



A Review of Glanders Regarded Iraq and Surrounding Countries

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Abstract

Glanders caused by *Burkholderia mallei* infected mainly the Equidae and it is really fatal zoonosis. It was known since ancient times. The horses, mules, and donkeys can get infection by skin abrasion, and during direct contact between animals or with water instruments, also the bacterium can be found in the soil. Since the disease was eradicated from most of the developed world, Glanders is endemic in several countries surrounding Iraq and other related regions of the middle east. Regardless a limited space of Glanders free regions when they're no cases of Glanders can be detected by sensitive and specific tests, the disease can appears as reemerge infection in those places, and that interfere with control, monitoring and strategies for prevention, so the reviewing of disease with special interest on Iraq and surrounding countries have an important.

Keyword: Glanders, Iraq, review.

Introduction

Glanders an extremely contagious disease and could be fatal zoonosis of Equidae, caused by *Burkholderia mallei*, a gram-negative, non-motile, and facultative intracellular microorganisms (1). It is soil resident and the horse's area extremely be the natural reservoir for infection (2). The sickness is informed to the planet Organization for Animal Health (OIE) (3). Asymptomatic or carrier animals are potential supply of infection for the healthy equine population and play a vital role in the spreading of the agent (4). Glanders mortality rate was 95% in untreated septicemic equine infections and fatality rate reach 50% in treated humans individuals (5). There is no vaccination or specific treatment programs available against *Burkholderia mallei* infection yet (4). The hazard can be explained as bio-weaponization, also Glanders is considered as a notifiable disease, and *Burkholderia mallei* was recognized as a Tier 1 select agent by Center for Disease Control (6). The aim of this study is a review of Glanders in Iraq and surrounding countries.

History of Glanders

First sign of Glanders as disease by Hippocrates whom reported clinical signs of the disease around 450-425 BC (5). And 100 years later Aristotle described the disease under the general term for epizootics, and named it 'melis', also mention the horse is the natural reservoir of infection and man an accidental host (3).

It the 4th century AD, Glanders had extended association with warfare, that when Apsyrus aiding as a veterinarian in the army of Constantine the Great (7). Moreover, the Roman military historian during the 5th

century AD named Vegetius, and was call 'malleus' to the disease (1).

Glanders was prominent during the Crusades and other military engagements eventually prompting the French King, Louis XV, to commission the first veterinary school at Lyons under Claude Bourgelat in 1761 to investigate Glanders in attempt to protect the French Army horses (8). In 1797, Viborg demonstrated the transmissible nature of Glanders but it was not until 1876 that it was accepted that Glanders was contagious (3) and in 1882 isolated the organism from the liver and spleen of an infected horse in Germany also named *Bacillus mallei* (9,10).

In 1956 named *Pfeifferella mallei* by Henning (11). In same year authors call it *Pseudomonas mallei* (12). Moreover, in 1966 named *Actinobacillus mallei* by Evans (13). Then after named *Malleomyces mallei* by Merchant & Packer (14). The common name nowadays is *Burkholderia mallei* by (15).

The etiology of Glanders

The Glanders caused by *Burkholderia mallei*, which can classify as: Kingdom; Bacteria, Phylum; pseudomonadata, Class; Betaproteobacteria, Order; Burkholderiales, Family; Burkholderiaceae, Genus; *Burkholderia* and Species; *B. mallei* (4).

This bacterium is developed from *Burkholderia pseudomallei* by selective reduction and deletions of genomes from the *B. pseudomallei* genome, but the *B. mallei* is non-motile, and shown as coccobacilli (16).

Transmission of Glanders

The most common source of infection is the ingestion of contaminated food or water, aerosols, and contaminated fomites brought to the animals via grooming equipment (4).

The bacteria can also enter the body through contact with lesions or abrasions of the skin or through the mucosa, in this case, a local infection with ulceration may develop spreading to other parts of the body in the course of the disease (5). The transmission was occurred from mare to its foal, also can occur from stallions to mares during mating. House flies *Musca domestica* take a role as a biological vector to transmit *Burkholderia mallei* (17). Moreover, poor husbandry and feeding conditions, as well as animal transport, can be a predispose factors. Unsanitary conditions and overcrowded stables are risk factors (4).

