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MICROBIOLOGY

# Identification of management factors associated with glanders's occurrence in equids in Brazilian Northeast region

**Jéssica de Crasto Souza Carvalho[1*] José Wilton Pinheiro Junior[2] Marcus Vinícius Dias Falcão[3] Karla Patrícia Chaves da Silva[4] Vania Lucia de Assis Santana[5] Rinaldo Aparecido Mota[2]**

[1]Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco (UFRPE), 52171-900, Recife, PE, Brasil. E-mail: jessicacrasto22@gmail.com. *Corresponding author.
[2]Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco (UFRPE), Recife, PE, Brasil.
[3]Centro Universitário Maurício de Nassau (UNINASSAU), Recife, PE, Brasil.
[4]Departamento de Medicina Veterinária, Centro de Ciências Agrárias, Universidade Federal de Alagoas (UFAL), Maceió, AL, Brasil.
[5]Ministério da Agricultura, Pecuária e Abastecimento (MAPA), Recife, PE, Brasil.

**ABSTRACT**: Glanders is a contagious zoonotic infectious disease characterized by an acute or chronic condition that mainly affects equids and is caused by *Burkholderia (B.) mallei*. From January 2005 to December 2017, 697 farms with positive cases of this disease were registered in Brazil, with the Northeast region accounting for 61.4% (428/697) of the total number of cases. This study conducted an epidemiological study of *B. mallei* infections that occurred in farms in the states of Pernambuco, Paraíba, Ceará and Alagoas. For this purpose, investigative questionnaires were applied to six farms where there were outbreaks of glanders and 11 farms where there were no reports of glanders between 2017 and 2021, and statistical analysis of the factors associated with the occurrence of glanders in Northeastern Brazil was performed. Factors such as the purpose of rearing (P = 0.023), lack of information on disease transmission modes (P = 0.034), failure to carry out periodic tests for glanders diagnosis (P = 0,029), no request for glanders tests prior to the purchase of new animals periodic tests for glanders diagnosis (P = 0.029), not requiring a negative test for glanders in the acquisition of animals (P = 0.06), use of extensive breeding systems(P = 0.027), and poor stall hygiene were associated with disease occurrence (P = 0.001). Understanding the factors related to the occurrence of glanders in the Northeast Region of Brazil helps to establish management measures that minimize bacteria transmission, thereby reducing the number of cases in the region and economic losses associated with glanders.
**Key words**: epidemiology, *Burkholderia mallei*, horses.

# Identificação dos fatores de manejo associados à ocorrência do Mormo em equídeos no Nordeste do Brasil

**RESUMO**: O mormo é uma doença infectocontagiosa, zoonótica, de caráter agudo ou crônico, que acomete principalmente os equídeos, causado por *Burkholderia (B.) mallei*. De janeiro de 2005 até dezembro de 2017, 697 focos da doença foram registrados no Brasil, sendo que a região Nordeste detém 61,4% (428/697) do total de focos. Objetivou-se realizar um estudo epidemiológico da infecção por *Burkholderia mallei* em propriedades nos estados de Pernambuco, Paraíba, Ceará e Alagoas. Para tal foi aplicado questionário investigativo em seis fazendas onde houve caso de Mormo e 11 fazendas em que não houve relato entre 2007 e 2011 e realizada análise estatística dos fatores de manejo associados à ocorrência do Mormo no Nordeste do Brasil. Observou-se associação significativa para as seguintes variáveis: objetivo da criação (comércio, esporte e trabalho nas fazendas) (P = 0,023); ausência de informação sobre as formas de transmissão da bactéria (P = 0,034); não realização de exames periódicos para o diagnóstico do mormo (P = 0,029); exigência de exame negativo na compra de equinos (P = 0,06); utilização do sistema de criação extensivo (P = 0,027) e deficiência de higienização das baias (P = 001). Os resultados obtidos devem ser utilizados na implementação de medidas de manejo que minimizem o risco de transmissão da bactéria, reduzindo os casos da doença na região e as perdas econômicas com o mormo.
**Palavras-chave**: epidemiologia, *Burkholderia mallei*, equídeo.

