

Re-emergence of glanders in India – Report of Maharashtra state

Praveen Malik · S. K. Khurana · S. K. Dwivedi

Received: 25 September 2008 / Accepted: 7 March 2009
© Association of Microbiologists of India 2009

Abstract Glanders, a notifiable highly contagious disease primarily of equids, is a disease of high zoonotic importance. Caused by gram-negative bacillus, *Burkholderia mallei*, the disease was restricted to certain pockets of India with sporadic cases. Recently, a major outbreak of glanders occurred in India starting from Maharashtra. Following clinical signs and symptoms and laboratory investigations on serum, nasal swab and pus swab samples, it was confirmed as glanders among equines in Pune and Panchgani areas of Maharashtra. One pus sample and three nasal swabs yielded *B. mallei* isolates while 23 serum samples were found positive for glanders by complement fixation test (CFT). The disease was successfully controlled in the state by following strategies for prevention of spread of the disease to other areas in accordance with Glanders and Farcy Act, 1899. Follow up of the occurrence in Maharashtra revealed negative status based on testing and physical surveillance on more than 3,500 equines thereafter. Investigations indicated that the nidus of infection may be present elsewhere in North India.

Keywords Glanders · *Burkholderia mallei* · Equines · Horse · Complement fixation test

Glanders, a highly contagious disease primarily of equids, is a disease of high zoonotic importance and thus poses a significant human health risk. The disease is amongst the oldest known disease of equines and is notifiable in India for more than a century. In India, the disease has to be dealt within the purview of ‘Glanders and Farcy Act 1899’ whereby the cases should not be treated and control is done by test and elimination policy.

Glanders is caused by the gram-negative aerobic, non-motile bacillus *Burkholderia mallei* (previously classified variously as *Pseudomonas*, *Pfeifferella*, *Loefflerella*, *Malleomyces* or *Actinobacillus*) of family *Burkholderiaceae* [1]. The organism is closely related to the causal agent of melioidosis, *Burkholderia pseudomallei*. Unlike other organisms in the *Pseudomonas* group, *B. mallei* have no flagellae and are therefore non-motile. In culture media, they vary in appearance depending on the age of the culture and type of medium.

Glanders is reported from Middle Eastern countries, the Indian subcontinent, Southeast Asia, parts of China and Mongolia, and Africa. Sporadic cases are also reported from South America. Glanders was believed to have been used during World War I and World War II on horses, mules and humans [2]. Thus the organism has got a scope of bio-terrorism in the present day scenario. Since 1990, the numbers of glanders outbreaks in race, military and pleasure horses are steadily increasing and are currently considered as a re-emerging disease [3].

Humans are accidental hosts and the disease usually results from occupational contact. Though the organism is susceptible to various antibiotics *in vitro*, treatment is difficult and needs longer course with combination of antibiotics upon early diagnosis. No vaccine for humans is available.

P. Malik (✉) · S. K. Khurana · S. K. Dwivedi
National Research Centre on Equines,
Sirsa Road, Hisar – 125001,
Haryana, India

E-mail: malik_p@indiatimes.com

Indian Army is also maintaining equines for transport of logistics. There is always a perceptible threat for the eruption of this dreaded disease amongst equines on the border, mainly due to work stress and cross border exposure. Considering that the disease in India was restricted to certain pockets with sporadic cases, it is important to detect and report any case of glanders in the field for applying early mitigation and control strategy. The dreaded disease amongst equines in Maharashtra state had re-emerged during 2006.

A total of 14 nasal swabs, 2 abscess swabs and 2 pus samples (Table 1) were collected following detailed clinical examination from Pune and Panchgani (Distt. Satara) areas of Maharashtra, upon information describing a disease problem in equines from Western Regional Disease Diagnostic Laboratory (WRDDL), Pune in last week of July 2006. Based on signs and symptoms, several clinical cases showing typical nasal/cutaneous form of glanders were found at Panchgani. Major clinical symptoms include nasal ulcers, nodular swellings all over the body, edematous swelling or wounds on legs, cough, dyspnea, reduced appetite and wasting. Three described forms i.e. cutaneous, nasal and pulmonary forms of glanders were not distinct in different animals in the present outbreak, similar to earlier findings.