Prevalence of Glanders in the world

The Glanders was reported endemic in Asian countries including; Bangladesh and Mongolia (18), as well as in horses of the Middle East (19). OIE record was involving that China and Russia as endemic regions for Glanders (5), also the disease reported in Vietnam, Korea and Afghanistan (20, 21). The horses of Mongolia can have diseases prevalence up to with percentage of (7.7 – 8.3 %) following examination by RBT and CFT, respectively. And they found there is relation of seropositive horses as a result from crossbreeding of Mongolian native horses with thoroughbred horses to be higher than that in Mongolian native horses endemic (22). More over the researchers in Punjab province of Pakistan found antibodies against *B. mallei* with CFT and then examination followed by western blot (23).

The Glanders was also endemic in India and had reported in several parts in a seroprevalence with indirect Elisa and CFT of (0.62% and 1.145%), and the scientists were recorded as a re-emerging cases in Maharashtra, Haryana and Punjab after a gap of 10 years of free areas from Glanders, they

thought that lack of awareness, little veterinary care and unrestricted movement of equids across state borders will led to the creation of the infection to these states (24). More than that a demonstration among Glanders outbreaks in Bahrain, there are two strains of *B. mallei* appeared as coming from similar geographical regions through import of diseased animals (8).

In Brazil Glanders seem to have serious problem, and every year recorded several new cases causing economic losses and affect the trade of animals (18).

The Glanders can be regained in the status of emerging disease, which related to a numerous recent out breaks throughout several countries in the world (18, 25). Such emerging disease generally in Asia and somewhat for Africa and south America regarded where endemic region for Glanders in horses and camels were provide reservoir for reintroduction of Glanders into those countries previously listed as Glanders-free (8).

As the official OIE reference laboratory for Glanders in the Arabic region, the Central Veterinary Laboratory (CVRL) in Dubai is the officially authorized institution for Glanders research, surveillance and eradication. All procedures involving animals were performed in strict accordance with the OIE guidelines for animal welfare using prescribed protocols (18, 26).

Glanders was eradicated from most of the developed world in the early 20th century, that is important signal, Glanders was eradicated from Western Europe, Northern American, Australia, Japan and others (27).

Prevalence of Glanders in the neighboring countries of Iraq

The Glanders is endemic in Turkey, and most outbreaks of the disease were registered in the southwest part (28). Four outbreaks of Glanders in horses have been reported, the first of them since 2017, and the other three of the outbreaks were reported on 2019 (29). That was occur following routine CFT screening detection. Those outbreaks are related to illegal animal transportation (6) The fourth outbreak was reported in Merkez on 2019, after a private veterinarian suspected the disease due to clinical signs and this was detected by mallein test (30).

Iranian region is reported to be endemic for Glanders, in which the infection appears in a number of horse breeding and horse-riding centers in two loci in Isfahan and Tehran cities (31, 32). Other report on the incidence and degradation of two horses in the Tafresh city was published in Markazi province since 2003, also in 2010, at the Tehran Eram Zoo, a male tiger imported from Russia died, which led to the elimination of Tehran Eram Zoo lions because of suspicion to Glanders (33). The source of infection was contaminated fresh donkey meat without a confirmatory mallein test, possibly imported from Iraq and Qatar, and had been consumed by zoo animals (34).

On other neighbor country, horses entered Kuwait from Syria was suspected of transmitting the infection with Glanders and were kept at the quarantine conditions, as well as a reoccurrence of the infection during 2011. In the CVRL research, 50 horses and one camel had Glanders-positive test, and the camel have *B. mallei* was isolated during investigating documents related to the importation of features. (18, 26). Due to the failure to eradicate the infection in its early stages in 2010, it is argued that continuous care and preventive measures should be

taken even after the onset of the infection (35).

