Ciência Rural, Santa Maria, v.53:10, e20210901, 2023 http://doi.org/10.1590/0103-8478cr20210901
ISSNe 1678-4596
MICROBIOLOGY

Historical highlights regarding glanders, from 2007 to 2017, in the state of Maranhão, Brazil

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ABSTRACT: Glanders is an infectious disease of equids caused by *Burkholderia mallei*, a facultative intracellular non-mobile Gram-negative bacterium that can be transmitted to other animals and humans. This study described glanders cases reported in the state of Maranhão, located within an Amazon-Cerrado transition region in northeastern Brazil, from 2007 to 2017. A database in an electronic spreadsheet (Microsoft Excel 2010) was developed containing information on the number of positive animals according to age, sex, purpose, year and month, municipality and mesoregion of origin of the animal. The descriptive analyzis was performed on data provided by the State Agency for Agricultural and Livestock Protection of the state of Maranhão (AGED), and by the official private laboratory. As the database did not have information data about negative animals, possible risk factors could not be evaluated. Among the total of 62,555 equids were evaluated by means of the complement fixation test (CFT), 59,036 were horses, 2,981 mules and 538 donkeys. Thirty-five samples (0.06%) reacted in the CFT. Five additional samples were by the western blot technique and three of them were positive. All the reactive horses (*Equus caballus*) were from rural areas. Results presented here indicate that glanders may be endemic in the state of Maranhão and is a public health concern.
Key words: horses. *Burkholderia mallei.* northeastern.

Destaques históricos do mormo, de 2007 a 2017, no Estado do Maranhão, Brasil

RESUMO: O mormo é uma doença infecciosa de equídeos causada por *Burkholderia mallei*, uma bactéria Gram-negativa intracelular, não móvel e facultativa que pode ser transmitida a outros animais e humanos. O objetivo deste estudo foi descrever os casos de mormo relatados no estado do Maranhão, em uma região de transição Amazônia-Cerrado do Nordeste do Brasil, no período de 2007 a 2017. Um banco de dados em uma planilha eletrônica (Microsoft Excel 2010) foi desenvolvido contendo informações sobre o número de animais positivos de acordo com a idade, sexo, finalidade, ano, mês, municípios e mesorregiões de origem do animal. A análise descritiva foi realizada sobre os dados fornecidos pela Agência Estadual de Defesa Agropecuária do Estado do Maranhão (AGED), e pelo laboratório privado oficial. Como o banco de dados não possui informações sobre animais negativos, possíveis fatores de risco não puderam ser avaliados. Entre o total de 62.555 equídeos foram avaliados com o teste de fixação do complemento (CFT), 59.036 eram cavalos, 2.981 mulas e 538 jumentos. Trinta e cinco (0,06%) amostras reagiram ao CFT. Cinco amostras adicionais foram testadas pela técnica de western blot e três foram positivas. Todos os animais positivos foram cavalos (*Equus caballus*) da área rural. Os resultados indicam que o mormo pode ser endêmico no estado do Maranhão e uma preocupação de saúde pública.
Palavras-chave: cavalos. *Burkholderia mallei.* Nordeste.

Glanders is an infectious disease of equids that can be transmitted to healthy animals and humans. It is characterized by chronic and acute stages, and leads to notable economic losses, with sociocultural importance. Although, the first records on glanders disease in Brazil dated from the beginning of the 19th century, through importation of equids from Europe, cases are now being detected again, as seen in the state of Maranhão (KORTEPETER et al., 2001; VAN ZANDT et al., 2013; CHAVES et al., 2016). These infectious and contagious conditions are caused by *Burkholderia mallei*, a facultative intracellular

Received 12.22.21 Approved 10.21.22 Returned by the author 01.21.23
CR-2021-0901.R4
Editors: Rudi Weiblen Juliana Felipetto Cargnelutti

non-mobile Gram-negative bacterium that has been used as a biological weapon in the past and could be used again in the future (WHITLOCK et al., 2007; NEUBAUER & MEYER, 1997; NEUBAUER et al., 2005). According to MANDELL & BENNETT, 2020, there is concern regarding release of high-dose aerosol production of *B. mallei*, which would cause pulmonary and septicemic disease, exacerbated by delays in diagnosis and treatment.

In Brazil, equids are used in a variety of activities such as tractive force, transportation, policing services, equine-assisted therapies and horse agribusiness. These activities are responsible for generating direct and indirect employment for 3.2 million people (ALMEIDA et al., 2006). Currently, the state of Maranhão has a herd of around 336,810 equids registered in this state (IBGE, 2017).

The Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA), through its National Equine Health Program (PNSE), has legislation that is enforced on farms with positive cases. Until the year 2017, i.e. the period covered by the present study, the main diagnostic methods established through the PNSE were the complement fixation test (CFT), mallein test (MT) and confirmatory immunoblotting (IB) test (MAPA, 2016). However, the current legislation establishes that the indirect enzyme-linked immuno sorbent assay (ELISAi) is the only screening test, with confirmatory IB; while the MT is to be used for animals aged up to 6 months (MAPA, 2018). Farms with glanders cases are interdicted, with immediate culling of animals with reactive to tests. There is not compensatory payment, while restrictions apply on buying and selling of individuals during the sanitization period. In addition, this interferes with sporting events because there is an embargo on transportation of equids (IBGE, 2017).

Glanders had been considered eradicated since 1968, which was the year when the last outbreaks of the disease were registered (FONSECA-RODRÍGUEZ et al., 2019). However, the disease reemerged in Brazil over the last decade, in equids in the northeastern region (states of Alagoas and Pernambuco). More recently still, cases have been confirmed in the northern region (states of Tocantins and Pará), with clinical signs, positive diagnostic samples and scientific investigations. There are still no reports of glanders in the northern-region states of Acre and Amapá (MAPA, 2016; FONSECA-RODRÍGUEZ et al., 2019; DE CARVALHO FILHO et al., 2012; ADEPEC, 2016; RESENDE et al., 2022).

Because glanders has been highlighted as an important re-emerging disease with a severe impact on animal and human health, and because there is a lack of systematic study over any period of time, we here present retrospective data on glanders from 2007 to 2017 a period in which the complement fixation test was still used as a screening test. These data were obtained through compilation of information in the databases of MAPA, AGED and the private official laboratory of the state of Maranhão.

A total of 62,555 equids were screened using the CFT, and this population comprised 59,036 horses, 2,981 mules and 538 donkeys. Out of these samples, 35 (0.06%) were reactive for glanders in the CFT, over the period from 2007 to 2017 (Figure 1).

In 2007, 4,116 equids were tested, of which two animals were considered positive (n = 2; 0.05%). In relation to the years 2008 to 2010, a total of 13,688 serum samples were tested in this period, and there were no reactive animals. In 2011, six animals were considered positive out of 6,171 samples (n = 6; 0.1%). In 2012, 5,976 equids were tested and three animals were reactive (n = 3; 0.05%). 2013 and 2014 showed the largest cases numbers of positive animals (n = 9; 0.15% and n = 8; 0.13%, respectively), among 12,326 tested equids. In the years 2015 and 2016, four (n = 4; 0.07%) and two (n = 2; 0.03%) samples were reactive, respectively, out of a total of 12,545 equid samples. In 2017, 7,733 equine samples were tested, among which one was positive (n = 1; 0.01%). Study on the distribution of reactive animals according to the month of the year over the study period showed that February, April and May presented the highest frequencies of occurrence, whereas in January and December there were no reactive animals in any of the ten years of the compiled database (Figure 2).

Over the ten years of observation, cases of glanders have been consistently reported every year, except in 2008, 2009 and 2010, with increasing frequency in the subsequent years. Perhaps, that increase is at least partially related to the growing number of tests performed by the official health surveillance actions that started in that period, covering almost all regions of the country. It is important to emphasize that control actions against this disease in Brazil have been regulated through normative instruction (NI) no. 24/2004-SDA/MAPA.

One important milestone in the increasing levels of diagnosing this disease was reached when it acquired prominence in the states of Pernambuco and Alagoas through a study by MOTA et al., 2000, after approximately 30 years without case reports in Brazil. More recently, the rise in glanders cases in Brazil between 2012 and 2015 was possibly due to equestrian events at the Olympic and Paralympic

Figure 1 - Absolute values (bars – green color) of the number of samples tested and frequencies (red line) of samples positive for glanders according to year in Maranhão, Brazil, from 2007 to 2017.

Games in 2016, despite major attention to sanitary and biosafety measures (BRASIL, 2016).

