cattle, sheep, rats and fowl are resistant to infection with *B. mallei*, but goats, camels, bears, wolfs and dogs can be infected. Both acute and chronic forms as well as latent infections are seen in mules (Minett, 1959; OIE, 2004; Pitt and Dance, 2005).

Mode of transmission in animals: The infection is acquired directly or indirectly from secretions and excretions of infected animals. The disease is chronic in horses and the organisms are found in the lesions and discharges of the skin and nasal mucosa (OIE, 2004, 2008). Acute form of the disease occurs in the mules and donkeys and the organisms are excreted in faeces, urine, saliva and tears (Hunting, 1913; Gulati and Gautam, 1962). Most common route of transmission is respiratory route and ingestion of feed and water contaminated by nasal

discharge or sputum of affected animals or direct contact with fomites. Infected animals or recovered animals are the important sources of infection. Severe and rapidly fatal pneumonia occurs after inhalational of *B. mallei.* Oral and cutaneous route of infections can also produce the disease. Dogs, cats, wild and zoo carnivores acquire the infection from ingestion of infected horse meat (Biberstein and Holzworth, 1987; Wittig *et al*., 2006; Pawaiya and Chauhan, 2008; Malik *et al*., 2010).

Epidemiology: The disease is common in Asia, Africa, South America, Eastern Europe and Middle East (Arun *et al*., 1999). In earlier times, it was more widespread worldwide, but now has been eradicated from most of the areas like Western Europe, Australia and Northern America (Wittig *et al*., 2006; Slater, 2013; Van Zandt *et al*., 2013). Glanders was successfully eradicated from Great Britain in 1928 (Stalheim, 1994). In 1934, glanders was officially eradicated in domestic animals in the United States of America (Gregory and Waag, 2007). Earlier it was an important disease, but now has become sporadic but due to recent outbreaks, it has regained the status as re-emerging disease (Khan *et al*., 2013). From 1998 to 2007, glanders were reported from Brazil, Eritrea, Ethiopia, former U.S.S.R., Iran, Iraq, Mongolia, Turkey and United Arab Emirates (OIE, 2008). In April 2010, Bahrain notified the first occurrence of the disease; in Brazil, the disease reappeared in 2009 (Van Zandt *et al*., 2013). The incidences of glanders have been reduced over past 100 years due to lesser dependence on horses, mules and donkeys for transportation purposes. Also, strict implementation of testing all these animals for glanders and destroying the positive ones has further reduced the occurrences of glanders.

Indian scenario: During 1808, East India Company appointed Dr. William Moorcroft, a veterinarian, as superintendent of their Bengal Study and he diagnosed glanders, strangles, bursati and anthrax in the horses (Gulati and Gautam, 1962). Major R. D. Verma of Remount Veterinary Corps of Indian Army has reported glanders during the period 1881-84 in the mail cart horses working on Bareilly Cantonment (Verma, 1981). More outbreaks of glanders in the Royal Artillery horses in Nuseerabad and Bombay led to the enactment of Glanders and Farcy Act, 1899 which is still in force in the country (Singh, 1964). The disease was reintroduced in horses that were imported during Indo China War in 1962 without subjecting them to proper testing that resulted in heavy mortality and morbidity among equines (Gulati and Gautam, 1962; Singh, 1964). Ray (1984) reported outbreak in army horses in Gauhati during 1979. The last three documented outbreaks are Saharanpur from Uttar Pradesh, Hissar and Karnal from Haryana during March, July and September months of 1984, respectively in equine populations (Misra *et al*., 1985). Kumar *et al*. (1999) reported the last confirmed case of glanders in a mule from Rohtak, Haryana before the current outbreak. After a long gap, the disease was re-emerged in July, 2006 at Pune and Panchgani area of Maharashtra state, where 23 animals were found positive and have killed about 120 equines. In 2007, disease outbreak occurred at Gautam Buddha Nagar (Anantpur) and Meerut (Mawana) districts of Uttar Pradesh (70 cases) and Kathgodam area in Nainital district of Uttarakhand (21 cases) (Table 1) (Malik *et al*., 2009, 2010, 2012).

