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Molecular characterization of *Burkholderia mallei* strains isolated from horses in Brazil (2014–2017)

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ABSTRACT

Glanders is an infectious zoonosis caused by *Burkholderia* (*B.*) *mallei* that mainly affects equids. The objective of this work was to provide additional knowledge on the diversity of the strains circulating in Brazil. Six *Burkholderia mallei* isolates obtained during necropsies of glanderous horses between 2014 and 2017 in two different states (Pernambuco and Alagoas) were analyzed by polymerase chain reaction–high-resolution melting (PCR-HRM). While four strains (9902 RSC, BM_campo 1, BM_campo 3 and UFAL2) clustered in the L3B2 branch, which already includes the Brazilian 16-2438_BM#8 strain, two strains (BM_campo 2.1 and BM_campo 2.2) clustered within the L3B3sB3 branch, which mostly includes older isolates, from Europe and the Middle East. Whole genome sequencing of two of these strains (UFAL2 and BM_campo 2.1), belonging to different branches, confirmed the HRM typing results and refined the links between the strains, including the description of the L3B3Sb3Gp1SbGp1 genotype, never reported so far for contemporary strains. These results suggest different glanders introduction events in Brazil, including a potential link with strains of European origin, related to colonization or trade.

1. Introduction

Burkholderia (B.) mallei is the causative agent of glanders, a zoonotic disease requiring compulsory notification to the World Organization for Animal Health (OIE) (OIE, 2018) and, in Brazil, to the official veterinary services (Brasil, 2018). In Brazil, the disease was first described by Pimentel (1938) in 1811, and the country was officially considered glanders-free in 1960, until the description of re-emergent cases in the 2000s in the states of Pernambuco and Alagoas (Mota et al., 2000). Nowadays, glanders cases have been recorded in 25 of the 27 Brazilian states, with an increase in the affected areas, although the incidence of the disease remains low (Fonseca-Rodríguez et al., 2019).

Equids are known to be susceptible to glanders and are carriers and disseminators (Mota, 2006; Neubauer et al., 2005). The disease can present in several clinical forms: cutaneous, respiratory, lymphatic, or asymptomatic. The respiratory form is characterized by chronic pneumonia with cough, epistaxis, and dyspnea. In the cutaneous and lymphatic forms, which are usually associated, rigid lymph nodes are observed in the abdominal region and on the lateral and medial faces of the limbs (Al-Ani et al., 1987; Mota *et al.,* 2006). In asymptomatic animals, characteristic lesions can be found during necropsies (Al-Ani and Roberson, 2007; Elschner et al., 2009). Limited clinical signs developing in the early stages of the disease also make clinical and laboratory diagnoses difficult. Serological tests such as the complement fixation test (CFT) or enzyme-linked immunosorbent assay (ELISA) and/or mallein-ization can be complemented by bacteriology or molecular biology.

Specific *B. mallei* molecular-based detection methods are available, including the OIE recommended and widely used PCR system targeting

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the *fli*P-IS407A sequence (Scholz et al., 2006; Tomaso et al., 2006). In contrast to the multi-locus sequence typing (MLST) scheme (Godoy et al., 2003), which fails to discriminate between *B. mallei* strains, the multiple-locus variable-number tandem repeat analysis (MLVA) method (U Ren et al., 2007) and more recently the polymerase chain reaction–high-resolution melting (PCR-HRM) method (Laroucau et al., 2018; Scholz et al., 2014) have been able to highlight the diversity of strains within the *B. mallei* species.

Few molecular typing data are available concerning circulating *B. mallei* strains in Brazil, with only one whole genomic sequence obtained from a strain isolated from a donkey in 2016 available to date (Girault et al., 2017). Included in the PCR-HRM development work, this strain has been localized to the L3B2 branch (Laroucau et al., 2018).

The present study aimed to characterize six new *B. mallei* isolates from cases diagnosed in Pernambuco and Alagoas states between 2014 and 2017, using PCR-HRM and whole genome sequencing (WGS). The aim was to provide additional knowledge about the strains circulating in the Northeast Region of Brazil.

