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Fatal Cowpox Virus Infection in Cotton-Top Tamarins (*Saguinus oedipus*) in Germany

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Abstract

Cowpox virus (CPXV) was isolated from a fatal outbreak among cotton-top tamarins. Samples from healthy common marmosets in contact were also CPXV genome positive. The CPXV isolated from the cotton-top tamarins exhibited a unique hemagglutinin sequence. Pathogenicity investigations using a Wistar rat model characterized the isolate as low pathogenic.

Key Words: Cowpox virus—*Orthopox*—Cotton-top tamarins—Hemagglutinin.

Introduction

Cowpox virus (CPXV) is a member of the virus family Poxviridae, belongs to the genus *Orthopoxvirus* within the subfamily Chordopoxvirinae and is endemic in Europe and Asia. Occasionally CPXV are identified as an etiological agent from diseased zoo animals, cats, or fancy rats (Vorou et al. 2008, Campe et al. 2009). In addition, descriptions of human infection after contact with diseased animals have increased lately, most likely because immunity against this zoonosis is increasingly absent because the smallpox-vaccinated population is declining. The suspected natural reservoirs of CPXV are voles and maybe other rodent species (Bennett et al. 1997). CPXV infections in New World monkeys and macaques have been reported from Germany and The Netherlands (Martina et al. 2006, Mätz-Rensing et al. 2006).

Case report

Four cotton-top tamarins were housed together for 1 year in the animal park Bad Liebenstein in Thuringia, Germany. In September of 2010, all animals died peracutely, and two monkeys were examined by gross pathology. Both animals showed extensive multifocal areas of alopecia and ulceration on the head, in particular at the muco-cutaneous junctions (Fig. 1A) and both on the palms and soles.

Skin scraping samples of cutaneous lesions that were tested using real-time PCR (adapted from Scaramozzino et al. 2007 using a TaqMan probe) scored positive for orthopox virus DNA. Virus isolation from the sample material was successfully performed with Vero cells (collection of cell lines in veterinary medicine, FLI, RIE228) and embryonated chicken eggs. For exclusion of a monkeypox virus (MPXV) infection, and for identification of the orthopox virus species involved, the hemagglutinin (HA) gene was sequenced (Nitsche et al. 2007). Analysis of the sequence (1143 bp; accession no. KC493623) identified the virus as CPXV and revealed a unique insertion of 75 bp within the HA gene of the cotton-top tamarin isolate, which resulted from duplication of nucleotides 475–649 with additional 4-nucleotide substitutions.

The closest homology was found with a human isolate from Norway (FJ769353), showing 98% sequence identity. In contrast, a CPXV isolate from an outbreak in a colony of New World monkeys from 2002 in Lower Saxony, Germany (HQ420898) was more distantly related (Fig. 1B). The exceptional phylogenetic allocation was confirmed from analyses of two additional genes—the vaccinia virus homolog F1L (759 bp; accession no. ; Fig. 1B) and the A-type inclusion body protein (*atip* KF709695) gene (3888 bp; accession no. KC894606; Fig. 1B).

Oropharyngeal swab samples from seven healthy common marmosets (*Callithrix jacchus*) housed in the same facility, but in different cages, were additionally analyzed and also scored positive for the CPXV genome (quantification cycle [Cq] values 28.9–35.5, corresponding to 6000 to 70 viral genome copies per microliter of PCR template). Sequencing of the HA gene revealed a 100% nucleotide identity between the cotton-top tamarin isolate and the marmoset sample. Organ samples (liver) of 23 different rodents (bank vole, yellow-necked mouse, house mouse, wild rat) trapped in the proximity of the facility 1 month after the death of the cotton-top tamarins scored negative for the *Orthopoxvirus* genome.

