10. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731–9. http://dx.doi.org/10.1093/molbev/msr121

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Alternative Routes of Zoonotic Vaccinia Virus Transmission, Brazil

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To the Editor: Vaccinia virus (VACV) causes exanthematous disease (bovine vaccinia) in Brazil. Outbreaks of this disease in humans have been reported since the late 1990s and have spread throughout Brazil (*1*). Natural human infections with VACV occur by close contact with infected cattle during milking. Lesions can spread to secondary body sites (forearms, arms, and face). Thus, person-to-person transmission occurs (*1*).

Moreover, virus can persist in household environments, remain infectious, and be transmitted by fomites (*2*). Although raw milk and cheese are potential sources of infection, no clinical cases have been associated with this transmission route (*3,4*). Data for person-to-person transmission in Brazil are scarce, but person-to-person transmission was recently reported (*5*). We report a possible case of person-to-person transmission of VACV.

This study was approved by the Research Ethics Committee of Universidade Federal de Minas Gerais (registration protocol FR-413704). In September 2012, during a serologic survey in a rural area of Serro City (18°36′17″S, 43°22′46″W), Minas Gerais, Brazil (online Technical Appendix Figure, panel A, http://wwwnc.cdc.gov/EID/article/21/12/14-1249-Techapp1.pdf), blood samples were obtained from a family of 5 persons (father, mother, and 3 daughters). The father and mother were 48 and 53 years of age, respectively, and had been vaccinated against smallpox. They reported contact with cows and horses (online Technical Appendix Table 1). Only the father had milked cows. The 3 daughters (13, 13, and 14 years of age) did not engage in any exposure activity. However, all family members had consumed raw milk and cheese.

Bovine vaccinia lesions were observed on the hand of the father (online Technical Appendix Figure, panel B). In 2011, he had vesicular disease (no laboratory diagnosis) with clinical and epidemiologic features (lesions) suggestive of bovine vaccinia on his hands and forearms and systemic symptoms (fever, headache, malaise, myalgia, lymphadenopathy, and abdominal pain). His symptoms were mild and without any systemic clinical features. Two lesions developed on his hands and dried swab samples were collected from both lesions. Swab samples were processed as described (*2*) and used for virus isolation and molecular diagnosis.

On the basis of previous studies that detected viral DNA in clinical samples from persons with bovine vaccinia (*1*), we used a quantitative PCR to amplify the *vgf* and *ha* genes of VACV (*3–5*), a standard PCR to detect the *ha* gene (*3–5*), and a seminested PCR to detect the *ati* gene (F.L. Assis, unpub. data). Serum samples were used for detection of virus-neutralizing antibodies (orthopoxvirus 50% plaque-reduction neutralization test) and molecular diagnostic studies (*1*). Virus isolation was attempted in Vero cells and chorioallantoic membrane. All results were negative.

The 50% plaque-reduction neutralization test showed that the father, mother, and 14-year-old daughter had neutralizing antibodies against orthopoxvirus (titers 800, 3,200, and 800 neutralizing units/mL, respectively). All family members had positive results by molecular diagnostic test for ≥1 virus gene (online Technical Appendix Table 1). To rule out infection with parapopoxvirus, a complementary PCR (*6*) was also performed, and all family members had negative results.

Quantitative PCR products for the *ha* gene from 3 virus-positive samples were sequenced in both directions in triplicate (Mega BACE Sequencer; GE Healthcare, Little Chalfont, UK). Sequences were aligned by using ClustalW (http://www.genome.jp/tools/clustalw/) and MEGA4.1 (http://www.megasoftware.net/) and showed 100% identity with each other (Figure). A phylogenetic tree was constructed by using the neighbor-joining method and 1,000-bootstrap replicates in the Tamura-3 parameter model (MEGA4.1). Sequences were grouped with VACV group 2 isolates. Sequences obtained were deposited in GenBank under accession nos. KP889223–5).

In Brazil, outbreaks of bovine vaccinia are associated with rural environments. However, some clinical and

