J. Vet. Med. OH Res. (2020). 2(1): 63-80 p-2664-2352 : ISSN : e-2664-2360
Website: www.lepvmbj.org DOI: 10.36111/jvmohr.2020.2(1).0017

SERO-SURVEILLANCE AND RISK FACTORS OF *BURKHOLDERIA MALLEI* INFECTION IN INDIGENOUS HORSES OF BANGLADESH WITH A BRIEF REVIEW ON VALIDATION OF SERODIAGNOSIS

A. C. Das,[1*] A. K. M. A. Rahman,[1] N. S. Tithy,[1] A. A. Maruf,[1] P. K. Bhattacharjee,[1] H. Singha,[3] H. Neubauer,[2] M. Elschner,[2] and M. S. Rahman[1**]

Academic Editor: M. A. Samad

[1]Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh [2]OIE and National Reference Laboratory for Glanders, Friedrich-Loeffler Institute, Federal Research Institute for Animal Health, Institute of Bacterial Infections and Zoonoses, Naumburger, 07743 Jena, Germany [3]National Research Centre on Equines, Sirsa Road, Hisar-125001, Haryana, India *Parts of MS thesis
**E-mail: prithul02@yahoo.co.uk

ABSTRACT

Background: Glanders is a highly contagious and fatal zoonotic reportable antique disease of solipeds caused by the Gram-negative bacterium *Burkholderia mallei*. This disease has been eradicated from most of the western developed countries in the 20th century and its occurrence was reduced in endemic developing nations but recent reports on the occurrence of clinical cases and outbreaks of this disease in both the eradicated and endemic countries indicates that it has regained the status of a re-emerging disease in the world. However, the information on the occurrence of *B. mallei* infection is almost lacking in Bangladesh.

Objective: This study was conducted on the sero-surveillance and risk factors of *B. mallei* infection in indigenous working horses in Bangladesh

Materials and Methods: A cross-sectional study on the sero-surveillance and risk factors of *B. mallei* infection was carried out in 125 indigenous horses in the districts of Mymensingh and Tangail during January to August 2019. Individual serum samples were screened using Complement fixation test (CFT) at the OIE and National Reference Laboratory for Glanders, Germany and Enzyme linked immunosorbent assay (ELISA) at the National Research Centre on Equines, Haryana, India. Risk factors were identified using multivariable logistic regression analysis.

Results: The overall sero-prevalence of *B. mallei* infection in indigenous horses was found to be 10.4% (95% CI: 5.9 -17.5). None of the 13 CFT positive sera was positive with ELISA. The odds of *B. mallei* infection were 6.1 times (95%CI: 1.7-28.9) higher in horses with the history of skin lesion than those without skin lesion. Significantly higher odds of *B. mallei* infection (odds ratio: 5.8; 95% CI: 1.4-39.7) were observed in horses with the history of parasitic infestation than those without parasitic infestation.

Conclusions: The relatively higher prevalence of *B. mallei* infection observed in this study should be interpreted with caution as all CFT positive samples negative with ELISA indicating some false positive reactions. Further studies are needed to test the accuracy of the serological tests for the detection of *B. mallei* infection in horses in Bangladesh.

Keywords: *Burkholderia mallei* infection, Carrier and latent infections, Sero-surveillance, Indigenous horses, CFT, ELISA, Bangladesh, Review, Validation, Serotests

Article Info: Article Code No. © LEP: JVMOHR/0017/2020
Received: 11 Feb. 2020 Revised: 10 March 2020 Accepted: 20 March 2020 Published: 30 June 2020

Citation: Das AC, Rahman AKMA, Tithy NS, Maruf AA, Bhattacharjee PK, Singha H, Neubauer H, Elschner M and Rahman MS (2020). Sero-surveillance and risk factors of *Burkholderia mallei* infection in indigenous horses of Bangladesh with a brief review on validation of serodiagnosis. *J. Vet. Med. OH Res.* 2 (1): 63-80 [DOI: 10.36111/jvmohr.2020.2(1).0017]

INTRODUCTION

Glanders is a highly contagious and fatal zoonotic disease, primarily affecting solipeds (horses, donkeys, asses and mules), caused by *Burkholderia mallei* and characterized by nodular lesions in the lungs and ulcerative and nodular lesions of the skin and respiratory mucosa.[1] It is an antique disease and Hippocrates was first described as a disease of horses in 450 BC and Aristotle grouped it under the generic term for epizootics ('melis' - Greek word or 'mallus' / 'malleus' in Latin word means severe disease) around 350 BC, from which the causative bacterium *Pseudomonas mallei* takes its name.[2-5] Many synonyms have been given to this disease including Cutaneous droes, Farcy (skin form), Farcy pipes, Farcy buds, Malleus or Equinia.[4,6] The glanders name has generally been accepted as the term of choice.[4] However, it has also been suggested that this disease is associated with enlargement and induration of the lymph nodes of the lower jaw and hence the name glanders from the French 'glandres' means glands.[7] Its bacterial etiology was established in 1882 when Friedrich Loffler and Wilhelm Schultz in Germany isolated and identified the causal agent from a horse dying of glanders, which they named the *Bacillus mallei* (now known as *Burkholderia mallei*). It has a 95% case fatality rate in untreated septicemia infections in equine and a 50% case fatality rate in treated human individuals, even diagnosed early.[8] It is regarded as a potential biological warfare or bioterrorism agent because of the high mortality rate and the small number of organisms needed to establish infection in humans and horses. Accordingly, this bacterium was one of the first biological warfare agents used in the 20th century which was used in the American Civil War and both the World Wars by infecting horses and subsequently soldiers.[9-11] Most recently, the former Soviet Union Army was also accused to have used weaponized *B. mallei* against opposition forces in the Afghan war between 1982 and 1984.[5,6,12] It is a notifiable disease under the Glanders and Farcy Act 1899 and currently, notification of OIE is compulsory for OIE member states. It is an important historic disease with high mortality rates in horses when large population of horses played a significant role in human transport and military campaigns and later on it is disappeared with the decreased of horse population and their uses. In addition, most of the developed nations have been eradicated this disease through large scale culling and slaughter, quarantine and pre-testing of animals for movement[13] but the regions of endemicity still exist in several countries in Eastern Europe, Asia, South America, Middle East and North Africa. However, recently several sporadic cases and outbreaks of glanders in horses have been reported from Afghanistan, Bahrain, Brazil, Eritrea, Germany, India, Iran, Kuwait, Lebanon, Mongolia, Myanmar, Nepal, Pakistan, Russia and Turkey.[14-20] It indicates that the glanders has regained the status of a re-emerging disease in both the endemic and previously eradicated countries.[5,21,22] Glanders is a primary disease of solipeds and humans but it also occurs in carnivores, small ruminants, whereas cattle and pigs are considered resistant.[23] However, the reservoirs of B. *mallei* are solipeds, particularly horses and the chronically affected horses can be asymptomatic carrier and latent but remain highly infectious.[16] So far, no clinical glanders case has been reported either in horses or humans from Bangladesh[24] but found seropositive horses.[25] This paper compares the CFT and i-ELISA for the sero-surveillance of *B. mallei* infection in indigenous horses in Bangladesh with a brief review on validation of the sero-diagnosis.

