Alveolar Echinococcosis Infection in a Monkey (Ateles geoffroyi) In Mashhad, Iran

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Abstract
Alveolar echinococcosis (AE), which is caused by ingestion of eggs of the fox tapeworm Echinococcus multilocularis, is the most potentially lethal parasitic infection because of its tendency to invade and proliferate in the liver and the difficulty in treatment. This article describes a case of alveolar echinococcosis found in Ateles geoffroyi in Mashhad, Iran. The cysts were characterized as an alveolar structure, composed of numerous small vesicles in liver, abdominal cavity, retroperitoneum and lungs. A characteristic feature of these vesicles was its exogenous tumor-like proliferation. These cysts were filled with numerous protoscoleces suggesting a potential role of this monkey in cycle of transmission. Up to now, this is probably the first report of alveolar echinococcosis in A. geoffroyi in the world.

Keywords: Alveolar echinococcosis, Ateles geoffroyi, Iran

Introduction

Echinococcus multilocularis, the causative agent of alveolar echinococcosis (AE), is one of the most dangerous zoonoses and widely distributed in the northern hemisphere (1). In untreated or incompletely treated patient the mortality rate is high (2). Transmission of this parasitic disease is mostly seen in the northern hemisphere. The parasite is geographically widespread being distributed from Alaska, across Canada and north central USA, through northern Europe and Eurasia to Japan (2). This spread may result in the emergence of alveolar echinococcosis (2). Since epidemiology, host ranges and the general level of knowledge about the parasite are different in various parts of the world, the current situation is discussed for Asia. Alveolar echinococcosis in Asia is widespread across the arctic, sub-arctic and temperate climate zones from Turkey to Japan (3). In Turkey, cases of human AE are most frequent in central and eastern Anatolia, but there is no information on the local transmission patterns (4). In China, AE is a serious public health problem in the western and central parts—including the Tibetan plateau—where it co-exists with cystic echinococcosis (5). In Japan, AE is restricted to the northern island of Hokkaido, where it was probably introduced accidentally with infected foxes from the Kurile Islands early in the 20th century (3). In Iran, AE is most frequent in the northwest region of country (6), although a case of human AE has been recently reported from Khorasan Province (7). It has been stated that the number of alveolar echinococcosis cases are on the increase due to the development of diag-

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nostic techniques and the widely spread health system in recent years (2).
Nowadays, the prevalence of *E. multilocularis* in red fox has increased based on recent research findings (8). It is possible that further understanding of biology and intermediate host of *E. multilocularis* may reduce transmission at local scales (8).
This report describes a new finding of AE in an *Ateles geoffroyi* thus demonstrating its susceptibility to infection with *E. multilocularis*.

**Case history**
A case of *A. geoffroyi* was brought to Small Animal Clinic Science of Veterinary Faculty of Ferdowsi University of Mashhad due to abdominal pain, chronic weight loss and anorexia on January 2008. Clinical examination was performed. Clinical symptoms were abdominal pain, jaundice, hepatomegaly, weight loss, and pleural pain.
Surgical finding was characterized an alveolar structure, composed of numerous small vesicles in liver, abdominal cavity, retroperitoneum and lungs (Fig. 1). A characteristic feature of these vesicles was its exogenous tumor-like proliferation. This monkey based on clinical parameters and the extrahepatic involvement of neighboring organs had poor prognostic considerations. After euthanizing, these vesicles were transported to parasitology and pathology lab for characterization of cysts.

**Parasitology findings**
Specimens of liver, abdominal cavity and lungs including all visible cystic lesions were carried to the Parasitology lab of Veterinary Faculty of Ferdowsi University of Mashhad. The specimens prepared from this mass were examined under the microscope (Fig. 2). Protoscoleces and germinative membranes were observed in this examination (9-13). Hooklets were detectable in the metacestode (Table 1).

**Pathology findings**

Pieces of liver and lung were fixed in 10% buffered, neutral formalin. After fixation for 24 h, tissues were embedded in paraffin; sections were cut at 5 mm and stained with hematoxylin and eosin. The different part of the liver contained multiple cysts characterized by laminated layer adjoining the inner germinal layer with multiple protoscoleces protruding from it. These cystic vesicles were surrounded by multiple layers of mature connective tissue, which was infiltrated by moderate numbers of lymphocytes, macrophages, and foreign body giant cells (Fig. 3-5). The liver tissue reaction showed bile ducts hyperplasia and mononuclear cell infiltration in portal area (Fig. 6).

