

Short Communication

Assessment of the effectiveness of the PPD-mallein produced in Brazil for diagnosing glanders in mules

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Abstract

To assess the potency of the PPD-mallein produced in Brazil, five animals were from a property identified as a focus of glanders. These animals had suggestive clinical signs of the disease and the other five, from a property free from glanders, showed no clinical signs and were serology negative (control group). PPD-mallein from *Burkholderia mallei* was obtained by precipitation with trichloroacetic acid and ammonium sulfate. The animals were inoculated according to the criteria established by Department of Agriculture, Livestock and Supply (MAPA) for the diagnosis of glanders. After 48 h of application of PPD-mallein, there was swelling in the area of application, presence of ocular secretion and tears in sick animals. The control group showed no inflammatory reaction at the site of inoculation of PPD-mallein. This immunogen produced in Brazil and still being tested was effective for identifying the infection in true positive animals and excluding the truly negative ones, being a new possibility for diagnosis and control of glanders

Key words: Mallein test, *Equus asinus*, diagnosis.

Glanders is a deadly bacterial disease of horses caused by *Burkholderia mallei*. The disease is usually chronic in horses and acute in mules (Al-Ani *et al.*, 1998). In Brazil, the re-emergence of the disease was identified at the end of the last century through the description of clinical, microbiological and serological tests in equine animals in the coastal region from state of Pernambuco and the diagnosis of glanders in Brazil follows the criteria laid down by MAPA Normative Instruction N° 24 for the control and eradication of the disease (Brasil, 2004). For diagnosis, the use of the complement fixation (CF) serologic test is recommended and those animals whose sera are anticomplementary or self-react with normal antigen in CF should be tested in the malleinization, using imported, mallein there is the possibility of nonspecific reaction in serological testing because of homologous antigens between *B. mallei* and *B. pseudomallei*. This reaction can result in a false-positive diagnosis, especially in animals imported from ar-

reas where melioidosis occurs in humans. This reaction is not observed in malleinization (Hagebock *et al.*, 1993).

Malleinization is similar to tuberculinization; mallein is a glycoprotein extracted from cultures of *B. mallei*, used as antigen in Intradermal-eyelid test in horses (Verma *et al.*, 1990, 1994; Neubauer *et al.*, 2005). This test is based on the detection of immune cellular response and provides greater specificity in the diagnosis of glanders. Considering the possibility of contributing to the diagnosis of glanders in Brazil, a Purified Protein Derivate (PPD) mallein was produced through precipitation and acid purification for use in malleinization tests. The objective of this study was to evaluate the potency of this immunogen in mules with clinical, positive serological and bacteriological diagnoses for glanders. The article was approved by the research ethics committee (n°009909-2011-35 UFAL)

For evaluation of PPD-mallein we used ten adult mules (*Equus asinus*), coming from the Coastal Region in

Pernambuco State. Five animals were from a property identified as a focus of glanders. These animals had suggestive clinical signs of the disease and the other five, from a property free from glanders, showed no clinical signs and were serology negative (control group). To confirm the diagnosis of the disease in the animals studied, the serological complement fixation technique was used, followed by the bacteriological examination. Purulent content from cutaneous animals with clinical signs were collected and sown in Petri dishes containing agar base enriched with 10% sheep blood and the plates were incubated at 37 °C for 72 h. The bacteria were subsequently identified through biochemical oxidase tests, catalase, Voges Proskauer (VP), indol, H₂S production, motility, arginine hydrolysis, D-glucose gas, gelatin liquefaction, urease, glucose, maltose, galactose, lactose, fructose, mannose, sucrose and mannitol (Mota *et al.*, 2000). During the clinical examination of sick animals, we noticed edema in the pelvic limbs and in the prepuce, lymphangitis in the neck, head and limbs, bilateral purulent nasal secretion, dyspnea, pulmonary stertors and progressive loss of weight. The clinical diagnosis was suggestive of glanders (Mota *et al.*, 2000; Rabelo *et al.*, 2006).