MATERIALS AND METHODS

A cross-sectional study using 125 randomly selected horses from six upazilas of two districts-Mymensingh (n = 83) and Jamalpur (n = 42) was carried out for a period of eight months from January to August 2019. Venous blood (5 to 7 ml / animal) was collected from each of the 125 randomly selected horses by using sterile disposable syringe. Samples were placed on a tray undisturbed for one hour at room temperature in a slightly inclined position to facilitate clotting and separation of serum. The clotted blood samples were then stored overnight at 4 °C. Sera were transferred to a fresh test tube and centrifuged at 2,500 rpm for 10 minutes. The clear supernatants were transferred to fresh tubes and sent equal volume to laboratory of Germany and India with adequate measures.

Primary data was collected through personal face-to-face interviews with the horse owners. For each sample, the following data were collected ① age group [< 6 years, ≥ 6 years], ② gender [male / female], ③ history of respiratory signs (nasal discharge and / cough) in last 3 months [yes / no], ④ parasitic infestation [yes / no], ⑤ history of skin lesions (nodules and / ulcers and / abrasions) in last 3 months [yes / no], ⑥ lameness and arthritis [yes / no] suggestive of glanders. Those risk factors were selected after informal discussion with equine owners.

Complement fixation test (CFT)

The CFT was performed at the OIE and National Reference Laboratory for Glanders, Friedrich-Loeffler-Institute, Germany (CFT) as described in the OIE manual.[1] Briefly, serum samples were diluted 1 : 5 in CFT buffer (Institute Virion / Serion GmbH), inactivated and two fold dilutions of them were mixed with Malleus CFT antigen (Ccpro GmbH) and five complement hemolytic units-50% of guinea pig complement (Institute Virion / Serion GmbH). Sera, complement and antigen were mixed in the plates and incubated overnight at 4 ^{0}C. A 2% suspension of sensitized (amboceptor from Institute Virion / Serion GmbH) sheep red blood cells (Labor Dr. Merk & Kollegen) were added and plates were incubated for 45 minutes at 37 ^{0}C and then centrifuged for 5 minutes at 600g. Samples with 100% hemolysis in a dilution of 1 : 5 were categorized as negative, samples showing 100% inhibition of hemolysis in a dilution of 1 : 5 were classified as positive. All suspicious test results (25-75% hemolysis) were classified as positive.[26]

Enzyme-linked Immuno-sorbant Assay (ELISA)

The indirect ELISA was performed at the National Research Centre on Equines, Haryana, India as described.[27] Briefly, the optimal concentration of ELISA reagents (TssB protein, serum, and secondary antibody dilution) was determined by checkerboard titration method using known glanders positive and negative equine serum. The optimal ELISA reagents concentration was assumed to be for those showing the highest discrimination between positive and negative serum. After optimization of the assay, 125 equine serum samples were assayed by ELISA. Pool of four positive and four negative serum samples were included in each ELISA plate along with field serum samples for monitoring the accuracy of the assay. The 96-well microtiter plates (Greiner Bio One, USA) were coated with TssB protein for overnight at 4 ^{0}C followed by washing with PBS-T and blocking with 6% skimmed milk in PBS-T for 1 hour at

37 ^{0}C. Serum samples were diluted in dilution buffer (2% (w/v) skimmed milk in PBS-T) and 100 μl of diluted serum was added in each well. The plates were incubated for 1 hour at 37 ^{0}C and washed five times with PBS-T. The diluted anti-horse conjugate (100 μl) was added in each well and incubated for 1 hour at 37 ^{0}C. Following washing steps, 100 μl of substrate solution containing 200 μmol of orthophenylene-diamine in citrate perborate buffer (Sigma-Aldrich, USA) was added in each well and plate was kept in dark at 37 ^{0}C for 10 minutes. Finally, the reaction was stopped by addition of 50 μl of 1MH_2SO_4 and the absorbance was read at 492 nm in a microtiter plate reader (Titertek Multiskan, Finland).

Statistical analysis

Data were processed and recorded by MS office excel (Office 10) and analyzed by Epi Info™ (Epi Info 7). Pearson chi-square test and Fisher Exact test (where expected count was less than 5) were used to evaluate the univariable association between seropositivity for *B. mallei* and its potential categorical predictors mentioned earlier. Multiple logistic regression analysis was used to identify risk factor for glanders. Variables with $p \leq 0.05$ in multivariable analysis were recorded as risk factor. Categories with lowest prevalence were used as reference.

RESULTS

Out of 125 horse serum samples tested, of which 13 (10.4 %, 95% CI: 5.9 –17.5) were found positive by using CFT and none of the samples was positive with ELISA (Table 1 & Photo 1 & Photo 2). The CFT positive animals were found in both of the Mymensingh (10.98%) and Jamalpur (9.30%) districts but no significant difference (OR: 1.20, CI: 0.34-4.15, $p > 0.05$) was

Table 1. Comparison of different serodiagnostic tests used for the detection of sero-surveillance of *Burkholderia mallei* infection of indigenous horses in Bangladesh

SN	Districts with Upazila	Complement fixation test (CFT) Earlier report[25]		Current study		Immunoblot Earlier report[25]		ELISA Current study	
		No. tested	Positive No. (%)	No. tested	Positive No. (%)	No. tested	Positive No. (%)	No. tested	Positive No. (%)
①	Fulpur	-	-	14	02 (14.29)	-	-	14	0
②	Muktagachha	-	-	22	03 (13.64)	-	-	22	0
③	Mymensingh Sador	-	-	35	03 (08.57)	-	-	35	0
④	Trishal	-	-	11	01 (09.09)	-	-	11	0
Total: Mymensingh		**115**	**043 (37.4)**	**083**	**09 (10.98)**	**115**	**11 (25.26)**	**83**	**0**
①	Jamalpur Sadar	-	-	23	02 (08.69)	-	-	23	0
②	Melandah	-	-	20	02 (10.00)	-	-	20	0
Total: Jamalpur		**110**	**042 (38.2)**	**042**	**04 (09.52)**	**110**	**13 (30.90)**	**42**	**0**
Total: Tangail		**076**	**020 (26.3)**	**-**	**-**	**076**	**02 (10.00)**	**-**	**-**
Overall		301	105 (34.9)	125	13 (10.40)	301	26 (08.64)	125	0

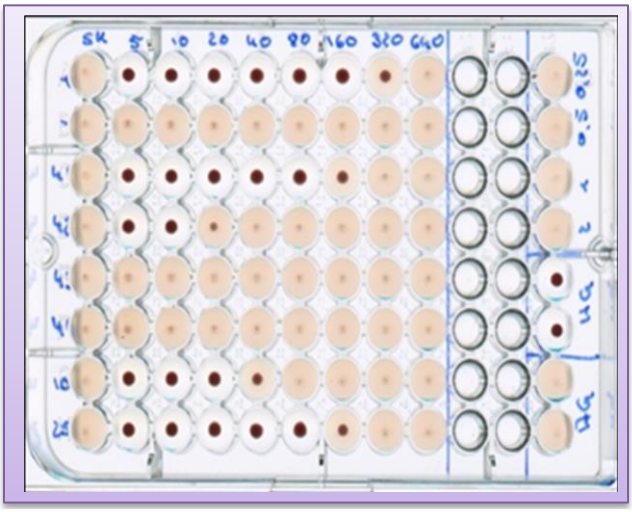

Fig. 1: CFT- the pattern of hemolysis at different dilution (1:5 to 1:640). Row 1= Negative control, 2 = Positive control, 3-4 & 7-8 Negative field samples and 5-6 = Positive field samples.