**PCR finding**
The metacestodes have been processed for genomic DNA isolation and subsequent PCR testing for *Echinococcus* and for actin as an internal quality control. Unfortunately, the genomic DNA isolates did not reach a quality that would have allowed appropriate PCR testing. Because the control target "actin" could not be amplified, the material did not allow any other PCR performance, and thus also the *Echinococcus* PCR was not feasible and negative, respectively.
As we have could not identify *E. multilocularis* based on PCR, we sent this sample to Prof. Dr. Bruno Gottstein (Institute of Parasitology, Vetsuisse Faculty and Faculty of Medicine, University of Bern) that is reference center for identification of this parasite. They could not isolate DNA from sample but they were able to isolate some hooks from protoscoleces, and they were measuring the hooks according to elder publications. The results unambiguously indicated that the hooks belong to *E. multilocularis*. 

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Fig. 1: Surgical finding showed alveolar structure, composed of numerous small vesicles in abdominal cavity of *Ateles geoffroyi*.

Fig. 2: The protoscoleces of *E. multilocularis* prepared from the specimens of the mass (Lactophenole-X320).

Fig. 3: Tissue reaction containing connective tissue formation and obvious inflammatory infiltration mostly giant cell and macrophage. Multiple cyst is visible in this section by arrow (A) (H&E×320).

Fig. 4: Multiple cystic vesicle was characterized by laminated layer (A) adjoining the inner germinal layer with multiple protoscolices (B) which was surrounded by mature connective tissue, moderate numbers of lymphocytes, macrophages, and foreign body giant cells (H&E×160).
Fig. 5: Section of protoscolex that a lot of inflammatory cell attached to surface of this (A) with hook of protoscolex (C) and hyaline layer of cyst (B) (H&E×640)

Fig. 6: Bile duct hyperplasia with infiltration of inflammatory cell around the cyst (H&E×320)

Table 1: Identified features of the examined protoscolex found in Ateles geoffroyi

<table>
<thead>
<tr>
<th></th>
<th>Total number</th>
<th>Total length(µ)</th>
<th>Blade length(µ)</th>
<th>Handle length(µ)</th>
<th>Blade distance(µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large rostellar hook</td>
<td>15</td>
<td>25</td>
<td>6</td>
<td>4</td>
<td>2.5</td>
</tr>
<tr>
<td>Small rostellar hook</td>
<td>15</td>
<td>17.5</td>
<td>5</td>
<td>2</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Discussion

The intermediate host of *E. multilocularis* is a variety of rodents and small lagomorphs. However, the species of host animals differ according to regional changes in mammalian fauna. Besides rodents, a number of non-rodent mammalian animals may also acquire an *E. multilocularis* infection by egg ingestion. In recent years, a variety of mammalian animal species have been described from European countries and Japan as aberrant hosts of the metacestode stage of *E. multilocularis*, including domestic dogs, domestic and wild pigs, horses, monkeys, and the nutria (Myocastor coypus) (14). Such hosts, which do not play a role in the transmission cycle, are denominated as aberrant hosts. From 1987 to 2000 in Zurich has diagnosed 10 cases of AE in dogs and 15 in captive monkeys (15). Vuitton et al. (1) has reported AE in 24 different Old World monkey species (15 cynomolgus monkeys, 5 rhesus monkeys, and 4 lion-tailed macaques) in northern Germany.

To our knowledge, this is probably the first documentation of AE in an *A. geoffroyi* throughout the world thus demonstrating its susceptibility to infection with *E. multilocularis*. The infection route in this monkey is unclear. It is proba-
bly assumed that this monkey exposed to the eggs of *E. multilocularis* by ingesting food or water contaminated with eggs. Such cases are not only of clinical interest, but also of epidemiological importance as they are indicators of environmental contamination with *E. multilocularis* eggs and the potential infection risk for humans. In contrast to the hydatid cysts of *E. granulosus*, the metacestode stage of *E. multilocularis* has the capacity to proliferate by external budding and to form root-like protrusions of tissue, enabling the parasite to progressively invade host organs (16).

It is important to note that AE has a long incubation period of about 5–15 years and clinical symptoms often occur in a rather late phase of the infection when large parts of the liver are already infiltrated by the parasite. Following primary infection, the liver is the predominantly affected organ (about 99%), but later on the parasite may spread to other organs by local or distant metastasis formation.

**Ethical considerations**

Ethical issues (including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

**Acknowledgements**

The authors declare that there is no conflict of interests.

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