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VETERINARY MICROBIOLOGY – SHORT COMMUNICATION

First record of *Burkholderia mallei* Turkey 10 strain originating from glanderous horses from Brazil

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Abstract

Burkholderia (B.) mallei is the causative agent of glanders in Equidae. This study describes the first record of the Turkey 10 strain of *B. mallei* in glanderous horses in Northeastern of Brazil. This description should contribute to the future actions of diagnosis, control, and eradication of this disease in Brazil.

Keywords Sequencing · Diagnosis · Glanders · Brazil

Glanders is a zoonotic disease caused by *Burkholderia (B.) mallei* with compulsory notification to the World Organization for Animal Health (OIE). While glanders was eradicated in Europe and North America during the first half of the last century by restrictive measures to sacrifice infected animals, in Asia, the Middle East, and South America, the disease is still endemic. In recent years, outbreaks of the disease have been reported and continue to grow numbers of countries, including Brazil, Pakistan, Iran, the United Arab Emirates, Lebanon, and India [13]. In Brazil, glanders was described in 1811 by Pimentel [15], and in 1960, the disease was officially considered eradicated. In 2000, Mota et al. registered the emergence of new cases of glanders in the states of Pernambuco and Alagoas. Nowadays, the disease is registered in all Brazilian regions, and little progress has been made in the epidemiological, molecular, and control aspects of this important disease.

The molecular methods have a potential for detecting *B. mallei* in animals with clinical signs of glanders, differing from *Burkholderia pseudomallei* infections, which determine similar clinical signs, whereas these *Burkholderia* species have similarity in their genomes greater than 90% [9, 11, 12, 16, 17, 19, 20]. Polymerase chain reaction (PCR) has been used in the clinical setting and in public health laboratories for the identification of *B. mallei* [8, 14] and when associated with genetic sequencing, it is possible to reduce time and to improve the efficiency of the reagents used [1, 3, 5].

Due to the high similarity between *B. mallei* and *B. pseudomallei* in phenotypic characteristics in microbiological culture, biochemical and molecular tests, and the impossibility of serological differentiation [11], it is necessary to correctly identify this bacterium and the strain involved in suspected cases of glanders. The objective of this study was to know the circulating strains of *B. mallei* isolated from clinical cases of glanders in horses from Brazil.

The entire experimental procedure is 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.

In total, six samples were collected from equine positive in official serological tests for glanders according to the Ministry of Agriculture, Livestock and Supply (MAPA) [2]. Five animals belonged to rural properties located in the Metropolitan Region of Recife (MRR), Pernambuco, and one animal came

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from the Metropolitan Region of Maceió (MRM), Alagoas. After euthanasia, the animals were submitted to necropsy and fragments of the piogranulomatous lesions were collected in the lung and liver, which were stored in sterile polypropylene bottles at room temperature and transported to the laboratory.

The isolation was done from the purulent material obtained from the interior of the piogranulomatous lesions and processed in a biological safety cabin. The purulent material was cultured using blood sheep agar containing 3% (*v*/*v*) glycerol at 37 °C for 48 h [16]. The suspected colonies were selected according to the phenotypic description provided by the OIE [13] and submitted to conventional PCR for confirmation of *B. mallei*.

DNA extraction was performed in suspected colonies after culturing using the genomic DNA purification Promega® kit for DNA extraction from Gram-negative bacteria following the manufacturer's recommendation. Conventional PCR was performed on SimpliAmp Applied Biosystems® thermal cycler looking for differences in the *fliP* sequences of *B. mallei* strain ATCC 23344^T and *B. pseudomallei* strain K96243. The oligonucleotides used were Bma-IS407-*fliP*-f (5′-TCA-GGT-TTG-TAT-GTC-GCT-CGG-3′) and Bma-IS407-*fliP*-r (5′ CTA-GGT-GAA-GCT-CTG-CGC-GAG-3′) in a final reaction volume of 12.5 μL, using Go-Taq green mastermix Promega® and primers at 10 pmol concentration. The thermal profile conditions were an initial denaturation of 94 °C for 3 min, 34 cycles of denaturation at 94 °C for 30 s, annealing at 67 °C for 45 s and extension at 72 °C for 1 min, ending with an extension final at 72 °C for 7 min and as positive control, using standard strain *B. mallei* ATCC 15310. From the PCR reaction product, 9 μL was subjected to agarose gel electrophoresis (1.5% *w*/*v* in TAE buffer) to verify the presence of the 989-bp band [17].

