Research Article

Serological Detection of *Burkhorderia mallei* in Equine

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Abstract | The current study was conducted to assess the application and demonstration of rapid, sensitive, and specific assays for confirmatory testing and to determine the prevalence of glanders in horses and donkeys. For this reason, a competitive enzyme-linked immunosorbent assay (cELISA) was applied to determine seropositivity against *Burkholderia mallei* in clinically confirmed cases of glanders from Baghdad city. Analysis of sera samples collected from horses (n=80) demonstrated that 36 horses were positive with a percentage of 22.5 % positivity rate. Of the donkey (n = 80), a total of 4 cases were identified as positive for glanders with a percentage positive rate of 2.5 %. However, no significant variations (p <0.05) in glanders seropositivity were observed between different sex or age groups. These findings highlight alarming seropositivity in equines and warrant future investigations on the prevalence of active infection.

Keywords | Glanders, *Burkholderia mallei*, Horse, ELISA, Epidemiology, Baghdad city.

Received | March 24, 2024; **Accepted** | April 06, 2024; **Published** | May 15, 2024
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Citation | Obayes AM, Hussein ZS (2024). Serological detection of burkhorderia mallei in equine. J. Anim. Health Prod. 12(2): 229-232.
DOI | http://dx.doi.org/10.17582/journal.jahp/2024/12.2.229.232
ISSN | 2308-2801

INTRODUCTION

Glanders or farcy is one of the most ancient known diseases of horses. Symptoms in equids were reported by Hippocrates around the year 425 BC. Aristotle described 'glanders' around 350 BC and named them 'malleus' (Groves and Harrington, 1994; Marr and Malloy, 1996). *Burkholderia mallei* is the etiologic agent of glanders, an infectious disease of solipeds (horse, mule, and donkey), carnivores including lions, tigers, domestic and feral cats, dogs, and hyenas after eating glanderous meat, also consider an infectious zoonotic disease of humans (Khan et al., 2013). This microorganism is a gram-negative bacillus bacterium causing severe impacts with a low infectious dose transmitted through direct contact with respiratory secretions, animal skin exudates, and fomite (Benanti et al., 2015; Quinn et al., 2015).

Despite the fact that the disease affects primarily Equidae, horses usually exhibit the chronic form of the disease, whereas mules as well as donkeys develop the acute form only and within a few days to weeks the animal is fatal (Theves,1993; Mota et al., 2010). However, carnivores may be infected particularly when they consume meat from infected horses (Alibasoglu et al., 1986). The clinical picture of equine glanders may manifest itself in three forms: pulmonary, nasal, and cutaneous; the course of disease may be acute, subacute, or chronic (Constable et al., 2016). Recording of clinical signs such as fever, inappetence, cough, dyspnea, nasal discharge, development, and location of ulcers, and the pulmonary form is seen almost in all clinical cases; the pulmocutaneous presentation is the most common form of glanders seen in natural disease outbreaks (Jubb et al.,1985). In the cutaneous form characterized by lymphangitis (farcy cords) and lymphadenitis (nodules,ulcers), lesions can occur in any part of the body (Khan et al., 2013).

While the disease is endemic in many countries, continued monitoring of the infection in equines is needed to risk assess the disease for cross-species transmission and its impact on animal health. This study was attempted to assess the potential of the disease in the country to determine seropositive rates in horses and donkeys.

MATERIALS AND METHODS

During the period, the period extended between April 2022 and May 2023. In this current study, 100 and sixty blood samples were collected randomly from 80 horses housed in the Equestrian Club in Baghdad city which is considered the largest reserve of horses in Iraq (Salah et al., 2016). Samples were also collected from 80 donkeys sacrificed to the predators at the Al-Zawraa Zoo in Baghdad city.

Blood samples were collected directly from the jugular vein using plain vacutainer tubes and serum was extracted, collected blood samples were transported immediately in cooled conditions to clinical pathology lab at the Department of Internal Veterinary Medicine/ Baghdad University to detect the presence of antibodies against *Burkholderia mallei* in their serum using a commercial cELISA kit (ID Screen® Glanders Double Antigen Multi-species) and the result data were used using the Statistical Analysis System program 9.6th ed. (SAS, 2018).

