



Current status of glanders in Brazil: recent advances and challenges

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Abstract

Glanders is an infectious disease that causes serious damage to the equine production chain in countries where it occurs endemically and poses a risk to public health. This study aimed to conduct an integrative review of the advances in the knowledge of glanders in Brazil over the last three decades since its re-emergence. Documentary research was conducted for the period between the years 2000 and 2022. SCOPUS and PUBMED databases were used to search for scientific articles, dissertations, and thesis, in addition to the Brazilian Digital Library of Thesis and Dissertations (BDTD). A total of 41 documents were retrieved, including 12 dissertations, five theses, and 24 scientific articles. The Federal Rural University of Pernambuco (UFRPE) group provided a noteworthy amount of material on this topic (25 documents, four theses, three dissertations, and 18 scientific articles). During this period, ten dissertations, one thesis, and six scientific articles published by other groups in other states of the Federation were also identified. It was concluded that there was a significant number of scientific publications with relevant data on the clinical, epidemiological, pathological, microbiological, serological, and molecular characteristics of glanders disease in Brazil. Additionally, training of human resources regarding this disease led to an increase in the nucleation of research groups, especially in the northeast region of Brazil. Despite significant advances, new research groups and specific funding are still needed for the development of more accurate diagnostic methods, immunizing production, training of veterinarians to recognize the disease, and more robust programs to control and eradicate the disease in Brazil.

Keywords Glanders disease · Lymphangitis · Equidae · *B. mallei*

Introduction

History of re-emergence of glanders in Brazil

Glanders is an infectious zoonotic disease that mainly affects Equidae. From a global perspective, it is still found in some countries in Asia, Africa, the Middle East, and South America [1]. Between 2020 and 2022, cases were reported from China, Iran, Nepal, and Turkey [1]. This disease is caused by a bacterium called *Burkholderia mallei* and is clinically characterized by fever, lymphangitis, skin nodules, cough, and a purulent runny nose [2].

In Brazil, this disease was introduced in 1811 through the importation of infected horses from Europe [3]. After its eradication from Brazil in 1968, new cases of the disease were described in Equidae in 1999, in the states of Pernambuco and Alagoas, in northeastern Brazil.

The historical background of the re-emergence of glanders in the country was obtained from clinical, epidemiological, serological, and microbiological records that confirmed the circulation of *B. mallei* in sugarcane mills in the states of Pernambuco and Alagoas. The field and laboratory studies were conducted under the leadership and coordination of researcher Dr. Rinaldo Mota, professor in the Department of Veterinary Medicine at the Federal Rural University of Pernambuco (UFRPE). Professor Marilene de Farias Brito from the Veterinary Institute of the Federal Rural University of Rio de Janeiro (UFRRJ) also participated in the study.

The animals in the studied foci presented a clinical picture of bronchopneumonia, purulent nasal secretions, nodulations, and ulcers in the cutaneous lymphatic chain. After collecting the secretions of cutaneous nodules (abscesses), it was possible

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to isolate a Gram-negative bacterium that was later confirmed to be *B. mallei* by biochemical tests. Strauss's test was also performed on guinea pigs, in addition to anatomic-histopathological and serological tests for antibody research. Lesions compatible with glanders and the presence of anti-*B. mallei* antibodies were confirmed. After achieving bacterial isolation and confirming the diagnosis, the disease was reported to the Ministry of Agriculture, Livestock and Food Supply (MAPA) of Brazil, and they, in turn, sent a technical team to evaluate the impact of the disease in these states.

This study was conducted in sugarcane mills in the Mata area of the states of Pernambuco and Alagoas, which had cases of a respiratory and lymphatic disease in mules that worked daily for traction of products from the sugarcane industry. The main complaint of the owners of the animals was discharge of nasal phlegm accompanied by ulcerated lesions on the skin, in addition to weight loss and death. Treatment with antimicrobials did not affect the clinical remission.

The team performed a thorough clinical examination of the animals and observed nodular lesions in the nasal mucosa; they harvested purulent material from skin abscesses by aspiration. This material was sent to the Laboratory of Bacterioses of Domestic Animals of the UFRPE for microbiological isolation. First, a blood agar culture medium (non-selective) was used to isolate Gram-positive and Gram-negative bacteria. After 48 h of incubation in aerobiosis at 37 °C, non-hemolytic whitish colonies similar to dew drops were observed. Gram-negative bacteria were detected using Gram's technique. After performing a battery of biochemical tests, the causative bacterium was suspected to be of the genus *Burkholderia* spp., hitherto unknown to Dr. Rinaldo Mota of UFRPE.

Insisting on the possibility of it being a new Equidae disease, the researcher searched for information and conducted a detailed survey on bacterial diseases of Equidae across the world, even for those that were already considered to be eradicated. In this bibliographic study, the researcher came across information about glanders, one of the oldest diseases of Equidae, which had a similar clinical and epidemiological description to the current disease presentation in the animals. The researcher then decided to perform other laboratory tests such as Strauss's test on male guinea pigs. The test results revealed lesions and clinical findings compatible with *B. mallei* infection. Professor Marilene de Farias Brito of UFRRJ also simultaneously identified the bacterium and confirmed

that the histopathological findings were compatible with those of glanders. Soon after, *B. mallei* infection was also confirmed by serological tests, which, together with previous findings, confirmed the re-emergence of glanders in Brazil.

The first article published in Brazil reporting the clinical, epidemiological, pathological, and diagnostic aspects of glanders in 2000 was a part of this ongoing study [4]. The authors concluded that urgent sanitary measures were needed to prevent the spread of the bacterium to Equidae from other states of the Federation. Research on the different aspects of the disease began that year because, at that time, we already had a scientific lapse of 30 years in the national literature on glanders disease; that is, we did not have robust information to support the control and eradication of the disease. Since the notification of the disease, the country has imposed international restrictions on the Equidae trade as well as on the participation of animals in various events. Altogether, we aim to present and discuss the advances in knowledge regarding glanders in Equidae in Brazil.

Materials and methods

The proposed methodology is an integrative review of glanders disease in Brazil between 2000 and 2022. For this purpose, the SCOPUS and PUBMED databases were used to search for scientific articles, and the Brazilian Digital Library of Thesis and Dissertations (BDTD) was used to search for dissertations and thesis. The combined terms used to search the databases are listed in Table 1. The survey was conducted on March 10, 2022.

The eligibility criterion adopted for the analysis was that the articles, dissertations, and thesis were related to the glanders theme, regardless of the type of study. For research on scientific productions (articles, thesis, and dissertations) of the group of the Federal Rural University of Pernambuco, the aforementioned bases and curriculum lattes of the main researcher (Rinaldo Aparecido Mota) were used. This last tool was included only for the main researcher's lattes to retrieve important articles, dissertations, and theses on the subject in question that were not indexed in the SCOPUS, PUBMED, and BDTD databases, as they have a historical value. The exclusion criteria were non-participation of Brazilian researchers in the research and records with duplicates in the databases (SCOPUS and PUBMED).