2. Material and methods

2.1. Animals

From December 2014 to October 2017, six samples from horses that were tested by CFT in the framework of Brazil's national surveillance and control of animal movement program tested positive, and the animals were subject to compulsory slaughter by the local veterinary service (Brasil, 2018) (Table 1). The entire experimental procedure was in accordance with the ethical principles adopted by the Ethics Committee for the Use of Animals from the Federal Rural University of Pernambuco, license number 004/2018.

Five horses came from four different farms in Recife, a city in Pernambuco (PE) State, while the sixth animal came from Satuba, a city in Alagoas (AL) State. Both states are located in the Northeast region of Brazil.

2.2. Isolates

Tissue samples collected during necropsies were cultured on blood sheep agar containing 3% (*v*/v) glycerol at 37 °C for 48 to 72 h (Schmoock et al., 2009; OIE, 2018). Colonies were confirmed as *B. mallei* by PCR targeting the *fli*P-IS407A sequence, using conventional (Scholz et al., 2006) and real-time PCR (Tomaso et al., 2006) methods. The isolates were frozen at −80 °C in a preservation liquid medium containing 50% glycerol.

2.3. Single nucleotide polymorphism (SNP) analysis and PCR-HRM markers

The PCR-HRM genotype of the isolates was determined as previously described (Girault et al., 2018). In short, a set of 15 markers was used for PCR amplification, followed by a high-resolution melting PCR analysis, allowing classification of the strains into three lineages (L1 to L3) and into branches, sub-branches and groups.

2.4. Whole genome sequencing

Genomic DNA from each *B. mallei* isolate was extracted using a Promega® genomic DNA purification kit for DNA extraction from Gram-negative bacteria, according to the manufacturer's instructions. For whole genome sequencing, DNA libraries were prepared using a Nextera XT kit (Illumina) and sequencing reactions were performed according to the manufacturer's instructions. The MiSeq run was carried out on the DNA preparation, with paired-end reads of 250 bp using MiSeq V2 reagents, and with a sequencing depth comprised between 72 and 82 (data not shown). The raw reads were trimmed using Trimmomatic-0.36 with the following parameters: Leading:3, Trailing:3, SlidingWindow:4:15, and Minlen:36, and assembled *de novo* using SPAdes 3.13 with the default parameters. MeDuSa was used to perform the contig scaffolding against the *B. mallei* 23,334 strain. The genome sequences were deposited in the European Nucleotide Archive (ENA) database under accession numbers ERS6071509 and ERS6071510 (Table 2).

2.5. Whole genome phylogenetic analysis

Publicly available genomic sequences of 21 *B. mallei* strains were used for comparison (Fig. 2). This set of strains includes at least one strain per lineage, sub-branch, and group identified so far, and all WGS available for strains related to L3B2 and L3B3sB3. The whole genome SNP (wgSNP) pipeline of BioNumerics software v7.6.1 (Applied Maths, Belgium) was used in order to detect SNPs on whole genome sequences and we performed cluster analyses on the resulting wgSNP matrix. Input for the wgSNP module was the raw data, except for the reference. Each genome file was processed with the ART-MountRainier-2016-06-05 simulation tool that generates synthetic paired-end reads with coverage 50 (Huang et al., 2012). These reads were aligned and mapped against the reference sequence *B. mallei* ATCC 23344, using the BWA algorithm implemented in BioNumerics with a minimum of 90% sequence identity. A phylogenetic tree was built using RAxML version 8.2.9 with the GTRGAMMA model and 1,000 bootstrap replicates based on the filtered SNP matrix (2278 SNPs) from BioNumerics (Stamatakis, 2014). Strain-specific SNPs were identified using the BioNumerics wgSNP module and then filtered using the following conditions: minimum 5× coverage to call an SNP, removal of positions with at least one ambiguous base, one unreliable base or non-informative SNP, and minimum inter-SNP distance of 25 bp.

3. Results and discussion

None of the six animals included in this study showed clinical signs, with the exception of animal No. 6, an 8-month-old foal from the city of Satuba, which showed clinical signs one month after having been in contact with other horses returning from a trade fair. The foal showed left hind limb edema and the presence of ulcerated lesions, disseminated lymphangitis, hemoptysis and severe dyspnea, in addition to apathy and enlarged lymph nodes (Fig. 1), corresponding to classic signs of glanders (Mota, 2006). Nevertheless, the remaining five animals had characteristic lesions found at necropsy (pulmonary and hepatic abscesses and pulmonary congestion), and *B. mallei* strains were successfully recovered from tissue samples from these six horses (Table 1).