Table 1: Re-emergence of glanders in India after 1985 outbreak, from 2006 to 2011*

Place	Month	Year	Total No. of serum samples screened in animals	No. of positive cases
Maharashtra	July-Aug	2006	720	23
Uttar Pradesh	Dec-March	2006-2007	1212	70
Punjab	Feb	2007	3228	3
Uttarakhand	March	2007	437	21
Andra Pradesh	September	2007	1821	16
Himachal Pradesh	Oct	2007	252	6
Haryana	Nov	2007	551	1
Chattisgarh	Oct-Jan	2009-2010	286	13
Himachal Pradesh	May	2010	314	4
Uttar Pradesh	Dec-Jan	2010-2011	121	7

*The serum samples were tested by using complement fixation test (CFT) as per OIE protocol. No human case was reported with any symptom simulating glanders. Table compiled according to data of Malik *et al.* (2009), Malik *et al.* (2010) and Malik *et al.* (2012)

GLANDERS AND FARCY ACT

In 1899 March 20, Governor General of India passed Glanders and Farcy Act, 1899 (Act 13 of 1899) for testing and destruction diseased horses with glanders and the outbreak is notifiable by the veterinary authorities. It was the first act on animal diseases to be propagated in India. It has been now substituted by the Prevention and Control of Infectious and Contagious Diseases in Animals Act, 2009 which was implemented in the country to prevent, control and eradicate the infectious and contagious diseases affecting animals and to meet the international obligations of India for facilitating the importation and exportation of animals animal products as well as to save the human life by introducing quarantine and the elimination of infected animals (Malik *et al*., 2012). Major problem in the enactment of the law, it provides about Rs. 50 per horse (equivalent to less than 1 US Dollar) for pittance as compensation to the owner for destroying suspected animals (Pawaiya and Chauhan, 2008). This discourages the owners even to disclose any sign of the disease in their animals. As a result, owners in

affected areas are not willing to get their animals tested for glanders which ultimately leads to existence of several silent disease carriers which is the major obstacle in eradicating the disease (Pawaiya and Chauhan, 2008). Canada was first country to successfully eradicated glanders as early as in 1938 and in those days they paid about 77 US Dollar per animal compensation for destroying the suspected equine populations (Derbyshire, 2002). The Act was applicable to whole India, but now Pakistan, Bangladesh and Myanmar are following the same rule.

Clinical signs: Symptoms of glanders are dependent upon the route of infection- characterized by pneumonia, septicaemia and chronic suppurative skin infections. The disease occurs in two forms:

- **Acute form:** Involvement of all three sites (pulmonary, cutaneous and nasal) is common in the acute form of glanders in donkeys (Jubb *et al*., 1993). It is characterized by high fever, cough, nasal discharge, ulcers on nasal mucosa and nodules on the skin of the lower limbs or abdomen. Death occurs due to septicaemia and anoxic anoxia (Al-Ani *et al*., 1998)
- **Chronic form:** It is characterized with three major manifestations such as pulmonary, skin (cutaneous) and nasal and is exacerbations of the chronic disease in horses. In pulmonary form, there is chronic pneumonia, coughing, frequent epistaxis and laboured breathing. In the nasal form, lesions appear on lower parts of the turbinates and cartilaginous nasal septum. They commence as a nodules (1 cm in diameter) and ulcers and heal with the formation of characteristic star-shaped scars. In early stages, serous discharge is usually unilateral. The nasal secretion is copious, purulent and greenish-yellow; frequently flecked with blood and fragments of desquamated epithelium. Enlargement of submaxillary lymph node is common. In skin form, there is subcutaneous nodules (1-2 cm in diameter) which ulcerate and discharge pus like dark honey. Thickened fistulous lymphatics radiate from the lesions and connect one to the other. Cutaneous lesions are more common in medial aspect of the hock, but can occur anywhere in the body. Lymphadenopathy and cording of lymphatics is common (Jones *et al*., 1997; Al-Ani *et al*., 1998; Colahan *et al*., 1999; Vegad and Katiyar, 2001; Malik *et al*., 2010, 2012)