One pus sample and three nasal swabs yielded isolates simulating *B. mallei* (Table 1) employing glycerol-dextrose agar (GDA - nutrient agar containing 5% glycerol and 1% dextrose) following standard protocol [4]. Organism was identified based on morphological, cultural and biochemical characteristics including motility test [5]. On GDA, there was a confluent, slightly cream-colored growth that was smooth, moist and viscid after 24 h incubation. With continued incubation, the growth thickened and became darker and tough. Colony boundaries did not appear to be smooth. The organisms were small gram-negative rods appeared to have bipolar staining character, especially in shorter rods. On blood agar, no hemolysis could be observed. No growth at 42°C and on McConkeys agar was observed. These findings were consistent with the literature [1, 6]. Cultural characteristics of *B. mallei* vary in appearance depending on the age of the culture, from young cultures and clinical samples are rods with bipolar staining, while from older cultures the organisms can be pleomorphic.

All the isolates were non-motile. *B. mallei* lacks flagellin [7] but motility associated flagellar genes of *B. mallei* were reported to be partially maintained [8]. The isolates were catalase and oxidase positive but did not reduce nitrates. Except weak fermentation of glucose, fructose and mannitol, the isolates looked inert to various sugar fermentation reactions including those of sucrose, lactose, galactose, sorbitol, mannose, maltose, ribose and rhamnose. Non-fermentation of carbohydrates by *B. mallei* has been reported [9].

The crude extracts of the isolated organisms obtained by boiling, containing approximately 30 µg of estimated protein, were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) using discontinuous buffer system of Laemmli [10]. The profile revealed a large number of bands ranging from <20 kD to >150 kD. However, the profile indicated all the isolates to be identical pointing to a common source of infection at both places. This was an important finding from epidemiological point of view and would be helpful in containment of the disease. Crude extracts from the isolates were also subjected to immunoblotting (western blotting) employing the standard protocol [11] using the horse sera (diluted to 1:750) collected from a naturally infected horse. Blots revealed reactive proteins at about 40 kD in all the isolates with positive sera of different affected horses while negative sera did not show these bands.

In addition, 720 serum samples were subjected to complement fixation test (CFT) as per standard protocol described by Office International des Epizooties (OIE) [12] till March 2008. Twenty-three equines from Pune (12) and Panchgani (11) were found to be positive by CFT. The titer in the positive animals ranged from 1:16 to 1:256 (Table 2). CFT was the most extensively used test for the diagnosis of glanders and reported to be highly sensitive by most workers [13, 14]. CFT has been found as the preferred diagnostic tool for eradication of glanders because of its capacity to detect clinically inapparent carriers and chronically infected horses. It is widely acceptable test for international trade as it has been reported to be 90–95% accurate [12]. Intracutaneous (IPD) mallein test using mallein protein purified derivative (PPD) was performed on the reactor

Table 1 Details of samples collected for bacteriological examination and isolates obtained

	Pune	Panchgani	Total
Pus	1 (1)	1	2 (1)
Nasal swab	1	13 (3)	14 (3)
Abscess swab	1	1	2
Total	3 (1)	15 (3)	18 (4)

Numbers in the brackets indicate number of *Burkholderia mallei* isolates.

Table 2 Results of CFT on serum samples collected/received from Maharashtra

No. of samples	Area of origin of samples	Titer*	Status
8	Pune	>1:64	Positive
2	Pune	1:32	Positive
2	Pune	1:16	Positive
39	Pune	<1:16	Negative
6	Panchgani	>1:64	Positive
3	Panchgani	1:32	Positive
2	Panchgani	1:16	Positive
1	Panchgani	<1:16	Negative
657	Different areas of Maharashtra including Pune and Satara	<1:8	Negative

*Antibody titers in the equine serum samples, determined by CFT.

animals for confirmation of disease before elimination by the local animal husbandry authorities. No human case has been reported from the area with any symptom simulating glanders.

In India, cases of glanders have earlier been reported from Bombay during 1950 and 1956. Sporadic incidences and outbreaks in certain pockets of India has been reported [15–19]. Verma [20] reported a detailed account of incidence and epidemiology of glanders on military farms in India and found high morbidity in the areas where horses, donkeys, and mules were thickly populated. Kumar et al. [21] reported a cutaneous glanders case in 1998 in mules from Rohtak, Haryana, which was the last confirmed case before the current outbreak.

Concerned authorities of Maharashtra State Animal Husbandry Department and Department of Animal Husbandry, Dairying and Fisheries (Ministry of Agriculture, Govt. of India) were informed. The animal owners and veterinarians were given tips on sanitary and hygienic precautions. Strategies for prevention of further spread of the disease to other areas and neighboring states as well action as per Glanders and Farcy Act (1899) were also suggested. Accordingly the state authorities reportedly eliminated 26 affected animals. Follow up of the occurrence revealed negative status based on testing on more than 657 samples from Maharashtra alone and more than 900 samples from other neighboring states thereafter. Since the disease appeared to have started subsequent to purchase of animals from Animal Fair in Jalna (Maharashtra) from traders coming from northern parts of India, it is considered that the nidus of infection may be present elsewhere in North India.