Table 1
Information about strains investigated in this study.

Animal	Month/Year	Location (city/state)	Farm	Host	Clinical signs	Collection source	Strain identification	PCR *FliP*	PCR-HRM genotype	WGS
1	12/2014	Recife/PE	A	Horse	No	Liver	9902 RSC	+	L3B2	No
2	03/2016	Recife/PE	B	Horse	No	?	BM_campo 1	+	L3B2	No
3	03/2016	Recife/PE	C	Horse	No	?	BM_campo 2.1	+	L3B3sB3	Yes
4	03/2016	Recife/PE	C	Horse	No	?	BM_campo 2.2	+	L3B3sB3	No
5	03/2016	Recife/PE	D	Horse	No	?	BM_campo 3	+	L3B2	No
6	10/2017	Satuba/AL	E	Horse	Yes	Lungs	UFAL2	+	L3B2	Yes

Table 2
WGS data.

Strain identification	Genome size (bp)	No. of contigs (scaffolds)	% G + C	N50	No. of CDS	Accession No.
BM_Campo 2.1	5,538,977	283	67.4	32,684	4640	ERS6071509
UFAL2	5,648,326	268	67.5	37,385	4739	ERS6071510

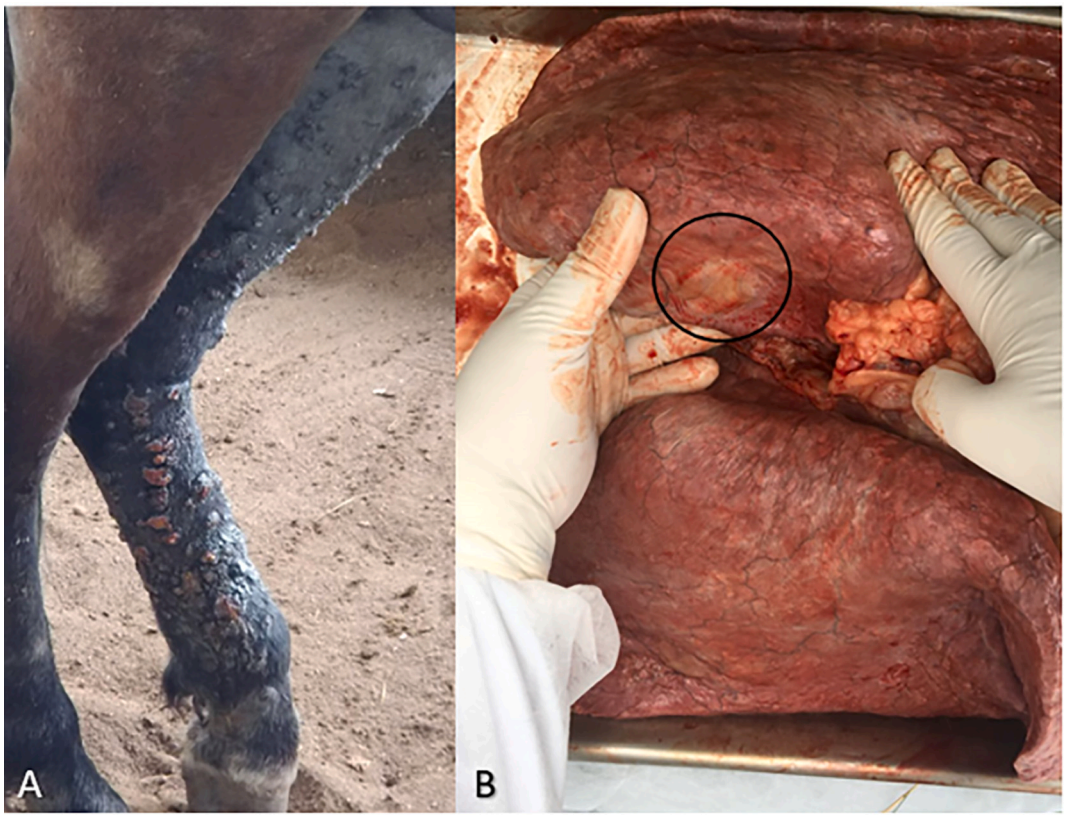

Fig. 1. Glanderous horse (No. 6), from Alagoas showed left hind limb edema with ulcerated lesions (A) and pictures of congestion in the lung parenchyma with nodules observed during the necropsy (B).