POST-MORTEM LESIONS

In acute cases, multiple petechial haemorrhages throughout the body along with catarrhal bronchopneumonia and enlarged bronchial lymph nodes are seen. In lungs, numerous gray, hard, small (2-10 mm) miliary nodules and diffuse pneumonia are found in one or more pulmonary lobes due to hematogenous dissemination. In chronic case, milliary nodules on lung, ulcers on upper respiratory tract, skin of lower limb and abdomen. Pyogranulomatous nodules develop in the nasal which subsequently ulcerate, releasing large amounts of *B. mallei* organisms into the nasal exudates. Characteristic small multiple nodules are found in the mucosa and surrounded by a narrow hyperaemic halo. The ulcerative lesions in mucosa are healed and replaced by typical stellate or star-shaped fibrous scars. In mild cases, few discrete foci are present in the posterior portions of the nasal cavity and the anterior portions may show only hyperaemia and catarrh. In experimental infections lesions occur in alimentary tract when large amount of organisms are given orally but in natural infections, lesions are rare in alimentary tract (Arun *et al*., 1999; Fritz *et al*., 1999; Malik *et al*., 2010, 2012).

Microscopic lesions: Each nodule is formed by severe cellular infiltration with an inner core of neutrophils and periphery of macrophages. The nodules consist of distinct or semi confluent suppurative cores separated by granulation tissue. Each nodule contains sloughed necrotic tissue results in crateriform ulcer with sharp margin and smooth base (Duval and White, 1907; Arun *et al*., 1999). In severe cases, ulcers may perforate the septum. In acute cases, ulcerative rhinitis, acute inflammation of nasal mucosa with marked thrombosis of large vessels, infiltration of mucosa and submucosa with neutrophils and macrophages. Lymphadenitis of submaxillary and retropharyngeal nodes is commonly seen (Duval and White, 1907; Jubb *et al*., 1993; Jones *et al*., 1997). Ulcerative and rarely pyogranulomatous nodules are seen in the tracheal mucosa. In lungs, pyogranulomatous lesions have necrotic centres containing dead or dying neutrophils; haemorrhagic and fibrinous exudates are seen in acute cases. As the lesions progress, necrotic centres become surrounded by epithelioid cells, giant cells and lymphocytes followed by fibroplastic encapsulation. The core may be gritty because of dystrophic calcification. Metastases to spleen through hematogenous route are

common, but less common in other viscera or locomotor organs and these lesions are similar to the pulmonary nodules. In glanders, cutaneous lesions are called equine farcy occurs as a result of severe suppurative lymphangitis characterized by cord-like thickening of the subcutaneous lymphatics referred to as 'farcy pipes' and enlarged lymph nodes in the region of legs, ventral abdomen, face and neck. Eventually, affected lymphatics rupture and release large amounts of tenacious, purulent exudate through sinuses to the surface of the skin. In male animals in addition to other lesions orchitis is also common (Mohammad *et al*., 1989; Kumar *et al*., 1999; Malik *et al*., 2010, 2012).