Table 1 Search strategy performed in the SCOPUS, PUBMED, BDTD databases on glanders between 2000 and 2022

Base	Combined search terms
SCOPUS	"Glanders" OR "muermo" OR "Burkholderia mallei" AND "Brazil" OR "Brasil"
PUBMED	("Glanders"[All Fields]) AND ("brazil"[All Fields]); (("burkholderia mallei"[All Fields]) AND ("brazil"[All Fields]))
BDTD	"Glanders" and " <i>burkholderia mallei</i> "; "Glanders"

For a better understanding and analysis of the evolution of knowledge regarding glanders in Brazil, a two-part analysis of the data was performed. First, we analyzed the results of the research conducted by the Laboratory of Infectious Diseases of Domestic Animals of UFRPE (they were the pioneers in studying this disease in Brazil). Second, we analyzed the results of other research groups.

Results

The results of database search are shown in Table 2.

After the preliminary reading of the titles and abstracts of the articles, dissertations, and theses retrieved from the search databases, six articles from the SCOPUS database and nine from the PUBMED database were excluded for data duplication. A total of 41 documents were obtained, including 12 dissertations, five theses, and 24 scientific articles. The UFRPE group contributed 25 documents, four theses, three dissertations, and 18 scientific articles (Tables 3 and 4). During this period, nine dissertations, one thesis, and six scientific articles published by other groups in other states of the Federation were also identified (Table 5).

^aThis thesis was retrieved from personal communication, since there are no records in the databases.

The first publication on the re-emergence of glanders in Brazil, in addition to the first dissertation and thesis, was published by the research group of the Laboratory of Infectious Diseases of Domestic Animals of UFRPE, a pioneer in the study of this disease in Brazil. The timeline of the records of the study is shown in Fig. 1.

Discussion

In the 22 years since the evidence of the re-emergence of glanders in Brazil, preceded by approximately 30 years of probable eradication of the disease, several scientific studies and training of human resources regarding this disease were conducted. It is important to highlight that after the MAPA was notified about this disease, specific legislation on the disease was published and included in the National Program

of Equidae Health, which contains guidelines to prevent, control, or eradicate diseases of Equidae. This normative instruction was updated and published by MAPA on January 16, 2018 [44].

To facilitate understanding, we hereby present a discussion on topics according to the historical evolution of the studies conducted by the UFRPE group led by Dr. Rinaldo Mota and other research groups in the country. Furthermore, we present the clinical, epidemiological, and pathological advances (experimental models). In addition, we also present the microbiological, molecular, and serological diagnosis, as well as future reflections for the advancement in the study of glanders disease in Brazil.

Advances in clinical, epidemiological, and pathological studies

Research on the clinical and epidemiological indicators of glanders was not initiated in Brazil. When the disease re-emerged, previous reports had already demonstrated the signs and some epidemiological indicators of glanders in Equidae. However, with the performance of new research, we have made significant advances in the study of the disease after its re-emergence [4].

Initially, the first thesis of the UFRPE research group was presented in 2003 in the Graduate Program in Veterinary Medicine of UFRPE. The central objective was to describe the frequencies of the clinical findings of glanders in mules working in sugarcane mills in the state of Pernambuco, Brazil. This study contributed in describing the clinical indicators at different stages of the disease. It also contributed to the differential diagnosis of respiratory and lymphatic diseases in Equidae in Brazil. In this study, lesions and clinical signs were restricted to the skin, respiratory, and lymphatic systems. From its re-emergence, glanders was considered as a prominent disease in the country. Furthermore, on conclusive diagnosis, the affected animals were euthanized [7].

Veterinarians working in the field and in the Animal Health Defense service also benefited greatly from this research. Until now, they did not have enough information about the clinical presentation of glanders disease in order to clinically diagnose suspected cases. The studies also allowed us to make

Table 2 Results of the search of SCOPUS, PUBMED, BDTD, and UFRPE* databases on glanders disease

Base	Article		Dissertation		Thesis	
	Recovered	Included	Recovered	Included	Recovered	Included
SCOPUS	22	16				
PUBMED	10	1				
BDTD			26	10	5	2
Lattes*	18	7	3	2	3	3
Total	50	24	29	12	8	5

*Researcher (Rinaldo Aparecido Mota).

Table 3 Main advances in the study of glanders from the presentation of theses and dissertations by the UFRPE group

Year	Type of intellectual production	Author	Title	Advances
2003	Dissertation	Silva [5]	Tipagem molecular de amostras de <i>Burkholderia mallei</i> isoladas de equídeos com mormo por Ribotipagem-PCR	First biochemical and molecular identification of <i>Burkholderia mallei</i> isolates in Brazil
2003	Thesis	Silva [6]	Estudo da infecção experimental em cobaias (<i>Cavia porcellus</i>) pela <i>Burkholderia mallei</i>	-First study on the experimental model of <i>Burkholderia mallei</i> infection in guinea pigs (Strauss test)
2003	Thesis	Rabelo [7]	Estudo epidemiológico, clínico, hematológico e bioquímico sérico de muarees naturalmente infectados pela <i>Burkholderia mallei</i>	-First clinical study and clinical pathology in mules naturally infected by <i>Burkholderia mallei</i>
2010	Thesis	Silva [16]	Produção e avaliação da proteína derivada (PPD) de <i>Burkholderia mallei</i> para o diagnóstico imuno-alérgico do mormo em equídeos	Production and evaluation of national mallein as an alternative for the diagnosis and control of Glanders in Brazil
2012	Dissertation	Teles [20]	Desenvolvimento e avaliação de um teste ELISA Indireto para o diagnóstico sorológico do mormo em equídeos	Standardization of an indirect ELISA as a Glanders diagnostic tool
2021	Thesis	Falcão [43]	Caracterização molecular e estudo do genoma de <i>Burkholderia mallei</i> isoladas de equídeos nos estados de Pernambuco e Alagoas, Brasil	Genetic characterization <i>B. mallei</i> isolates obtained from Glanders Equidae in the states of Pernambuco and Alagoas
2022	Dissertation	Carvalho [41]	Identificação dos fatores de manejo associados à ocorrência do Mormo em equídeos, no Nordeste do Brasil	First study on the identification and understanding of factors associated with the occurrence of Glanders in Brazil

photographic records (Fig. 2) of the main clinical findings that were extensively used by the authors for disseminating disease information as teaching material for undergraduate and graduate students in Brazilian universities and other countries. Moreover, it was also used in health defense training in the states of the Federation to identify the disease [4].

The UFRPE team also performed an epidemiological study of the disease, where a low prevalence and high lethality for Equidae (nearly 100%) was observed in positive transit animals. Additionally, they noticed differences in clinical manifestation, the intensity of symptoms, and forms of presentation between each of the studied species (horses, mules, and donkeys), with mules and donkeys having greater susceptibility. Some aspects of the chain of transmission of bacteria in herds were also defined. The disease was characterized as more frequent in animals that were elderly, stabled, or submitted to intensive and/or semi-extensive management, who share food and water in collective troughs and are subjected to inadequate hygienic-sanitary management [4].

