Cowpox Virus Transmission from Rats to Monkeys, the Netherlands

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We report an outbreak of cowpox virus among monkeys at a sanctuary for exotic animals. Serologic analysis and polymerase chain reaction were performed on blood and swab samples from different rodent species trapped at the sanctuary during the outbreak. Sequence comparison and serologic results showed that brown rats (*Rattus norvegicus*) transmitted the virus to monkeys.

Cowpox virus (CPXV) is a member of the genus *Orthopoxvirus*, family *Poxviridae*, and is antigenically and genetically related to variola virus, vaccinia virus, and monkeypox virus (MPXV). With the eradication of smallpox, routine vaccination with vaccinia virus ceased, which created a niche for animal poxviruses to infect humans. However, cowpox is a rare zoonosis, and infection of immunocompetent persons usually results in localized lesions mainly on fingers, hands, or face. However, in immunocompromised patients, severe generalized infections have been documented (*1*,*2*).

The reservoir hosts of CPXV are wild rodents; cows, domestic cats, and humans are incidental hosts. In Europe, bank voles (*Clethrionomys glareolus*) and wood mice (*Apodemus sylvaticus*) constitute the main reservoirs (*3*), whereas CPXV was sporadically detected in rats (*Rattus norvegicus*) (*4*,*5*). Domestic cats play a role in transmission of CPXV to humans (*6*,*7*). Direct transmission of CPXV from rodents to humans has also been documented (*3*,*5*). In the United States, prairie dogs (*Cynomys ludovicianus*) have been suggested as a potential reservoir for MPXV and are susceptible to CPXV infection by wild rodents (*8*). We report an outbreak of CPXV in nonhuman primates through contact with infected brown rats.

The Study

In September 2003, three Barbary macaques (*Macaca sylvanus*) at a sanctuary for exotic animals in Almere, the Netherlands, showed multifocal gingival, buccal, and lingual lesions. Typical intranuclear inclusions were found by histologic analysis, and poxlike particles were found by transmission electron microscopy of 6 biopsy specimens from buccal lesions of the same animals. Because of concerns that these macaques were infected with MPXV, additional biopsy specimens of poxlike lesions were obtained for virus isolation and polymerase chain reaction (PCR) studies.

Vero cells were infected with homogenized biopsy samples from the 3 macaques, and cells were monitored daily for appearance of cytopathic changes. Three days after infection, cells showed cytopathic effects characterized by plaques of rounded cells with prominent cytoplasmic bridging and syncytia formation. To confirm the isolation of a poxvirus, an immunofluorescence test was conducted with human antivaccinia serum (diluted 1:1,000) and goat antihuman immunoglobulin G (IgG) (diluted 1:500, Dako, Roskilde, Denmark). Diffuse cytoplasmic fluorescence confirmed an orthopoxvirus.

Isolates were further characterized by PCR and sequence analysis with primers for the hemagglutinin gene (*9*). Melting curve and sequence analyses confirmed the presence of an orthopoxvirus, most likely CPXV (Figure). Because this PCR assay was designed to differentiate variola virus from other orthopoxviruses but not among nonvariola orthopoxviruses, we developed a CPXV-specific PCR by using nested primer sets within the A-type inclusion protein (ATI) gene. PCR was conducted by using external primers (ATIF1) 5′-GAACTTAATAAGTGTTTCGATA-3′ (forward primer) and (ATIR1) 5′-CAGTAACGTCGGACGATGGAGG-3′ (reverse primer) with nested forward primer ATIF2 5′-GAGGAAGTTAAGAGATTGCGTC-3′ and reverse primer ATIR1. The nucleotide sequences are available from GenBank. Nucleotide sequencing confirmed that the virus isolated from Barbary macaques was a CPXV.

Since all macaques were in the center before disease manifested and they had not been in contact with other animals, other monkeys were tested for CPXV infection by serologic analysis. Serum samples from 16 Barbary macaques (*Macaca sylvanus*), 2 pig-tailed macaques (*M. nemestrina*), 2 squirrel monkeys (*Saimiri sciureus*), 2 Japanese macaques (*M. fuscata*), 6 cynomolgus macaques (*M. fascicularis*), 2 Hamadryas baboons (*Papio hamadryas*), 4 rhesus macaques (*M. mulatta*), and 1 vervet (*Cercopithecus aethiops*) were tested with a virus neutralization test (VNT) using a CPXV strain isolated in this study (CPXVmac). Neutralizing titers were determined after 5 days on the basis of complete reduction of a cytopathic effect. At the end of the outbreak, neutralizing serum antibodies were detected in 9 Barbary macaques,

2 pig-tailed macaques, 1 Japanese macaque, 3 cynomolgus macaques, and 1 rhesus monkey. This finding suggested that that all of these animals had been exposed to CXPV. Retrospective serosurveillance showed that only 1 Barbary macaque was seropositive at the start of the outbreak. No swab samples from the animals were available for culture or PCR analysis.

To identify the possible reservoir of the CPXV infection, animals known to be susceptible to CPXV and housed at the sanctuary at the time of the outbreak were tested. These included 4 domestic cats (*Felis catus*), 2 red squir-