Pathogenesis: Mucous membranes of eye and nose, gastrointestinal tract and the integument are the natural routes of entry of *B. mallei* (Howe, 1949; Fritz *et al*., 1999; Pitt and Dance, 2005). Bacteria penetrate the mucosa from the oropharynx or intestine and crosses via lymph vessels to regional lymph nodes and then to the blood stream and internal organs, particularly the lungs. From there it spreads through blood to cause nasal, cutaneous and nodal lesions (Howe, 1949; Jubb *et al*., 1993). Through cutaneous entry, the organism moves to the lymphatic tracts resulting in Lymphangitis (Kovalev, 1971). Terminal signs are mainly bronchopneumonia and death is due to anoxic anoxia. In human, the lesions occur in spleen, liver, lymph nodes, skin, skeletal muscles, bones, joints and less commonly in brain, meninges, nose and eye (Howe, 1949; Georgiades and Fishman, 2001). Previously, it was thought that glanders does not affect bone in animals and humans (Duval and White, 1907; Gaiger, 1913) but in later studies bone lesions have been described in mules (Gulati and Gautam, 1962), human (Steele, 1979) and experimental hamsters (Fritz *et al*., 1999). The main protective mechanism of *B. mallei* includes intracellular localization and the presence of capsule and capsular lipopolysaccharide to escape phagocytosis (Fritz *et al*., 2000; DeShazer *et al*., 2001). Major virulence factor produced by the glanders bacilli *in vivo* during infections are functional type III secretion system (TTSS) and over-expression of type VI secretion (T6S) protein (Moore *et al*., 2004). Recent *in vitro* studies showed that *B. mallei* organisms significantly influenced the activity of murine macrophages with inducible nitric oxide synthase (iNOS) activity which is critical for the clearance of bacteria from activated macrophages (Brett *et al*., 2007; Schell *et al*., 2007). Romero *et al*. (2006) reported that *B. mallei* have an exceptionally high level of genomic alterations upon its short term passage through several mammalian hosts including human. It is the first and only bacterial pathogen to have such ability as till now only RNA viruses were known to possess capacity for consequential rapid genomic variation as a major component of their strategy for escaping the host immune response. This high and rapid genomic variation may upregulate the virulence gene expression in *B. mallei* during *in vivo* infection. They also detected the mechanism by which *B. mallei* escape the immune recognition and phagocytic clearance *in vivo* by a mutant gene encoding penicillin-binding protein (PBP-1c) that is involved in cell wall synthesis and β-lactum resistance. *B. mallei* are maintained as a population of variant or mutant organisms inside the host but not as a clonal population like other organisms. Such genomic instability upon passage could have implications for vaccine development and treatment of glanders (Romero *et al*., 2006).

Zoonotic potential of glanders and its public health significance: Glander is a zoonotic disease (Dvorak and Spickler, 2008; Malik *et al*., 2010; Varga *et al*., 2012). Even though infection rates of 30% in horses in China during World War II and 5-25% in Mongolia, few or no human cases occurred (Romero *et al*., 2006). In 1793, a first human case of glanders was reported in the French veterinarian Dr. Charles Vial de Sainbel, the first Principal of London Veterinary College and subsequently he died (Hunting, 1913; Wilkinson, 1981). Recently, clinical glanders appeared in a 33 year old microbiologist at the U.S. Army Medical Research Institute (USAMRIID) for Infectious Diseases in March 2000 and treated successfully (Srinivasan *et al*., 2001). In India, information on human glanders is scanty despite many reported cases of disease in equines. The only authentic case was of Gaiger (1913), a veterinary pathologist at Punjab Veterinary College, Lahore who contracted the disease while autopsying an infected horse and himself underwent 45 operations including amputation of an arm before he died. This bacterium has been listed as potential agent for biological warfare and bioterrorism under CDC category B (Wittig *et al*., 2006). Man is also susceptible to infection which is usually fatal with a very high mortality rate of 90-95% in untreated septicaemic infections and 50% mortality rate in treated humans. Transmission of *B. mallei* from animals to humans is generally not seen commonly. Person to person transmission is also a rare event. Human epidemics have not occurred but isolated outbreaks have been documented.

Occupational exposure is the main risk factor to veterinarians, farmers, horse traders/fanciers, laboratory workers and other personnels working in stable, slaughterhouses and soldiers. Close contact with high concentrations of virulent bacterium poses a higher risk

for infection. Ingestion of *B. mallei* contaminated food and water is not an important route of human infections (Kovalev, 1971; Van Zandt *et al*., 2013). Routes of transmission *B. mallei* in humans include direct invasion of cut, abraded or lacerated skin, inhalation and by attack to mucous membranes (nasal, oral and conjunctiva) (Dvorak and Spickler, 2008; Van Zandt *et al*., 2013). The organism is unable to penetrate normal/intact skin. Zoonotic infections arise from contact with infected animals or with *B. mallei* cultures in laboratory (CDC, 2000; Varga *et al*., 2012; Van Zandt *et al*., 2013). In two cases sexually transmission of glanders also reported. Humans can develop four forms of clinical disease, i.e., acute, chronic, pulmonary, septicemic and disseminated forms of the disease are observed. Clinical signs of Glanders in humans are non-specific which is the main factor hindering accurate diagnosis and treatment. Incubation period of 1-14 days is observed in acute form while in chronic form it is up to 12 weeks (Whitlock *et al*., 2007). A localized infection is seen with swelling of the affected area and a weeping discharge (ulcerating and draining abscesses) in the initial phase, followed by an acute pulmonary infection/pneumonic disease. Septicemia is seen in glanders. The infection is fatal at 2-4 weeks in untreated cases.

