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Laboratory-acquired Buffalopox Virus Infection, India

To the Editor: In India, buffalopox virus (BPXV), a variant of vaccinia virus, is associated with severe disease outbreaks among buffaloes (1,2), cattle (3), and humans in contact with these animals (1,4). Most human BPXV infections occur in animal attendants and milkers (1,4). A similar type of vaccinia virus infection has also been reported from rural areas in Brazil (5). We report a case of laboratory-acquired infection with BPXV in a researcher in India.

Clinical signs, symptoms, diagnosis, and management of this case highlight the need for observance and enforcement of strict biosafety measures within the laboratory.

A 28-year-old man (researcher) who was freeze-drying BPXV isolates in a laboratory in Hisar, India sustained a cut on his right palm through nitrile gloves by accidental piercing of shrapnel from a broken ampule. The virus being freeze-dried ($10^{5.5}$ 50% tissue culture infectious doses/mL) was isolated from a buffalo in Jalgaon, India, in 2010. The injured site on the palm was immediately cleaned with 70% ethanol and treated with povidone-iodine solution. No untoward reaction was observed ≤ 2 days postinjury. Erythema appeared at the injury site on postinjury day 3. Subsequently, a small vesicle developed on postinjury day 5 (Figure, panel A). This vesicle progressed into a pustule with a central area of necrosis by postinjury day 7 (Figure, panel B). On postinjury day 9, symptoms worsened (onset of high fever and general malaise and pain at the affected site), and the researcher sought medical care.

Physical examination showed high fever (104°F), unilateral axillary lymphadenopathy, and edema of the palm (Figure, panel C). Amoxicillin (500 mg, 2×/d), cephalexin (500 mg, 2×/d), and analgesic/antipyretic (paracetamol, 500 mg, 2×/d) were prescribed to control secondary complications caused by bacterial infection and pain.

The next day, the entire palm became cyanotic and edema increased (Figure, panel D). The researcher was then referred to a specialty hospital where the lesion was surgically excised on postinjury day 11 (Figure, panel E) under axial block anesthesia. Blood, necrotic tissue, pustular material, and swab specimens were obtained for laboratory examination. Postsurgery treatment included cleaning of the surgical site on alternate days and oral medication (amoxicillin/clavulanic acid, 625 mg; ibuprofen, 400 mg;

paracetamol, 325 mg; and rabeprazole, 20 mg) for 5 days.

On postinjury day 19, the surgeon advised the patient to take cefuroxime (500 mg/d for 5 days) and use a topical ointment containing mupirocin to prevent a delay in healing (Figure, panel F). The lesion healed slowly, and by postinjury day 30, thickening and blackening of the skin was observed (Figure, panel G) that extended to a wider area by postinjury day 38, and the skin started to peel off by postinjury day 50. The entire skin of the palm sloughed off with complete healing by postinjury day 85, leaving a 20-mm blackened eschar over the area (Figure, panel H).

Clinical samples were subjected to laboratory examination. Virus was isolated from tissue samples in a Vero cell line during the first passage. BPXV infection was confirmed by PCR amplification of orthopoxvirus-specific A type inclusion gene (552 bp) and a BPXV-specific C18L gene (368 bp) from tissue material and the laboratory-isolated virus (BPXV/Human/Lab/11), according to procedures described by Singh et al. (6). Sequences of these 2 genes were submitted to GenBank under accession nos. JN653284.1 and JN653278.1, respectively. Phylogenetic analysis showed 95%–100% nt similarity of the laboratory isolate (BPXV/Human/Lab/11) with other BPXVs from India (online Technical Appendix Figure, wwwnc.cdc.gov/EID/article/20/2/13-0358-Techapp1.pdf). Antibodies against BPXV were detected in patient serum samples by using an indirect immunoperoxidase test and a 50% plaque-reduction neutralization test according to methods reported by Bera et al. (7). Serum of the infected patient showed 50% plaque-reduction neutralization test titers of 256 and 512 on postinjury days 11 and 28, respectively. These findings confirmed BPXV infection because the patient had not been vaccinated against smallpox.

Pre-freezing of the glass ampule to -80°C caused a hairline crack in



Figure. Progression of lesion on palm of researcher caused by buffalopox virus infection, India. A) Small vesicle on postinjury day 5. B) Pustule with a central area of necrosis on postinjury day 7. C) Pustule with edema on postinjury day 9. D) Increase in edema on postinjury day 10 (cyanosis is not apparent). E) Lesion after surgical excision on postinjury day 11. F) Lesion on postinjury day 19 (healing was erratic). G) Blackening and thickening around surgical site on postinjury day 30, which then extended to a wider circumference. H) Healed lesion with a black eschar on postinjury day 85.

the glass. The ampule broke while being introduced into the freeze-drying manifold and pierced the palm of the researcher. As a follow-up measure, the freeze drying procedure was reviewed and the pre-freezing temperature was reduced to -60°C . Measures were also taken to ensure use of better-quality ampules. Surgical and contact material associated with the lesion was placed in biohazard bags for autoclaving before disposal. In addition, laboratory and hospital staff was apprised of the risk associated with BPXV transmission.