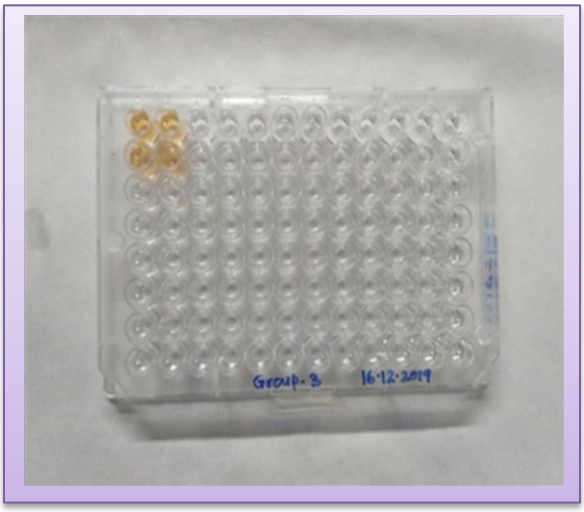

Fig. 2 : ELISA test to detect *B. mallei* antibodies. Row 1 & 2 (1st 2 colored wells) - Positive control. Other all tested wells- Negative results

found between the two districts (Table 1). Table 1 also shows that the Fulpur upazila had the highest sero-prevalence of 14.29%, followed by Muktagacha (13.64%), Melandah (10%), Trishal (9.09%), Jamalpur Sadar (8.69%) and Mymensingh Sadar (8.57%).

The univariate analysis reveals significant ($p < 0.05$) association of sero-positive *B. mallei* with history of respiratory signs, history of parasitic infestation and history of skin lesions (Table 2). Respiratory signs within last 3 months were included in the study and coughing, sneezing, dyspnea, nasal discharge, epistaxis, abnormal lung sound were considered as respiratory illness. Presence of nodules, ulceration, superficial or deep abscess or any kind of skin injury was recorded as skin lesions in the study. It was also evident from the analysis that animals with endo-parasitic and ecto-parasitic infestation were more likely to be seropositive. The odds of *B. mallei* infection were 6.1 times (95% CI : 1.7-28.9) higher in horses with the history of skin lesions than those without skin lesions (Table 2). Significantly higher odds of *B. mallei* infection (Odds ratio: 5.8 ; 95% CI: 1.4-39.7) were observed in horses with the history of parasitic infestation than those without parasitic infestation (Table 2).

DISCUSSION

The world's horse population is likely to be around 60 million[28] but it seems to be quite low in Bangladesh in comparison to other livestock animal species and it might be the reason that the horse population does not appear in both the national and international livestock statistics.[28-32] However, some year-wise horse population of Bangladesh has been reported to be varied from 43 to 46 thousands during the period from 1969 to 1990.[33,34]

Horses are very sparsely distributed throughout Bangladesh with a noticeable concentration in greater Sylhet, Tangail, Kishorgonj and greater Dhaka districts. The socio-economic status of horse owner in the districts of Mymensingh, Tangail, Sherpur and Jamalpur showed that they

Table 2. Risk factors associated with *Burkholderia mallei* infection based on Pearson's Chi-square test

SN	Variables	Sub-variables	Total No. tested	CFT +ve No. (%)	CFT -ve No. (%)	Odds ratio (OR)	95% CI of OR	p value
1	Age (years)	< 6	42	07 (16.7)	35 (83.3)	2.6	0.8 - 8.5	0.11
		≥ 6	83	06 (07.2)	77 (92.8)	Reference	-	-
2	Gender	Male	69	08 (11.6)	61 (88.4)	1.3	0.41- 4.7	0.62
		Female	56	05 (08.9)	51 (91.1)	Reference	-	-
3	History of RS	Yes	26	06 (23.1)	20 (76.9)	3.9	1.20 - 13.2	0.02
		No	99	07 (07.1)	92 (92.9)	Reference	-	-
4	History of PI	Yes	72	11 (16.4)	56 (83.6)	5.5	1.4 - 36.5	0.03
		No	53	02 (03.4)	56 (96.6)	Reference	-	-
5	History skin lesions	Yes	51	10 (19.6)	41 (80.4)	5.8	1.7- 26.8	0.01
		No	74	03 (04.1)	71 (95.9)	Reference	-	-
6	Lameness & arthritis	Yes	03	01 (33.3)	02 (66.7)	4.58	0.39 -54.37	0.28
		No	122	12 (09.8)	11 (90.2)	Reference	-	-

RS = Respiratory signs PI = Parasitic infestation

are mainly used for pulling cart, transportation, land tillage and sport purposes for their livelihood.[35] Horses are also used as horse-drawn carriages,[36] horse rent for wedding / marriage ceremony,[37] horseback riding[38] and racing[39] in Bangladesh. In addition, there are some horse establishments like President's Guard Regiment, National Police Head Quarter, Remount Veterinary and Farm Core (RVFC), Sarda Police Academy, Bangladesh Military Academy where small number of exotic horses are maintained for various purposes. Recently, six trained horses have been imported from India for the Bangladesh Police through Benapole Port on February 4, 2020.[40]

The horses of Bangladesh cannot be defined under any specific breed and these indigenous and non-descriptive horses have been influenced genetically by Arabian and Persian horses which have been immigrated through India from the west but their numbers are not known.[41] However, Bangladeshi horses are observed to be close to those of the Thai native horse which are direct descendants of the Chinese South Western mountain ponies. Thus, it can be said that the native horses of Bangladesh have belonged to the lineage of the South East Asian ponies rather than that of Arabian and Persian horses.[34] However, it claims that Bangladesh has only two breeds of horses: the Bangladesh native horse and the Rajshahi pony. The Bangladesh native is of the South-East Asian pony type and is said to have been influenced by Arabian and Persian horses migrating from the west through India. The Rajshahi pony is of unknown origin which is described as dwarf, standing 109-113 cm tall and is found mainly in Rajshahi, Jessore, Tangail, Mymensingh, Sylhet and Dhaka districts.[42]

Glanders is caused by the bacterium *Burkholderia mallei*[43] and has been variously classified in the past as *Bacillus mallei*,[44] *Loefflerella mallei* and then *Pfeifferella mallei*,[2] *Malleomyces*,[45] *Pseudomonas*[46-48] and *Actinobacillus*.[49] The new name *Burkholderia mallei* was based on the 16s rRNA sequences, DNA-DNA homology values, cellular lipid and fatty acid composition and phenotypic characteristics.[43] The characteristics of 14 equine isolates of *B. mallei* have been reported.[50]

Glanders is a highly communicable disease of equids but acute in donkeys and mules whereas mostly the chronic in horses and the recovered animals remain carrier.[51-52] This infection has also been reported in sheep and goats,[53] wild animals, canids and felids.[54-56] Guinea pigs and hamsters are reported to be highly susceptible and caused mortality with 24 hours following inoculation[4] whereas pigs and cattle have reported to be resistant.[23]