In 2003, another thesis in the area of pathology was presented in the Graduate Program in Veterinary Medicine at UFRPE, which dealt with the intraperitoneal inoculation of different field isolates of *B. mallei* in guinea pigs (Strauss's test). In this study, the clinicopathological response pattern was defined for different field isolates of *B. mallei*, in addition to a detailed study of the hematological response of inoculated animals. The pattern of macro- and microscopic lesions revealed differences in the intensity of the response of the animals according to the different bacterial isolates. Moreover, an experimental infection model was established in guinea pigs that contributed to other studies related to the diagnosis of the disease [6].

In 2019, we published a study on space–time analysis of the disease in the country with data from the registration of glanders outbreaks obtained from the World Organization of Animal Health [37]. More recently, in 2022, a dissertation presented in the Graduate Program in Animal Bioscience of UFRPE specifically focused on the study of management factors associated with the occurrence of glanders in Brazil. Variables associated with glanders were identified: the objective of use of the animal (for example: trade, sport, or farm work), lack of information on the forms of transmission of the bacterium, non-periodic examinations for the diagnosis of glanders, requirement of negative test results on the purchase of horses, and use of the extensive rearing system at the lowest frequency of cleaning of the bays. The results should assist in the implementation of management measures that minimize the risk of transmission of the bacterium and reduce cases of the disease in the region [41].

Advances in microbiological, molecular, and serological diagnosis

The beginning of microbiological research involving *B. mallei* preceded the diagnosis of glanders re-emergence in 2000,

Table 4 Main advances in the study of glanders based on the scientific articles published by the UFRPE group

Year	Author	Title	Advances
2000	Mota et al. [4]	Glanders in equidae in the states of Pernambuco and Alagoas	First record of the re-emergence of Glanders in Brazil
2004	Mota et al. [8]	Influence of bacteria isolated from upper airway lesions on microbiological diagnosis of Glanders in equidae	Advancement and improvement in the microbiological diagnosis of Glanders
2004	Rabelo et al. [9]	Hematological and biochemical aspects in mules naturally infected by <i>Burkholderia mallei</i>	First hematological and biochemical study of mules naturally infected with <i>Burkholderia mallei</i>
2005	Mota et al. [10]	Biochemical characterization and in vitro antimicrobial sensitivity profile of <i>Burkholderia mallei</i> samples isolated from equidae from the northeastern region of Brazil	First antimicrobial sensitivity profile study of <i>Burkholderia mallei</i> field isolates
2006	Mota [2]	Etiological, epidemiological and clinical aspects of Glanders	Literature review as support for scientific dissemination of the Glanders
2006	Rabelo et al. [11]	Clinical indicators in mules naturally infected by <i>Burkholderia mallei</i>	First study on clinical indicators in mules naturally infected with <i>Burkholderia mallei</i>
2007	Mota et al. [12]	Serum Proteinogram in mules naturally infected by the <i>Burkholderia mallei</i>	First study on serum proteinogram in mules naturally infected with <i>Burkholderia mallei</i>
2008	Mota et al. [13]	Clinical alterations in guinea pigs (<i>Cavia porcellus</i>) experimentally inoculated with <i>Burkholderia mallei</i> field isolates of Glanders equidae	Establishment of an experimental model of <i>Burkholderia mallei</i> infection in guinea pigs
2009	Schmoock et al. [14]	DNA microarray-based detection and identification of <i>Burkholderia mallei</i> , <i>Burkholderia pseudomallei</i> , and <i>Burkholderia</i> spp.	Development of a rapid molecular assay for differentiation of <i>Burkholderia</i> spp. species
2009	Silva et al. [15]	Phenotypic and molecular characterization of <i>Burkholderia mallei</i> samples isolated in northeastern Brazil	First biochemical and molecular identification of <i>Burkholderia mallei</i> isolates in Brazil
2010	Mota et al. [18]	Glanders in donkeys (<i>Equus asinus</i>) in the state of Pernambuco, Brazil: a case report	First record of clinical and histopathological findings of Glanders in donkeys in Brazil
2012	Teles et al. [23]	Development and evaluation of an indirect ELISA test for the serological diagnosis of Glanders in equidae	Standardization of an indirect ELISA as a Glanders diagnostic tool
2013	Silva et al. [24]	Assessment of the effectiveness of the PPD-mallein produced in Brazil for diagnosing Glanders in mules	Production and evaluation of a national malleina as an alternative for the diagnosis and control of Glanders in Brazil
2014	Silva et al. [26]	Production and partial purification of PPD-mallein for diagnosis of Glanders in equidae	-Production and evaluation of national mallein as an alternative for the diagnosis and control of Glanders in Brazil
2019	Falcão et al. [34]	First record of <i>Burkholderia mallei</i> Turkey 10 strain originating from glan-derous horses from Brazil	First registration of <i>Burkholderia mallei</i> turkey 10 strain in horses with Glanders in Brazil
2019	Fonseca-Rodríguez et al. [37]	Spatiotemporal Analysis of Glanders in Brazil	Epidemiological analysis of the distribution of Glanders foci in Brazil
2021	Rocha et al. [40]	Monitoring the outbreak of equine Glanders in Alagoas, Brazil: clinical, immunological, molecular, and anatomopathological findings	Clinical, serological and molecular monitoring of horses in a disease focus
2022	Falcão et al. [42]	Molecular characterization of <i>Burkholderia mallei</i> strains isolated from Brazil (2014–2017)	Genetic characterization <i>B. mallei</i> isolates obtained from Glanders Equidae in the states of Pernambuco and Alagoas

Table 5 Results of research conducted in the SCOPUS, PUBMED, BDTD databases between 2000 and 2022

