**Echinococcus multilocularis Infection of Several Old World Monkey Species in a Breeding Enclosure**

Dennis Tappe,* Klaus Brehm, Matthias Frosch, Anja Blankenburg, Annette Schroed, Franz-Josef Kaup, and Kerstin Mätz-Rensing

Consilary Laboratory for Echinococcosis, Institute of Hygiene and Microbiology, University of Würzburg, Germany; German Primate Center, Department of Infectious Pathology, Göttingen, Germany

Abstract. *Echinococcus multilocularis*, the causative agent of alveolar echinococcosis, is spreading geographically in Europe, and prevalence rates in foxes, the final host, are increasing. Concomitantly, the rate of newly diagnosed human infections has already doubled in Germany. We report a cluster of alveolar echinococcosis in 24 animals of different Old World monkey species (15 cynomolgus monkeys, 5 rhesus monkeys, and 4 lion-tailed macaques) in northern Germany. The cluster described is the largest ever recorded in a single center. Cynomolgus monkeys were very susceptible and constituted the monkey species at highest risk, indicating that this species could act as a sentinel animal for the transmission of alveolar echinococcosis in zoological gardens or similar institutions.

INTRODUCTION

The fox-tapeworm *Echinococcus multilocularis* is widely distributed in the northern hemisphere and is the causative agent of alveolar echinococcosis (AE), one of the most dangerous zoonoses. The sylvatic life cycle involves red foxes (*Vulpes vulpes*) as final hosts and small rodents as intermediate hosts. Many mammal species, including humans and rarely nonhuman primates, can become intermediate hosts by accidental ingestion of eggs deposited with feces. AE develops subsequently with the metacestode stage of the parasite growing in the intermediate host. AE predominantly affects the liver and is often fatal. Recent reports from Switzerland and Japan indicate that the disease may be a threat for animals living in zoological gardens. We report the largest cluster of cases of AE recorded in a single center, affecting cynomolgus monkeys (*Macaca fascicularis*), rhesus monkeys (*Macaca mulatta*), and lion-tailed macaques (*Macaca silenus*) in a breeding facility in northern Germany, in the period from 1994 to 2006.

STUDY

The animals were kept in an open-air enclosure of the German Primate Center located at the edge of a forest in Lower Saxony, northern Germany. In 1994, the first rhesus monkey (*M. mulatta*) developed AE after the housing changed from entirely indoors to largely outdoors. The animal showed progressive abdominal enlargement, apathy, and anorexia and was euthanized. Hepatomegaly with fibrotic transformation and infiltrative cyst formation was noted at necropsy. Gross aspect and histopathological findings were consistent with infection by the metacestode stage of *E. multilocularis*. Since then, an additional three *M. mulatta*, five *M. fascicularis*, and three *M. silenus* have developed similar symptoms and died as a consequence of AE. The main pathologic findings in all 12 cases were severe hepatomegaly with fibrous transformation of the liver tissue, mainly in the right and middle lobes. Cystic metacestode tissue with a diameter up to 20 cm and a weight up to 4000 g could be demonstrated in the liver (Figure 1A).

A generalized subacute peritonitis was a concomitant finding in all animals investigated. The mesenteric lymph nodes appeared enlarged. Multilocular cysts were occasionally present in the lungs, the pancreas, and the heart (Table 1). These cysts were usually smaller than the total cystic mass present in the liver. Histologically, the liver parenchyma was destroyed by infiltrative growing cysts of different sizes. The cysts were surrounded by chronic inflammatory cell infiltration with foreign body-type giant cells. The cysts contained multiple protoscolices (Figure 1B).