This pathogen cause major destructive effect on human health because of its ability to cause opportunistic infections in diabetic and perhaps otherwise immunocompromised persons (Estes *et al*., 2010). Symptoms in humans include low-grade fever and chills, malaise, fatigue, myalgias, backache, headache, rigors, chest pain and lymphadenopathy. Others may be excessive lacrimation, photophobia, inflammation/swelling of the nose, copious nasal discharge, facial swelling and pneumonic signs of bronchitis with cough and mucopurulent sputum, dyspnea. Papular lesions erupting on the body are seen in cutaneous forms. Glanders node is seen as a single blister developing into an ulcer, discharge and lymphangitis. In pulmonary infection, pneumonia, pulmonary abscess, pleuritis and plural effusions are observed. Dissemination of the infection gives rise to septicemia, internal organs (spleen, liver and lungs) gets colonized with the bacteria and abscesses develop with septic shock and high mortality. The septicaemic form of glanders has a high mortality rate in humans; the case fatality rate is 95% in untreated cases and in treated cases it is more than 50%. When treated with antibiotics, mortality rate for localized disease is 20%. The overall mortality rate is 40%. However, survival is possible if the infected person is treated early and aggressively with multiple systemic antibiotic therapies (Srinivasan *et al*., 2001; Bossi *et al*., 2004; Rega, 2007).

Biological warfare and bioterrorism of glanders: *Burkhoideria mallei* are a group B biothreat agent and are a host-adapted pathogen (Chandler and Landrigan, 2004). Due to the high mortality rate in humans and the small number of organisms required to establish infection, it is regarded as a potential biological warfare or bioterrorism agent along with closely related *B. pseudomallei*, the causative agent of melioidosis. During the 1st World War, large numbers of Russian horses and mules on the Eastern Front was intentionally infected with glanders by Germans (Wheelis, 1998; Alibek and Handelman, 1999). This severely affected the troop and supply convoys, artillery movement which are dependent on horses and mules. Subsequently, the human cases in Russia increased with the infections during and after World War I (Bossi *et al*., 2004). During World War II Japan intentionally infected horses, civilians and prisoners of war with glanders at the Pinfang Institute, China (Rega, 2007). In China, during World War II, 30% of the tested horses were infected with glanders, but human cases were rare. The U.S. studied this agent as a possible biological warfare weapon in 1943-44, but they did not weaponize it. The Soviet Union is also interested in glanders as a potential biological warfare agent after World War II. If this organism is aerosolized during a biological attack or in a laboratory accident, the morbidity rate could reach very high. Its use as a biological weapon is now banned under the international Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological and Toxin Weapons and on Their Destruction (Rosebury and Kabat, 1947; Woods, 2005; Wittig *et al*., 2006; Pawaiya and Chauhan, 2008).

Diagnosis: Isolation of *B. mallei* and its authenticated identification employing diagnostic tests are required for definitive diagnosis of glanders. Diagnosis is based on the following methods.

Laboratory diagnosis

Samples to be taken: Clinical samples to be collected include pus from open ulcers, lungs, choanal and organ abscesses, nasal mucosa or necropsy material. Nasal swabs, lymph node biopsy, serum sample 2-5 mL aseptically are collected in sterile vial and send it on ice with cold chain for laboratory testing. Samples are often contaminated with other bacterial species like *Pseudomonas* and *Pasteurella* which makes isolation very difficult. Subcutaneous abscesses contain good numbers of the pathogen whereas ulcers are usually free of *B. mallei*. Glanders is a zoonotic disease; all samples must be handled with great care in a laboratory that meets the requirements for containment group-3 pathogens.

Serious concerns exist over the conditions and justification for carrying out necropsy of suspected cases (Malik *et al*., 2009, 2012).

Demonstration of organism in smear: Organisms are in enough number in smear prepared from pus. It should be stained by methylene blue or Gram stain. Bacteria are Gram-negative rods with rounded ends.