Type of intellectual production	Author	Institutions	Year	Title
Article	Mota et al. [4]	UFRPE	2000	Glanders in equidae in the states of Pernambuco and Alagoas
Thesis ^a	Santos	UFMG	2002	Aspectos clínicos, morfológicos e imunohistoquímico do Mormo em equídeos naturalmente infectados pela <i>Burkholderia mallei</i>
Dissertation	Silva [5]	UFRPE	2003	Tipagem molecular de amostras de <i>Burkholderia mallei</i> isoladas de equídeos com mormo por Ribotipagem-PCR
Thesis	Silva [6]	UFRPE	2003	Estudo da infecção experimental em cobaias (<i>Cavia porcellus</i>) pela <i>Burkholderia mallei</i>
Thesis	Rabelo [7]	UFRPE	2003	Estudo epidemiológico, clínico, hematológico e bioquímico sérico de muarens naturalmente infectados pela <i>Burkholderia mallei</i>
Article	Mota et al. [8]	UFRPE	2004	Influence of bacteria isolated from upper airway lesions on microbiological diagnosis of Glanders in equidae
Article	Rabelo et al. [9]	UFRPE	2004	Hematological and biochemical aspects in mules naturally infected by <i>Burkholderia mallei</i>
Article	Mota et al. [10]	UFRPE	2005	Biochemical characterization and in vitro antimicrobial sensitivity profile of <i>Burkholderia mallei</i> samples isolated from equidae from the northeastern region of Brazil
Article	Mota [2]	UFRPE	2006	Etiological, epidemiological and clinical aspects of Glanders
Article	Rabelo et al. [11]	UFRPE	2006	Clinical indicators in mules naturally infected by <i>Burkholderia mallei</i>
Article	Mota et al. [12]	UFRPE	2007	Serum Proteinogram in mules naturally infected by the <i>Burkholderia mallei</i>
Article	Mota et al. [13]	UFRPE	2008	Clinical alterations in guinea pigs (<i>Cavia porcellus</i>) experimentally inoculated with <i>Burkholderia mallei</i> field isolates of Glanders equidae
Article	Schmoock et al. [14]	Friedrich-Loeffler-Institut	2009	DNA microarray-based detection and identification of <i>Burkholderia mallei</i> , <i>Burkholderia pseudomallei</i> , and <i>Burkholderia</i> spp.
Article	Silva et al. [15]	UFRPE	2009	Phenotypic and molecular characterization of <i>Burkholderia mallei</i> samples isolated in northeastern Brazil
Thesis	Silva [16]	UFRPE	2010	Produção e avaliação da proteína derivada (PPD) de <i>Burkholderia mallei</i> para o diagnóstico imuno-alérgico do mormo em equídeos
Dissertation	Moraes [17]	UNB	2011	Prevalência de mormo e anemia infecciosa equina em equídeos de tração do Distrito Federal
Article	Mota et al. [18]	UFRPE	2010	Glanders in donkeys (<i>Equus asinus</i>) in the state of Pernambuco, Brazil: a case report
Article	Carvalho Filho et al. [19]	UFMG	2012	Development and validation of a method for purification of mallein for the diagnosis of Glanders in equines
Dissertation	Teles [20]	UFRPE	2012	Desenvolvimento e avaliação de um teste ELISA Indireto para o diagnóstico sorológico do mormo em equídeos
Dissertation	Carvalho Filho [21]	UFMG	2012	Desenvolvimento e avaliação de um novo método para produção de maleína para diagnóstico de mormo
Dissertation	Souza [22]	UFRPE	2012	Diagnóstico do mormo através da técnica de fixação do complemento utilizando-se diferentes antígenos e métodos de incubação

Table 5 (continued)

Type of intellectual production	Author	Institutions	Year	Title
Article	Teles et al. [23]	UFRPE	2012	Development and evaluation of an indirect ELISA test for the serological diagnosis of Glanders in equidae
Article	Silva et al. [24]	UFRPE	2013	Assessment of the effectiveness of the PPD-mallein produced in Brazil for diagnosing Glanders in mules
Dissertation	Silva [25]	UFRPE	2014	Avaliação da reação em cadeia de polimerase (PCR) e ELISA indireto como método de diagnóstico da <i>Burkholderia mallei</i> (Mormo)
Article	Silva et al. [26]	UFRPE	2014	Production and partial purification of PPD-malein for diagnosis of Glanders in equidae
Dissertation	Oliveira [27]	UFPR	2016	Desenvolvimento e padronização de teste para diagnóstico de mormo por soroaaglutinação em placa
Article	Girault et al. [28]	Paris-Est University	2017	First draft genome for a <i>Burkholderia mallei</i> isolate originating from a glanderous mule from Brazil
Dissertation	Gouveia Filho [29]	UFRPE	2017	Desempenho da fixação do complemento para diagnóstico do mormo utilizando antígeno de cepas brasileiras de <i>Burkholderia mallei</i>
Article	Laroucau et al. [30]	Paris-Est University	2018	First molecular characterisation of a Brazilian <i>Burkholderia mallei</i> strain isolated from a mule in 2016
Dissertation	Rosado [31]	UFPB	2018	Caracterização epidemiológica do mormo em equídeos no Estado da Paraíba com base em dados secundários
Dissertation	Rocha [32]	UFAL	2018	Infecção por <i>Burkholderia mallei</i> em equídeos e cobaias (<i>Cavia porcellus</i>): avaliação da resposta humoral e estudo clínico
Thesis	Silva [33]	UFRPE	2018	Avaliação do diagnóstico do mormo
Article	Falcão et al. [34]	UFRPE	2019	First record of <i>Burkholderia mallei</i> Turkey 10 strain originating from glanderous horses from Brazil
Dissertation	Silva [35]	USP	2019	Gerenciamento por processos de negócios na gestão e no controle epidemiológico do Mormo no Brasil
Article	Cárdenas et al. [36]	College of Veterinary Medicine, Raleigh, North Carolina	2019	<i>Burkholderia mallei</i> : The dynamics of networks and disease transmission
Article	Fonseca-Rodríguez et al. [37]	UFRPE	2019	Spatiotemporal Analysis of Glanders in Brazil
Article	Abreu et al. [38]	Instituto Biológico de São Paulo	2020	Systematic monitoring of Glanders-infected horses by complement fixation test, bacterial isolation, and PCR
Article	Fonseca Júnior et al. [39]	LFDA-MG	2021	Validation of three qPCR for the detection of <i>Burkholderia mallei</i> in equine tissue samples
Article	Rocha et al. [40]	UFAL	2021	Monitoring the outbreak of equine Glanders in Alagoas, Brazil: clinical, immunological, molecular, and anatomopathological findings
Dissertation	Carvalho [41]	UFRPE	2022	Análise de fatores de risco para o mormo, detecção de gente de virulência TTSS e genes de resistência a B-lactâmicos em isolados de <i>Burkholderia mallei</i> do Nordeste do Brasil
Article	Falcão et al. [42]	UFRPE	2022	Molecular characterization of <i>Burkholderia mallei</i> strains isolated from Brazil (2014 – 2017)

when the researcher responsible for proving the existence of the disease in the country isolated the bacterium from closed cutaneous nodules of mules with clinical signs of the disease and performed phenotypic characterization of the bacterium obtained in different culture media. Since then, imperative studies have been conducted that have significantly advanced

the microbiological diagnosis through isolation of bacterium and its phenotypic and molecular characterization [4].

The first dissertation on the microbiology of glanders was presented in 2003 at the Graduate Program in Veterinary Medicine at UFRPE. In this study, the biochemical characteristics of *B. mallei*, different media of bacterial cultivation,

Fig. 1 Timeline of the records of the studies on the glanders carried out by the research group of the Laboratory of Infectious Diseases of Domestic Animals of UFRPE, a pioneer in the study of glanders in Brazil