From our descriptive study, the highest percentages of positive cases of glanders were concentrated in the months of February, April and May, and especially in May, which showed the highest frequency of cases in the state of Maranhão over the years. The larger demand for assessments through laboratory tests from April to July in the state of Maranhão may be associated with equestrian events

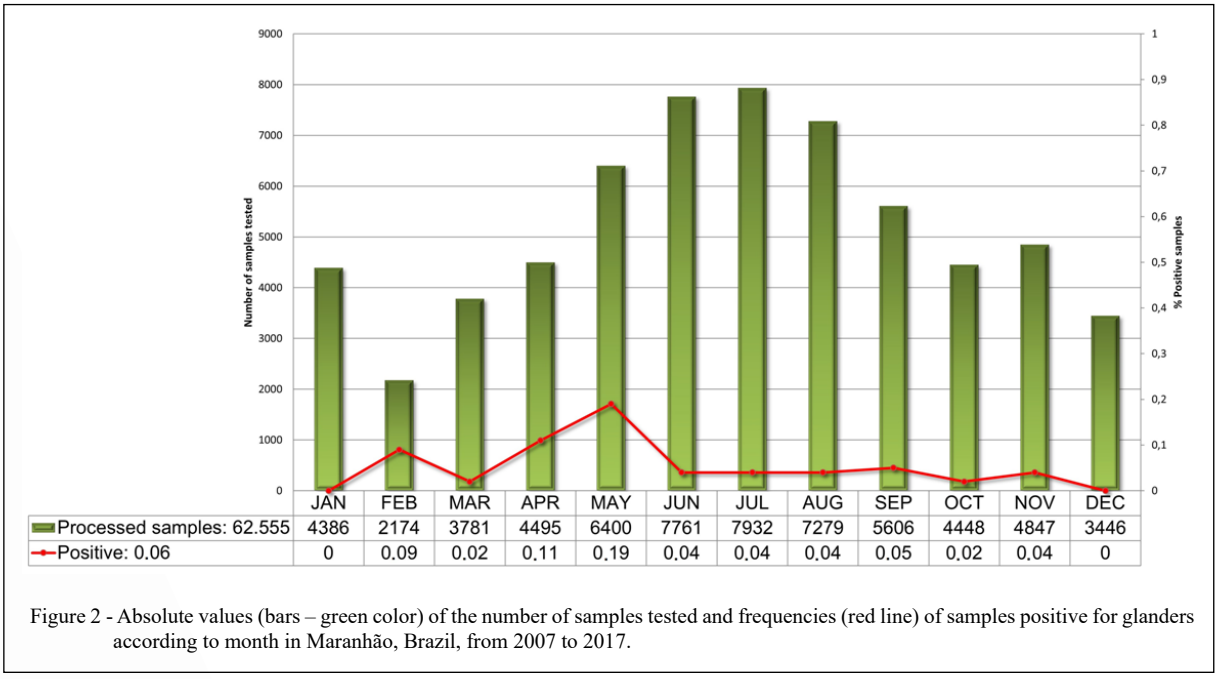

Figure 2 - Absolute values (bars – green color) of the number of samples tested and frequencies (red line) of samples positive for glanders according to month in Maranhão, Brazil, from 2007 to 2017.

over this period (i.e. rodeo events and barrel racing); and consequently, the need for transportation of animals. For permits for animal transportation to be issued, negative tests with a validity of 60 days are obligatory.

The overall frequency of occurrence (0.06%; 35/62555) was lower than what was reported from the state of Pará by RESENDE et al., 2022 (2.31%; 9/388). Although, Pará and Maranhão are neighboring states, the techniques used may have been the main difference between the results, since the technique performed in Pará was ELISAi, a technique with greater sensitivity than the CFT. However, the results from our retrospective study are similar to those from a survey carried out in the Maranhão lowlands (Baixada Maranhense), in which the CFT showed that 0.48% of the horses were positive (CHAVES et al., 2016).

Throughout the study period, horses were the only equids that were reactive to the tests. All of the cases were among horses living in rural areas, of both sexes, that were reared for sports activity and breeding. The breeds that were reactive to tests were quarter horse, mangalarga marchador, appaloosa, paint horse and hybrids, and the ages of these animals ranged from 1.5 to 18 years. These findings are similar to those of other studies in the northern and northeastern regions of Brazil, but cases among mules and donkeys were reported in some of those studies (LAROUCAU et al., 2018; MOTA et al., 2010, 2000; RESENDE et al., 2022).

The spatial distribution of reactive samples showed that glanders cases occurred in the following mesoregions of the state of Maranhão: north: 0.07% (16/22577); west: 0.05% (9/18215); center: 0.06% (5/8494); and east: 0.05% (5/8977). Conversely, no cases were reported from the southern mesoregion, which had the lowest number of processed samples, totaling 4,292. According to AGED, the highest concentration of equids is in the southwest of the state of Maranhão, with approximately 40,081 registered animals. However, throughout the study period, the northern mesoregion had the highest rate of glanders occurrence with 16 positive cases, which may have been related to higher numbers of animal transportation permits issued. This was followed by the western mesoregion with nine positive cases, and the eastern and center mesoregions, each with five positive cases.