Isolation and identification of agent: Bacteria are aerobic and facultative anaerobic. It requires 72 h incubation on glycerol agar.

Laboratory animal inoculation: Guinea-pigs, hamsters and cats are the suitable experimental models. Suspected sample should be inoculated intraperitoneally in male guinea pig. In positive cases, there is development of severe orchitis and inflammation of scrotal sac (Strauss reaction). This reaction is not confirmatory to glanders, because some other organism may also cause this reaction, so the material from infected testes should be processed for bacteriological identification.

MALLEIN TEST

It is a prescribed test for international trade. It is used to detect chronic infections, carriers or occult cases. The major disadvantage, it is not 100% specific and a fair margin of error is always associated with the test (Verma, 1975). Mallein is Purified Protein Derivative (PPD) that is prepared from protein fractions of *B. mallei* after heat treatment. Recently, ultrafiltration in a Tangential Flow Filtration system (TFF) was used as new method of production of mullein (De Carvalho Filho *et al*., 2012). Mallein test is an allergic test which is most frequently used, along with complement fixation test (De Carvalho Filho *et al*., 2012), for diagnosing glanders in endemic areas, wherein a delayed type hypersensitivity reaction as observed during tuberculosis/tuberculin testing can be seen. This test is not recommended for diagnosis of glanders in humans:

- **Intradermo-palpebral test:** It is the most sensitive, reliable and specific test. About 0.1 mL of mallein is injected intradermaly into the lower eyelid using tuberculin syringe. Reading of the test should be taken at 48 h. Positive reaction is marked oedematous swelling on eyelid with blepharospasm and severe purulent conjunctivitis
- **The ophthalmic test:** Less reliable in comparison to intradermo-palpebral test. Few drops of mallein are instilled into the eye at the canthus. In positive cases, eyelids and face become swollen along with discharge from the eye
- **The subcutaneous test:** This not a preferred test because it interferes in serological diagnosis. At the time of testing the rectal temperature of horse should be under 102°F. About 10 cm^2 skin in the middle of the neck is clipped and 2.5 mL of dilute mallein are injected subcutaneously. The positive reaction is characterized by high temperature (104°F or more) during the first 15 h along with painful swelling at site of injection

Serological tests: Various tests viz., Complement Fixation Test (CFT), agglutination test, indirect hemagglutination assay (IHA), ELISA, avidin-biotin dot ELISA. Among these, mallein and CFT are prescribed tests for international trade of equines. CFT is 90-95% accurate and positive result is obtained within one week of infection and remains positive in chronic cases. CFT has been the preferred and most extensively used diagnostic tool because of its capacity to detect clinically inapparent carriers and chronically infected horses (Neubauer *et al*., 2005). However, ELISA has been found to be comparable with CGT in terms of sensitivity and specificity (Sprague *et al*., 2009). However, none of these procedures are sensitive enough to differentiate serologically between *B. mallei* and *B. pseudomallei*.

Molecular tests: Use of molecular tests like PCR (Scholz *et al*., 2006; Grishkina and Samygin, 2010) and real time PCR (Thibault *et al*., 2004a; U'Ren *et al*., 2005; Ulrich *et al*., 2006; Zhang *et al*., 2012) are found suitable for determination of generic, inter and intraspecies characteristics of bacteria. Recently, multiplex PCR and qPCR has been developed for rapid and reliable detection and differentiation of *Burkholderia mallei* and *Burkholderia pseudomallei* (*B. pseudomallei* is the cause of melioidosis in humans and animals) (Lee *et al*., 2005; Koh *et al*., 2012; Janse *et al*., 2013). A 5' nuclease real-time PCR assay is used for rapid identification and simultaneous screening of *B. mallei* and *B. pseudomallei* and is also used for rapid and most specific identification and detection of *B. mallei* in clinical samples by targeting flagellin P gene, fliP. The assay is most sensitive which detects 60 fg of *B. mallei* DNA in the clinical samples from diseased horses. The technique has not yet been fully validated for wide acceptance (Tomaso *et al*., 2004, 2006). RAPD, ribotyping as well as of plasmid and DNA microrestriction analyses, Intact cell Matrix-assisted Laser Desorption/Ionisation mass spectrometric typing has also been described recently for rapid identification/detection of *B. mallei* (Antonov and Iliukhin, 2005; Karger *et al*., 2012).