The PCR-HRM typing scheme based on 15 SNP signatures (Girault et al., 2018) was able to group four of these *B. mallei* isolates (9902 RSC, BM_campo 1, BM_campo 3 and UFAL2*)* in the L3B2 branch*,* which already includes the Brazilian 16-2438_BM#8 strain (Laroucau et al., 2018), alongside the Indian BMQ strain (Table 1, Fig. 2). The 16-2438_BM#8 strain was previously isolated from a glanderous donkey from Paudalho, a city in the state of Pernambuco. The location of these four new strains in this L3B2 branch suggests active circulation of related strains in this state, but also in the neighboring state of Alagoas, since the UFAL2 strain isolated from a horse from Satuba also belongs to this L3B2 branch. Animal trade, exhibition fairs, auctions and sport are activities that may explain the transmission and the circulation of the disease between these two neighboring states, as previously suggested by Fonseca-Rodríguez et al. (2019). Interestingly, the BM_campo 2.1 and BM_campo 2.2 strains, both isolated from horses belonging to the same farm, grouped together within the L3B3sB3 branch, which includes several older strains of *B. mallei*: 11 (Turkey/1949), NCTC10247 (Turkey/1960), NCTC_10229 (Hungary/1961), 2,002,734,299 (Hungary/1961), Ivan (Hungary/1961), 2,002,721,280 (Iran/1972), and SR0952700I (unknown origin). Both the BM_campo 2.1 and BM_campo 2.2 strains were isolated in 2016 from two asymptomatic but serologically positive horses. This is the first description of contemporary strains belonging to this L3B3sB3 group. Pimentel (1938) suggested that glanders may have been introduced to Brazil by infected animals imported from Europe at the beginning of the 19th century; these two strains may have links with strains from Europe and the Middle East isolated in this period.

For further phylogenetic analysis of the Brazilian isolates, the UFAL2 and BM_campo 2.1 strains, which belong to the L3B2 and L3B3sB3 branches, respectively, were subjected to whole genome sequencing. These data were used to construct a tree based on 2278 SNPs, together with 21 other selected *B. mallei* strains. Of note, the selection includes one strain per branch, except for the L3B2 and L3B3sB3 branches for which all available genomes were integrated. The PCR-HRM typing results for these two strains were confirmed by SNP analysis (Fig. 2), which provided additional information. UFAL2 clustered close to the 16-2438_BM#8 strain, in a distinct branch from the BMQ strain. While within the L3B3sB3 branch, two groups (Gp1 and Gp2), and two subgroups within the Gp1 group are observed, the BM_campo 2.1 strain clusters in the L3B3sB3Gp1SbGp1 group, in close proximity to Hungarian strains (NCTC_10229, 2,002,734,299, Ivan), all of which were isolated in 1961 and to SR0952700I, of unknown origin. Interestingly, based on the 2278 SNPs selected for analysis, only a few differences were observed between these strains isolated 50 years apart, suggesting a potentially common origin and a low rate of mutation for this species. This was also recently observed from the analysis of ten contemporary Indian strains, which were found to be a very homogeneous clade (Harisankar et al., 2021).