This organism can invade its host through mucous membrane, gastro-intestinal tract and the integument. Glanders is pathologically characterized by ulcerating nodular lesions of the skin and mucous membrane and clinically characterized by fever, malaise, depression, cough, anorexia and weight loss.[5]

Glanders is primarily classified into two forms include clinical and sub-clinical but it can also be classified into four forms: cutaneous, pulmonary, nasal and asymptomatic carrier.[57]

Cutaneous glanders may result from skin injury or may be due to a secondary manifestation of the respiratory form. It consists of nodules, pustules and ulcers that occur over any part of the body but most frequently observed on the legs.[57,58] The lesions appear as nodules in chains along the lymphatic vessels, which tend to break down and form crater-like ulcers discharging thick yellowish viscid and sticky purulent material heavily laden with the organism. This is usually referred to as farcy pipes. It induces a neutrophilic leukocytosis and severe anemia probably because of depressed erythropoietic activity of the bone marrow.[59]

The pulmonary form is most commonly associated with the formation of round, greyish, firm, encapsulated nodules embedded throughout the lung tissue and clinically characterized by acute broncho-pneumonia with cough and high fever.[60]

The nasal form is the formation of nodules or ulcers in the upper air passages commonly on the lower parts of the turbinate and on the cartilaginous nasal septum. It is clinically characterized by bloody muco-purulent nasal discharge when those nodules rupture.

The asymptomatic carrier form of glanders develops after a period of illness of some months. The affected animal makes an apparent recovery but persists as an occult case. Mallein test is positive but no obvious skin lesions can be seen.[57]

Equine glanders may be diagnosed based on clinical signs, the Mallein test, serological tests and bacterial isolation.[52,61-63] The isolation and identification of *B. mallei* bacterium from cutaneous lesions, lymph nodes, and nasal and respiratory exudates are considered to be the 'gold standard' method for diagnosis of glanders.[26]

The majority of the glanders cases (approximately 90%) occur as asymptomatic latent and carrier forms under field condition that make the difficulty to diagnose these sub-clinical glanders on the bacteriological method.[1] Accordingly, various immune-diagnostic tests have been used for the diagnosis of the asymptomatic glanders which include the Agar-gel immunodiffusion (AGID) test,[64] Counter-immuno-electro-phoresis (CIE) test,[65] Indirect

fluorescent antibody (IFA) test,[66] Indirect haemagglutination test (IHA) test,[50,67,68] Rose Bengal test,[68] Complement fixation test = CFT,[64,68-71] Enzyme linked immunosorbent assay = ELISA,[69,72-75] and Immunoblot.[76]

The accuracy of the sero-diagnosis tests is challenging due to false-positive and false-negative test results.[26,75] However, there is a general agreement that the CFT is superior to other serological tests.[1,64,73,77] CFT can detect carriers and chronically infected animals and still the only OIE mandatory serological test for international trade of equids and also recommended for sero-surveillance.[1,5] The CFT is known to have high sensitivity but it gives a considerable number of false-positive results.[26,78] It may give a positive cross-reaction in horses suffering from strangles, equine influenza or petechial fever and in emaciated horses not suffering from glanders.[79] These limitations of the CFT might be due to use of non-standardized antigen that caused different diagnostic accuracies. The CFT antigens have also been evaluated and the selection of antigens used for CFT has also to be updated and standardized.[18,80] CFT and other conventional serological tests have used crude whole cell preparation of *B. mallei* antigens which caused to false-positive results because of cross-reactive antigens.[70,78]

ELISA has been developed for the diagnosis of glanders in horses.[69,70,72,73,81] The ELISA has also been compared with CFT, AGID and HA test with culture results, and the results showed that ELISA correctly identified 100% of confirmed clinical cases of horses with glanders where other tests gave only 90.0% positive reaction.[73] Accordingly, it has been suggested that ELISA could be routinely adopted as a highly sensitive diagnostic test for glanders. However, these serological tests are not only incapable of discriminating between *B. mallei* and *B. pseudomallei* antibodies, they are also unable to differentiate between malleinized and naturally infected animals.

The accuracy of the CFT which is prescribed for international trade by the OIE which has also been compared with different ELISA based on recombinant antigens (TssA, TssB, BimA and Hcp1), the IDVet ELISA is based on a semi-purified fraction of *B. mallei* and Western blot (WB) with use of a purified LPS-containing *B. mallei*-antigen. ELISA based on TssA, TssB and BimA antigens had significantly lower sensitivity compared to CFT while the sensitivity of the Hcp1-ELISA, the IDVet-ELISA and the WB did not differ significantly from that of the CFT.[26]

The low sensitivity and specificity of the conventional sero-tests including the CFT and ELISA has been linked with crude antigens of whole cells. Therefore, the false-positive results with crude antigens lead to financial losses to animal owners and false-negative results can turn a risk into a possible threat.[82] To improve the sensitivity and specificity of the sero-diagnosis and reduce cross-reaction with closely related melioidosis, the purified recombinant proteins of *B. mallei* has been reported in an indirect ELISA format with two recombinant proteins, 0375H and 0375TH that exhibited 100% sensitivity and specificity for the diagnosis of glanders.[82]

The Western blot (WB) and ELISA have also been evaluated to overcome the disadvantages of the CFT but these tests have not been fully validated in large scale studies.[27,76,83-85] Comparative evaluation CFT, WB and five different ELISAs have been reported that the WB and all ELISAs except BimA antigen had significantly more specific than the CFT, whereas ELISAs based on TssA, TssB and BimA antigens had significantly lower sensitivity compared

to CFT and the sensitivities of the Hcp1-ELISA, the IDVet-ELISA and the WB did not differ significantly from that of the CFT.[26] However, all these evaluated serological diagnostic tests are unable to differentiate between *B. mallei* and *B. pseudomallei* in the regions and country where both the close phylogenetic bacteria prevalent endemically. However, combined use of both serological and molecular detection methods increases the detection rate of glanders.[43,86,87]

The CFT and indirect ELISA were used for the detection of antibodies against *B. mallei* infection in indigenous horse serum of Bangladesh. Of the 125 serum samples of apparently healthy indigenous horses screened with CFT and i-ELISA, of which only 10.4% animals found positive with CFT but none of the samples was positive with i-ELISA. These findings support the best test sensitivity results for the CFT followed by WB, Hcp1- and IDVet-ELISAs for the serodiagnosis of glanders in horses.[26] The lack of specificity of the CFT especially in areas where false positive rate is very high that results in constant obstacles to trade in horses.[88] However, the comparative sensitivities and specificities of the recommended sero-tests for *B. mallei* infection in horses have been reported as 98.0% and 96.4% with CFT, 96.8% and 99.4% with the WB, 95.3% and 99.6% with the Hcp1-ELISA and 92.5% and 99.5% with the IDVet-ELISA, respectively.[26] Therefore, a confirmatory test for *B. mallei* infection with both the higher sensitivity and higher specificity would be developed to meet OIE requirements to avoid an introduction of infected animals into disease free populations at national and international levels.[79]

The CFT and Mallein test subcutaneously, intracutaneously or ophthalmically and the resulting fever, swelling or reflux of pus from the eye in positive animals have historically been used as indirect diagnosis methods of equine glanders for the eradication of this disease.[5]

Glanders which is eradicated from most of the Western Europe and Northern American countries including Australia, Japan and some other countries in the last century, it has regained the status of a re-emerging disease because of the numerous recent outbreaks throughout South American and Asian countries.[4,5,85,89-91] However, cases of glanders still periodically occur in South America, Eastern Europe, certain parts of Asia, the Middle East, northern Africa and various Mediterranean regions and Indo-Pakistan sub-continent.[92-95] The major outbreaks of this disease were reported between 1976 to 1982, followed by sporadic cases in 1988, 1990 and 1998 in India and remained free for eight years and re-emerging of outbreaks occurred during 2006 to 2011 in India.[22,96] The re-emerging of glanders cases have been reported in Maharastra, Haryana and Punjab after a gap of 10 years with an overall sero-prevalence of 0.62% to 1.15% with Hcp1 indirect ELISA followed by confirmatory diagnosis by CFT during 2015 to 2018.[97] Lack of awareness, inadequate veterinary care and unrestricted movement of equids across state borders might have led to the introduction and establishment of the infection in these states. This base line data on glanders are suggesting for devising surveillance and control strategies.