Diagnosis of glanders in humans: It is mainly diagnosed by isolation and identification of *B. mallei*. In septicemic form, blood cultures may be negative until just before death. Commonly used serologic tests are agglutination and complement fixation tests (Cravitz and Miller, 1950). High background titers can be found in normal serum and cross-reactions may occur with *B. pseudomallei*, the causative agent of Melioidosis (Neubauer *et al*., 2005). Positive reactions in agglutination tests develop only after 7 to 10 days. In imaging studies, chest radiography and computer assisted tomography (CT) may demonstrate miliary nodules, bilateral bronchopneumonia, cavitating lesions, segmental and lobar infiltrates (Georgiades and Fishman, 2001).

Advances in diagnostic tools and techniques need to be exploited fully for rapid and confirmatory diagnosis of the disease especially heightening the surveillance and monitoring programmes which would help design effective disease prevention and control programmes (Schmitt and Henderson, 2005; Bollo, 2007; Deb and Chakraborty, 2012; Dhama *et al*., 2012b, 2013d, e; Deb *et al*., 2013).

Treatment

In animals: According to Glander and Farcy Act, 1899 affected animals must be destroyed and disposed off safely and is a notifiable disease (Malik *et al*., 2010). For eradication, affected animals should not be treated because it may result in carrier state. Sodium sulfadiazine is reported to be effective in hamsters. Doxycycline and ciprofloxacin have also been found to be useful. Therapeutic regimens for glanders require prolonged course of treatment, usually treatment should be continued for 20 days or more (Al-Izzi and Al-Bassam, 1989; Manzeniuk *et al*., 1994; Saqib *et al*., 2012). Use of formalin killed *B. mallei* and sulfadiazine, or mallein and sulfadimidine, are found effective in horses (Batmanov, 1993). Use of drugs in acute form of glanders in equines increases the lifespan (Iliukhin *et al*., 2012). Use of antibiotics in liposomal forms of antibiotics was found to be more effective in *in vitro* studies (Iliukhin *et al*., 2012). The treatment in horses includes single or combination use of antibiotics like Ceftazidime, Sulfadiazine, Trimethoprim+Sulfamethoxazol, Gentamicin, Imipenem etc (Lehavi *et al*., 2002). As the cost of therapy is too high, so it can only be used in precious horses, but it should not replace the "test and slaughter policy" (Saqib *et al*., 2012).

In humans: Recently, the prophylactic usefulness of Co-trimoxazole for *B. mallei* has been suggested in humans. Treatment with sulfonamides (trimethoprim-sulfamethoxazole, TMP-SMX) has been recommended.

Table 2: Antibiotics for treatment of glanders in humans

Drug	Dose	Interval
Intensive IV therapy (mg kg^{-1})		
Imipenem	25	up to 1 g every 6 h
Meropenem	25	up to 1 g every 8 h
Ceftazidime	50	up to 2 g every 6 h
Oral eradication therapy		
TMP-SMX	8/40 mg kg^{-1} up to 320 mg/1600 mg	every 12 h
Doxycycline	2.5 mg kg^{-1} up to 100 mg	every 12 h
Amoxicillin-clavulanate	500 mg or 875 mg for adults	every 8 h

Recently, Piperacillin/tazobactum as an alternative for currently used drug ceftazidime (Table 2) for the treatment of both glanders and melioidosis in view of the emergence of ceftazidime-resistant clinical isolates in Southeast Asia (Thibault *et al*., 2004b; Van Zandt *et al*., 2013).