The lack of glanders cases in the southern mesoregion may have been related to the quantity of examinations performed during the evaluated period. The highest quantities of samples sent to the laboratory were from the northern and west mesoregions, and this was related to animal transportation for equestrian events, which are very common in those places; and to animal transportation between the northern and northeastern regions of Brazil (Figure 3).

Regarding the biome, presence of glanders was clearly evident in both the Amazon biome (n = 25; 0.04%) and the Cerrado biome (n = 10; 0.01%).

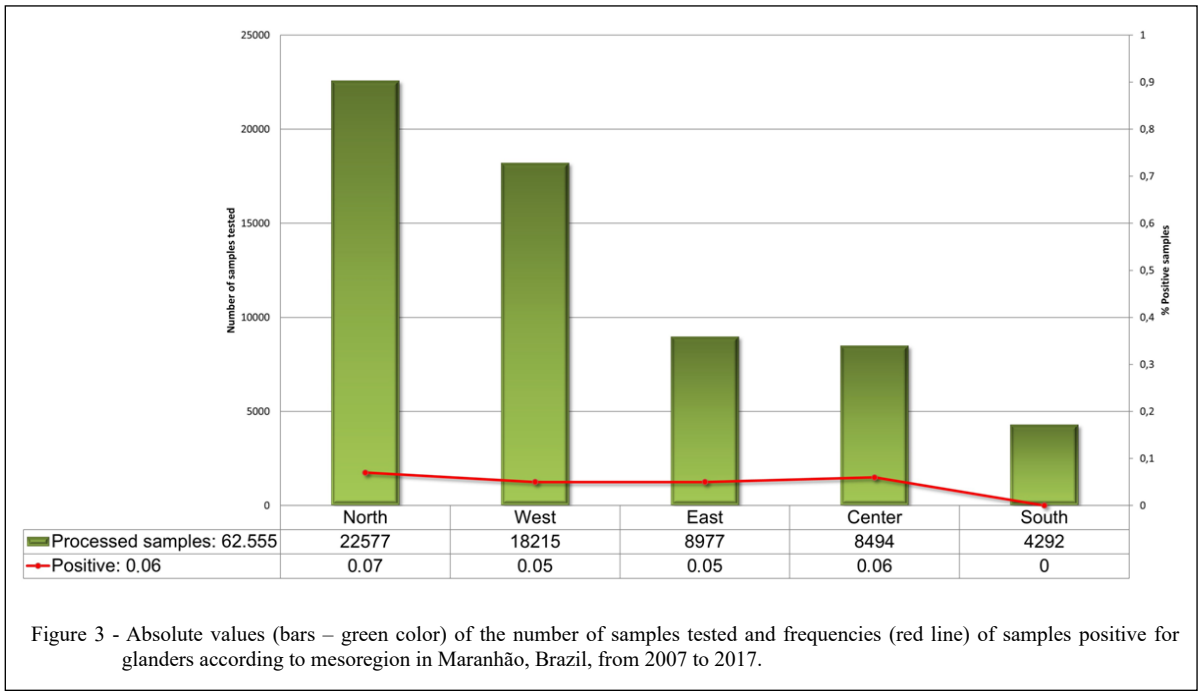

Figure 3 - Absolute values (bars – green color) of the number of samples tested and frequencies (red line) of samples positive for glanders according to mesoregion in Maranhão, Brazil, from 2007 to 2017.

The cases were concentrated in the center and the north of the state (Figure 4).

Out of the 35 samples that were positive in the CFT, only 18 samples underwent two consecutive mallein tests with an interval of 45 days, in accordance with the current MAPA legislation. Overall, the samples were 100% test-positive, showing eyelid edema and purulent secretion on the eyeball. Another test was available in the years 2016 and 2017, and a total of five samples were thus tested by means of the western blot technique. From these tests, two samples were positive in 2016 and one in 2017.

The state of Maranhão is of great importance for Brazilian agribusiness. In this context, investigation of compulsorily notifiable diseases and fast detection and identification of the etiological agent involved are important for minimizing transference and dissemination of this zoonosis in Brazil (CHAVES et al., 2016). In addition, systematized retrospective studies on data collected in official files have become increasingly important within the field of animal health, since it is through these surveys that the prevalence of a disease can be defined in terms of epidemiological, clinical and anatomopathological data (CHAVES et al., 2016).

A total of 35 horses were considered positive through the CFT. This number was significant because of the importance of this disease in relation to animal and human health, and consequently to social and economic issues. Conversely, mules and donkeys did not react in the CFT. These findings are similar to those of previous studies, with regard to comparing frequencies in other places in Brazil (MACHADO et al., 2013) and in other countries, for example, India (MALIK et al., 2015).

