In conclusion, this case indicates an unusual complication and condition in travel medicine with no straightforward diagnosis. However, it illustrates that TBRF should be systematically considered in the differential diagnosis of acute meningoencephalitis in travelers, even if microscopic results are negative, to prompt appropriate empirical treatment and molecular or serologic testing.

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References


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Serologic Evidence of Orthopoxvirus Infection in Buffaloes, Brazil

To the Editor: Since 1999, several exanthematous vaccinia virus (VACV) outbreaks affecting dairy cattle and rural workers have been reported in Brazil (1,2). VACV, the prototype of the genus Orthopoxvirus (OPV), exhibits serologic cross-reactivity with other OPV species and was used during the World Health Organization smallpox eradication campaign (3). The origin of VACV in Brazil is unknown, although some studies have suggested that VACV strains used during the campaign may be related to outbreaks of bovine vaccinia (BV) (2). In Brazil, BV affects the milk industry and public health services (1,2,4,5). During outbreaks, dairy cattle developed lesions on the teats and udders, causing a decrease in milk production (1,2,4,5).

Another VACV subspecies, buffalopox virus (BPXV), has been isolated from buffaloes (Bubalus bubalis) in rural areas in India and causes clinical signs that resemble those seen during BV outbreaks in Brazil (6). Recent genetic analysis of BPXV samples confirmed its close relationship to VACV-like viruses, although each virus has distinct genetic signatures (1,2,6). Until recently, buffalo herds have been almost exclusive to northern Brazil. However, the buffalo market has experienced great expansion in this country, and today, there are herds in all geographic regions of Brazil. These buffalo herds are hypothetically at risk for VACV infection, on the basis of the outbreaks caused by BPXV that have been described in India (6). To assess the risk for OPV infection in milk buffaloes in Brazil, we conducted a serosurvey of herds from southeastern Brazil, the region most affected by BV.
During October 2010, we screened milk buffalo herds in rural areas of Minas Gerais State, Brazil. Serum samples were collected from 48 female buffaloes used for milk production; these animals belonged to 3 neighboring properties in Carmo da Mata city (20°33′S, 44°52′15″W), which is in the same mesoregion where the VACV Passatempo virus strain was isolated during an outbreak in 2003 (5). Since then, several outbreaks have been reported in this area.

Serum samples were inactivated, and an OPV plaque-reduction neutralization test (PRNT) was performed (7). The serum titer was defined as the highest dilution that inhibited >70% of viral plaques relative to the level of inhibition of the negative controls. Samples also underwent ELISA for OPV IgG as described (4). Bovine serum samples were used as positive and negative controls (1, 4). OPV-PRNT specificity (98.4%) and sensitivity (93.5%) were confirmed by using receiver-operating characteristic analysis as described (8). The tests were performed in duplicate.

Of the 48 buffalo serum samples, 15 (31.25%) contained neutralizing antibodies against OPV; of these, 6 (40%) had titers of 20, 5 (33.3%) had titers of 40, and 4 (26.6%) had titers ≥80 (Table). The ELISA yielded results similar to those of the PRNT; of the 48 serum samples, 17 (35.41%) were IgG positive (Table). A total of 14 samples were coincident in the PRNT and the ELISA, including most of those with high titers by PRNT. To detect viral DNA, we conducted nested PCR to amplify the viral growth factor gene (9) and real-time PCR to amplify the A56R gene (10); results were negative for all 48 serum samples.

We detected antibodies against OPV in buffaloes in Brazil 10 years after the first reported VACV outbreak in cattle in southeastern Brazil (1). Because PRNT and ELISA indicate the presence of OPV antibodies in a nonspecific manner (OPV serologic cross-reaction), it was not possible to determine the species responsible for these results. However, seropositive buffaloes may have been exposed to VACV, the only OPV known to be circulating in Brazil (1, 2, 4, 5, 8).

The management of milk buffaloes in Brazil is similar to that of dairy cows, including manual milking (1, 4, 5). Cow milkers usually work on ≥2 farms, and the farm infrastructure commonly is unsophisticated (1, 4, 5). These conditions were shown to be favorable for the spread of VACV among cattle, which suggests that the same conditions could lead to the introduction of VACV into buffalo herds. Because some BV outbreaks are not reported by the farmers, it is not possible to know exactly how or when a buffalo herd in the study area was exposed to the virus. However, milkers who work with both cattle and buffalo may be a route of viral transmission, although other sources of exposure are possible (8). Although no exanthematous VACV outbreaks have been described in milk buffaloes in Brazil, our results suggest that buffalo herds may be exposed to VACV in BV-affected areas and therefore may be at risk for VACV infection. Further research is needed to determine routes of infection, including whether humans working as milkers contribute to virus transmission.