## INTRODUCTION

Glanders is an infectious, zoonotic disease caused by *Burkholderia mallei* that mainly affects equids. However, it can also affect humans, carnivores, and eventually, small ruminants (MOTA, 2006).

The disease persists in several countries in Asia, Africa, South America, and the Middle East (WHITLOCK, 2007). Due to a control program, it has been eradicated in the United States, Western Europe, Canada, and Australia (VARGAS et al., 2015). From January 2005 to December 2016, 697 farms with

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glanders cases were recorded in Brazil, with the Northeast region accounting for 61.4% (428/697) of the total and emphasis on the state of Pernambuco, which had the highest incidence in the Northeast. It is suspected that this zoonosis has never been eradicated in the national territory (FONSECA-RODRIGUEZ; PINHEIRO-JUNIOR; MOTA, 2019).

Brazil has the largest number of horses (*Equus caballus*) in Latin America and the third most globally, with approximately 6 million animals. When donkeys (*Equus asininus*) and mules (*Equus asininus* × *Equus caballus*) are included, Brazil has approximately 8 million heads, resulting in an average of 7.3 billion reais annually (IBGE, 2017). The mandatory notification of this disease in Brazil places export restrictions on both live animals and meat. This disease is included in the list of passive diseases of health defense actions requiring mandatory euthanasia without compensation and is part of the National Equine Health Plan from the Ministry of Agriculture, Livestock and Food Supply (MAPA) (MOTA, 2006; MOTA AND RIBEIRO, 2016; VARGAS et al., 2015).

Few studies have identified the factors associated with glanders. Never the less they highlighted the movement of equidae for participation in sporting events, hygienic-sanitary management; the introduction of infected animals to farms free from the disease (AL-ANI & ROBERSON, 2007), proximity between households and large numbers of animals (GHORIA et al., 2017), and sharing sources of food, water, pastures, and materials for common use among animals (MOTA, 2006).

Identification of the factors associated with glanders throughout the country is an important epidemiological tool for the understanding of this disease in the national territory and for guidance in the adoption of measures to control and eradicate this zoonosis. To date, no other study has been carried out in Brazil to identify the factors associated with disease outbreaks. Therefore, this study conducted an epidemiological study of infections by the bacterium *Burkholderia mallei* occurring on farms in the states of Pernambuco and Alagoas in order to identify the appropriate management strategies for the occurrence of glanders in equids in the Northeast Region of Brazil.

## MATERIALS AND METHODS

*Study design*

We chose to use a case and control design. The selected farms were defined by screening the farms with positive cases of glanders over a maximum of five years (2017–2021). The control farms (negative for glanders) belonged to the same region as the positive farms. Questionnaires were administered to 17 farms in the state of Pernambuco, Alagoas, Ceará and Paraiba (6 positive and 11 negative). The positive farms were identified through the records of the Ministry of Agriculture, Livestock and Food Supply (MAPA) (Table 1).

*Administration of investigative questionnaire*

Investigative questionnaires were applied to farm owners to collect information about sanitary management, periodic examinations, presence of clinical signs of glanders, disease perception, technical assistance, technological level of creation, and completion of a consent form for the use and disclosure of data. The questionnaire was based on variables related to the epidemiology of glanders, according to previously published studies on the disease in other countries.

*Analysis of factors associated with the occurrence of glanders*

A mixed generalized linear model was used to analyze the factors associated with the occurrence of glanders. The dependent variable consisted of the occurrence (1) or not (0) of glanders. The independent variables included purpose of rearing, knowledge about the disease, periodic examinations for glanders diagnosis, provision of technical assistance, animal management, sharing tools, acquisition of animals with or without the Animal Transit guide, quarantine information, breeding system, cleaning of environments, participation in events, and farm size.

To identify the factors associated with glanders, a univariate analysis of the variables of interest was performed using Pearson's chi-square test or Fisher's exact test, adopting a significance level of 5%.

