A case of an Echinococcus ortleppi infestation in a red-shanked douc langur (Pygathrix nemaeus) in northern Vietnam

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Summary

An Echinococcus ortleppi infestation was demonstrated in a red-shanked douc langur (Pygathrix nemaeus) at the Endangered Primate Rescue Center, Cuc Phuong National Park, Vietnam. In pathology, four parasitic cysts were found within both lungs. In parasitology, Echinococcus ortleppi was identified by polymerase chain reaction and mitochondrial gene sequencing. This is the first record of E. ortleppi from a non-human primate, and, to the authors' knowledge, the first ever isolate of the E. granulosus-assemblage from a monkey that has been molecularly characterized using strain-specific methods. The presence of E. ortleppi, in particular, is rather unexpected, as the nearest region where that species has been recorded is the Indian subcontinent.

Một trường hợp nhiễm Echinococcus ortleppi ở Chà vá chần nâu (Pygathrix nemaeus) ở Bắc Việt Nam

Tóm tắt

Một trường hợp chưa chần nâu (Pygathrix nemaeus) tại Trung tâm Cứu hộ Linh trưởng, VQG Cúc Phuong, Việt Nam được phát hiện nhiễm Echinococcus ortleppi.

Trong bệnh lý học, bốn nang bả kí sinh đã được phát hiện ở cả hai là phải. Đối với ngành kí sinh trung, Echinococcus ortleppi được phát hiện bằng phương pháp gen PCR và kỹ thuật phân tích gen trong ty thể. Ngoài trừ được phát hiện ở người, đây là ghi nhận đầu tiên loại Echinococcus ortleppi ký sinh ở một loại linh trưởng khác, và đối với sự hiểu biết của các tác giả, đây là loại được mô tả là E. granulosus đầu tiên kỹ sinh trên một loài kí sinh được xác định về chất phân tử sử dụng các phương pháp chưng-dác hiệu. Cách riêng, sự hiện diện của loại E. ortleppi nằm ngoài đủ kích đố khư xúc gần nhất mà loại này được phát hiện là tiều luc địa Ân Đô.

Introduction

Cystic echinococcosis

Cystic echinococcosis (CE), or hydatidosis, is a parasitic disease caused by various taxa of the cestode genus Echinococcus. It is typically transmitted between domestic dogs or wild canids as definitive hosts, harboring tapeworms in their intestines, and domestic or wild ungulates as
intermediate hosts, where the cystic metacestodes grow in various organs and may cause severe symptoms or death due to the occupying space in the organs. Apart from ungulates, a wide range of other mammals, including humans and non-human primates, are known to be susceptible as, mainly aberrant, intermediate hosts.

CE was previously ascribed to one diverse species, the dog tapeworm (*E. granulosus*). By now it is apparent that a number of independent species with distinct genetic, morphological and biological features were hidden under that name, and the *E. granulosus*-assemblage have recently been split into *E. granulosus* sensu stricto, *E. equinus*, *E. ortleppi*, *E. canadensis* and *E. felidis* (Nakao et al., 2007; Hüttner et al., 2008; Saarma et al., 2009). Diagnostic morphological characters are not known for the cyst stage for any of these species, and the various molecular tools for differentiation of these taxa have only recently been developed. Therefore, relatively few data are available on the geographical distribution and host range of these forms (Jenkins et al., 2004), although a large body of epidemiological information was collected in the past on the *E. granulosus* assemblage as a whole (Eckert et al., 2001).

*E. ortleppi* has been known as the ‘cattle strain’ (G5) of *E. granulosus*, because the transmission of this parasite seems to occur preferentially between dogs and cattle (Eckert and Thompson, 1988; Thompson and McManus, 2002). Originally described from South Africa, it is (or was) widespread in Europe, and was also recorded from South America, sub-Saharan Africa and some Asian countries (India, Sri Lanka and Nepal) (Thompson & McManus, 2002; Dinkel et al., 2004).

Endangered Primate Rescue Center

The Endangered Primate Rescue Center was established in 1993 to house highly endangered primates confiscated from the illegal wildlife trade. Vietnamese endemic species are a major focus of the center’s work. Over the years the center has developed as a breeding facility for several species. The final stage of this program will be the release of captive bred offspring into protected areas to strengthen dramatically declining wild populations. Currently the centre keeps more than 150 animals of 15 taxa, six of which are only successfully kept in captivity at the EPRC (Nadler, 2008).