According to MOTA et al., 2000, age is a relevant factor in the emergence of the clinical form of natural infection, with higher prevalence among animals that are senile and debilitated through stressful conditions. In addition, predisposing factors such as poor diet, stress, housing in contaminated environments and low-sensitivity tests used for screening are potential causes of occurrence and dispersal of glanders (FONSECA-RODRÍGUEZ et al., 2019).

The main reactive horse breeds were the quarter horse and mangalarga marchador. These breeds are predominant in the state of Maranhão and are known for their great versatility in competitions and field working (COSTA et al., 2016; LAGE et al., 2009). According to the data, glanders has been

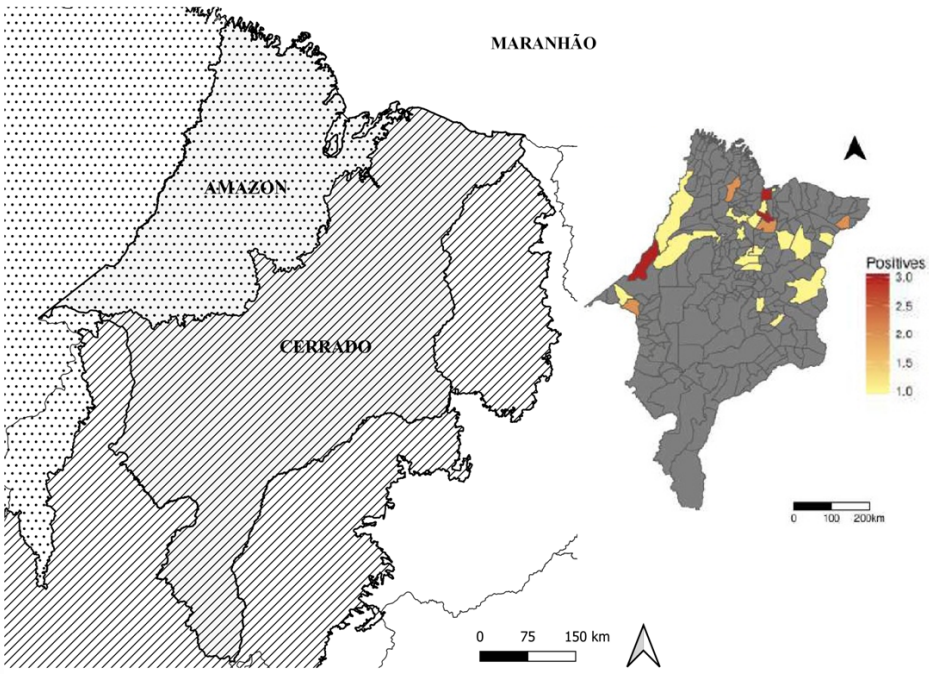

Figure 4 - Absolute values of the number of samples positive for glanders according to municipalities in Maranhão, Brazil, from 2007 to 2017. The gray color on the map means municipalities without glanders cases.

endemic in the state of Maranhão for the last ten years. This is a matter of public health concern, especially in rural areas, and intensification of surveillance at regional events with regard to transit of equids must be maintained as a preventive measure.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

ACKNOWLEDGEMENTS

We are grateful to the institutions that provided the data for this survey: State Agency for Agricultural and Livestock Protection of the state of Maranhão (AGED); Federal Superintendence of Agriculture in the state of Maranhão; Ministry of Agriculture, Livestock and Supply; and the official private laboratory. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Estado do Maranhão (FAPEMA) e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brasil - Finance codes (DCR-03526/16) and UEMA (22-2018- PPG/UEMA).

AUTHORS' CONTRIBUTIONS

E. C. S. Vieira, N. P. Chaves, and F. B. Costa contributed to the study design, study execution, data analysis and interpretation, preparation of the manuscript and final approval. F. A. Melo, A. K. S. M. S., D. P Chaves and R. A. M. contributed to the study execution, data interpretation and final approval. O. S. Baquero contributed to data analysis, interpretation, and final approval. V. C. S. Coimbra, E. T. K. C. Mesquita and J. C. A. Ferreira contributed to the data acquisition and final approval.

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Ciência Rural, Santa Maria, v.53:11, 2023 10.1590/01038478crerr20210901
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Erratum

Erratum

In the article "Historical highlights regarding glanders, from 2007 to 2017, in the state of Maranhão, Brazil" published in Ciência Rural, volume 53, number 10, DOI http://dx.doi.org/10.1590/0103-8478cr20210901.

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