**Table. Results of testing for orthopoxvirus seropositivity in milk buffalo herds, Minas Gerais, Brazil, October 2010**

<table>
<thead>
<tr>
<th>Test</th>
<th>No. (%) samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRNT</td>
<td></td>
</tr>
<tr>
<td>Total positive</td>
<td>15 (31.2)</td>
</tr>
<tr>
<td>Titer</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td>40</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>80</td>
<td>2 (13.3)</td>
</tr>
<tr>
<td>160</td>
<td>2 (13.3)</td>
</tr>
<tr>
<td>Total negative</td>
<td>33 (68.7)</td>
</tr>
<tr>
<td>ELISA</td>
<td></td>
</tr>
<tr>
<td>Total positive</td>
<td>17 (35.4)</td>
</tr>
<tr>
<td>Total negative</td>
<td>31 (64.6)</td>
</tr>
<tr>
<td>PRNT and ELISA positive</td>
<td>14 (29.2)</td>
</tr>
</tbody>
</table>

*Serum samples were collected from 48 female buffaloes used for milk production. A positive titer was defined as the highest dilution that inhibited >70% of viral plaques relative to the level of inhibition of the negative controls. Samples also underwent ELISA for orthopoxvirus IgG as described (4). PRNT, plaque-reduction neutralization test.

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Methicillin-Susceptible Staphylococcus aureus ST398, New York and New Jersey, USA

To the Editor: Clinical infections with livestock-associated Staphylococcus aureus sequence type (ST) 398 have been reported in Europe, Canada, and the People’s Republic of China (1), as well as the Caribbean (2,3), and Colombia (4). Most reports describe infection with methicillin-resistant S. aureus; relatively few describe infection with methicillin-susceptible S. aureus (MSSA). In the United States, colonization of healthy adults by ST398 has been reported in Iowa (5) and in New York, New York (2); MSSA infections have been anecdotally reported in St. Louis, Missouri (6), and The Bronx, New York (7). We describe 8 infections with MSSA ST398 in the New York City area during a 7-year period (2004–2010). Five infections with a related ST (ST291) from clonal complex (CC) 398 also were identified. These findings highlight the emergence of clinical infections with 2 distinct CC398 sequence types in the New York City area.

Retrospective typing of 4,167 clinical S. aureus isolates from various studies involving inpatients and outpatients in the New York City area identified 13 meca-negative isolates with CC398-associated spa types (Table). Nine isolates were obtained from cultures of outpatients with skin and soft tissue infections; samples were submitted by physicians in the community. One isolate was associated with recurring skin and soft tissue infections in multiple body sites (BK21466); another was associated with genital infection (BK21732). Of the 4 ST398 isolates derived from bloodstream infections in hospitalized patients, 3 were recovered from intravenous drug users, 1 of whom died 1 day after admission for variceal bleeding (BK26722). Unlike the multidrug-resistant ST398 MSSA recently described in Colombia (4), most isolates in this study were susceptible to all antimicrobial drugs tested except penicillin, although several strains exhibited resistance to clindamycin and erythromycin. One isolate (BK23527) was submitted as oxacillin resistant (MIC = 4 μg/mL) but lacked the meca gene, which suggested that another mechanism was contributing to the resistance phenotype.

Multilocus sequence typing confirmed 8 isolates as ST398 (3–35–19–2–20–26–39); 5 isolates were assigned to ST291 (3–37–19–2–20–26–32), a double-locus variant of ST398 (online Appendix Figure, panel A, wwwnc.cdc.gov/EID/article/18/4/11-1419-FA1.htm). Most of the ST398 strains were spa type 109 (t571), described in MSSA carriage isolates from New York City (2) and MSSA infections from China (1), France (8), Martinique (3), the Dominican Republic (2,3), and Colombia (4). BURP (based upon repeat pattern) analysis clustered all of the spa types into spa-CC t571 (online Appendix Figure, panel B). ST398 isolates clustered with spa type 109 (t571), whereas ST291 isolates clustered with spa type 865 (t2313). Pulsed-field gel electrophoresis was also performed on the 11 available isolates. Although the ST291 isolates were sensitive to digestion with Smal, pulsed-field gel electrophoresis was performed with ClaI to compare