Horse, donkeys and mules are the only known natural reservoir of *B. mallei*. Asymptomatic or carrier animals are the potential source of reintroduction of the infection into glanders free areas and countries that caused trade restrictions with animals and products from regions or outbreak areas or countries.[88] Recently it has been reported that *B. mallei* can persist as latent infection similar to *B. pseudomallei* which may not be detected by the currently used diagnostic tests.[88] The CFT has been recommended by the OIE for international trade in equids but this

test has been reported to have varying sensitivities and specificity depending on the antigen and methodology used. False positives are problematic for the horse owners and veterinary authority whereas false negatives may allow the reintroduction of *B. mallei* into free areas and countries. The limitations of the currently available diagnostic tests and epidemiological knowledge on glanders indicate that infection with *B. mallei* remains a major risk in the context of international movement of equids.[88]

The persistence of *B. mallei* infection in horse population poses a potential threat to occupational exposed human especially equine handlers and Veterinarians. The positive sera with positive isolation of *B. mallei* are very difficult to collect from field cases because to pose a zoonotic risk and spread of infection to equine population and accordingly, the clinically glanders positive cases are usually destroyed immediately before samples can be collected.[26] However, the WB has been used to exclude false-positive CFT results as a confirmatory test on CFT-positive sera and use of a combination of tests can enhance the accuracy for sero-diagnosis.[26] However, the replacement of CFT with the WB or any of the ELISAs cannot be recommended for testing equids for national and international trade purposes.[26] Under these situations, the importation of horses from glanders endemic region of India for the Bangladesh Police might be dangerous like importation of horses from Brazil to Germany.[40,98]

The present study also represents identification of various risk factors associated with sero-positivity of *B. mallei* infection of horse in Bangladesh. The results of this study suggest that the presence of *B. mallei* infection related symptoms, history of lesions on skin, age, presence of parasitic infection and history of respiratory signs are highly significantly associated with *B. mallei* infection sero-positive horses of Mymensingh and Jamalpur districts in Bangladesh.

Of the 125 serum samples tested with CFT and i-ELISA, of which only 13 (10.4%) horses showed positive to *B. mallei* infection with CFT but none with ELISA. The CFT was performed at the OIE and National Reference Laboratory for Glanders, Germany and i-ELISA at the National Research Centre on Equines, India. This difference of results of these two tests conducted in two different countries might be due to different antigens used, sample was preserved for longer period of time, longer transportation period of the samples and also presence of different antigenic variance of the *B. mallei* organisms in the country where the sample were tested or other epidemiological factors.[12] However, comparatively higher sero-positivity of *B. mallei* infection in horses in Bangladesh has been reported in our earlier report with CFT (34.9%) and immunoblot (24.8%) techniques.[25] The presence parasitic infection and history of skin lesions were found to be significantly ($p < 0.05$) associated with *B. mallei* infection. The lower sero-prevalence was recorded in the adult age (≥ 6 years) group (6.02%) than the young (< 6 years) age group (19.05%) of horses. This could be due to the fact that CFT gives a false-negative result in older animals due to poor immune response and more susceptible to the infection.[4] Animals with history of lesions on skin were found at 5.77 times higher risk than the animals with no skin lesions in the conducted study. This may correspond to the fact that disease is chronic in horses evidenced by the skin nodules and hematological features.[58] Also horse with parasitic infestation were found apparently about four times more likely to be *B. mallei* seropositive as compared to horse without parasitic infestation. This may be due to most the farmers do not treat their horses with anthelmintic regularly and hardly use

any disinfectants to clean the water troughs and stables. Communal water troughs and stables serve as a reservoir of *B. mallei* infection.[99] The secretions of the affected animals may contaminate the water and transfer the infection to others in-contact animals.

CONCLUSIONS AND RECOMMENDATION

The risk factors associated with *B. mallei* infection in horses can be strengthened by increasing the number of samples and also ensuring a more representative sample including mules and donkeys. The relatively higher sero-prevalence of *B. mallei* infection recorded in indigenous horses should be interpreted with caution as all the CFT positive samples were negative with ELISA indicates some CFT positive samples might be false positive. Further studies are needed with highly sensitive and specific test to detect the accurate sero-prevalence status of *B. mallei* infection in indigenous horses in Bangladesh. The risk factors associated with *B. mallei* infection in indigenous horses should be emphasis on further studies on sero-surveillance, disease awareness and control. However, counter-measures against *B. mallei* infection include early detection of disease in susceptible animals, stringent quarantine measures, testing and safe destruction of infected carcasses, adequate compensation to the animal owners, disinfection of infected premises and awareness about glanders and the zoonotic implications through veterinary extension services.

CONFLICT OF INTEREST

No conflict of interest to declare

ACKNOWLEDGEMENTS

The research work was supported under the special allocation for science and technology for the financial year 2019 to 2020 (Sanction Order 39.00.0000.09.090.18-19/BS-332, date 14.01.2019), Ministry of Science of Technology, Government of the Peoples' Republic of Bangladesh, Bangladesh Secretariat, Dhaka. The authors are grateful to Prof. Dr. M. A. Samad, Editor, JVMOHR for writing assistance and editing this article.

REFERENCES

01. OIE (2018). Glanders and melioidosis. Chapter 3.5.11. OIE Terrestrial Manual 2018. 1350-1362. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.05.11_GLANDERS. pdf

02. Henning MW (1956). *Animal Diseases in South Africa*. 3rd edn., Central News Agency, Pretoria, South Africa. Pp. 159-181

03. Sharrer T (1995). The great glanders epizootic, 1861-1866- A civil war legacy. *Agricultural History* 69: 79-97 [jstor.org/stable/3744026?seq=1]

04. Al-Ani FK and Robertson J (2007). Glanders in horses: A review of the literature. *Veterinarski Arhiv* 77: 203-218

05. Khan I, Wieler LH, Melzer F, Elschner MC, Muhammad G, Ali S, Sprague LD, Neubauer H and Saqib M (2013). Glanders in animals: A review on epidemiology, clinical presentation, diagnosis and countermeasures. *Transboundary and Emerging Diseases* 60: 204-221 [doi: 10.1111/j.1865-1682.2012.01342.x]