## RESULTS AND DISCUSSION

Questionnaires were administered at six farms with glanders, and eleven farms without glanders were used as negative controls from 2017 to 2021 in the states of Pernambuco, Ceará, Alagoas, and Paraiba. The farm maps were obtained from the MAPA, which provided data on the establishments where the disease was located in the respective states and the contact information of the owners. It is important to highlight the consequences in the positive cases for glanders, such as euthanasia and interdiction of farms, considering that there was great resistance on the part of the farm owners who opposed

Table 1 - Positive control (focus) and negative control (non-focus) farms for glanders from 2017 to 2021 in Northeastern Brazil.

| Localization | ----------Result---------- | ----------No of animals---------- |
|---|---|---|
| 1. União dos Palmares– AL* | Negative | 45 |
| 2. Angelim – PE** | Negative | 200 |
| 3. Rio Largo – AL | Negative | 52 |
| 4. Viçosa – AL | Negative | 41 |
| 5. Mata Norte - Itambé PE | Negative | 44 |
| 6. Gravatá – PE | Negative | 134 |
| 7. João Pessoa – PB*** | Negative | 4 |
| 8. São Bento do Una – PE | Negative | 20 |
| 9. Recife – PE | Negative | 4 |
| 10. Recife – PE | Negative | 13 |
| 11. Recife – PE | Negative | 9 |
| 12. Porto Cavo – AL | Positive | 11 |
| 13. São Luiz do Quintude – AL | Positive | 12 |
| 14. Palmeira dos Índios – AL | Positive | 1 |
| 15. Camocim – CE**** | Positive | 7 |
| 16. Limoeiro – PE | Positive | 40 |
| 17. Recife –PE | Positive | 20 |

*Alagoas.
**Pernambuco.
***Paraíba.
****Ceará.

participating in this research, making it difficult to collect information leading to a smaller number of farms with glanders outbreaks in this study.

The univariate analysis of the factors studied demonstrated that the objective of the animals destined for commerce, sport, and work; the absence of information about the methods of bacterium transmission; failure to carry out periodic examinations for the diagnosis of glanders; not making glanders tests mandatory before the purchase of new animals; use of an extensive rearing systems; and the low frequency of stall cleaning were factors associated with the occurrence of glanders on the farms (Table 2).

Global equid transport increases annually and is directly associated with live animal trade, sporting events, and exhibitions (BRASIL, 2018). Thus, quarantine periods and diagnostic tests are the main objectives to prevent the spread of *B. mallei* (SAID et al., 2016). The transit of asymptomatic animals is a relevant risk factor for the spread of the etiological agent of glanders, especially considering the Brazilian scenario where equid trade is frequent for use in sporting events and exhibitions (KHAN et al., 2013).

According to the MAPA, it is mandatory to utilize the Animal Transit Guide for interstate and intra-state transit of equids, with a negative result for glanders required within the validity period (BRASIL, 2018). However, in some places, the transit of animals to participate mainly in fairs or horseback riding occurs without the Animal Transit Guide. This is also exacerbated by the system's inability to inspect all movement of equidae.

The accreditation of tests with high sensitivity and specificity that provide veracity to the positive results is of fundamental importance to avoid the displacement of false negative animals, which could result in the spread of the agent throughout the territory and at the level of international borders and cause damage to the equid production chain (ELSCHNER et al., 2017),

In this way, the points addressed in the questionnaire, the objective of raising the horse and the requirement of a negative test for the acquisition of these animals, which were correlated with the positive cases, justify how the lack of control in the movement of animals directly interferes with the spread of the bacteria between the farms.

The intense animal's transit results in a high frequency of glanders cases in the Northeastern Region of Brazil, and through molecular analyses, high heterogeneity was observed between the strains

Table 2 - Analysis of the association between epidemiological variables and the occurrence of glanders in equids in the Northeast Region of Brazil from 2017 to 2021.