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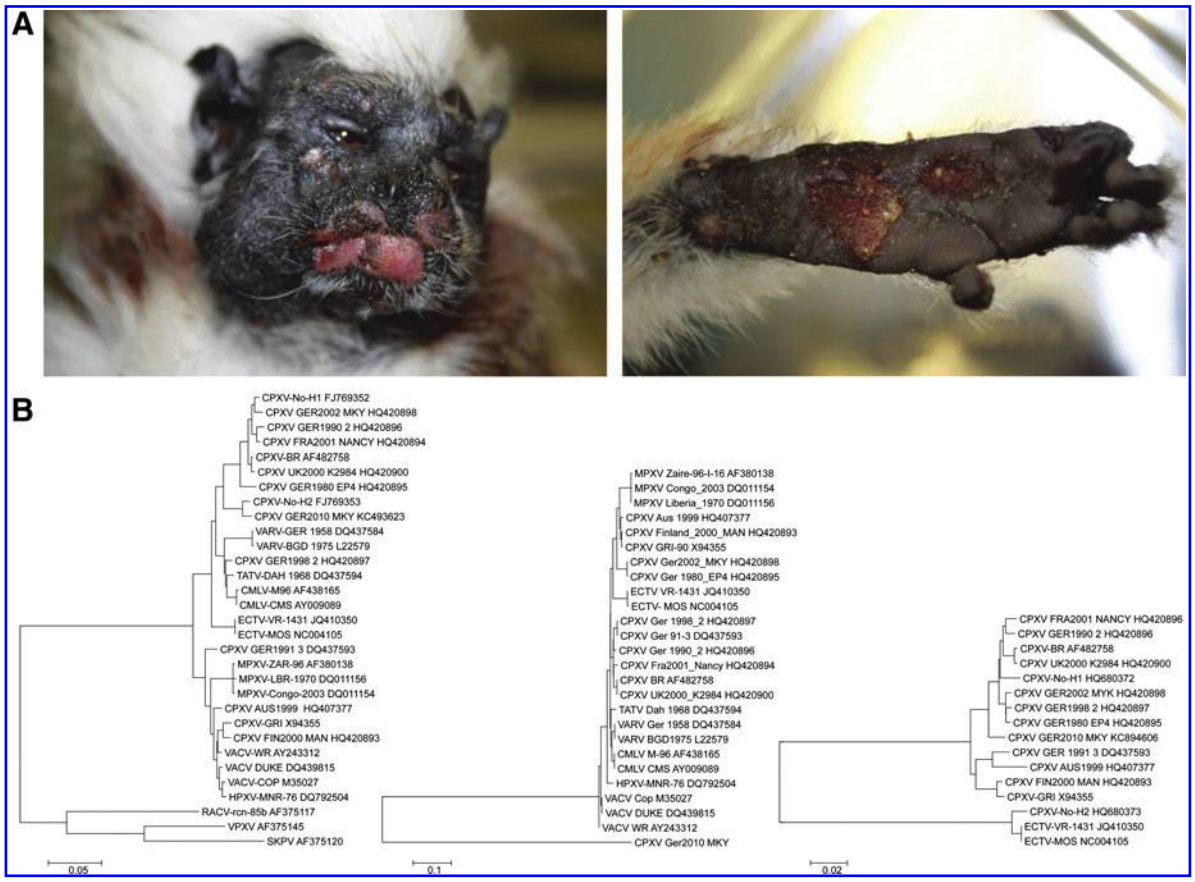

FIG. 1. (**A**) Typical skin lesions on the face and on the sole of a cotton-top tamarin. (**B**) Phylogenetic tree based on the nucleotide sequence of the hemagglutinin gene, the vaccinia virus homolog F1L gene, and the *atip* gene. The evolutionary history was inferred using the neighbor-joining method. The bootstrap consensus tree inferred from 1000 replicates. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011). Abbreviations used are: CMLV, camelpox virus; CPXV, cowpox virus; ECTV, ectromelia virus; HPXV, horsepox virus; MPXV, monkeypox virus; RACV, racoonpox virus; SKPV, skunkpox virus; TATV, taterapox virus; VACV, vaccinia virus; VARV, variola virus; VPXV, volepox virus.