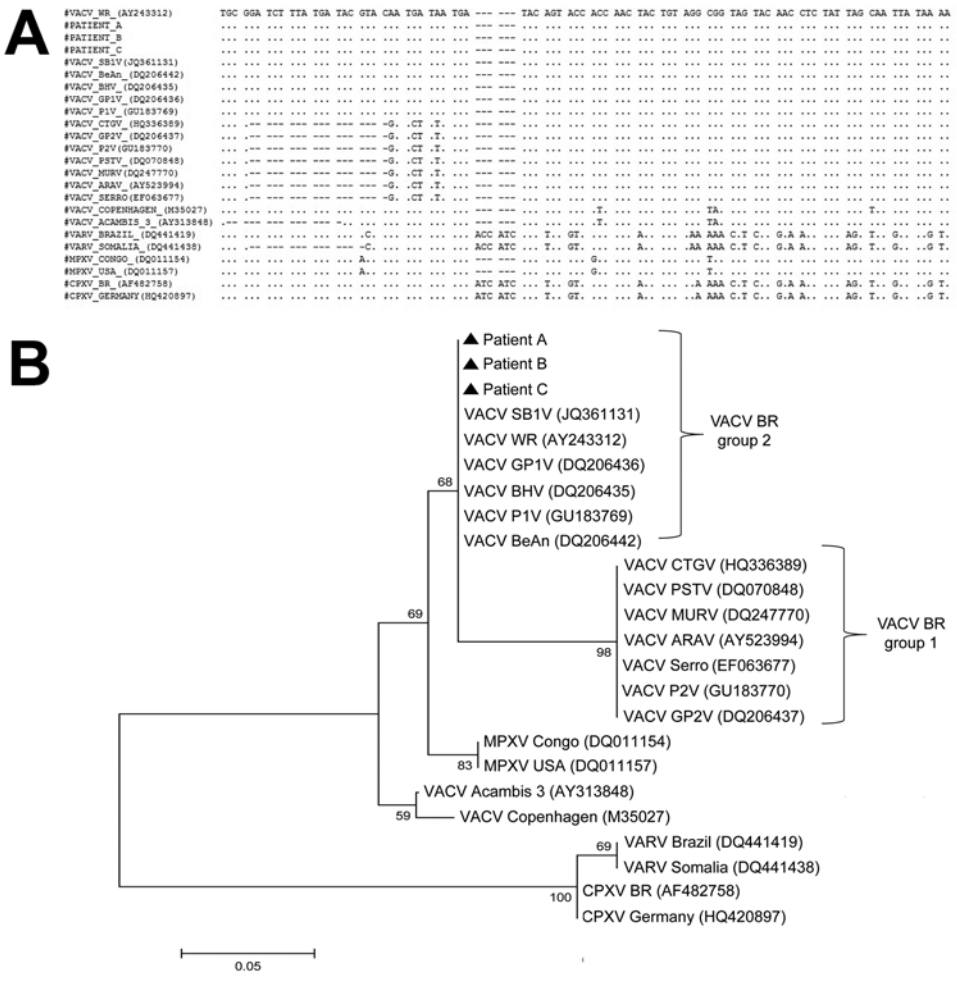

Figure. A) Nucleotide sequence of vaccinia virus (VACV) hemagglutinin gene and homologous sequences of several orthopoxviruses, Brazil. Dots indicate sequence identity; dashes indicate deletions. VARV, variola virus; MPXV, monkeypox virus; CPXV, cowpox virus. B) Consensus phylogenetic tree based on nucleotide sequences of orthopoxvirus hemagglutinin genes. Tree was constructed with hemagglutinin gene sequences by using the neighbor-joining method with 1,000 bootstrap replicates and the Tamura 3-parameter model in MEGA4 (http://www.megasoftware.net/). Strains had the deletion region conserved and were grouped with other VACV (group 2) isolated in Brazil. Numbers along branches are bootstrap values. Scale bar indicates nucleotide substitutions per site.

epidemiologic aspects remain unclear. The infection of the father was associated with direct contact with cattle. Immunity conferred by smallpox vaccination did not prevent infection; this lack of immune response has been demonstrated in other studies in Brazil (*7*). Long-term protection might require multiple virus exposures, and severity of poxvirus infections might be influenced by the immunologic state of the host and virulence of virus strains (*1*,*8*,*9*).

The mother and 2 daughters with virus DNA in blood samples and the 14-year-old daughter with high titers of virus-neutralizing antibodies suggest that alternative routes (other than milking) for VACV infection of humans should be considered. These alternative routes can include person-to-person or environmental transmission because the 2 daughters did not report any exposure activities related to milking or contact with cattle (online Technical Appendix Tables 1, 2). Persistence of VACV in household environments has been reported (*2*,*10*). The family also consumed raw milk and cheese, a common practice in the region. Therefore, infection with VACV through raw milk and cheese consumption should also be considered. The patients did not report oral lesions or a history of skin/mucosal lesions.

In conclusion, person-to-person transmission of VACV in these cases might have been caused by direct contact between the father and family members, contact with virus in the home, or consumption of unpasteurized milk and cheese. Additional studies are necessary to elucidate the role of these transmission pathways in spread of VACV in Brazil.