06. Zandt KEV, Greer MT and Gelhaus HC (2013). Glanders: an overview of infection in humans. *Orphanet Journal of Rare Diseases* 8: 131 [doi: 10.1186/1750-1172-8-131]

07. Shiel WC (2018). Medical definition of glanders. Medicinenet.com/script/main/art.asp?articlekey=13712

08. Judy BM, Whitlock GC, Torres AG and Estes DM (2009). Comparison of the *in vitro* and *in vivo* susceptibilities of *Burkholderia mallei* to ceftazidime and levofloxacin. *BMC Microbiology* 9: 88 [doi: 10.1186/1471-2180-9-88]

09. Neubauer H, Maeyer H and Finke EJ (1997). Human glanders. *International Review of the Armed Forces Medical Services* 70: 258-265

10. Wheelis M (1998). First shots fired in biological warfare. *Nature* 395(6699): 213

11. Whitlock GC, Estes DM and Torres AG (2007). Glanders: off to the races with *Burkholderia mallei*. *FEMS Microbiology Letters* 277: 115-122 [doi: 10.1111/j.1574-6968.2007.00949.x]

12. Gilad J, Harary I, Dushnitsky T, Schwartz D and Amsalem Y (2007). *Burkholderia mallei* and *Burkholderia pseudomallei* as bioterrorism agents: national aspects of emergency preparedness. *Israeli Medical Association Journal* 9: 499-503

13. Derbyshire JB (2002). The eradication of glanders in Canada. *Canadian Veterinary Journal* 43: 722-726

14. Robert H, Lopez M and Hancock R (2010). International disease monitoring, April to June. *Veterinary Record* 167: 192-195

15. Saqib M, Muhammad G, Naureen A, Hussain M, Asi MN, Mansoor MK, Toufeer M, Khan I, Neubauer H and Sprague LD (2012). Effectiveness of an antimicrobial treatment scheme in a confined glanders outbreak. *BMC Veterinary Research* 8, Article NO. 214 [doi: 10.1186/1746-6148-8-214]

16. Scholz HC, Pearson T, Hornstra H, Projahn M, Terzioglu R, Wernery R, Georgi E, Riehm JM, Wagner DM, Keim PS, Joseph M, Johnson B, Kinne J, Jose S, Hepp CM, Witte A and Wernery U (2014). Genotyping of *Burkholderia mallei* from an outbreak of glanders in Bahrain suggests multiple introduction events. *PLoS Neglected Tropical Diseases* 8 (9): e3195 [doi: 10.1371/journal.pntd.0003195]

17. Neubauer H, Elschner MC and Elisabeth LT (2016). Equine glanders: A diagnostic approach in Germany. Researchgate.net/publication/292143603_Equine_glanders_A_diagnostic_approach_in_Germany

18. Malik P (2016). Harmonising diagnostic testing for glanders in equids. *Veterinary Record* 178: 630-631 [doi: 10.1136/vr.i3093]

19. Adhikari N, Acharya KP and Wilson RT (2019). The potential for an outbreak of glanders in Nepal. *Tropical Medicine and Health* 47:57 [doi: 1186/s41182-019-0185-2]

20. Perrin L, Gauntlett F and Roberts H (2020). Preliminary outbreak assessment: Glanders in Turkey. assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/857218/glanders-turkey-poa-jan2020.pdf

21. Pawaiya RVS and Chauhan RS (2008). A review of glanders- a re-emerging zoonosis in India. Indian *Journal of Veterinary Pathology* 32: 1-14

22. Verma AK, Saminathan M, Neha, Tiwari R, Dhama K and Singh SV (2014). Glanders- A re-emerging zoonotic disease: A Review. *Journal of Biological Sciences* 14: 38-51

23. OIE (2020). Glanders. oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_ cards/GLANDERS.pdf

24. Sikder S, Rahman AMK, Rima UK, Alim MA, Das S, Das BC and Faruque MR (2012). Equine diseases: A retrospective prevalence study in farm conditions in Bangladesh. *Wayamba Journal of Animal Science.* Article No. 1329045675 [http://www.wayambajournal.com]

25. Rahman MS, Bhattacharjee PK, Sarker RR, Parvin MS, Tasnin S, Sarker MAS, Neubauer H, Khatun F, Wares MA, Nishidale I and Elschner MC (2018). Glanders in horses in some selected areas of Bangladesh and comparison between CFT and immunobloat used for the screening of glanders. *Indian Journal of Animal Research* pp.1-4 [doi: 10.18805/ijar.B-976]

26. Elschner MC, Laroucau K, Singha H, Tripathi BN, Saqib M, Gardner I, Saini S, Kumar S, El-Adawy H, Melzer F, Khan I, Malik P, Sauter-Louis C and Neubauer H (2019). Evaluation of the comparative accuracy of the complement fixation test, Western blot and five enzyme-linked immunosorbent assays for serodiagnosis of glanders. *PLoS ONE* 14 (4): e02214963 [doi: 10.1371/journal.pone.0214963]

27. Singha H, Malik P, Goyal SK, Khurana SK, Mukhopadhyay C, Eshwara VK and Singh RK (2014). Optimization and validation of indirect ELISA using truncated TssB protein for the serodiagnosis of glanders amongst equines. *The Scientific World Journal*. Article ID 469407 [doi: 10.1155/2014/469407]

28. Clarkson N (2017). World horse population likely to be over 60 million, figures suggest. horsetalk.co.nz/2017/07//10/world-horse-population-60-million/

29. FAO (2005). Livestock Sector Brief: Bangladesh. fao.org/ag/againfo/resources/en/publications/sector_briefs/lsb_BGD.pdf

30. Banglapedia (2015). Livestock. en.banglapedia.org/index.php?title=Livestock

31. Khadka R (2010). Global horse population with respect to breeds and risk status. fao.org/tempref/AG/Reserved/DAD-Net/Rupak_Khadka_Thesis.pdf

32. DLS (2017). Livestock economy as a glance, DLS. dls.portal.gov.bd

33. FAO (1969-90). FAOs Livestock Statistics. Rome, Italy

34. Bhuiyan AKFH (2014). Farm Animal Genetic Resources in Bangladesh: Diversity, Conservation and Management. In book: Farm Animal Genetic Resources in SAARC Countries: Diversity, Conservation and Management (pp.1-74). SAARC Agriculture Center (SAC), Dhaka 1215, Bangladesh

35. Alam MP, Bhuiyan MSA and Bhuiyan AKMFH (2015). The socio-economic status of horse owners in the rural areas of Bangladesh. *The Agriculturists* 13: 46-52

36. Xinhua (2019). Horse-drawn carriages still in use in old quarter of Dhaka, Bangladesh. xinhuanet.com/English/asiapacific/2019-01/30/c_137785495_2.htm

37. ClickBD (2019). Horse or Ghora rent in Dhaka. clickbd.com/Bangladesh/2419836-horse-or-ghora-rent-in-dhaka.html

38. Anon (2020). Bangladesh (Dhaka): Horseback riding, riding stable, riding academy, trailriding, horse riding vacation. ride77.com/en/bangladesh

39. Rouf KMA (2018). 14-year-old girl from rural Bangladesh making waves in horse racing. dhakatribune.com/Bangladesh/nation/2018/03/08/14-year-old-girl-rural-bangladesh-making-waves-horse-racing/