The center is actively monitoring the situation nationwide. Intense follow ups are also being continued on samples from different states of the country. Follow up of

the occurrence by testing on more samples from various states by National Research Centre on Equines (NRCE) and physical surveillance by local authorities thereafter was done on more than 3,500 equines. Vaid et al. [22] reported that many cases of glanders are latent and clinically inapparent, so systematic testing is essential to identify all infected animals in an outbreak. The disease was successfully controlled in the state of Maharashtra mainly due to efforts of NRCE in coordination with WRDDL and Government of Maharashtra.

Acknowledgements The authors are thankful to Director, NRCE, Hisar for extending necessary facilities for the work; Commissioner (AH), Maharashtra and Joint Director and his team, WRDDL, Pune for cooperation and assistance in collection of samples; Director (DRDE) and Joint Director (HCF), DRDE Gwalior for providing containment facilities for the work.

References

- Garrity GM, Bell JA and Lilburn T (2005) Family I. *Burkholderiaceae famnov.* In: *Bergey's Manual of Systematic Bacteriology*. 2nd edition, Vol. 2, Brenner DJ, Krieg NR, Stanley JT (Eds.), Springer-Verlag, New York, USA, pp 575–600
- Woods JB (Ed.) (2005) Glanders and melioidosis. In: *USAMRIID's Medical Management of Biological Casualties Handbook*. 6th edition, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland, USA, pp 32–39
- 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, *Dtsch Tierarztl Wochenschr* 113:323–330

4. Misra VC and Verma RD (1989) Research on epizootic, diagnosis and control of glanders with a view to eradicate the disease from India, Project Report, ICAR Research Scheme, pp 54
5. Steele JH (1979) Glanders. In : *CRC Handbook Series in Zoonoses*. Vol. 1, Steele JH (Ed.), CRC Press, Florida, pp 339–362
6. Quinn PJ, Carter ME, Markey BK and Carter GR (1994) *Clinical Veterinary Microbiology*, CV Mosby, St. Louis, pp 237–242
7. Anuntagool N and Sirisinha S (2002) Antigenic relatedness between *Burkholderia pseudomallei* and *Burkholderia mallei*. *Microbiol Immunol* 46:143–150
8. Iihara H, Niwa T, Shah MM, Nhung PH, Song SX, Hayashi M, Ohkusu K, Itoh Y, Makino S and Ezaki T (2007) Rapid multiplex immunofluorescent assay to detect antibodies against *Burkholderia pseudomallei* and taxonomically closely related non-fermenters. *Japanese J Infect Dis* 60: 230–234
9. Al-Ani FK, Al-Rawashdeh OF, Ali AH and Hassan FK (1998) Glanders in horses: clinical, biochemical and serological studies in Iraq. *Vet Arhiv* 68:155–162
10. Laemmli K (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage, T₄. *Nature* 227: 680–685
11. Sambrook J, Fritsch EF and Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*. 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York
12. Anonymous (2006) Glanders. In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. http://www.oie.int/eng/normes/MANUAL/A_00086.htm.
13. Sen GP, Singh G and Joshi TP (1968) Comparative efficacy of serological tests in the diagnosis of glanders. *Indian Vet J* 45:286–292
14. Verma RD (1980) Studies on Serodiagnosis of glanders with special reference to serodiagnostic procedures, PhD Thesis, Jiwaji University, Gwalior, India, pp 181
15. Ray DK (1984) Incidence of glanders in the horses of mounted platoon of 4th AP Bn, Kahilipara, Gauhati-19: A case history. *Indian Vet J* 61:264
16. Misra VC, Kaushik RK, Dhingra PN and Satija KC (1985) Emergence of glanders epidemic in civilian equines of northern India. *J Remount Vet Corps* 24:110–115
17. Anonymous (1988) Glanders Annual Report - National Research Centre on Equines, Hisar, pp 48–51
18. Anonymous (1989-1990) Glanders Annual Report - National Research Centre on Equines, Hisar, pp 58–59
19. Anonymous (1990-1991) Glanders Annual Report - National Research Centre on Equines, Hisar, pp 49
20. Verma RD (1981) Glanders in India with special reference to incidence and epidemiology. *Indian Vet J* 58:177–183
21. Kumar S, Malik P, Jindal N and Garg DN (1999) Cutaneous glanders in a mule: A case study. *J Remount Vet Corps* 38:131–133
22. Vaid MY, Muneer MA and Naem M (1981) Studies on the incidence of glanders at Lahore. *Pakistan Vet J* 1:75.