Prevalence of Glanders in Iraq

A several studies were reported incidence of Glanders in Iraq, since 1948 the disease has been reported in the Baghdad province (36). Then after Al-Kafawi, et al., (37) has study the hematological changes in Arabian horses infected with Glanders. The clinical signs were variable and ranged from asymptomatic carrier to obvious pneumonia, both the nasal and skin forms were giving a characteristic pathognomonic lesion such as nodules ruptured leaving on the cartilaginous nasal septum as well as the chains of cutaneous nodules ruptures giving rise ulcers with thick yellowish sticky purulent honey like material were observed. Moreover, (38) were describe three horses with advance Glanders, also they isolate *B mallei* from unopened glanderous lung nodules. Also, Al-Ani and Al-Delaimi on 1988 study Glanders in horses with concerning clinical, epidemiological and hematological findings (39). Then Al-Izzi, & Al-Bassam (40) studies in vitro susceptibility of *B mallei* to antimicrobial agents. Furthermore, the researcher in the college of Veterinary Medicine in Baghdad studies the histopathological and electron microscope (41). Moreover, Elisa test was adapted by group scientists for detection of antibody to *B mallei* infection in horses (42).

Otherwise, the outcomes of sensitivity tests for antibiotics showed that *B. mallei* is susceptible to Baytril, gentamicin, erythromycin, oxytetracycline and ampicillin. diseased horses appeared to develop an antibody titer of 1:80 and over (43).

There is register of an outbreak of Glanders in Iraq by Al-Jeboori and Habasha (44),

whom found the disease in Diyala equestrian club, there were thirteen horses infected with *B mallei*, that ten of them appeared ill and react positively to mallein test and antibody titer at range of 80 – 640. Other researcher explained that horses lived in eight different Iraqi provinces; Al-Muthanna, Baghdad, Al-Najaf, Babylon, Al Qadsyia, Diyala, Karbala and Wasit, were found infection in (2.97%) by cELISA technique against *Burkholderia mallei* antibodies, and all those positive horses were noted in Baghdad and Muthanna governorate (45).

Recently during 2022 Hmood and Al-Amery (46) use IDvet Elisa and then CFT against Glanders antibodies, and they found four horses positive for the disease in Maysan , also they conclude that Glanders is remerging in Maysan governerat, while Basrah and ThiQar governorates have horses free from Glanders in the south of Iraq, accordingly they suggest more confirmation to establish free zone area.

Pathogenesis of Glanders

The *Burkholderia mallei* was a high virulence bacterium, can be directly transmitted to invade nasal, conjunctival, and oral mucous membranes, the gastrointestinal tract, and the integument, resulting in either septicemia or bacteremia as an acute form of the infection (2). The incubation time has always been a matter of intensive debate which arrange between 6 days to several months (47).

The ability of *Burkholderia mallei* to enter, survive and multiplicity within a variety of host cells is a key component in the pathogenesis of Glanders (4). Adherence and invasion of host cells are important first steps in the pathogenesis of *B. mallei* infection, also *Burkholderia* capsular polysaccharide

and liposaccharides have been shown to reduce cytokine and nitric oxide production in macrophages and reduce the stimulation of pattern recognition receptors such as Toll-like receptors, that may attribute change or delay the recognition of *B mallei* by the innate immune system (48).

The *B. mallei* enter the mucosa, move via lymph vessels, and then reach the lymphatic sentinels. Afterward, proliferation in the lymph nodes, spread hematogenous. Then develop granulomas and ulcers in different tissues, nodules, and fulminating ulcers on the mucous membranes of the nasal passages, larynx, and upper lip (47). In pulmonary Glanders, lungs may show nodular foci underneath the pleura and diffuse military granulomatous nodules in the lungs and severe bronchopneumonia (49).

Clinical Findings of Glanders

The clinical picture of equine Glanders may manifested itself in three forms: pulmonary, nasal, and cutaneous; also, the course of the disease may be acute, subacute, or chronic (4). However, the distinction between the various clinical presentations of Glanders is difficult because all three forms frequently coincide, while the pulmonary form is seen almost in all clinical cases (50, 51).