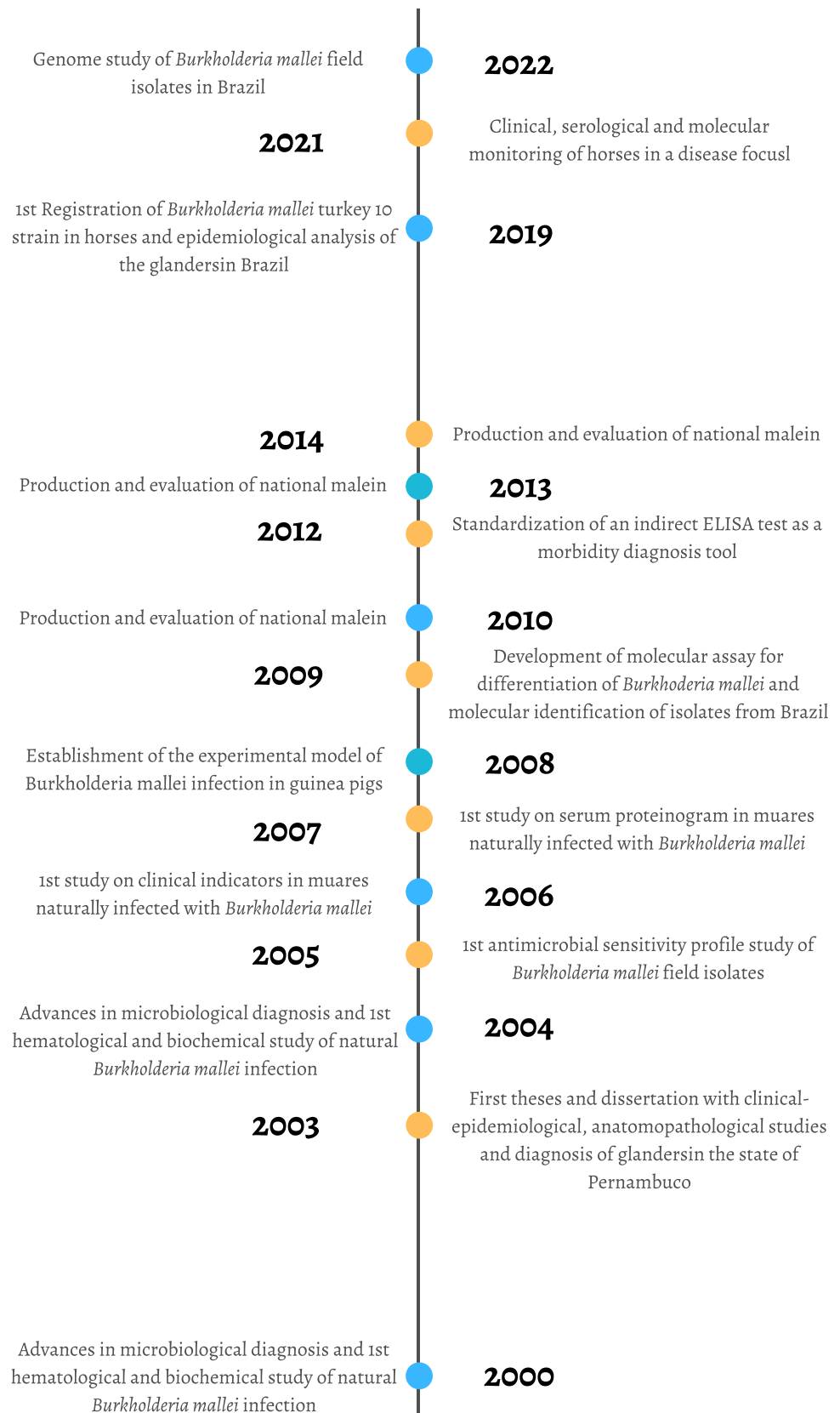


Fig. 2 Clinical, epidemiological, and pathological findings of glanders' cases in Equidae in Brazil. Glanders: nodules in lymphatic vessels and skin ulcers (a); respiratory clinical presentation of glanders in mules: unilateral purulent nasal secretion in mules (b); cachexia and purulent nasal secretion in mules (c); nodules and thickening of the lymphatic vessels of the neck in donkeys (d); risk factors for glanders: confinement and feeding in collective troughs (e); pulmonary granuloma in mule necropsy (f); liver nodules were distributed in the liver of mules (g); and proliferative inflammatory reaction in the nasal septum of mules (h)



phenotypic tests of antimicrobial sensitivity, and molecular characterization of *B. mallei* isolates recovered from Equidae from the states of Pernambuco and Alagoas were demonstrated. Molecular differences were demonstrated in the circulating strains from northeastern Brazil. The conclusion was that there are no effective treatment protocols for glanders, but the study of antimicrobial resistance of *B. mallei* isolates can help in the treatment of this disease in humans [5].

The first published study on the microbiological diagnosis of glanders was in 2004. It allowed us to conclude that other bacteria isolated from lesions of the upper airways of Equidae influence the isolation of *B. mallei* and consequently the microbiological diagnosis of glanders. This study recommended that instead of samples of nasal swabs, samples of closed skin nodules should be collected for microbiological diagnosis purposes, which result in much higher sensitivity and specificity. This study was a hallmark in microbiological diagnosis and decisively confirmed the re-emergence of the disease in the country. The researchers who studied the disease previously did not reach a conclusive diagnosis; this was because they always collected biological material for bacterial isolation of the nasal cavity of sick animals which did not obtain satisfactory microbiological results [8].

In 2009, we participated in a research study where a rapid oligonucleotide microarray assay based on genetic markers was conducted for the precise identification and differentiation of *B. mallei* and *B. pseudomallei*, namely, glanders and melioidosis agents, respectively. This study was conducted in partnership with the Institute of Bacterial Infections and Zoonosis of the Federal Institute of Animal Health Research, Germany and allowed the advancement in the differentiation of these species that cause similar clinicopathological diseases in Equidae [14].

In 2019, a study was published on the first record of the Turkey 10 lineage of *B. mallei* in horses in northeastern Brazil. This study confirmed the circulation of a single line of *B. mallei* in the northeastern region of the country, and this result will contribute to future improvements in diagnostic techniques [34]. Abreu et al. [38] performed isolation of *B. mallei* in four animals, in addition to standardizing the PCR to improve the molecular detection of the bacteria in clinical samples from positive animals monitored for glanders. In 2021, another thesis was presented in the Graduate Program in Animal Bioscience at UFRPE, which aimed to genetically characterize *B. mallei* isolates obtained from glanders affected Equidae in the states of Pernambuco and Alagoas. This research was partnered with the National Food Security Agency of France. Sequencing revealed a correlation between the circulating strains from the two states, in addition to a genetic variation between strains of Pernambuco. This suggested multiple events of the introduction of the bacterium in Brazil. This is the first description of an isolate with genetic similarity with other strains reported in Europe, which suggested more than one event of the introduction of the disease in the country [43].

The identification of the same strain of circulating *B. mallei* is important for the development and improvement of serological diagnostic techniques and development of specific molecular markers for the detection of Brazilian strains. In addition, the identification of the same strain also contributed to for the development and improvement of serological diagnostic techniques and development of specific molecular markers for the detection of Brazilian strains, in addition to the production of vaccines, since treatment is not recommended by the legislation that is in force in Brazil and the World Organisation for Animal Health (OIE). The results of this research are of great impact to the scientific community because they nullify any hypothesis about the possibility that the causative bacteria of glanders are not present in the country. Some previous discussions in this regard have been put forward over the years by a small group of veterinarians and owners of horses but without a scientific basis. This is in contrast to the vast amount of scientific evidence that proves the occurrence of the disease in the country [42].