To determine the pathogenicity of the cotton-top tamarin virus isolate, Wistar rats (6 weeks of age, of either sex) were inoculated intranasally with 10^4 tissue culture infectious dose 50 ($TCID_{50}$) per animal or 10^6 $TCID_{50}$/animal, respectively. (The animal trials gained governmental approval under the registration number LVL MV/TSD/7221.3-2.1-022/10-1.) Four rats per dose group were inoculated. After 24 h, two additional animals per dose group were included to serve as control rats for the viral transmission between the animals. Oropharyngeal swab samples taken from the animals throughout the study were negative by real-time PCR, and only one inoculated rat per dose group seroconverted. Therefore, the level of pathogenicity was estimated to be low in comparison to a 2009 CPXV isolate from a rat that was recently characterized (Kalthoff et al. 2011).

Discussion

CPXV was described as an etiological agent with predominant clinical signs of sudden death and skin lesions in cotton-top tamarins and common marmosets once in Germany (Mätz-Rensing et al. 2006). Interestingly, in our case, human cases were not reported while common marmosets housed in the same facility remained healthy, but CPXV DNA could be detected in swab samples (from marmosets). Paired serum samples were not available, but pre-existing immunity may be an explanation for the absence of clinical signs. The alternative possibility that cotton-top tamarins and common marmosets exhibit different susceptibilities to CPXV infection cannot be ruled out. Samples from rodents that were trapped in the vicinity of the housing tested negative, but due to the delay between the outbreak and the period of sampling, this result does not exclude rodents as being the transmitting host. Interestingly, 2010 was a year in which a high vole density was observed in Germany, so it could be predicted that transmission rates of rodent-transmitted pathogens would rise, as was recently demonstrated for hantaviruses (Ettinger et al. 2012).

Phylogenetic analysis of the HA gene, vaccinia virus homolog F1L gene, and *atip* gene sequences did not cluster the cotton-top tamarin isolate together with the monkey isolate

from an outbreak in 2002 (HQ420898), but displayed a more unique allocation. Phylogenetic studies of the full genome sequence are part of future investigations.

The reported outbreak is a further indication of a high vulnerability of New World monkeys, especially cotton-top tamarins, to different CPXV isolates. However, the characterization of CPXV isolates from different hosts in a reference model species allows a pathogenicity-based classification. Here the pathogenicity determination of the cotton-top tamarin isolate categorized it as low pathogenic for the model species Wistar rats.

In conclusion, the pathogenicity factors of CPXV need to be studied further and any lesions in New World monkeys should be tested for the presence of CPXV. Finally, the risk of further transmission of CPXV to humans needs to be taken into account and the role of the described 75-bp HA insertion needs to be elucidated.

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Author Disclosure Statement

No competing financial interests exist for any of the authors.

References

Bennett M, Crouch AJ, Begon M, Duffy B, et al. Cowpox in British voles and mice. J Comp Pathol 1997; 116:35–44.

Campe H, Zimmermann P, Glos K, Bayer M, et al. Cowpox virus transmission from pet rats to humans, Germany. Emerg Infect Dis 2009; 15:777–780.

Ettinger J, Hofmann J, Enders M, Tewald F, et al. Multiple synchronous Puumala hantavirus outbreaks, Germany, 2010. Emerg Infect Dis 2012; 18:1461–1464.

Kalthoff D, König P, Meyer H, Beer M, et al. Experimental cowpox virus infection in rats. Vet Microbiol 2011; 153:382–386.

Mätz-Rensing K, Ellerbrok H, Ehlers B, Pauli G, et al. Fatal poxvirus outbreak in a colony of New World monkeys. Vet Pathol 2006; 43:212–218.

Martina BE, van Doornum G, Dorrestein GM, Niesters HG, et al. Cowpox virus transmission from rats to monkeys, the Netherlands. Emerg. Infect Dis 2006; 12:1005–1007.

Nitsche A, Kurth A, Pauli G. Viremia in human Cowpox virus infection. J Clin Virol 2007; 40:160–162.

Scaramozzino N, Ferrier-Rembert A, Favier AL, Rothlisberger C, et al. Real-time PCR to identify variola virus or other human pathogenic orthopox viruses. Clin Chem 2007; 53: 606–613.

Tamura K., Peterson D., Peterson N., Stecher G., et al. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011; 28:2731–2739.

Vorou RM, Papavassiliou VG, Pierroutsakos IN. Cowpox virus infection: An emerging health threat. Curr Opin Infect Dis 2008; 21:153–156.

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