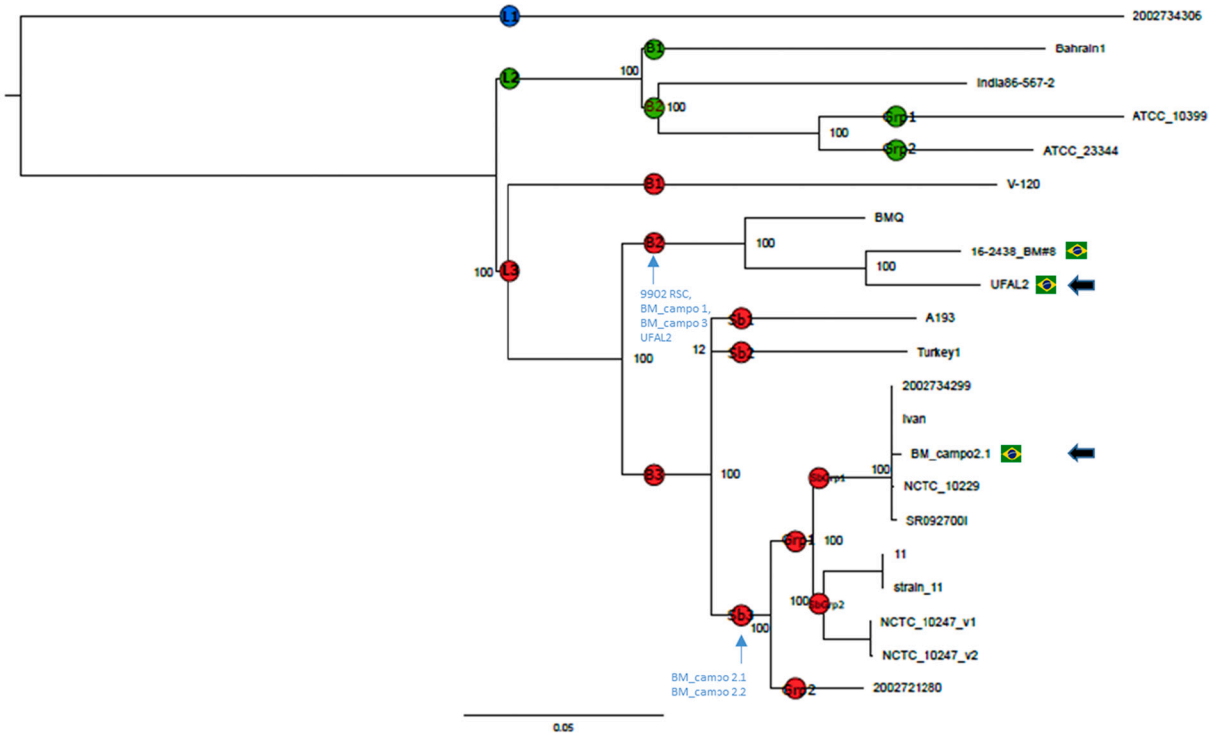

Fig. 2. SNP-based phylogenetic tree of *Burkholderia mallei* incorporating two newly sequenced strains from Brazil (UFAL2 and BM_campo2.1). A representative strain of each lineage (L1 to L3), branch, sub-branch and group was included, as well as all available genomes related to branches L3B2 and L3B3sB3, as previously determined (Girault et al., 2018). New subgroups were introduced: L3B3Sb3Gp1, L3B3Sb3Gp1SbGpr1, L3B3Sb3Gp1SbGpr2, and L3B3Sb3Gp2. PCR-HRM clustering results for the six Brazilian *B. mallei* DNAs included in the study, without preliminary WGS information, are shown in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Among the six horses included in this study, only animal No. 6 showed clinical signs. It was a young animal. The strain isolated from this animal (UFAL 2) is closely related to strains isolated from asymptomatic (9902 RSC, BM_campo 1, BM_campo 3) or clinical (16-2438_BM#8) (Laroucau et al., 2018) cases of glanders in Brazil. Inoculation routes and strains are probably important in terms of infection, but it is worth mentioning that the incubation period of the disease is variable, so the other animals could be in chronic or asymptomatic forms, therefore without obvious clinical signs (Al-Ani et al., 1987; Al-Ani and Roberson, 2007; Jubb et al., 1993; Mota, 2006).

More typing data are needed to describe the diversity of *B. mallei* strains circulating in Brazil. One of the limitations is access to strains, as cultivation of *B. mallei* from field samples is highly dependent on the bacterial load (Mota et al., 2004). The PCR-HRM typing method offers many advantages, including the ability to be applied directly to biological samples (Laroucau et al., 2020), and also the possibility of integrating new SNP markers, depending on the availability of WGS sequences and the degree of identification required. However, the design of new markers must consider the inherent limitations of the high GC rate of the genome, which means that strategies must be used to compensate for this constraint in order to target a specific SNP, as was recently the case for specific markers designed for Indian strains (Harisankar et al., 2021).