Regarding serological and immunoallergic diagnosis, we highlight the contribution of our group with a thesis presented in the Graduate Program in Animal Bioscience of UFRPE in 2010. It aimed to produce and evaluate a national purified protein derivative (PPD) malein using a local *B. mallei* isolate as an alternative for the diagnosis and control of glanders in Brazil. This PPD-malein was evaluated in guinea pigs and demonstrated good sensitivity and specificity when applied to horses. The horses were experimentally infected with inactivated *B. mallei* and compared with the control group [16]. In 2012, a dissertation was presented in the same program that aimed to standardize and evaluate an indirect ELISA for the diagnosis of glanders. This test was the first to use an isolate of *B. mallei* originating from an equine in Brazil [20]. The standardization and evaluation of indirect ELISA was the starting point for new studies that improved this diagnostic technique. In 2015, it was registered in the MAPA of Brazil and included in the National Equidae Health Program as a screening technique for glanders in place of the complement fixation technique (which was used only for animals in international transit). Notably, ELISA technique presented better sensitivity and specificity than the complement fixation test. The PPD-malein produced was not used in the National Program for the control and eradication of glanders, as it was replaced by western blotting as a confirmatory technique. PPD-malein is currently used in Brazil for foals up to 6 months of age [44].

Advances made by other research groups on glanders in Brazil

Ten dissertations on glanders were presented, the first in the Graduate Program in Animal Health at the University of Brasília to understand the epidemiological situation of

glanders and equine infectious anemia in traction Equidae of the Federal District in 2011 [17]. In 2012, another dissertation was undertaken at the Veterinary School of the Federal University of Minas Gerais to develop and evaluate a new method for the production of malein for the diagnosis of glanders [19]. In the same year, the dissertation was presented in the Graduate Program in Veterinary Medicine of UFRPE, by another research group, to evaluate the results of the complement fixation test against two commercially available antigens, using two methods of incubation in equine sera from a property in the state of Pernambuco [20]. In 2014, another dissertation was presented in the Graduate Program in Tropical Animal Science of UFRPE to evaluate polymerase chain reaction (PCR) and indirect ELISA as diagnostic methods for *B. mallei* (Glanders) [25]. In 2016, another dissertation was presented in the Graduate Program in Veterinary Sciences of the Federal University of Paraná to develop and standardize a test for the diagnosis of glanders by plaque seragglutination [27].

In 2017, a dissertation was presented in the Graduate Program in Veterinary Medicine, UFRPE, to evaluate antigens (Biovotech, Recife Brazil) produced with Brazilian strains of *B. mallei* for the diagnosis of glanders compared to the c.c.pro antigen (GmbH, Oberdorla, Germany), which employs strains from Yugoslavia, India, and Indonesia [29]. In 2018, a dissertation was presented in the Graduate Program in Veterinary Science of the Federal University of Paraíba to study the epidemiological characterization of glanders in Equidae in the state of Paraíba based on secondary data. In 2018, a thesis was presented in the Graduate Program in Tropical Animal Science that phylogenetically identified the strains circulating in the national territory for animals suspected of glanders [31]. In 2019, the last dissertation on this theme was presented in the Graduate Program in Sciences of the University of São Paulo that contributed to the analysis of the critical points caused by this disease's legal/sanitary threat within the business chain [35].

Training of human resources related to the glanders disease was impressively performed in the northeastern region of Brazil, even for groups that were not part of the team of the main researcher of UFRPE. Only six dissertations were conducted in other states: one in the federal district, one in Paraná, one in São Paulo, one in Minas Gerais, one in Paraíba, and one in Alagoas. Although these dissertations and theses had relevant findings, most were not published as scientific articles, except for a dissertation by the Federal University of Minas Gerais group, which advanced the scientific knowledge in the field. In a previous study, we produced and evaluated PPD-malein for glanders [21]. Another article published by the same group, but which was not part of a dissertation or thesis, presented the results of the validation of three qPCR for the detection of *B. mallei* in tissue samples of horses. It is noteworthy that knowledge on the

subject should be made available to the scientific community in journals after peer review and subsequent publication. Thus, unpublished results are not discussed in depth in this article [39].

In 2017 and 2018, two other important scientific contributions were published together with a group from France. These studies presented the genomic sequence of the *B. mallei* 16-2438_BM#8 strain isolated from a mule in Pernambuco, northeastern Brazil. They also presented the first molecular characterization based on multiple locus variable-number tandem repeat analysis and single nucleotide polymorphism analysis of a *B. mallei* strain isolated from a mule found dead in northeastern Brazil in 2016 [28, 30]. In 2020, the findings of a study conducted by the research group of the Biological Institute, São Paulo, in which systematic monitoring of horses infected with glanders was performed by complement fixation test, bacterial isolation, and PCR, were published. This study concluded that the cold complement fixation test with United States Department of Agriculture (USDA) and c.c.pro antigens in combination with PCR increase the sensitivity of diagnosis and may be useful in the diagnosis of chronic glanders [38].

It is noteworthy that the main scientific articles published outside the UFRPE group had the participation of some foreign researchers, who were the main contributors of these studies in the area of microbial genetics and in the diagnosis of glanders.

Challenges for future studies

In recent decades, there have been important advances in the clinical-epidemiological, microbiological, and molecular diagnosis of glanders in Brazil. Notably, relevant training of human resources led to the increase of capillarity and nucleation of new research groups in the country, which may contribute to research in this area. Despite the advances observed in this area, it is still important to nucleate new research groups and approve specific funding lines for research. This is necessary to improve the diagnosis and production of immunizers, because the country will not be able to move forward given the few financial resources and increased competition in the field of research. This disease has a strong impact on the equine production chain and causes severe economic obstacles to the commercialization of animals and their products. Additionally, there are restrictions on the movement of animals in the country and abroad. Moreover, it is an important zoonosis neglected in the country and needs to be better understood and studied. Further study on this is needed because human health professionals do not yet have sufficient knowledge, and the disease may be underreported.

At present, we have a sound scientific base and highly qualified researchers who support research in the area of

vaccines. Nevertheless, there are still no investments that further the advances in research in this area. Health education programs based on studies of factors associated with glanders should also be implemented in the country to contribute to disease control and eradication.

In recent decades, the scientific advances of glanders in Brazil have transformed the scenario of knowledge on the subject, even under deficient financial and human resources for the execution of research projects. Much of the knowledge and training of human resources in the area was generated in the northeast region of Brazil, more specifically by the UFRPE group. It is important to highlight that in Brazil, the disease can present with varied clinical and epidemiological characteristics, with a predominance of the acute form in donkeys and mules in the northeast region. However, in the Southeast region, the disease is usually chronic and affects horses more frequently. Even in the face of all the scientific evidence that proves the existence of glanders in Brazil, there continue to be difficulties that lead to legal issues of glanders cases that compromise scientific research and confirmation of the disease by direct methods. International partnerships with important research centers in Germany and France have also been established and are important for the study of glanders in Brazil.

It is important to continue training veterinarians for the clinical identification of the disease. In addition, it is crucial to sensitize the surveillance and control agencies of the Ministry of Agriculture to conduct a seroepidemiological survey on the disease in the country to obtain results in all states of the Federation. This will help to plan and structure control and eradication endeavors.