PCR amplified products were purified using the Quiacki purification® kit following the manufacturer's recommendations and forwarded to the Central Laboratory (LABCEN) of the Center of Biological Sciences (CCB) from Federal University of Pernambuco (UFPE).

It was then performed a bidirectionally sequenced by standard protocols using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems®) following the manufacturer's recommendations using a 96-well plate and cycled in a Veriti thermocycler (Applied Biosystems®) for 40 cycles of 15 s at 96 °C, 15 s at 56 °C, and 4 min at 60 °C in an automated ABI-PRISM 3130 sequencer (Applied Biosystems®). Subsequently, the sequences acquired were analyzed with aid of software BioEdit® [7] and MEGA 5 to compare with the database found in the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST).

All six samples were submitted to GenBank which provided the following accession numbers: MK947136, MK947137, MK947138, MK947139, MK947140, and MK947141. The sequencing analysis results obtaining all six samples sequences were the same; the Turkey 10 strain of *B. mallei* then confirms the etiology of glanders (Fig. 1).

Glanders was described in Brazil in the nineteenth century, and since then, several measures have been suggested and implemented to control this disease [2, 10, 15]. Between 1960 and 1999 in Brazil, glanders was probably underreported and/or new sources of infection may have been introduced with the importation of animals from all over the world [10]. After confirmation of the disease in the country, there were some advances related to bacterial identification, including the use of a strain in the standardization of serological and intradermal diagnostic techniques. Silva et al. [18] studied animals from Pernambuco and Alagoas and observed four groups of genotypes with distinct biochemical characteristics, indicating differences of the circulating strains in the study area. Subsequently, Girault et al. [6] carried out the first draft of the genome of *B. mallei* strain 16-2438_BM#8 isolated from a mule in Pernambuco and concluded that a comparative genomic analysis of strains from different geographic regions and different hosts is necessary to improve the knowledge about this reemerging disease in the country.

The results obtained in our study indicate the circulation of the same strain of *B. mallei* in the studied animals. This finding is important from epidemiology aspect of glanders and was probably due to the strong trade in animals between the two states and also the participation of animals from fairs and agricultural events. This strain is also reported in Turkey [4], raising the alert level for the global distribution of this bacterium.

The identification of the same *B. mallei* strain circulating in different regions is an important finding to develop and to improve serological and molecular diagnostic techniques, with the development of specific molecular markers for strains

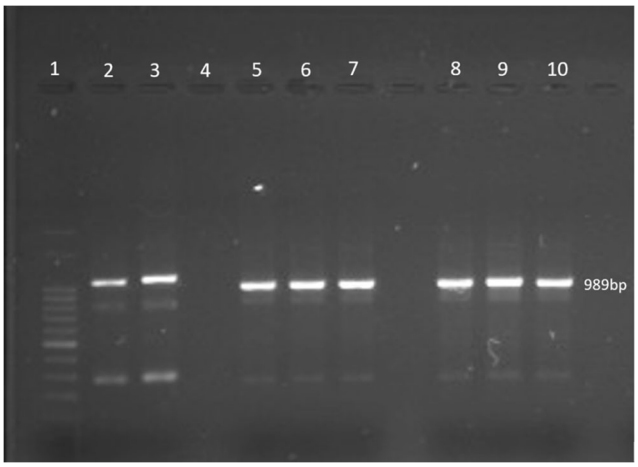

Fig. 1 Specific PCR for *B. mallei* agarose gel. Line 1, molecular marker; lines 2 and 3, positive controls; and line 4, negative control. Lines 5, 6, 7, 8, 9, and 10 are positive samples

detection, and also to contribute to vaccine production, since the treatment is not recommended by the OIE.