Nasal form of Glanders

Under stress conditions like starvation, poor nutrition, extreme weather, severe workload, overcrowding, the lesions of nodules and ulcers will develop in the nasal passages and give rise to a sticky yellow discharge from one or both nostrils, accompanied by enlarged, tortuous firm submaxillary lymph nodes (lymphadenitis), which rarely suppurate. Perforation of the septum has

frequently been observed in chronic cases (4). On healing, nasal ulcers leave small, whitish, characteristic stellate star shaped scars indurated to touch and remain almost for an indefinite time (2).

The Cutaneous form (Farcy)

The skin (cutaneous) form of the Glanders commonly considered by multiple papules or pustular nodules, especially in the hind limbs, abdomen, and sometimes all over the body. So the cutaneous Glanders may result from skin injury or may be due to a secondary manifestation of the respiratory form (41). These nodules usually appear in chains along the course of the lymphatic vessels, and usually referred to as farcy pipes like (4).

Pulmonary form of Glanders

This form is most common following Glanders infection for equine, is characterized by the formation of round, greyish, firm, encapsulated nodules embedded throughout the lung tissue. Cough and high fever reflect the fulminating bronchopneumonia that characterizes the acute form (38). Lung lesions may erupt and lead to acute bronchopneumonia. Exacerbation to nasal and cutaneous forms of Glanders may be observed (5).

Acute or septicemic form of disease

The acute form is most frequent in donkeys and mules and it is fatal within a few days to weeks (52). However, the susceptibility of mules to Glanders has been suggested to be somewhat less compared to horses and donkeys, acute Glanders begins with chill and high fever, depression, anorexia, and emaciation, it is clinically characterized by bouts of coughing and watery nasal discharge from one or both nostrils, nodules

and ulcers develop rapidly on the nasal septum and turbinate bones, and copious thick sticky yellowish white mucopurulent to hemorrhagic discharge, that leading to labored breathing, as well as submaxillary lymph nodes uni- or bilaterally enlarged and usually indurated (4). In some cases, edema of the glottis can develop, death occurs within a few days to weeks because of respiratory failure associated with bronchopneumonia and septicemia (2).

Chronic form of Glanders

Chronic Glanders usually develops in horses (53). That runs over a long period of months to years, with episodic worsening and successive improving of body conditions. The pulmonary form usually manifests in chronic Glanders or may remain latent for a variable period of time without significant clinical signs particularly when lesions in lungs and other internal organs are sporadic (54). Chronic Glanders is usually fatal although a few cases may recover but remain carriers for life, the disease may likely to occur with advancing age (38).

Mallein test

The test is a hypersensitive skin test based on a water-soluble protein extracted from the microorganism, but it is not recommended via the World Organization for Animal Health because of animal welfare concerns (5). Moreover, the test of poor sensitivity and considerable time consumption make cultural isolation a less preferred method for Glanders diagnosis, and the test is not accepted for trade testing (4). Furthermore, malleinised animals may undergo seroconversion and then show positive reactions as an additional diagnostic method, like CFT (55). However, the mallein test

may be useful for Glanders eradication in remote endemic regions (22).

Isolation and identification

The gold standard method for the diagnosis of Glanders is the isolation and identification of *B. mallei* from clinical samples (56). It has to be stressed that the characteristic properties of the infectious agent may undergo changes during infection resulting in a hindrance to the use of classical methods of microbiological diagnosis (57). Bacterial isolation and identification of *B. mallei* from cutaneous lesions and nasal exudates are considered to be the perfect reference. As approximately (90%) of early infections occur in a non-clinical or latent form, and that clinical and bacteriological diagnoses are difficult (58). *B. mallei* can be grown on routine culture media including nutrients, blood, and MacConkey agar. Viscid, smooth, and creamy colonies of *B. mallei* can be obtained after 48h at 37C° on these routine culture media. In the presence of nitrogen, the organism can grow as an anaerobic and facultative anaerobe (47). On brain heart infusion agar supplemented with 3% glycerin has been used to propagate the organism in large quantities (59).

