California, USA); Courtney Edwards, Teresa R. Fields, Matthew Johnson, and Vaneet Arora (Kentucky Department for Public Health); Erica Reaves, Jeanette P. Dill, Katie Nixon, Linda S. Thomas, Victoria N. Stone, and Xiaorong Qian (Tennessee Department of Health); Joseph Yglesias and Ian Stryker (Florida Department of Health, Bureau of Public Health Laboratories); Andrea Leapley (Florida Department of Health, Bureau of Epidemiology); Daryl Lamson, Patrick Bryant, and Kirsten St. George (New York State Department of Health); Sharmila Talekar, Oxana Mazurova, Kim Kilgour, and Elizabeth Franko (Georgia Department of Public Health Laboratory); and state departments of health reporting Sanger sequences as part of technical assistance, including Will Probert, Carlos Gonzalez, and Jill K Hacker (California Department of Public Health [CDPH]) and the CDPH Hepatitis A Epidemiology Team; Michigan State Department of Health; Elizabeth Cebelinski (Minnesota Department of Health); and Jacquelyn Woods (US Food and Drug Administration).

About the Author

Dr. Ramachandran is a senior scientist in the National Center for HIV, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia, USA. Her research interests include hepatitis surveillance, outbreak response, public health technical assistance, and strategic partnerships.

References


Address for correspondence: Sumathi Ramachandran, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop A33, Atlanta, GA 30329-4027, USA; email: dcqf6@cdc.gov

Molecular Typing of Burkholderia mallei Isolates from Equids with Glanders, India

Harisankar Singha, Mandy C. Elschner, Praveen Malik, Sheetal Saini, Bhupendra N. Tripathi, Katja Mertens-Scholz, Hanka Brangsch, Falk Melzer, Raj K. Singh, Heinrich Neubauer

Author affiliations: Indian Council of Agricultural Research—National Research Centre on Equines, Haryana, India (H. Singh, S. Saini); Friedrich-Loeffler-Institut Federal Research Institute for Animal Health, Institute for Bacterial Infections and Zoonoses, Jena, Germany (M.C. Elschner, K. Mertens-Scholz, H. Brangsch, F. Melzer, H. Neubauer); Ministry of Fisheries, Animal Husbandry & Dairying, Government of India, New Delhi, India (P. Malik); Indian Council of Agricultural Research, Krishi Bhawan, New Delhi (B.N. Tripathi); Indian Council of Agricultural Research—Indian Veterinary Research Institute, Izatnagar, India (R.K. Singh)

DOI: https://doi.org/10.3201/eid2706.203232

These first authors contributed equally to this work.
We collected 10 Burkholderia mallei isolates from equids in 9 districts in India during glanders outbreaks in 2013–2016. Multilocus variable-number tandem-repeat analysis showed 7 outbreak area–related genotypes. The study highlights the utility of this analysis for epidemiologically tracing of specific B. mallei isolates during outbreaks.

Burkholderia mallei is the etiologic agent of the contagious and fatal infection in equids known as glanders. It is one of the most ancient diseases and is distributed worldwide. B. mallei infections are frequently reported in South America, the Middle East, South Asia, and some countries in Africa. Equine glanders is a notifiable zoonotic disease; surveillance measures are enforced by the World Organisation for Animal Health (1).

Since 2006, equine glanders has been reported in India with consistently higher numbers from the Uttar Pradesh state (2,3). Regular glanders surveillance programs revealed presence of the disease in 14 states and, during 2015–2018, fresh B. mallei infections were reported in 6 states: Jammu and Kashmir, Gujarat, Rajasthan, Delhi, Madhya Pradesh, and Tamil Nadu (4). Epidemiologic investigations indicated that trading of equids from Uttar Pradesh to other states played a major role in spreading glanders (2). However, B. mallei isolates were not genotyped, which is necessary for understanding the epidemiologic association between glanders outbreaks across India.

Our study describes molecular typing of 10 B. mallei isolates recovered from horses (n = 4) and mules (n = 6) during 2013–2016 (Table; Appendix 1 Figure, https://wwwnc.cdc.gov/EID/article/27/6/20-3232-App1.pdf). All the affected equids were used for cart pulling and kept in small household stables. Five isolates (3324, 3478, 3701, 3711, and 3712), originating from 3 horses and 2 mules, were from adjoining districts of Uttar Pradesh state, which is regarded as a glanders hotspot zone (2). Three isolates (3076, 3081, 3595) from mules were located in 2 districts of Himachal Pradesh. Available information from the equine keeper suggested that these animals were traded from Uttar Pradesh and were responsible for the reported glanders incidence in this state. One isolate was recovered from a mule (3880) in Gujarat and 1 from a horse (3897) in Haryana state; both animals had no recent travel history.