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Compliance with ethical standards

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References

1. Bauernfeind A, Roller C, Meyer D, Jungwirth R, Schneider I (1998) Molecular procedure for rapid detection of *Burkholderia mallei* and *Burkholderia pseudomallei*. J Clin Microbiol 36:2737–2741
2. 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
3. Cárdenas NC, Galvis JOA, Farinati AA, Grisi-Filho JHH, Diehl GN, Machado G (2018) *Burkholderia mallei*: The dynamics of networks and disease transmission. Transbound Emerg Dis. https://doi.org/10.1111/tbed.13071
4. Daligault HE, Johnson SL, Davenport KW, Minogue TD, Bishop-Lilly KA, Broomall SM, Bruce DC, Coyne SR, Frey KG, Gibbons HS, Jaissle J, Koroleva GI, Ladner JT, Lo C-C, Munk C, Wolcott MJ, Palacios GF, Redden CL, Rosenzweig CN, Scholz MB, Chain PS (2016) Draft genomes for eight *Burkholderia mallei* isolates from Turkey. Genome Announc 4(1)
5. Diniz MC, Farias KM, Pacheco ACLP, Viana DA, Araújo-Filho R, Lima APS, Costa RB, Oliveira DM (2008) Análise Genômica de *Burkholderia mallei* e *Burkholderia pseudomallei*: dois Patógenos de Primeira Grandeza e de Genomas surpreendentemente complexos. Rev Bras Hig San Anim 2(1):1–34
6. 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 Annunc 5(28)
7. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98
8. Laroucau K, Santana VLA, Girault G, Martin B, Silveira PPM, Machado MB, 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. Infect Genet Evol 57:117–120
9. Lee MA, Wang D, Yap EH (2005) Detection and differentiation of *Burkholderia pseudomallei, Burkholderia mallei* and *Burkholderia thailandensis* by multiplex PCR. FEMS Immunol Med Microbiol 43:413–417
10. Mota RA, Brito MF, Castro FJC, Massa M (2000) Mormo em equídeos nos estados de Pernambuco e Alagoas. Pesqui Vet Bras 20(4):155–159
11. Neubauer H, Sprague LD, Zacharia R, Tomaso H, Al Dahouk S, Wernery R et al (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
12. Nierman WC, DeShazer D, Kim HS, Tettelin H, Nelson KE, Feldblyum T, Ulrich RL, Ronning CM, Brinkac LM, Daugherty SC, Davidsen TD, Deboy RT, Dimitrov G, Dodson RJ, Durkin AS, Gwinn ML, Haft DH, Khouri H, Kolonay JF, Madupu R, Mohammoud Y, Nelson WC, Radune D, Romero CM, Sarria S, Selengut J, Shamblin C, Sullivan SA, White O, Yu Y, Zafar N, Zhou L, Fraser CM (2004) Structural flexibility in the *Burkholderia mallei* genome. Proc Natl Acad Sci U S A 101(39):14246–14251
13. OIE (2018) Organização Mundial de Saúde Animal. Terretrial manual, chapter 2.5.11 – glanders and melioidoses. Disponível em : < http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.05.11_GLANDERS.pdf> Acesso em: 20/12/2018
14. Patel JB (2001) 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. Mol Diagn 6:313–321
15. Pimentel W (1938) História e organização do serviço veterinário do exército. Revta Milit Med Vet 1(4):283–322
16. Schmoock G, Ehricht 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
17. Scholz HC, Joseph M, Tomaso H, al Dahouk S, Witte A, Kinne J, Hagen RM, Wernery R, Wernery U, Neubauer H (2006) Detection pf 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
18. Silva KPC, Mota RA, Cunha AP, Silva LBG, Leal NC, Cavalcante YVN, Teles JAA, Pereira MCC, Freitas NS (2009) Caracterização fenotípica e molecular de amostras de *Burkholderia mallei* isoladas na Região Nordeste do Brasil. Pesqui Vet Bras 29(5):439–444
19. Thibault FM, Valade E, Vidal DR (2004) Identification and discriminaton of *Burkholderia pseudomallei, B. mallei* and *B. thailandensis* by *real time* PCR targeting type III secretion system genes. J Clin Microbiol 42:5871–5874
20. U'ren JM, Van Ert MN, Schupp JM et al (2005) Use of real-time PCR TaqMan assay for rapid identification of *Burkholderia pseudomallei* and *Burkholderia mallei*. J Clin Microbiol 43:5771–5774