As many asymptomatic animals were suspected to be among the various monkey groups, sera of all animals that once lived in the enclosure were screened for anti-*Echinococcus* antibodies. Two different ELISAs were used in parallel: one using a crude larval antigen preparation from laboratory *E. multilocularis* and another using the recombinant EM10 antigen, an immunodominant *E. multilocularis*-specific cytoskeleton protein (sensitivity 93%, specificity 97–100%). Human sera of confirmed AE cases and sera of the deceased monkeys served as controls. Additionally, a commercially available western blot (LDBIO, Lyon, France) was used for the sera of the animals that tested positive in the ELISAs, confirming the results. A total of 130 rhesus monkeys (*M. mulatta*), 45 cynomolgus monkeys (*M. fascicularis*), 13 lion-tailed macaques (*M. silenus*), and 14 hamadryas baboons (*Papio hamadryas*) were tested, including the deceased animals. Among the entire population, 5 of the 130 rhesus monkeys (3.8%), 15 of the 45 cynomolgus monkeys (33.3%), and 4 of the 13 lion-tailed macaques (30.8%) were found to have positive serology at the end of the study period (March 2006). All monkeys that were initially seronegative were tested twice during the study period, and seroconversion was observed in 3 rhesus monkeys, 3 cynomolgus monkeys, and 1 lion-tailed macaque. Of the seropositive animals, 12 were still alive at the end of the study period and the developing hepatic AE lesions were controlled yearly by abdominal ultrasound. The animals were not treated and survived until today. The highest percentage of infection was found among the group of cynomolgus monkeys, the second highest among the lion-tailed macaques, and the lowest among the rhesus monkeys. In contrast, none of the baboons had a positive serology. There was no sex preference of seropositivity (male:female ratio, 1.3:1).

Wild foxes have been noticed frequently in the surroundings and on the Primate Center grounds. Direct contamina-
tion of the individual outdoor cages by entering foxes seems unlikely, as foxes cannot penetrate the cages' fencings. However, shredded branches and mulch, which are used for environmental enrichment within the cages, have been stored outdoors on the Primate Center grounds and could have been fecally contaminated. Moreover, branches taken from the local woods have been used for environmental enrichment. Foxes might also have contaminated the direct surroundings of individual cages. As a preventive measure, collected branches were pre-cleaned using a high-pressure washer. Mulch and shredded branches were stored out of reach of foxes, and fox bait containing praziquantel were dispersed.

DISCUSSION

Many monkey species are susceptible to infection with *E. multilocularis*. In recently described smaller outbreaks of AE, only a few species were affected (five cynomolgus monkeys and two gorillas, 12 cynomolgus monkeys, or 12 Japanese monkeys). We describe the largest cluster of AE cases reported in captive monkeys, affecting 24 animals of three different Old World monkey species over a period of 12 years. Cynomolgus monkeys were the species at highest risk and presumably more susceptible to infection than other monkey species. The percentage of infected cynomolgus monkeys among colonies of captive primates was also high (>50%) in a report from Switzerland. In contrast, none of the baboons showed clinical signs of AE and no anti-*Echinococcus* antibodies were detectable either, although they had lived in the same enclosure for the same time period. Accordingly, no reports about AE in baboons have been published. As baboons are used to feeding on the ground, they would even have a higher risk of infection than the macaques. There were no differences observable in the behavior of the captive baboons compared with the macaques, e.g., no noticeable avoidance of branches or possibly contaminated soil. In contrast to AE, cases of cystic echinococcosis caused by the closely related *E. granulosus* have been described in baboons. However, baboons were shown to be relatively resistant to experimental *E. granulosus* infection, and a similar resistance to *E. multilocularis* infection can be assumed. The fact that *E. granulosus* is endemic in areas where wild baboons live, whereas *E. multilocularis* is not present in these regions, could also account for the lack of recorded AE cases in these monkeys.

The histologic patterns of infection in cynomolgus monkeys, rhesus monkeys, and lion-tailed macaques were shown to be similar to those found in the typical rodent intermediate host (e.g., presence of protoscolices, little necrosis). This report is the first description of AE in lion-tailed macaques.