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References

1. Kroon EG, Mota BE, Abrahão JS, da Fonseca FG, de Souza Trindade G. Zoonotic Brazilian vaccinia virus: from field to therapy. Antiviral Res. 2011;92:150–63. http://dx.doi.org/10.1016/j.antiviral.2011.08.018
2. Assis FL, Borges IA, Mesquita VS, Ferreira PC, Trindade GS, Kroon EG, et al. Vaccinia virus in household environment during bovine vaccinia outbreak, Brazil. Emerg Infect Dis. 2013;19:2045–7.
3. Abrahão JS, Oliveira TM, Campos RK, Madureira MC, Kroon EG, Lobato ZI. Bovine vaccinia outbreaks: detection and isolation of vaccinia virus in milk samples. Foodborne Pathog Dis. 2009;6:1141–6. http://dx.doi.org/10.1089/fpd.2009.0324
4. de Oliveira TM, Rehfeld IS, Siqueira JM, Abrahão JS, Campos RK, dos Santos AK, et al. Vaccinia virus is not inactivated after thermal treatment and cheese production using experimentally contaminated milk. Foodborne Pathog Dis. 2010;7:1491–6. http://dx.doi.org/10.1089/fpd.2010.0597
5. Pereira Oliveira G, Tavares Silva Fernandes A, Lopes de Assis F, Augusto Alves P, Moreira Franco Luiz AP, Barcelos Figueiredo L, et al. Intrafamilial transmission of vaccinia virus during a bovine vaccinia outbreak in Brazil: a new insight in viral transmission chain. Am J Trop Med Hyg. 2014;90:1021–3. http://dx.doi.org/10.4269/ajtmh.13-0621
6. Inoshima Y, Morooka A, Sentsui H. Detection and diagnosis of parapoxvirus by the polymerase chain reaction. J Virol Methods. 2000;84:201–8. http://dx.doi.org/10.1016/S0166-0934(99)00144-5
7. Silva-Fernandes AT, Travassos CE, Ferreira JM, Abrahão JS, Rocha ES, Viana-Ferreira F, et al. Natural human infections with *Vaccinia virus* during bovine vaccínia outbreaks. J Clin Virol. 2009;44:308–13. http://dx.doi.org/10.1016/j.jcv.2009.01.007
8. Gallwitz S, Schutzbank T, Heberling RL, Kalter SS, Galpin JE. Smallpox: residual antibody after vaccination. J Clin Microbiol. 2003;41:4068–70. http://dx.doi.org/10.1128/JCM.41.9.4068-4070.2003
9. Hammarlund E, Lewis MW, Hansen SG, Strelow LI, Nelson JA, Sexton GJ, et al. Duration of antiviral immunity after smallpox vaccination. Nat Med. 2003;9:1131–7. http://dx.doi.org/10.1038/nm917
10. Lederman E, Miramontes R, Openshaw J, Olson VA, Karem KL, Marcinak J, et al. Eczema vaccinatum resulting from the transmission of vaccinia virus from a smallpox vaccinee: an investigation of potential fomites in the home environment. Vaccine. 2009;27:375–7. http://dx.doi.org/10.1016/j.vaccine.2008.11.019

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Hunter Island Group Phlebovirus in Ticks, Australia

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To the Editor: A recent article described the isolation and subsequent analysis of a tickborne phlebovirus: Hunter Island Group virus (HIGV), associated with an albatross disease event that occurred in 2002 on Albatross Island, 6 kilometers off the northwest coast of Tasmania, Australia (*1*). The authors present HIGV as a novel isolate; however, new data and historical records demonstrate that the virus was originally isolated in 1983. Provisionally named Albatross Island virus (ABIV), the virus was classified as unidentified because of its uniqueness and dissimilarity to any known virus in Australia. ABIV and HIGV were isolated from ticks of the same species, *Ixodes eudyptidis*, collected from the nests of shy albatross (*Thalassarche cauta*) on Albatross Island, the only island inhabited by albatross within the Hunter Island Group Important Bird Area. At the time of collections, many immature albatross were dying. Records from this time indicate that postmortem blood samples were collected from the birds, and subsequent virus neutralization studies conducted soon after demonstrated that 50% of these samples were ABIV positive. Ensuing testing of samples collected in the next 2 years also identified a positive sample from a black noddy in Queensland (Table). ABIV was subsequently sent for testing at the Arbovirus World Reference Laboratory and, more than a decade later, to the Australian Animal Health Laboratory, Commonwealth Scientific and Industrial Research Organisation, where it was identified as a bunyavirus but remained largely uncharacterized.

We recently sequenced the genome of ABIV by using high-throughput sequencing and have compiled near complete sequences for the large (L), medium (M), and small (S) segments (GenBank accession nos. KM198925–7). Overall, ABIV shares 99% nt identity with HIGV, and thus they can be considered isolates of the same virus. The translated nucleocapsid and S segment nonstructural proteins of both viruses are identical, and the polymerases and glycoproteins share 99% identity. There are 26 nt changes across the whole genome (1 in S, 8 in M, 17 in L), but only 7 of these translate into an amino acid change (3 in the Gn/Gc polyprotein, 4 in the polymerase protein). Predictive protein analysis indicates that at least 1 of the 3 aa changes occurs in the ectodomain of the Gn protein, which could affect virus–host interactions. Of the remaining changes, 14 are silent mutations and 5 occur in noncoding regions.

In light of the genomic similarity of these 2 viruses, we suggest that the species name Albatross Island virus encompass both isolates, ABIV and HIGV, thereby representing the name of the original 1983 isolate and the location