40. Star D (2020). Trained horses imported from India for Bangladesh police. thedailystar.net/country/news/ trained-horses-imported-india-bangladesh-police-1863850

41. Nozawa K, Amano T, Namikawa T, Hasnath MA, Mostafa KG and Faruque MO (1984). Gene constitution of the native horses in Bangladesh. In: T. Amano (Ed). 1984. Genetic studies in breed differentiation of the native domestic animals in Bangladesh, Tokyo University of Agriculture, pp. 43-56

42. Porter V, Alderson L, Hall JG and Sponenberg DP (2016). Mason's World Encyclopedia of Livestock Breeds and Breeding, 2 Volumes, CABI, pp 1107

43. Yabuuchi E, Kosako Y, Oyaizu H, Yano I, Hotty H, Hashimoto Y, Ezaki T and Arakawa M (1992). Proposal of *Burkholderia* genus and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus. *Journal of Microbiology and Immunology* 36: 1251-1275

44. Sarker-Tyson M and Titball RW (2009). *Burkholderia mallei* and *Burkholderia pseudomallei*. In: Vaccines for Biodefense and Emerging and Neglected Diseass. Sciencedirect.com/topics/ veterinary-science-and-veterinary-medicine/glanders

45. Merchant IA and Packer RA (1967). *Veterinary Bacteriology and Virology*. 7th edn. Iowa State University Press, Ames, Iowa. Pp. 364-369

46. Wetmore PW and Gochenour WS (1956). Comparative studies on the genus *Malleomyces* and selected *Pseudomonas* species. *Journal of Bacteriology* 72: 79-89

47. Smith PB and Chery WB (1957). Identification of malleomyces by species bacteriophages. *Journal of Bacteriology* 74: 668-672

48. Rogul M, Brendle JJ, Haapala DK and Alexander AD (1970). Nucleic acid similarities among *Pseudomonas pseudomallei*, *Pseudomonas multivorans* and *Actinobacillus mallei*. *Journal of Bacteriology* 101: 827-853

49. Evans D (1966). Utilization of carbohydrates by *Actinobacillus mallei*. *Canadian Journal of Microbiology* 12: 625-639

50. Al-Ani FK, Al-Rawsashdeh OF, Ali HA and Hassan FK (1998). Glanders in horses: clinical, biochemical and serological studies in Iraq. *Veterinary Archive* 68 : 155-162

51. Mahadevan S, Dravidamani S and Dare BJ (1987). Glanders in horses. *Centaur* 3: 135-138

52. Theves G (1993). Glanders in Luxembourg and elsewhere from the 13th Century to the end of the 19th Century or the arduous advancement of ideas on an animal and human disease. *Annals of Medicine and Veterinary* 137: 469-481

53. Hu TP, Kung CD and Au BH (1958). Studies on experimental infection of cattle, sheep and goats with glanders bacilli. *Acta Veterinary Zootech Sinica* 3: 36-43

54. Galati P, Puccini V and Contente F (1973). An outbreak of glanders in lions: histopathological findings. *Acta Medica Veterinaria* 10: 26-27

55. Alibasoglu M, Yesildere T, Calislar T, Inal T and Calsikan U (1986). Glanders outbreak in lions in the Istanbul zoological garden. *Berliner und Munchener tierartztliche Woschenschrift* 99: 57-63

56. Khaki P, Mosavari N, Khajeh NS, Emam M, Ahouran M, Hashemi S, Taheri MM, Jahanpeyma D and Nikkhah S (2012). Glanders outbreak at Tehran Zoo, Iran. *Iran Journal of Microbiology* 4: 3-7 [pmid: 22783454]

57. Al-Ani FK, Al-Delami AK and Ali AH (1987). Glanders in horses: clinical and epidemiological studies in Iraq. *Pakistan Veterinary Journal* 79: 126-129

58. Muhammad G, Khan MZ and Athar M (1998). Clinico-microbiological and therapeutic aspects of glanders in equines. *Journal of Equine Science* 9: 93-96

59. Al-Kafawi AA, Al-Ani FK, Al-Bassam LS and Youkob AY (1977). Hematological changes in Arabian horses infected with glanders. *Veterinary Record* 101: 427

60. Zubaidy AJ and Al-Ani FK (1978). Pathology of glanders in horses in Iraq. *Veterinary Pathology* 15: 566-568

61. Allen H (1929). The diagnosis of glanders. *Journal of the Royal Army Veterinary Crops* 1: 241-245

62. Uppal PK and Yadav MP (1987). Equine respiratory diseases and their diagnosis. *Centaur* 4: 67-72

63. Pritchard DC (1995). Glanders. *Equine Veterinary Education* 7: 29-32

64. Sen GP, Singh G and Joshi TP (1968). Comparative efficacy of serological tests in the diagnosis of glanders. *Indian Veterinary Journal* 45: 286-292

65. Jana AM, Gupta AK, Pandya G, Verma RD and Rao KM (1982). Rapid diagnosis of glanders in equine by counterimmunoelectrophoresis. *Indian Veterinary Journal* 59: 5-9

66. Ma CI, Fan SM, Wang X, Jiang L and Fang SQ (1986). Diagnosis of glanders in horses by the indirect florescent antibody technique. *Chinese Journal of Veterinary Science and Technology* 9: 3-5

67. Gangulee PC, Sen GP and Sharma GL (1966). Serological diagnosis of glanders by haemagglutination test. *Indian Veterinary Journal* 43: 386-391

68. Naureen A, Saqib M, Muhammad G, Hussain MH and Asi MN (2007). Comparative evaluation of Rose Bengal plate agglutination test, mallein and some conventional serological tests for diagnosis of spontaneous equine glanders. *Journal of Veterinary Diagnostic Investigation* 19: 362-367

69. Verma RD, Sharma JK, Venkateswaran KS and Batra HV (1990). Development of an avidin-biotin dot enzyme-linked immunosorbent assay and its comparison with other serological tests for diagnosis of glanders in equines. *Veterinary Microbiology* 25: 77-85

70. Sprague LD, Zachariah R, Neubauer H, Wernery R, Joseph M, Scholz C and Wernery U (2009). Prevalence-dependent use of serological tests for diagnosis glanders in horses. *BMC Veterinary Research* 5:32 [doi: 10.1186/1746-6148-5-32]

71. Khan I, Wieler LH, Melzer F, Gwida M, de Assis Santana VL, de Souza MMA, Saqib M, Elschner MC and Neubauer H (2011). Comparative evaluation of three commercially available complement fixation test antigens for the diagnosis of glanders. *Veterinary Record* 169: 495 [doi: 10.1136/vrd5410]

72. Niederwohrmeier B and Bohm R (1990). Detection specificity of an optimal solid-phase enzyme immunoassay for *Pseudomonas aeruginosa* and *Pseudomonas mallei*. *Zentralbl. Veterinarmed* B 37: 684-695

73. Al-Ani FK, Al-Omran AH and Al-Zubaidy FS (1993). A micro-enzyme-linked immunosorbent assay (ELISA) for detection of antibody to *Pseudomonas mallei* infection in horses. *Pakistan Veterinary Journal* 13: 70-73