Laboratory animal inoculation

The Straous reaction is performed by the intra-peritoneal injection of male guinea pigs with suspected materials to help in diagnosis (43). Swelling and peri- Orchitis occurs 3 to 7 days post-inoculation (60).

Serological test of Glanders

Complement Fixation Test (CFT)

This tool was commonly used in serological diagnosis of the disease over 100 years ago (61), which was highly sensitive and be used

in reliable culling of positive Equidae, whereas contributed to the elimination of the Glanders in numerous worldwide districts, also it was agreed by the OIE for international trade of equines, moreover, the test have considerable number of false positive outcomes (5,62).

Enzyme Linked Immunosorbent Assay (ELISA)

The Elisa founded as a semi-purified portion of *B. mallei*, which is used as a purified LPS from the bacterium *B. mallei*-antigen. The test with sensitivity and specificity was estimated by using cut-off values, that were suggested by the pioneer's developers (63). Moreover, ELISAs based on TssA, TssB, and BimA antigens had significantly sensitivity in compared with CFT (48).

Another serological test

Agar-gel immunodiffusion (AGID) test (51), Counter immunoelectrophoresis (CIE) test (64), Fluorescent antibody (IFA) test (65), and Indirect Hemagglutination (IHA) test (66) were used as either screening or multi test strategies (2). More over the rapid agglutination assay called Rose Bengal plate test was used antigens purified *B. mallei* culture (48), as well as Bacteriophage specific to detected *B. malle* (67).

DNA-sequencing and Polymerase Chain Reaction (PCR)

By using primers to amplify part of the bacterial genome have been developed to differentiate between Glanders, melioidosis, and other related organisms (68). The use of 16S rRNA gene sequencing to rapidly identify *B. mallei*, *B. pseudomallei*, and differentiate them from closely related organisms by a molecular method, has been used (66).

Glanders in humans

Glanders well known zoonotic disease, so humans can get the infection through prolonged contact with *B. mallei*-infected equines (5). Equine attendants, veterinarians, slaughterhouses, and laboratory workers face the occupational risk of acquiring the infection (69).

The bacteria that cause Glanders are transmitted to humans through contact with tissues or body fluids of infected animals. *B. mallei* enter the body through cuts or skin abrasion or through mucosal surfaces such as the eyes & nose. Also, can inhale via infected aerosols or dust contaminated by infected animals. Sporadic cases were documented in veterinarians, horse caretakers, and laboratorian (5). Cases of human-to-human transmission have not been reported. The symptoms of Glanders in men commonly include increased body temperature with chills and sweating, moreover muscle aches and discharge, chest pain, headache, nasal discharge, and sweating. The severity of symptoms depends on the type of infection (70).

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مراجعة للرعام في العراق والدول المجاورة

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مرض الرعام المتسبب بـ *Burkholderia mallei* يصيب بشكل رئيسي الفصيلة الخيلية وهو من الامراض المشتركة المميتة حقاً. وهو معروف منذ العصور القديمة. ويمكن أن تصاب الخيول والبغال والحمير بالعدوى بسبب تآكل الجلد، وأثناء التماس المباشر بين الحيوانات أو بأدوات المياه، ويمكن أيضاً حياة البكتيريا في التربة. منذ أن تم استئصال المرض من معظم دول العالم المتقدمة، إلا ان الرعام مستوطن في العديد من البلدان المحيطة بالعراق والمناطق الأخرى ذات الصلة بالشرق الأوسط، مع بعض المساحات المحدودة من المناطق الخالية من الرعام عندما لا تكون هناك حالات مرضية يمكن اكتشافها بواسطة فحوصات حساسة وخاصة، ويظهر المرض على شكل عدوى عائدة الظهور في تلك الأماكن، وهذا يتداخل مع السيطرة والمراقبة واستراتيجيات الوقاية من المرض، لذا فإن مراجعة المرض مع الاهتمام الخاص بالعراق والدول المجاورة بذات أهمية

الكلمات المفتاحية: الرعام، العراق، مراجعة.