The isolates were recovered from different types of biologic samples (Table) as described previously (3) and identified as B. mallei by real-time PCR (1). Genomic DNA was extracted by using the PureLink genomic DNA isolation kit (Invitrogen, https://www.thermofisher.com) and used for PCR-based multilocus sequence typing (MLST) and multilocus variable-number tandem-repeat (VNTR) analysis (MLVA). We typed all 10 B. mallei isolates as sequence type (ST) 40 by the B. pseudomallei MLST scheme and ST734 by the B. cepacia MLST scheme (5–6); Appendix 2 Tables 1, 2, https://wwwnc.cdc.gov/EID/article/27/6/20-3232-App2.xlsx).

We conducted MLVA by PCR amplification and sequencing of 23 loci using previously described primers (7). We determined sequence length and repeat number for each locus using Geneious software version 6.1.8 (https://www.geneious.com). A distance matrix giving the number of VNTR loci differing between isolates was used for analysis applying the minimum-evolution method implemented in MEGA X software version 10.0.5 (https://www.megasoftware.net).

MLVA assigned the 10 isolates to 7 genotypes, indicating considerable variability among B. mallei isolates in India (Figure, panel A). Identical MLVA patterns were observed for isolates 3076 and 3081 from Himachal Pradesh and isolate 3324 from Uttar Pradesh state, which is regarded as a glanders hotspot zone (2).

<table>
<thead>
<tr>
<th>B. mallei isolate</th>
<th>Place of origin (district, state)</th>
<th>Year isolated</th>
<th>Host species</th>
<th>Salient clinical signs</th>
<th>Sample type</th>
</tr>
</thead>
<tbody>
<tr>
<td>India3076</td>
<td>Solan, Himachal Pradesh</td>
<td>2013</td>
<td>Mule</td>
<td>Blood tinged nasal discharge, respiratory distress</td>
<td>Nasal swab</td>
</tr>
<tr>
<td>India3081</td>
<td>Solan, Himachal Pradesh</td>
<td>2013</td>
<td>Mule</td>
<td>Respiratory distress, nasal discharge, cutaneous nodules</td>
<td>Nasal swab</td>
</tr>
<tr>
<td>India3324</td>
<td>Hardoi, Uttar Pradesh</td>
<td>2014</td>
<td>Horse</td>
<td>Nasal discharge, hind limb ulcer, liver abscess</td>
<td>Liver abscess</td>
</tr>
<tr>
<td>India3478</td>
<td>Agra, Uttar Pradesh</td>
<td>2014</td>
<td>Horse</td>
<td>Hind limb ulceration, lacrimation</td>
<td>Lesion swab</td>
</tr>
<tr>
<td>India3595</td>
<td>Mandi, Himachal Pradesh</td>
<td>2015</td>
<td>Mule</td>
<td>Labored breathing, nasal discharge</td>
<td>Nasal swab</td>
</tr>
<tr>
<td>India3701</td>
<td>Kasganj, Uttar Pradesh</td>
<td>2015</td>
<td>Mule</td>
<td>Nasal discharge, cutaneous nodules</td>
<td>Lesion swab</td>
</tr>
<tr>
<td>India3711</td>
<td>Etah, Uttar Pradesh</td>
<td>2015</td>
<td>Mule</td>
<td>Respiratory distress, cutaneous nodules</td>
<td>Nasal swab</td>
</tr>
<tr>
<td>India3712</td>
<td>Ghaziabad, Uttar Pradesh</td>
<td>2015</td>
<td>Horse</td>
<td>Ulcerous nodules on body surface</td>
<td>Nasal swab</td>
</tr>
<tr>
<td>India3880</td>
<td>Banaskantha, Gujarat</td>
<td>2016</td>
<td>Mule</td>
<td>Mucopurulent nasal discharge</td>
<td>Nasal swab</td>
</tr>
<tr>
<td>India3897</td>
<td>Yamunanagar, Haryana</td>
<td>2016</td>
<td>Horse</td>
<td>Ulcerous nodules on hind limb and forelimb, purulent nasal discharge</td>
<td>Lesion swab</td>
</tr>
</tbody>
</table>
Pradesh. These findings correlate with epidemiologic investigations regarding the spread of a particular strain of *B. mallei* by equine movement, emphasizing the need to control equine trade between states. An identical pattern was also observed for *B. mallei* 3701 and 3712, which were isolated from Kasganj and Ghaziabad districts, 190 km apart in Uttar Pradesh state.