Declarations

Ethics approval No ethical approval was required as this is a review article with no original research data.

Conflict of interest The authors declare no competing interests.

References

- OIE (2022) World Organization for Animal Health. <https://wahis.oie.int/#/dashboards/country-or-disease-dashboard>. Accessed 10 March 2022
- Mota RA (2006) Aspectos etiopatológicos, epidemiológicos e clínicos do mormo. *Vet Zootec* 13:117–124. Available from: <https://rvz.emnuvens.com.br/rvz/article/view/260/131>
- Pimentel W (1938) History and organization of the army veterinary service. *Milit Vet Med J* 1(4):283–322
- Mota RA, Brito MF, Castro FJ, Massa M (2000) Glanders in horses in the states of Pernambuco and Alagoas. *Pesq Vet Bras* 20(4):155–159. <https://doi.org/10.1590/S0100-736X2000000400005>
- Silva KPC (2003) Tipagem molecular de amostras de *Burkholderia mallei* isoladas de equídeos com mormo por Ribotipagem-PCR. Dissertation, Federal Rural University of Pernambuco. Not available for on line evaluation
- Silva LBG (2003) Diagnóstico microbiológico do mormo em equídeos e infecção experimental em cobaias (*Cavia porcellus*) pela *Burkholderia mallei*: aspectos clínicos e anátomo-histopatológicos. Doctoral Thesis, Federal Rural University of Pernambuco. Not available for on line evaluation
- Rabelo SSA (2003) Estudo clínico, hematológico e bioquímico sérico de muarees naturalmente infectados pela *Burkholderia mallei*. Doctoral Thesis, Federal Rural University of Pernambuco, Recife. Not available for on line evaluation
- Mota RA, Farias AFA, Silva LBG, Rabelo SSA, Soares PC, Cunha AP (2004) Influência das bactérias isoladas de lesões das vias áreas superiores no diagnóstico microbiológico do mormo em equídeos. *Vet Not* 10:41–46
- Rabelo SSA, Mota RA, Nascimento Sobrinho ES, Cunha AP, Silva Neto JB, Teixeira MN, SoaresCarneiro PCAS (2004) Hematological and biochemical aspects in mules naturally infected by *Burkholderia mallei*. *Cienc Vet Trop* 2:98–105
- Mota RA, Silva LBG, Silva KPC, Silva Neto JB, Cunha AP, Nascimento Sobrinho ES (2005) Caracterización bioquímica y perfil de sensibilidad antimicrobiana in vitro de muestras de *Burkholderia mallei* aisladas de équidos de la región Nordeste de Brasil. *Arq Inst Biol* 72:7–11. <https://doi.org/10.1590/1808-1657v72p0072005>
- Rabelo SSA, Soares PC, Silva LBG, Cunha AP, Nascimento Sobrinho ES, Pinheiro Junior JW, Barbosa MAG, Mota RA (2006) Clinical indicators in mules naturally infected by *Burkholderia mallei*. *Vet Anim Sci* 13:54–62
- Mota RA, Rabelo SSA, Cunha AP, Pinheiro Junior JW, Rego EW, Soares PC, Oliveira VLA, Valença MIB (2007) Proteinograma sérico em muarees naturalmente infectados pela *Burkholderia mallei*. *Braz J Vet Res Anim Sci* 44(2):69–76. <https://doi.org/10.11606/issn.1678-4456.bjvras.2007.26643>
- Mota RA, Silva LBG, Cunha AP, Nascimento Sobrinho ES, Pinheiro Junior JW, Rabelo SSA, Oliveira AAF (2008) Alterações clínicas em cobaias (*Cavia porcellus*) inoculados experimentalmente com isolados de campo de *Burkholderia mallei* de equídeos com mormo. *Med Vet (UFRPE)* 2:1–9. <http://www.journals.ufrpe.br/index.php/medicinaveterinaria/article/view/700/579>
- Schmoock G, Ehrlich R, Melzer F, Rassbach A, Scholz HC, Neubauer H, Sachse K, Mota RA, Saqib M, Elschner M (2009) DNA microarray-based detection and identification of *Burkholderia mallei*, *Burkholderia pseudomallei* and *Burkholderia* spp. *Mol cell probes* 23:178–187. <https://doi.org/10.1016/j.mcp.2009.04.001>
- Silva KPC, Mota RA, Cunha AP, Silva LBG, Leal NC, Cavalcante YVN, Teles JAA, Pereira MCC, Freitas NS (2009) Phenotypic and molecular characterization of *Burkholderia mallei* samples isolated in northeastern Brazil. *Pesq Vet Bras* 29(5):439–444. <https://doi.org/10.1590/S0100-736X2009000500015>
- Silva KPC (2010) Produção e avaliação da proteína derivada (PPD) de *Burkholderia mallei* para o diagnóstico imuno-alérgico do mormo em equídeos. Doctoral Thesis, Federal Rural University of Pernambuco. <http://www.tede2.ufrpe.br:8080/tede2/handle/tede2/5700>
- Moraes DDA (2011) Prevalência de mormo e anemia infecciosa equina em equídeos de tração do Distrito Federal. Dissertation, University of Brasília. https://repositorio.unb.br/bitstream/10482/10231/1/2011_DaniellaDianeseAlvesdeMoraes.pdf
- Mota RA, da Fonseca Oliveira AA, da Silva AM, Junior JW, da Silva LB, de Farias BM, Rabelo SS (2010) Glanders in donkeys (*Equus asinus*) in the state of Pernambuco, Brazil: a case report. *Braz J Microbiol* 41(1):146–149. <https://doi.org/10.1590/S1517-83822010000100021>
- Carvalho Filho MB, Ramos RM, Fonseca AA Jr, Orzil LL, Sales ML, Santana VLA, Souza MMA, Machado ER, Lopes Filho