### Table 1

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Monkey species</th>
<th>Sex (M/F)</th>
<th>Organ affected (necropsy findings)*</th>
<th>Year of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>M. mulatta</em></td>
<td>M</td>
<td>Liver, lung, mediastinum</td>
<td>October 1994</td>
</tr>
<tr>
<td>2</td>
<td><em>M. silenus</em></td>
<td>F</td>
<td>Liver, lymph nodes, mediastinum</td>
<td>April 1999</td>
</tr>
<tr>
<td>3</td>
<td><em>M. silenus</em></td>
<td>F</td>
<td>Liver</td>
<td>December 1999</td>
</tr>
<tr>
<td>4</td>
<td><em>M. silenus</em></td>
<td>M</td>
<td>Liver, lung, mediastinum, mesenterium, heart</td>
<td>August 2000</td>
</tr>
<tr>
<td>5</td>
<td><em>M. fascicularis</em></td>
<td>M</td>
<td>Liver, lung, mediastinum</td>
<td>August 2002</td>
</tr>
<tr>
<td>6</td>
<td><em>M. fascicularis</em></td>
<td>M</td>
<td>Liver, lung, mediastinum, pancreas</td>
<td>October 2002</td>
</tr>
<tr>
<td>7</td>
<td><em>M. fascicularis</em></td>
<td>M</td>
<td>Liver, mediastinum, pancreas</td>
<td>November 2002</td>
</tr>
<tr>
<td>8</td>
<td><em>M. fascicularis</em></td>
<td>F</td>
<td>Liver</td>
<td>January 2004</td>
</tr>
<tr>
<td>9</td>
<td><em>M. fascicularis</em></td>
<td>F</td>
<td>Liver</td>
<td>August 2004</td>
</tr>
<tr>
<td>10</td>
<td><em>M. fascicularis</em></td>
<td>F</td>
<td>Liver, lung</td>
<td>August 2004</td>
</tr>
<tr>
<td>11</td>
<td><em>M. fascicularis</em></td>
<td>M</td>
<td>Liver</td>
<td>July 2005</td>
</tr>
<tr>
<td>12</td>
<td><em>M. mulatta</em></td>
<td>F</td>
<td>Liver, lung, mediastinum, pancreas</td>
<td>March 2006</td>
</tr>
</tbody>
</table>

* The liver is the organ primarily affected, harboring the bulk of metacestode tissue. Dissemination occurs later, with smaller cysts in various organs.
confirms and expands previous data obtained from cynomolgus monkeys\(^2,4\) and a rhesus monkey.\(^8\) However, the findings contrast with AE cases in great apes, such as gorillas\(^9,12\) and orang-utans,\(^13\) in whom pathologic changes resemble those found in humans (e.g., usually no protoscolices, widespread necrosis).

Since the 1990s, \textit{E. multilocularis} has been spreading geographically, and increasing infection rates of red foxes have been noted in western and eastern European countries.\(^1,4,15\) For the last decade, fox population densities have been increasing drastically, and the raccoon dog, a neozootic, highly susceptible final host, has migrated from Asia to Germany and Poland.\(^15\) Today, \textit{E. multilocularis} has been reported from Denmark, the Netherlands, Belgium, central France and Germany to Lithuania, Poland, Slovakia, Hungary and Northern Italy.\(^1,15\) The presence of the parasite in southeastern European countries is likely.\(^1,15\) In northern Germany, Denmark, and Poland, prevalence rates in foxes are usually <5%,\(^16\) but focal areas of higher prevalence exist. In southern Germany and Switzerland, infection rates often exceed 50% and approach 100% locally.\(^16\) Animals in zoological gardens and in institutional colonies in the northern hemisphere are at risk, and AE must be considered as an emerging disease also in captive animals. Cynomolgus monkeys are very susceptible to AE and may thus indicate previously unknown areas of high transmission in zoos. In the scenario described, the increase of AE cases over the years in captive monkeys reflects the transition from sporadic cases to an endemic situation. Human caretakers for these animals are also at risk of infection via the same routes as the primates. With respect to the raised prevalence rates in foxes and an increase of fox population densities, an upsurge of human echinococcosis has already been noticed.\(^13\)

Received May 3, 2007. Accepted for publication May 20, 2007.

Acknowledgment: The authors thank Mechthild Schulze for carrying out the serology tests.

Authors’ addresses: Dennis Tappe, Klaus Brehm, and Matthias Frosch, Conradi Laboratory for Echinococcosis, University of Giessen, Germany. Additional correspondence addresses: Anja Blankenburg, Annette Schrod, Franz-Josef Kaup, and Kerstin Blankenburg, Department of Infectious Pathology, Kellnerweg 4, 37077 Goettingen, Germany.

REFERENCES