Knowledge of genotypes is an additional asset in the disease control strategy, making it possible to establish links between the various cases and to identify chains of contamination and transmission.

4. Conclusions

Two *B. mallei* genotypes in circulation in Brazil were described in this study, with the description of a new one, never reported for contemporary strains. These results suggest different introduction events regarding glanders in Brazil, including a potential link with strains of European origin, in connection with colonization or trade.

Ethical approval

The entire experimental procedure is in accordance with the ethical principles adopted by the Ethics Committee on Animal Use from the Federal Rural University of Pernambuco, license number 004/2018.

CRediT authorship contribution statement

Marcus V.D. Falcão: Conceptualization, Methodology, Formal analysis, Writing – original draft, Investigation. **Karine Laroucau:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Investigation. **Fabien Vorimore:** Data curation, Formal analysis. **Thomas Deshayes:** Data curation, Investigation. **Vania L.A. Santana:** Methodology, Data curation. **Karla P.C. Silva:** Investigation, Data curation. **Sergio A. do Nascimento:** Investigation, Data curation. **Roberto S. de Castro:** Investigation, Data curation. **Flabio R. Araújo:** Writing – review & editing. **Rinaldo A. Mota:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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References

Al-Ani, F.K., Roberson, J., 2007. Glanders in horses: a review of the literature. Vet. Arski 77, 203–218.

Al-Ani, F.K., Al-Delami, A.K., Ali, A.H., 1987. Glanders in horse: clinical and epidemiological studies in Iraq. Pak. Vet. J. 7, 126–129.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa n° 6 de 16 de janeiro de, 2018. Diretrizes Gerais para Prevenção, Controle e Erradicação do Mormo no Território Nacional. Diário Oficial da União de 17 de janeiro de 2018, Brasília-DF.

Elschner, M., Klaus, C., Liebler-Tenorio, E., Schmoock, G., Wohlsein, P., Tinschmann, O., Lange, E., Kaden, V., Klopfleisch, R., Melzer, F., Rassback, A., Neubauer, H., 2009. *Burkholderia mallei* infection in a horse imported from Brazil. Eq. Vet. Edu 21, 147–150.

Fonseca-Rodríguez, O., Júnior, J.W.P., Mota, R.A., 2019. Spatiotemporal analysis of glanders in Brazil. J. Equine Vet. Sci 18, 14–19.

Girault, G., Woudstra, C., Martin, B., Vorimore, F., Santana, V.L.A., Fach, P., Madani, N., Laroucau, K., 2017. First draft genome for a *Burkholderia mallei* isolate originating from a glanderous mule from Brazil. Genom. Announc. 5 (28).

Girault, G., Wattiau, P., Saqib, M., Martin, B., Vorimore, F., Singha, H., Engelsma, M., Roest, H.J., Spicic, S., Grunow, R., Vicari, N., De Keersmaecker, S.C.J., Roosens, N.H. C., Fabbi, M., Tripathi, B.N., Zientara, S., Madani, N., Laroucau, K., 2018. High-resolution melting PCR analysis for rapid genotyping of *Burkholderia mallei*. Infect. Genet. Evol. 63, 1–4.

Godoy, D., Randle, G., Simpson, A.J., Aanensen, D.M., Pitt, T.L., Kinoshita, R., Spratt, B. G., 2003. Multilocus sequence typing and evolutionary relationships amongs the causative agents of melioidosis and glanders, *Burkholderia pseudomallei* and *Burkholderia mallei*. J. Clin. Microbiol. 41, 2068–2079.

Harisankar, et al., 2021. Submitted.

Huang, W., Li, L., Myers, J.R., Marth, G.T., 2012. ART: a next-generation sequencing read simulator. Bioinformatics 28 (4), 593–594. https://doi.org/10.1093/bioinformatics/btr708.

Jubb, K.V.F., Kennedy, P.C., Plamer, N., 1993. Pathology of Domestic Animals, 4th ed. Academic Press.