74. Katz JB, Dewald R and Nicholson J (2000). Procedurally similar competitive immunoassay systems for the serodiagnosis of *Babesia equi*, *Babesia caballi*, *Trypanosoma equiperdum* and *Burkholderia mallei* infection in horses. *Journal of Veterinary Diagnostic Investigation* 12: 46-50

75. Kumar S, Malik P, Verma SK, Pal V, Gautam V, Mukhopadhyay C and Rai GP (2011). Use of a recombinant Burkholderia intracellular motility a protein for immunodiagnosis of glanders. *Clinical and Vaccine Immunology* 18: 1456-1461 [pmid:21752949]

76. Katz JB, Chieves LP and Hennager SG (1999). Serodiagnosis of equine piroplasmosis, dourine and glanders using an arrayed immunobloating method. *Journal of Veterinary Diagnostic Investigation* 11: 292-294 [pmid: 10353365]

77. Verma RD (1990). A micro-complement fixation test for identification of *Pseudomonas mallei*. *Journal of Remount Veterinary Crops* 29: 1-5

78. Neubauer H, Sprague LD, Zacharia R, Tomaso H, Al-Dahouk S, Wemery R, Wernery U and Scholz HC (2005). Serodiagnosis of *Burkholderia mallei* infections in horses: state-of-the-art and perspectives. *Journal of Veterinary Medicine* 52: 201-205 [pmid:16115091]

79. Jacobson RH (1998). Validation of serological assays for diagnosis of infectious diseases. *Revue scientifique et technique Off international Epizootics* 17: 469-486

80. Laroucau K, Colaneri C, Jay M, Corde Y, Drapeau A, Durand B, Zientara S and Beck C (2016). Interlaboratory ring trial to evaluate CFT proficiency of European laboratories for diagnosis of glanders in equids. *Veterinary Record* 178: 632 [doi: 10.vr.103617]

81. Hagebock JM, Schlater LK, Frerichs WN and Olson DP (1993). Serologic responses to the mallein test for glanders in solipods. *Journal of Veterinary Diagnostic Investigation* 5: 97-99

82. Pal V, Kumar S, Malik P and Rai GP (2012). Evaluation of recombinant proteins of Burkholderia mallei for serodiagnosis of glanders. *Clinical and Vaccine Immunology* 19: 1193-1198 [doi: 10.1128/CVI.00137-12]

83. Elschner MC, Scholz HC, Melzer F, Saqib M, Marten P, Rassbach A, Dietzsch M, Schmook G, de Assis-Santana VL, de Souza MM, Wernery R, Wemery U and Neubauer H (2011). Use of a Western blot technique for the serodiagnosis of glanders. *BMC Veterinary Research* 7:4 [pmid:21247488]

84. Elschner MC, Liebler-Tenorio E and Neubauer H (2016). The diagnostic approach to equine glanders in Germany. *Equine Disease Quarterly* 25(1) [https//equimanagement.cm/news/ diagnostic-approach-equine-glanders-germany-31089]

85. Ghori MT, Khan MS, Khan JA, Rabbani M, Shabbir MZ, Chaudhry HR, Ali MA, Muhammad J, Elschner MC and Jayarao BM (2017). Seroprevalence and risk factors of glanders in working equines- findings of a cross-sectional study in Punjab province of Pakistan. *Acta Tropica* 176: 134-139 [doi: 10.1016/j.actatropica.2017.07.031; pmid:28760480]

86. Gee JE, Sacchi CT, Glass MB, De BK, Weyant RS, Levett PN and Whitney AM (2003). Use of 16SrRNA gene sequencing for rapid identification and differentiation of *Burkholderia pseudomallei* and *B. mallei. Journal of Clinical Microbiology* 41: 4647-4654

87. Godoy D, Randle G, Simpson AJ, Aanensen DM, Pitt TL, Kinoshita R and Spratt BG (2003). Multilocus sequence typing and evolutionary relationships among the causative agents of meliodosis and glanders, *Burkholderia pseudomallei* and *Burkholderia mallei*. *Journal of Clinical Microbiology* 41: 2068-2079

88. Kettle AN and Wernery U (2016). Glanders and the risk for its introduction through the international movement of horses. *Equine Veterinary Journal* 48: 654-658 [doi: 10.1111/evj.12599]

89. Arun S, Neubauer H, Gurel A, Ayyildiz G, Kuscu B, Yesildere T, Meyer H and Hermanns W (1999). Equine glanders in Turkey. *Veterinary Record* 144: 255-258 [doi: 10.1136/vr.144.10.255]

90. Wittig MB, Wohlsein P, Hagen RM, Al-Dahouk S, Tomaso H, Scholz HC, Nikolaou K, Wernery R, Wernery U, Kinne J, Elschner M and Neubauer H (2006). Glanders- a comprehensive review. DTW. *Deutsche Tierarztliche Wochenschrift* 113: 323-330

91. Malik P, Singha H, Goyal SK, Khurana SK, Tripathi BN, Datt A, Singh D, Sharma N and Jain S (2015). Incidence of *Burkholderia mallei* infection among indigenous equines in India. *Veterinary Record* 2: e000129.pmid:26457190

92. Vaid MY, Muneer MA and Naeem M (1981). Studies on the incidence of glanders at Lahore. *Pakistan Veterinary Journal* 1: 75

93. Ray DK (1984). Incidence of glanders in the horses of mounted platoon of 4th A.P. Bn. Kahilpara, Gauhati- A case history. *Indian Veterinary Journal* 61: 264

94. Krishna L, Gupta VK, and Masand MR (1992). Pathomorphological study of possible glanders in solipeds in Himachal Pradesh. *Indian Veterinary Journal* 69: 211-214

95. Nagal KB, Joshi VB, Katoch RC, Sharma M and Kumar RK (1995). Glanders outbreaks in Himachal Pradesh. *Centaur* 12: 30-35

96. Malik P, Khurana SK and Dwivedi SK (2010). Re-emergence of glanders in India- Report of Maharashtra state. *Indian Journal of Microbiology* 50: 345-348 [doi: 10.1007/s12088-010-0027-8]

97. Singha H, Shanmugasundaram K, Tripathi BN, Saini S, Khurana S, Kanani A, Shah N, Mital A, Kanwar P, Bhatt L, Limaye V, Khasa V, Arora R, Gupta S, Sangha S, Sharma H, Agarwal SK, Tapasen J, Parnam S, Dubey P, Baalasundaram SK, Mandal BN, Virmani N, Gulati BR and Malik P (2020). Serological surveillance and clinical investigation of glanders among indigenous equines in India from 2015 to 2018. *Transboundary and Emerging Diseases* [doi: 10.1111/tbed.13475]

98. Elschner MC, Klaus CU, Liebler-Tenorio E, Schmoock G, Wohlsein P, Tinschmann O, Lange E, Kaden V, Klopffeischen R, Melzer F, Rassback A and Neubauer H (2009). *Burkholderia mallei* infection in a horse imported from Brazil. *Equine Veterinary Education* 21: 147-150 [doi: 10.2746/095777309 ×401071]

99. Hornstra H, Pearson T, Georgia S, Liguori A, Dale J, Price E, O'Neill M, DeShazer D, Muhammad G, Saquib M, Naureen A and Krim PS (2009). Molecular epidemiology of glanders, Pakistan. *Emerging Infectious Diseases* 15**:** 2036-2039 [doi: 10.3201/eid1512.090738]