Material

Animal and keeping conditions

This paper reports on a female red-shanked douc langur (*Pygathix nemaeus*) from the Endangered Primate Rescue Center, Cuc Phuong National Park, Vietnam. The animal was born on 17 April 2004 and died on 29 September 2008. It was caged since its birth in a group with its mother, a wild born female which died in 2007, a wild born adult male and another adult female, born at the EPRC in 2001.

The group of monkeys lived in an open cage (10m x 5.5m x 3.2m) made of wire mesh, metal pillars a concrete floor. The cage is furnished with bamboo poles. About forty similar cages are situated in a park-like area surrounded by a fence.  

Only the animal keepers have access to the cages. Visitors are required to stay on a foot path about 3.5m distance from the wire mesh of the animal cages.

The EPRC keeps a disciplined watch dog which does not approach the cages very closely. Wild mammals which occasionally have closer contact with the cages include squirrels (*Callosciurus* sp.) and mongoose (*Herpestes javanicus*).
Clinical history

The langur became apathetic only two days before it died, but continued to ingest foods. On the morning of 29 September the animal ate but was found dead at 1 pm. The body was frozen at -20°C for further pathological investigations to determine the cause of death.

Methods

Pathology

Necropsy was performed immediately after the thawing of the carcass. Photos were taken and tissues of interest were fixed in 70% ethanol.

Parasitology

Parasitological identification of the parasite was performed by polymerase chain reaction and subsequent sequencing of parts of the mitochondrial 12S rRNA, nad1 and cox1 genes.

DNA extraction

DNA was isolated from ethanol fixed cyst material as described by Dinkel et al. (1998): About 0.5 g of the cyst wall was cut into small pieces and digested in the presence of 2 mg/ml proteinase K in 500 μl of 10 mM Tris-HCl (pH 7.5), 10 mM EDTA, 50 mM NaCl, 2% sodium dodecyl sulfate and 20 mM dithiothreitol. DNA was extracted with phenol chloroform isoamyl alcohol (25:24:1) and ethanol precipitation. After drying, the DNA was suspended in 200 μl TE-buffer (pH 7.6).

Polymerase chain reaction

A cestode specific PCR (cs PCR) was done as described previously (Dinkel et al., 1998; Dinkel et al., 2004) in 50 μl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM of MgCl2, 200 μM of each dNTP, 20 pmol of each primer and 1.25 units of Ampli-Taq Polymerase (Applied Biosystems). Amplification was done for 40 cycles (denaturation for 30 s at 94°C, annealing for 1 min at 55°C and elongation for 30 s at 72°C). For identification of genotypes and species of Echinococcus a semi-nested PCR assay specific for E. canadensis G6/7 and E. ortleppi as described in Dinkel et al. (2004) was performed. For the first PCR (g5/6/7), the 50 μl reaction mixture consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM of MgCl2, 200 μM of each dNTP, 25 pmol of each primer, and 1.25 units of Ampli-Taq Polymerase (Applied Biosystems) for 40 cycles (denaturation for 30 s at 94°C, annealing for 1 min at 53°C and elongation for 40 s at 72°C). To discriminate between E. ortleppi and E. canadensis G6/7, the semi-nested PCRs for E. ortleppi (g5 PCR) and E. canadensis G6/7 (g6/7 PCR) were used in a second step, both in a 50μl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM of MgCl2, 200 μM of each dNTP, 25 pmol of each primer, and 1.25 units of Ampli-Taq Polymerase (Applied Biosystems) for 40 cycles (denaturation for 30 s at 94°C, annealing for 1 min at 60°C and elongation for 30 s at 72°C). For all PCRs, target sequence for amplification is a part of the mitochondrial 12S rRNA gene.

For subsequent gene sequencing, two additional PCRs were performed as described in Bowles et al. (1992) and Bowles & McManus (1993) with the target sequences of a part of the mitochondrial cox1 and nad1 genes.

All amplification products were resolved on a 1.5% ethidium bromide stained agarose gel.
Mitochondrial gene sequencing

Amplification products of the cox 1, nad 1 and cs PCR were purified over QIAquick™ columns and cycle sequencing was performed on the Gene Amp 9700 (Applied Biosystems) with the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) for 25 cycles (denaturation for 10 s at 94°C and annealing for 4 min at 60°C). Electrophoresis was carried out on the ABI Prism 310 Genetic Analyzer (Applied Biosystems) and nucleotide sequence analysis was made using the National Center for Biotechnology Information BLAST programs and databases.