Laroucau, K., Santana, V.L.A., Girault, G., Martin, B., Silveira, P.P.M., Brasil, M.M., Joseph, M., Wernery, R., Wernery, U., Zientara, S., Madani, N., 2018. First molecular characterization of a Brazilian *Burkholderia mallei* strain isolated from a mule in 2016. Infc. Gene. Evol. 57, 117–120.

Laroucau, K., Aaziz, R., Vorimore, F., Varghese, K., Deshayes, T., Bertin, C., Delannoy, S., Sami, A.M., Al Batel, M., El Shorbagy, M., Almutawaa, K.A.W., Alanezi, S.J., Alazemi, Y.S.N., Guernier-Cambert, V., Wernery, U., 2020. A genetic variant of *Burkholderia mallei* detected in Kuwait: consequences for the PCR diagnosis of glanders. Transbound. Emerg. Dis. https://doi.org/10.1111/tbed.13777.

Mota, R.A., 2006. Aspectos etiopatologicos, epidemiológicos e Clínicos do Mormo. Vet. Zootec. 13 (2), 117–124.

Mota, R.A., Brito, M.F., Castro, F.J.C., Massa, M., 2000. Mormo em equídeos nos Estados de Pernambuco e Alagoas. Pesqui. Vet. Bras. 20 (4), 155–159.

Mota, R.A., Farias, A.F.A., Silva, L.B.G., Rabelo, S.S.A., Soares, P.C., Cunha, A.P., 2004. Influência de bactérias isoladas das vias aéreas superiores no diagnóstico microbiológico do mormo em equídeos. Vet. Not 10 (1), 41–46.

Neubauer, H., Sprague, L.D., Zacharia, R., Tomaso, H., Al, Dahouk S., Wernery, R., Wernery, U., Scholz, H.C., 2005. Serodiagnosis of *Burkholderia mallei* infections in horses: state-of-the-art and perspectives. J. Vet. Med. B Infect. Dis Vet. Public Health 52, 201–205.

OIE (2018, November 14) World Animal Health. Terretrial Manual, Chapter 2.5.11 – Glanders and Melioidoses. Retrieved from https://www.oie.int/fileadmin/Home/fr/Health_standards/tahm/3.05.11_GLANDERS.pdf.

Pimentel, W., 1938. História e organização do serviço veterinário do exército. Revta. Milit. Med. Vet 1 (4), 283–322.

Schmoock, G., Ehricht, R., Melzer, F., Rassbach, A., Scholz, H.C., Neubauer, H., Sachse, K., Mota, R.A., 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.

Scholz, H.C., Joseph, M., Tomaso, H., al Dahouk, S., Witte, A., Kinne, J., Hagen, R.M., Wernery, R., Wernery, U., Neubauer, H., 2006. Detection of the reemerging agent *Burkholderia mallei* in a recent outbreak of glanders in the United Arab Emirates by a newly developed fliP–based polymerase chain reaction assay. Diag. Infec. Dis 54 (4), 241–247.

Scholz, H.C., Pearson, T., Hornstra, H., Projahn, M., Terzioglu, R., Wernery, R., Georgi, E., Riehm, J.M., Wagner, D.M., Keim, P.S., Joseph, M., Johnson, B., Kinne, J., Jose, S., Hepp, C.M., Witte, A., Wernery, U., 2014. Genotyping of *Burkholderia mallei* from an outbreak of glanders in Bahrain suggests multiple introduction events. PLoS Negl. Trop. Dis. 8 (9).

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.

Tomaso, H., Scholz, H.C., Al, Dahouk S., Eickhoff, M., Treu, T.M., Wernery, R., Wernery, U., Neubauer, H., 2006. Development of a 5′-nuclease real-time PCR assay targeting fliP for the rapid identification of *Burkholderia mallei* in clinical samples. Clin. Chem. 52, 307–310.

U Ren, J.M., Schupp, J.M., Pearson, T., Hornstra, H., Friedman, C.L., Smith, K.L., 2007. Tandem repeat regions within the *Burkholderia pseudomallei* genome and their application for high resolution genotyping. BMC Microbiol. 7 (23).