- PR, Leite RC, Reis JKP (2012) Development and validation of a method for purification of mallein for the diagnosis of glanders in equines. *BMC Vet Res* 8:154. <https://doi.org/10.1186/1746-6148-8-154>
20. Teles JAA (2012) Desenvolvimento e avaliação de um teste ELISA indireto para o diagnóstico sorológico do mormo em equídeos. Dissertation, Federal Rural University of Pernambuco. <http://www.tede2.ufrpe.br:8080/tede2/handle/tede2/4636>
 21. Carvalho Filho MB (2012) Desenvolvimento e avaliação de um novo método para produção de maleína para diagnóstico de mormo. Dissertation, Federal University of Minas Gerais. <http://hdl.handle.net/1843/BUOS-8VWMFM>
 22. Souza MMA (2012) Diagnóstico do mormo através da técnica de fixação do complemento utilizando-se diferentes antígenos e métodos de incubação. Dissertation, Federal Rural University of Pernambuco. <http://www.tede2.ufrpe.br:8080/tede2/handle/tede2/5776>
 23. Teles JAA, Campos AC, da Silva KP, Santos AS, Santana VLA, Castro RSD, Mota RA (2012) Standardization and evaluation of an indirect ELISA for the serological diagnosis of glanders in horses. *Pesq Vet Bras* 32(9):838–842. <https://doi.org/10.1590/S0100-736X2012000900004>
 24. da Silva KP, de Campos Takaki GM, da Silva LB, Saukas TN, Santos AS, Mota RA (2013) Assessment of the effectiveness of the PPD-mallein produced in Brazil for diagnosing Glanders in mules. *Braz J Microbiol* 44:179–188. <https://doi.org/10.1590/S1517-83822013005000022>
 25. Silva CMSL (2014) Avaliação da reação em cadeia de polimerase (PCR) e Elisa indireto como método de diagnóstico da Burkholderia mallei (Mormo). Dissertation, Federal Rural University of Pernambuco. <http://www.tede2.ufrpe.br:8080/tede2/handle/tede2/5041>
 26. Silva KPC, Takaki GMC, Teles JAA, Dantas AFM, Costa MM, Felix PW, Mota RA (2014) Production and partial purification of PPD-mallein for diagnosis of Glanders in equidae. *Pesq Vet Bras* 34:57–61. <https://doi.org/10.1590/S0100-736X2014000100010>
 27. Oliveira RC (2016) Desenvolvimento e padronização de teste para diagnóstico de mormo por soroaglutinação em placa. Dissertation, Federal University of Paraná. <http://hdl.handle.net/1884/43352>
 28. Girault G, Woudstra C, Martin B, Vorimore F, Santana VLA, Fach P, Madani N, Laroucau K (2017) First draft genome for a Burkholderia mallei isolate originating from a glanderous mule from Brazil. *Genome Announc* 5(28):e00579–e617. <https://doi.org/10.1128/genomeA.00579-17>
 29. Gouveia Filho PCB (2017) Desempenho da fixação do complemento para diagnóstico do mormo utilizando antígeno de cepas brasileiras de Burkholderia mallei. Dissertation, Federal Rural University of Pernambuco. <http://www.tede2.ufrpe.br:8080/tede2/bitstream/tede2/7134/2/Paulo%20Castelo%20Branco%20de%20Gouveia%20Filho.pdf>
 30. Laroucau K, Santana VLA, Girault G, Martin B, Miranda da Silveira PP, Brasil Machado M et al (2018) First molecular characterisation of a Brazilian Burkholderia mallei strain isolated from a mule in 2016. *Infect Genet Evolution* 57:117–120. <https://doi.org/10.1016/j.meegid.2017.11.014>
 31. Rosado F (2018) Caracterização epidemiológica do mormo em equídeos no Estado da Paraíba com base em dados secundários. Dissertation, Federal University of Paraíba. Available from: <https://repositorio.ufpb.br/jspui/handle/123456789/15432>
 32. Rocha LO (2018) Infecção por Burkholderia mallei em Equídeos e cobaios (Cavia porcellus): avaliação da resposta humoral e estudo clínico. Dissertation, Federal University of Alagoas. Available from: <http://www.repositorio.ufal.br/handle/riufal/6036>
 33. Silva CMSL (2018) Avaliação do diagnóstico do mormo. Doctoral Thesis, Federal Rural University of Pernambuco. 2018. Available from: <http://www.tede2.ufrpe.br:8080/tede2/handle/tede2/7288>
 34. Falcão MVD, Silveira PPM, Santana VLA, Da Rocha LO, Chaves KP, Mota RA (2019) First record of Burkholderia mallei Turkey 10 strain originating from glanderous horses from Brazil. *Braz J Microbiol* 50(4):1125–1127. <https://doi.org/10.1007/s42770-019-00113-2>
 35. Silva RLB (2019) Gerenciamento por processos de negócios na gestão e no controle epidemiológico do Mormo no Brasil. Dissertation, University of São Paulo. Available from: <https://www.teses.usp.br/teses/disponiveis/74/74134/tede-13082019-111700/publico/ME9527720COR.pdf>
 36. Cárdenas NC, Galvis JOA, Farinati AA, Grisi-Filho JHH, Diehl GN, Machado G (2019) Burkholderia mallei: the dynamics of networks and disease transmission. *Trans emerg disease* 66:715–728. <https://doi.org/10.1111/tbed.13071>
 37. Fonseca-Rodríguez O, Pinheiro Júnior JW, Mota RA (2019) Spatiotemporal analysis of glanders in Brazil. *J Equine Vet Sci* 78:14–19. <https://doi.org/10.1016/j.jevs.2019.03.216>
 38. Abreu DC, Gomes AS, Tessler DK, Chiebao DP, Fava CD, Romaldini AHCN, Araujo MC, Pompei J, Marques GF, Harakava R, Pituco EM, Nassar AFC (2020) Systematic monitoring of Glanders-infected horses by complement fixation test, bacterial isolation, and PCR. *Vet Anim Sci* 10:100147. <https://doi.org/10.1016/j.vas.2020.100147>
 39. Fonseca Júnior AA, Pinto CA, Alencar CAS, Bueno BL, Dos Reis JKP, de Carvalho Filho MB (2021) Validation of three qPCR for the detection of Burkholderia mallei in equine tissue samples. *Arch Microbiol* 203(7):3965–3971. <https://doi.org/10.1007/s00203-021-02367-7>
 40. Rocha LO, Lima LAR, Albuquerque RMS, Lages SLS, Nunes ACBT, Castro RS, Mota RA, Silva KPC, Falcão MVD (2021) Monitoring the outbreak of equine Glanders in Alagoas, Brazil: clinical, immunological, molecular, and anatomopathological findings. *Cienc Rural* 51(12). <https://doi.org/10.1590/0103-8478r20200834>
 41. Carvalho JCS (2022) Identificação dos fatores de manejo associados à ocorrência do Mormo em equídeos no Nordeste do Brasil. Dissertation, Federal Rural University of Pernambuco. Not available for on line evaluation
 42. Falcão MVD, Laroucau K, Vorimore F, Deshayes T, Santana VLA, Silva KPC, do Nascimento SA, de Castro RS, Araújo FR, Mota RA, (2022) Molecular characterization of Burkholderia mallei strains isolated from horses in Brazil (2014–2017). *Infect Genet Evolution* 99:105250. <https://doi.org/10.1016/j.meegid.2022.105250>
 43. Falcão MVD (2021) Caracterização molecular e estudo do genoma de Burkholderia mallei isoladas de equídeos nos estados de Pernambuco e Alagoas, Brasil. Doctoral Thesis, Federal Rural University of Pernambuco. https://sucupira.capes.gov.br/sucupira/public/consultas/coleta/trabalhoConclusao/viewTrabalhoConclusao.jsf?popup=true&id_trabalho=10965133
 44. Brasil (2018) Portaria nº 06, de 16 de janeiro de 2018. Minist. Agric.Pec.eAbastecimento. https://www.in.gov.br/web/guest/materia/-/asset_publisher/Kujrw0TZC2Mb/content/id/1892934/do1-2018-01-17-instrucao-normativa-n-6-de-16-de-janeiro-de-2018-1892930

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