| FACTORS | -------Positive Farms------- | | ------Negative Farms------ | | -----P-value----- |
|---|---|---|---|---|---|
| | F.A.* | F.R.** (%) | F.A. | F.R. (%) | |
| 1. Purpose of creation | | | | | 0.023*** |
| Reproduction | - | - | 6 | 100 | |
| Competition | - | - | 1 | 100 | |
| Commerce, sport, and | 3 | 42.86 | 4 | 57.14 | |
| farm work | 3 | 100.00 | - | - | |
| 2. Knowledge about methods of bacterium transmission | | | | | |
| Yes | 1 | 10 | 9 | 90 | 0.034*** |
| No | 5 | 71.43 | 2 | 28.57 | |
| 3. Tests conducted for glanders diagnosis | | | | | |
| Yes | 3 | 21.43 | 11 | 78.57 | 0.029*** |
| No | 3 | 100 | - | - | |
| 4. Requirement of negative examination in the purchase of horses | | | | | |
| Yes | 2 | 15.38 | 11 | 84.62 | 0.06*** |
| No | 4 | 100 | - | - | |
| 5. Use of intensive breeding system | | | | | |
| Yes | 2 | 16.67 | 10 | 83.33 | 0.027*** |
| No | 4 | 80 | 1 | 20 | |
| 6. Frequency of facility cleaning | | | | | |
| Weekly | 2 | 100 | - | - | 0.001*** |
| Once a day | - | - | 3 | 100 | |
| More than once a day | - | - | 8 | 100 | |

*Absolute frequency.
**Relative frequency.
***Result values.

of *B. mallei*, demonstrating genetic diversity and dispersion of this pathogen in this region (SILVA et al., 2009). FALCÃO et al. (2019), through molecular sequencing, identified the Turkey 10 strain in equids in Northeastern Brazil, more specifically in the states of Pernambuco and Alagoas, demonstrating the strong impact of animal trade between the two states and the participation of animals at fairs and agricultural events as factors in the spread of *B. mallei* strains, considering that the same strain was found in different distinct places.

A study conducted to evaluate the risk factors for glanders in Punjab Province, Pakistan, an endemic disease region, reported that the increased seropositivity of the animals was directly related to locations where *B. mallei* was detected in the soil, including environmental contamination, as a risk factor for disease. Seropositive equidae circulating in large grazing areas can excrete *B. mallei* in the environment, resulting in the seroconversion of healthy animals that graze together (GHORI et al., 2017). Therefore, the extended creation system, as observed in our study, has direct interference in the epidemiology of the disease, leading to an increase in the number of disease focus areas in the country.

Lack of hygiene in stalls is another factor that related to glanders occurrence, as daily hygiene practices act directly as a protective factor to reduce the number of cases (KHAN et al., 2012; MALIK et al., 2015). Humid environments and the presence of houseflies, vectors of the bacteria, are favorable for the multiplication of *B. mallei* (LOPEZ et al., 2003). This microorganism is sensitive to the action of sunlight, heat, and common disinfectants and can barely survive in contaminated environments for more than 2 weeks (NEUBAUER et al., 1997); however, in favorable environments, it can survive for a few months (NEUBAUER et al., 1997; OIE, 2018).

Drinking fountains are a potential source of infection, considering that this bacterium can live for up to 100 days in water; therefore, animals become infected by ingesting water contaminated with nasal

secretions from infected animals (MALIK et at., 2015). In addition to skin exudates from glanders-positive animals, these secretions contain a considerable number of active bacteria that can easily contaminate, in addition to drinking and feeding troughs, brushes, harnesses, whips, spurs, and other items that can spread the bacteria (GREGORY & WAAG, 2007).

The measures recommended by MAPA for the control and eradication of glanders in the national territory are directed towards the diagnosis of animals and the sanitation of farms that are positive for glanders. However, it still lacks instructions on correct management measures and guidance to producers, based on scientific studies to prevent the occurrence of new cases of the disease.

## CONCLUSION

The identification and understanding of the factors associated with the occurrence of glanders in the Northeast Region of Brazil facilitate the establishment of management measures that minimize the transmission of the bacteria and reduce the number of cases of the disease. In this way, actions for greater clarification of equine breeders on the purpose and type of breeding, information on the disease, periodic examinations, and cleaning of facilities should be disclosed to reduce the number of glanders cases, in addition to the diagnostic measures and transit methods already used by the MAPA.

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## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

## BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

This project was approved by the Ethics Committee on the Use of Animals of the Federal University of Alagoas (license number 92/2016), the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (number A23EF3A), and the Plataforma Brasil (Certificate of Presentation of Ethical Appreciation, number 41426720.5.0000.9547).

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