After the confirmation of infection by bacterial isolation and antibody test, we used the PPD-mallein produced experimentally in Brazil (Silva, 2010) to confirm the disease. We applied 0.1 mL of immunogen intradermally in the lower eyelid of the right eye of each animal. To prove the effectiveness of mallein, five mules serologically and bacteriologically negative for glanders were inoculated in the same conditions. The reading was performed after 48 h and followed the criteria established in IN 24 (Brasil, 2004; OIE, 2010) that characterize as a positive reaction the presence of persistent edema with or without observation of ocular secretion. The five sick animals were reactive to the test and showed progressive and persistent edema, starting 24 h later and with a peak 48 h after inoculation; ocular secretion was noticed in some animals, but not in others. In the control group, neither an inflammatory reaction nor any other clinical signs in the inoculated area were noticed (Figure 1).

The PPD-mallein tested induced an inflammatory response in the sick animals, which developed an allergic reaction and exhibited Delayed-Type Hypersensitivity reactions of (DTH), as seen in Figure 2. The intradermo-palpebral positive reaction, besides being characterized by ocular edema can also be associated with other findings such as photosensitivity, tear shedding and suppuration within 48 h after inoculation (Hagebock *et al.*, 1993). These findings were also observed in positive animals in this study. The variation in the pattern of inflammatory response after inoculation of PPD-mallein in infected animals is directly related to the degree of infection in each equine. During the phases of bacteremia, when a more intense circulation of the infectious agent occurs, the antigenic stimulus is stronger, generating a greater effect of



Figure 1 - Control group without inflammatory reaction or any other clinical signs were observed in the inoculated left eye.

cytokines such as IL-2 that reinforces the activation of Th-1 lymphocytes activated against *B. mallei*, thus enhancing DTH reactions (Amemiya *et al.*, 2002).

Malleinization has greater specificity in the diagnosis of glanders as compared to other techniques, and its greater sensitivity should be considered for confirmation of positive cases as well as for clarification of doubts about the reaction of CF (Naureen *et al.*, 2007). The test is inexpensive, easy to apply and commonly used in endemic areas for identifying horses carrying the infection after the completion of serology. The disadvantage is that the test may cause transient seroconversions in non-infected healthy animals as long as 45 days after the test. Thus, sera from negative tested animals become reagents in the CF (Huitema, 1969; Hagebock *et al.*, 1993). Another limitation of the mallein test includes the negative results in horses in the disease's advanced stage due to the development of anergy due to peripheral immune tolerance (Verma *et al.*, 1981; Jana *et al.*, 1982).

A study conducted in healthy and infected horses found that the mallein test is perfectly reliable, even in cases when the disease is beginning (Naureen *et al.*, 2008). With a positive predictive value of 92% in the acute and chronic phases of the disease and a negative predictive value of 96% in advanced cases, it is the most indicated test to clarify the suspected cases of glanders in the CF. Other tests, as agglutination reaction, hemagglutination inhibition



Figure 2 - Animal (*Equus asinus*) mallein-test reagent, progressive and persistent swelling right lower eyelid, with a peak 48 h after inoculation, ocular discharge was observed in some animals.

and immunoelectrophoresis are inferior in specificity and sensitivity as compared to the mallein test (Verma *et al.*, 1994).

In this study, despite the small number of animals tested, the mallein produced was effective for identifying the true positive animals as well as those animals negative for glanders with 100% (5/5) of agreement among them, being a new possibility for the diagnosis and control of glanders in the country. However, it is necessary to apply this immunogen in a larger number of positive and negative animals in order to eliminate possible interpretation doubts in the diagnosis of this disease. Additionally, future prospects for standardization and validation of a quick serological test with high sensitivity applicable to routine diagnosis should be considered to reduce nonspecific reactions and increase the correlation between serological and mallein tests.

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