Results

Necropsy

At necropsy, four parasitic cysts approximately the size of table tennis balls were found within both lungs occupying at least 70 % of the lung tissue (Fig. 1). The cysts were filled with clear watery fluid and the walls of the cysts were yellowish-white and smooth. No protoscolices were found. The wall of each cyst contained a small ovoid thickened area the size of about 2mm.

In the left thoracic cavity, one cyst had been disrupted resulting in a severe fibrinous-exsudative pleuritis with some yellowish watery fluid (Fig. 2).

Residual lung tissue was severely atelectatic due to compression of the parasitic cysts.

Parasitology

Using the specific PCR system, DNA isolated from the cyst material was found to belong to E. ortleppi. This result was confirmed by gene sequencing of parts of the mitochondrial 12S rRNA, cox1 and nad1 genes. The sequences obtained showed 99% identity with published sequences of E. ortleppi on GenBank™.

Discussion

This is the first record of E. ortleppi from a non-human primate, and, to the authors’ knowledge, the first isolate of the E. granulosus-assemblage from a monkey that has ever been molecularly characterized using strain-specific methods. Non-human primates are known to be susceptible to cystic echinococcosis, but are usually accidental intermediate hosts which are not substantially involved in maintaining the transmission of the parasites. Natural infections are known from baboons (Papio sp.) in Kenya and Mozambique (Macpherson & Wachira, 1997), probably as a spill-over from domestic or wildlife cycles involving canids and ungulates. Accidental infections of primates in captivity were apparently common in the past, although detailed reports are few (reviewed in: Toft, 1986). Rhesus monkeys and baboons had been successfully used for experimental infections with CE (Hutchison, 1966; Macpherson et al., 1986).

From a geographical view, this is an interesting record from a region where only spurious information on echinococcosis (in general) is available: CE was recorded sporadically in Southeast Asia, but there are no details known on life cycles, species, or frequency (Eckert et al., 2001; Schantz et al., 1995; Segal & Humphrey, 1968). The presence of E. ortleppi, in particular, is rather unexpected, as the nearest region where that species has been recorded is the Indian subcontinent (Thompson & McManus, 2002). There is no doubt that the animal acquired the infection locally. However, it would be a matter of interest if a transmission cycle of E. ortleppi is autochthonous in the area, or whether the parasite was introduced from elsewhere, e.g. via the cattle trade. As to the
Fig. 1. Lungs of a red-shanked douc langur (*Pygathrix nemaeus*) with yellowish-white cysts of *Echinococcus ortleppi*. Photo: R. Plesker.

Fig. 2. Caudal view of the thorax of a red-shanked douc langur (*Pygathrix nemaeus*) infested with *Echinococcus ortleppi*. The right side shows a disrupted parasitic cyst (white) and a fibrinous pleuritis with yellowish frozen fluid in the thorax. Photo: R. Plesker.
immediate infection route to the captive monkey, faeces from a domestic dog are certainly the source of the parasite eggs, as wild canids are not known to be present in the immediate vicinity of the animal enclosure. Apart from faeces deposited immediately outside the cage, the contamination of vegetative matter (branches, leaves, fruits) brought as food or environmental enrichment from outside is a distinct possibility. The latter route seems to be important for the infection of primates in European and Japanese zoos with *E. multilocularis* (Sato et al., 2005; Tappe et al., 2007).

From the data available, *E. ortleppi* shows a strong predilection for cattle as intermediate hosts, where it produces large cysts predominantly in the lungs. In Switzerland, 95% of these cysts were fertile (Eckert & Thompson, 1988). Infection in other animals, e.g. sheep, goats, water buffaloes, domestic pigs and even zebra are known (Zhang et al., 2000; Dinkel et al., 2004; Obwaller et al., 2004), but the epidemiological role of these hosts is unclear. Only a few human cases are on record from the Netherlands, Argentina and Mexico (Bowles et al., 1992; Kamenetzky et al., 2002; Maravilla et al., 2004). Whether the small number of human cases is due to low exposure or indicates a certain degree of resistance to this species is not known. In the absence of such data, the presence of this parasite in the area should be a matter of concern for those involved in public health issues.

Cyst location in the lungs appears to be a typical feature of *E. ortleppi* in ruminants. The case referenced here suggests that this might also be the case in primates. In contrast, a massive zoo-borne infection of a colobus monkey and baboons in the USA with unspecified ‘*E. granulosus*’ produced masses of cysts in all organs posterior to the diaphragm, but did not involve the lungs (Myers et al., 1965), and a more recent case of unspecified CE in a captive pig-tailed macaque also showed cysts only in the liver (Plesker et al., 2001).

References