Reporting of laboratory-acquired infections is crucial because infections could also spread to other personnel. Strict biosafety practices and laboratory guidelines are useful in minimizing laboratory-acquired infections. Guidelines, no matter how stringent, are not sufficient on their own. Laboratory-acquired infections occur because humans or machines are not infallible. Thus, laboratories should have emergency procedures in place to deal with such situations.

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Recurrence of Animal Rabies, Greece, 2012

To the Editor: Rabies is caused by 12 recognized virus species within the *Lyssavirus* genus (family *Rhabdoviridae*) (1) and each year causes 55,000 deaths worldwide among humans. In Europe, the main reservoir and vector of rabies is the red fox (*Vulpes vulpes*), followed by the raccoon dog (*Nyctereutes procyonoides*) in central and Baltic Europe (2). Among these virus species, the rabies virus (former genotype 1, classical rabies virus) is maintained in reservoir mammals, mainly carnivores. Despite the successful oral vaccination campaigns of wildlife that eliminated rabies in large parts of Europe (2), the disease still occurs in Europe; 4,884 cases in animals were recorded in 2012.

Until 2012, Greece had been free of rabies since 1987; the last case occurred in a dog. During 1971–1987, a total of 248 cases were recorded in domestic animals, of which only 6 occurred during 1981–1987 (3). The wide compulsory vaccination of dogs, along with control of stray dogs, were the main measures leading to the elimination of rabies in Greece.

Greece shares land borders with Turkey, Former Yugoslav Republic of Macedonia (FYROM), and Bulgaria, where rabies is reported in wild and domestic animals (4; www.who-rabies-bulletin.org/). After the fox rabies case reported in FYROM in 2011, ≈0.3 km from Greece (4), rabies surveillance was improved along

the northern and eastern land borders of Greece, and wild and domestic animals found dead or suspected of having rabies were collected. This program was approved by the European Commission. The first rabies case was diagnosed on October 19, 2012, in a red fox in Palaioakastro, 60 km from the Albanian border. The animal was wandering in the village during daytime and attacked a dog before being killed by local hunters and sent to the National Reference Laboratory for Animal Rabies (Athens, Greece) for rabies testing. The second case, isolated in Ieropigi, 4 km from the Albanian border, was a shepherd dog demonstrating aggressive behavior against dogs and sheep of the flock. Seven additional cases (6 foxes, 1 dog) were reported in December 2012 in 2 prefectures of northern Greece that share borders with FYROM (5).

In 2012, a total of 237 domestic and wild animals were submitted to the National Reference Laboratory for Animal Rabies for rabies testing. Samples were tested by the fluorescent antibody test, rabies tissue culture infection test, real-time reverse transcription PCR, and heminested reverse transcription PCR, as described (6,7). In brief, viral RNA was extracted (Viral RNA Mini Kit, QIAGEN, Hilden, Germany) from 140 μL of homogenized brain suspension supernatant and subjected to the partial nucleoprotein (N) gene amplification (positions 71–644 compared with PV strain genome). The PCR products were bidirectionally sequenced by using the same primers in a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The phylogenetic tree was constructed by using the neighbor-joining method with 1,000 replicates using MEGA5 (8).

Of 237 animals tested, 9 (7 foxes, 2 dogs) were rabies positive by fluorescent antibody test, rabies tissue culture infection test, and PCR. Positive samples were subjected to sequencing analysis of the N gene. The sequence

analysis of the first 567 nt of the N gene of the 3 isolates (GR64C/12, GR112C/12, GR238C/12) showed 99.8% nt identity with the N gene of a rabies virus (GenBank accession no. JQ973884) isolated from a red fox in FYROM in 2011. Nucleotide identity was 100% between the 6 isolates from Greece (GR177C/12, GR187C/12, GR217C/12, GR231C/12, GR236C/12, GR242C/12) and isolate JQ973884, as well as a Serbia strain (GenBank accession no. JF973785). This perfect nucleotide identity shown with the Serbia isolate collected >15 years ago suggests the persistence of some viral strains over time in the Balkans, in accordance with previous studies (4,9). Amino acid identity was perfect among all 9 isolates from Greece.

Phylogenetic analysis of the partial sequences of the N gene of isolates from Greece compared with representative sequences from the Balkans (Figure) showed that isolates from Greece resolved within the East Europe (EE) group of the cosmopolitan lineage. The EE group encompasses the 9 Greek isolates with referenced viral sequences from FYROM, Bulgaria, Serbia, Bosnia Herzegovina, and Montenegro. Within the EE group, <1.5%-nt divergence exists between all analyzed N gene sequences (567 nt).