The isolates 3897 and 3880 from Haryana and Gujarat differ clearly from the isolates from Himachal Pradesh–Uttar Pradesh cluster (Figure, panel A). However, isolates 3712, 3880, and 3897 were previously grouped into the L2B2sB2 branch by HRM-PCR analysis (8), which indicates superiority of MLVA for better epidemiologic resolution of glanders outbreaks.

Comparative MLVA between old and recent isolates from India revealed that most of the earlier isolates Mukteswar, BMQ, NCTC3708, NCTC3709, and India 86–567–2 are distantly related, whereas the isolate SAVP1 showed the highest similarity to the new isolates (Figure, panel A; Appendix 2 Table 3).

Further analysis of these *B. mallei* isolates plus 77 from other countries revealed that the 10 recent isolates of our study form a cluster that is most similar to isolates from Pakistan, followed by isolates from Turkey (Figure, panel B). This finding suggests that *B. mallei* strains prevalent in geographically close countries might have originated from an ancestral clone and gradually disseminated to different areas. Of interest, adoption of a strict regulatory movement policy at the beginning of the 19th century for control and eradication of glanders might have resulted in establishing specific *B. mallei* lineages at different ecologic settings. Our finding confirms previous observations regarding circulation of different *B. mallei* MLVA types in the Middle East (9,10).

In summary, MLVA proved useful as a genetic tool for classifying *B. mallei* isolates and tracing possible infection chains of glanders outbreaks in equids. VNTR information from more *B. mallei* isolates from India and other countries would be helpful to draw an epidemiologic conclusion between outbreaks.

**Acknowledgments**

We thank the staff of the involved field veterinary hospitals for their cooperation during field investigation and sample collection. We thank Sita Ram and Gurudutt Sharma for assistance with the collection and processing of clinical samples.

The World Organisation for Animal Health, Paris, provided funding under the Laboratories Twinning Programme.

**Figure.** Minimum evolution trees based on 23 VNTR loci of 10 *Burkholderia mallei* isolates from Himachal Pradesh, Uttar Pradesh, Gujarat, and Haryana states, India, compared with reference sequences. A) Comparison of Himachal Pradesh–Uttar Pradesh cluster isolates (blue circles) with 6 older *B. mallei* isolates from India. B) Comparison of Himachal Pradesh–Uttar Pradesh cluster isolates (red branches) with 77 previously published *B. mallei* isolates, including the 6 others from India (blue branches). Scale bars indicate allelic differences.
About the Authors

Dr. Singha is in charge of the National Reference Laboratory for Glanders at the Indian Council of Agricultural Research — National Research Centre on Equines, Hisar, Haryana, India. His primary research interests are diagnosis of glanders, host–pathogen interaction, and molecular typing of *Burkholderia mallei*.

Dr. Elschner is in charge of the National Reference Laboratory for Glanders at the Friedrich-Loeffler-Institute, Federal Research Institute of Animal Health, Institute for Bacterial Infections and Zoonoses, Jena, Germany. She also works for the OIE Reference Laboratory for Glanders. Her primary research interest is diagnosis of glanders.

References


Atypical *Brucella inopinata*–Like Species in 2 Marine Toads

Raisa A. Glabman, Kimberly A. Thompson, Rinosh Mani, Ryan Colburn, Dalen W. Agnew

Author affiliations: National Institutes of Health, Bethesda, Maryland, USA (R.A. Glabman); Michigan State University, East Lansing, Michigan, USA (R.A. Glabman, K.A. Thompson), R. Mani, R. Colburn, D.W. Agnew; Binder Park Zoo, Battle Creek, Michigan, USA (K.A. Thompson); John Ball Zoo, Grand Rapids, Michigan, USA (R. Colburn)

DOI: https://doi.org/10.3201/eid2706.200401

We describe the isolation of atypical *Brucella inopinata*–like species and unique clinicopathologic findings in 2 adult marine toads (*Rhinella marina*), including oophoritis in 1 toad. These findings represent a novel emerging disease in toads and a possible zoonotic pathogen.

Brucellosis is a worldwide zoonosis caused by gram-negative, intracellular *Brucella* coccobacilli. Expanding from 6 species classically associated with abortion in mammals (*B. melitensis*, *B. suis*, *B. abortus*, *B. ovis*, *B. canis*, and *B. neotomae*), the genus now includes novel strains from marine mammals (*B. ceti*, *B. pinnipedialis*), baboons (*B. papionis*), and foxes (*B. vulpis*). Two of these (*B. ceti*, *B. pinnipedialis*) are also considered atypical *Brucella* species similar to *B. microti* and *B. inopinata* (1). Atypical *Brucella* lesions in humans, wild mammals, amphibians, and fish range from localized manifestations to systemic infection with high death rates (2–8); however, reproductive lesions more