RESULTS AND DISCUSSION

Current results show that 25% (40/160) of the tested animals were seropositive and most of them suffered from several clinical signs, such as nasal discharge, skin lesions, and cough (Figure 1 and Table 1). 22.5% (36/80) of the horses examined were seropositive for Burkholderia mallei, which confirmed many previous findings in Iraq regarding this bacterium as an endemic disease (Al-Ani et al., 1998; Hussein, 2018). Such high infection rates were expected outcomes since the veterinary health authorities in Iraq have stopped culling and destroying infected animals, as well as the inconsiderate importation of horses from neighboring countries without convenient inspection or quarantine, and with the lack or inadequate diagnostic capabilities, most infected horses were treated as ambiguous chronic pneumonia with a wide range of antibiotics that created numerous carrier states. As for the examined donkeys, these animals were of advanced age and suffered from numerous incurable chronic diseases, which is why they are used as a cheap source of meat to feed the predators in Al-Zawraa Zoo These reasons play a role in the spread of the disease, however, despite the study revealed a lower seropositivity for Burkholderia mallei 2.5% (4/80), it is still a potential source for spreading the disease mainly for human in contact because they are a cheap means of transportation and travel long distances, particularly in rural areas and for susceptible predators that were feed on them. in Iraq, donkeys are raised individually and in small numbers and this is probably the reason for the low infection rate that is recorded in this study considering that infected donkeys commonly develop acute lethal forms of the disease (Silva et al., 2013; Sial et al., 2020)

There was an increased probability that glanders is mainly a disease of horses when found the seropositivity to *B.mallei* was relatively higher in donkeys (4.1%;8/194) compared to that in horses (2.1%; 4/194) in Pakistan, that is why the current study involved this animal to exhibit the role that may play in the epidemiology of this disease in Iraq. Statistical analysis revealed no significant differences (P <0.05) between the genders of the animals tested (Table 2), which confirms previous results by Hussien (2018) that used the same applied test in the same study area, eliminated gender as a risk factor in Glander disease taking into consideration that a large portion of the sampled animals was aged and suffering from chronic diseases. The current study reveals no association between seropositivity to *B.mallei* and the age of both studied animals (Table 3) and the result agrees with some previous studies that roll out the age factor (Neubauer et al., 2005; Ghori et al., 2017), however, large-scale survey designed to cover different age is needed to determine which age is at risk infection.

Table 1: Positivity rates of the cELISA in a horse and donkeys samples

Host	No. of samples examined	ELISA	
		No. of positive	Percentage (%)
horse	80	36	22.5
donkey	80	4	2.5
Total	160	40	25
X^2	34.133333		
P value	0.00**		

*: significant differences in (P0.05).
NS: Nonsignificant differences at (P0.05).

Figure 1: Showing clinical sings of glanders (A) nasal and (B) cutaneous forms

CONCLUSIONS

The disease is widely endemic, especially in the breeding place of horses in the Equestrian Club in Baghdad, and

Table 2: ELISA results showing effect of gender on the incidence of Glanders

Gender	No. of donkey samples examined	No. of positive	Percentage of total (%)	No. of horse samples examined	No. of positive	Percentage of total (%)
male	41	2	2.5	52	27	33.75
female	39	2	2.5	28	9	11.25
Total	80	4	5	80	36	45
X^2	0.002633			2.8771230.019488		
P value	0.959075NS			0.089846 NS		

Table 3: The results of the ELISA test show the incidence of glanders in different age groups.

Age (year)	No. of donkey samples examined	No. of positive	Percentage of total (%)	Age (year)	No. of horse samples examined	No. of positive	Percentage of total (%)
1≥2.5 years	36	1	1.2	2≥3 years	26	12	15
2.5>3.5 years	21	2	2.4	3>5 years	27	10	12.5
3.5yeas and more than	23	1	1.2	5years and more than	26	14	17
Total	80	4	4.9	Total	80	36	45
X^2	1.056642			X^2	0.964632		
P value	0.589594NS			P value	0.617 NS		

outbreaks of glanders have increased significantly in several parts of Iraq, Furthermore, donkeys do not play an important role in spreading the disease in Iraq.

ACKNOWLEDGEMENTS

The author is deeply intended to acknowledge the staff of the Equestrian Club in Baghdad.

NOVELTY STATEMENT

The novelty of the study is to focus on the prevalence of glanders disease among the equine family and the comparison of infections of horses with donkeys in Baghdad province, which represents a large part of Iraq, using accurate examination ELISA.

AUTHORS' CONTRIBUTION

These authors each contributed equally.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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