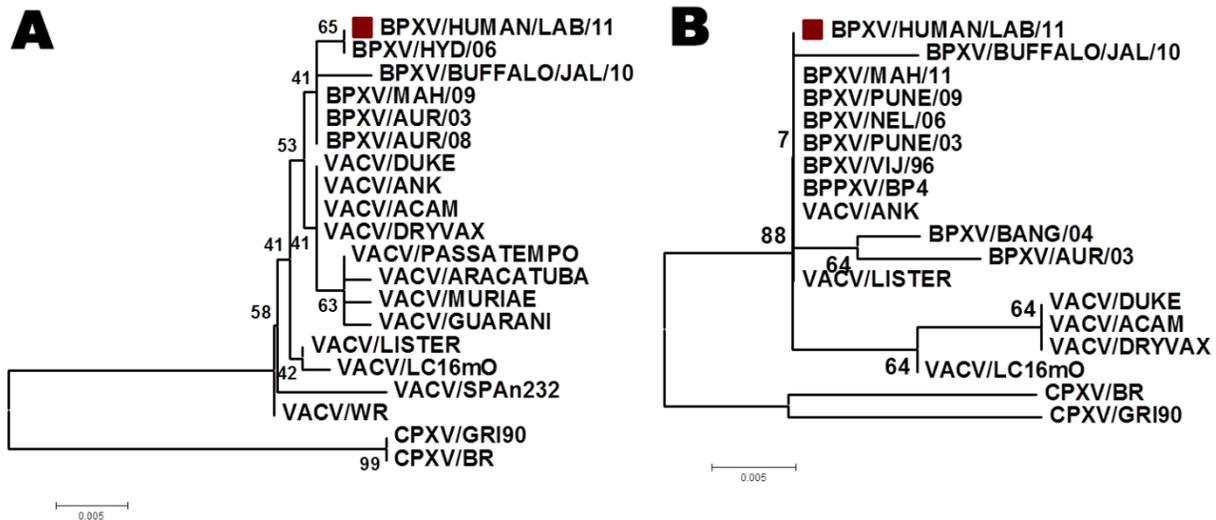
The perfect nucleotide identity shown between the 6 isolates from Greece and 2 strains from Serbia and FYROM demonstrates a close genetic relationship between them. This finding supports the hypothesis of movement of rabies-infected hosts in the western Balkan countries.

Greece was rabies free for 25 years. In the 2012 outbreak, the rabies cycle appears to be sylvatic, whereas until 1987, dogs were the main reservoir of rabies. Measures including public awareness campaigns and intensified vaccination of stray animals and shepherd dogs, combined with control of stray dogs and cats, already have been implemented. In accordance with the

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Technical Appendix

Phylogenetic Trees of A-Type Inclusion Genes and C18L Genes of Buffalopox Virus and Related Viruses



Technical Appendix Figure. Phylogenetic trees of A type inclusion genes (A) and C18L genes (B) of buffalopox virus and related viruses. Trees were constructed by using MEGA5 software (<http://megasoftware.net/>). Boxes indicate strain isolated in this study. Values along branches indicate bootstrap confidence calculated per 1,000 bootstrap values. Scale bars indicate nucleotide substitutions per site. BPXV, buffalopox virus; LAB/11, laboratory human isolate in 2011; HYD/06, buffalo isolate from Hyderabad in 2006; JAL/10, buffalo isolate from Jalgaon in 2010; MAH/09, human isolate from Maharashtra in 2009; AUR/03 and AUR/08, buffalo isolates from Aurangabad in 2003 and 2008, respectively; VACV, vaccinia virus; DUKE, Duke strain; ANK, Ankara strain; ACAM, vaccinia virus strain Acambis; DRYVAX, Dryvax strain; PASSATEMPO, vaccinia-like virus strain Passatempo from Brazil; ARACATUBA, vaccinia-like virus strain Aracatuba from Brazil; MURIAE, vaccinia virus strain from Brazil; GUARANI, vaccinia virus strain from Brazil; Lister, vaccinia virus strain; LC16mO, vaccinia virus strain; SPAn232, vaccinia virus strain from Brazil; WR, vaccinia virus strain Western Reserve; CPXV, cowpox virus; GRI90, cowpox virus strain; BR, Brington Red; MAH/11, human isolate from Maharashtra in 2011; PUNE/03 and PUNE/09, buffalo isolates from Pune in 2003 and 2009, respectively; NEL, buffalo isolate from Nellore in 2006; VIJ/96, buffalo isolate from Vijaywada in 1996; BP4, reference strain of buffalopox virus; BANG/04, buffalo isolate from Bangladesh in 2004.