PREVENTION AND CONTROL

No human or veterinary vaccines are available for immunization/prevention of Glanders (Estes *et al*., 2010; Burtnick *et al*., 2012). The immune evading strategy and genomic fluidity of this complex pathogen have made the use of live vaccine unsatisfactory (Nierman *et al*., 2004; Romero *et al*., 2006). Efforts need to be made for identifying broadly protective antigens, efficient vaccine delivery/adjuvant systems and an exploring protection from both acute and chronic infections which would altogether pave way for the development of effective vaccine for *B. mallei* (Bondi and Goldberg, 2008). Experimentally, two live attenuated strains of *B. mallei*, a capsule mutant and a branched-chain amino acid auxotroph which is genetically engineered mutant of *B. mallei* using newly constructed allelic exchange vector, as vaccines in mice. The auxotrophic mutant was found to enhance the Th1 response and with a survival rate of 25% for one month post-challenge in comparison to control where none survived beyond five days (Ulrich *et al*., 2005). The 6-deoxy-heptan Capsular Polysaccharide (CPS) of *B. mallei*, having both a pathogenic determinant and a protective antigen, is being exploited for developments of novel vaccine against glanders (Burtnick *et al*., 2012).

Clinical and serological recovery is rare, recovered animals are also not immune, so every animal positive for glanders should be destroyed and remaining animals should be retested at intervals of 3 weeks until all reactors have been removed:

- In case of death due to glanders, carcass should not be opened. It must be buried or incinerated (Khan *et al*., 2013)
- Adequate compensation to the owners for destroying the horses

- Manure, bedding and feed residue should be burned or buried
- Follow vigorous disinfection programme for premises, feed and water trough etc
- The entire suspect, in contact animals must be isolated, properly tested and positive animal should be destroyed
- There should be restriction of the movement of horses
- Strict isolation, proper hygiene and sanitation procedures should be adopted
- Disinfection of surroundings of dead or infected animals should be done as *B. mallei* is highly susceptible to common disinfectants like benzalkonium chloride, iodine, mercuric chloride in alcohol, potassium permanganate, 1% sodium hypochlorite, 70% ethanol and 2% glutaraldehyde. It is less susceptible to phenolic disinfectants. It also destroyed by heating to 55°C for 10 min or by ultraviolet irradiation
- Contaminated material should be cleaned with a solution of 1 part household bleach (0.5% sodium hypochlorite solution) to 9 parts water
- Veterinarians, animal handlers and persons in contact with infected animals should follow appropriate biosafety measures, wear gloves and masks during animal handling
- Awareness programmes about the glanders need to be carried out from time to time (Khan *et al*., 2013)

Employing the new generation developments and progress in vaccines and vaccinology, safer and effective vaccines need to be focused for countering this important disease having high zoonotic potential (Paul-Pierre, 2009; Dhama *et al*., 2008, 2013f). Judicious application of drugs and alternative and novel/emerging therapeutic modalities should also be kept in mind for treating glanders (Mahima *et al*., 2012b; Dhama *et al*., 2013g, h; Tiwari *et al*., 2013a, b, c). Nowadays, one health and one medicine approach is being given due importance to counter deadly pathogens, emerging/re-emerging infectious diseases and their zoonotic threats which need to be applied from all aspects for combating glanders (Kahn *et al*., 2007; Dhama *et al*., 2013i).

CONCLUSION AND FUTURE PERSPECTIVES

Glanders is a very important disease of solipeds (horses, donkeys, mules) having high zoonotic significance. The causative bacterium (*B. mallei*) has the potential to be used as biological weapon in biowarfares/terrorism. The disease is highly contagious and fatal in nature and therefore, active surveillance of Glanders in animals is essential. Timely recognition of *B. mallei* is a key factor for adapting suitable therapy, as glanders is a rapidly progressive disease and shows resistant to several antibiotics. For this notifiable disease, regulatory measures call for culling/disposal of diseased animals, however in certain circumstances where wild life conservation activities are being followed and in cases of extremely valuable breeding stocks, effective treatment regimens and post-exposure prophylaxis are the need of the hour. Due to a lack of an effective vaccine for glanders, long courses of various antibiotics being required to eliminate/eradicate this pathogen and its potential threat to be used as a biological weapon, the development of effective glanders medical countermeasures and effective novel vaccines are the need of the hour. An international veterinary certificate is required attesting that the animals showed no clinical signs of glanders and were kept in an exporting country free of the disease for at least 6 months prior to shipment. In areas that are at risk or where the disease is endemic cooperation of horse owners with veterinarians is essential for disease detection and control.

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