



CASE REPORT

Echinococcus granulosus “*Sensu stricto*” in a Captive Ring-Tailed Lemur (*Lemur catta*) in Northern Italy

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ABSTRACT

Cystic echinococcosis (CE) by *Echinococcus granulosus* (Eg) infection was seen in a 13 years old male lemur, found dead in a zoo in Northern Italy. Necropsy revealed several transparent cysts in the lungs and in the abdominal cavity. Free-floating cysts of varying sizes were found in the peritoneal cavity, and no protoscolex was seen microscopically. Histologically, a multifocal severe parasitic granulomatous pneumonia was observed. Confirmation of *E. granulosus* “*sensu stricto*” was reached by PCR and sequencing. In view of the absence of definitive host in the zoo, located in non-endemic region for CE, it is speculated that infection introduced through translocation of lemur from endemic region (Southern Italy zoo).

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INTRODUCTION

Echinococcus spp., are tiny flatworms belonging to the family Taeniidae and widespread all over the world. Its classification and taxonomy has been very obscuring and controversial and as far as could be ascertained, other than the ‘lion strain’ 10 distinct genotypes (G1-10) of *E. granulosus* have been reported on the basis of mitochondrial DNA sequences (Lavikainen *et al.*, 2003). Currently, based on several morphological, biological and biomolecular criteria, 9 species are ascribed to the genus *Echinococcus*: *E. granulosus* “*sensu stricto*” (s.s.) (G1-G3), *E. multilocularis*, *E. vogeli*, *E. oligarthrus*, *E. shiquicus*, *E. equinus* (G4), *E. ortleppi* (G5), *E. felidis* and *E. canadensis* (G6-G10) (Nakao *et al.*, 2007). The life cycle of *E. granulosus* is indirect and involves as definitive hosts domestic and wild canids, and several species of domestic and wild mammals (humans included) as intermediate hosts for the tissue-invading metacestode stage, the hydatid cyst.

Cystic echinococcosis (CE), represents a public health problem and plays an important socio-economic role in many areas of the world and in particular in the Mediterranean Region (MR) where it is currently considered among the five most frequently diagnosed zoonosis, along with brucellosis, rabies, leishmaniasis and food-borne zoonotic infection (Sadjjadi, 2006).

Cystic echinococcosis is endemic in different regions of Italy. Prevalence in livestock is high in Sicily and Sardinia, medium-high in the central and southern regions, and negligible in the remaining areas (Varcasia *et al.* 2011). The reports of CE in wild intermediate hosts are very rare. Our report is referred to the metacestodosis in a captive Ring-tailed lemur (*Lemur catta*). This non-human, arboreal primate is endemic to islands of Madagascar. It is an omnivorous, terrestrial and diurnal animal. The Ring-tailed lemur is highly social, living in groups of up to 30 individuals and it is an endangered species according to the IUCN Red List of Threatened Species™ (Andriaholinirina *et al.*, 2014).

Case history and clinical examination: A 13 years old male lemur, host of a colony in a zoo of the province of Ravenna (Northern Italy: 44°19’35.5’’N; 12°16’29.9’’E) was found dead. The colony originated from animals previously imported from another zoo in Southern Italy and was composed of 12 animals. The diet was based on vegetables and fruits industrially produced in the area and tap water.

During the necropsy of the dead animal, innumerable transparent cysts were observed in the lungs and in the abdominal cavity. The cysts were of different size from 4 cm to few mm, many were free floating in the peritoneal cavity (Fig.1). The microscopic exam of cystic fluid revealed non-fertile cysts (without any protoscolices).



Fig. 1: Cysts of different size found during necropsy.

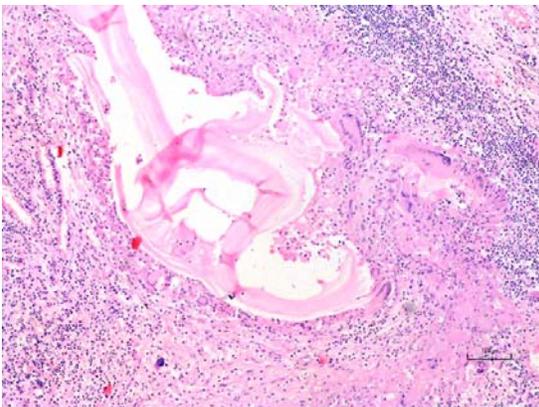


Fig. 2: Crumpled fragments of the parasitic membrane surrounded by severe granulomatous reaction, with multinucleated giant cells, palisading epithelioid cells, macrophages and lymphocytes. Lung, HE, bar=100µm.

Histologically, the lung cysts were outlined by a 100-200µm thick lamellar hyaline layer, and were internally lined by a thin germinal layer, but did not contain any protoscolices. Externally, the cysts were surrounded by an infiltrate of mixed inflammatory cells, including multinucleated giant cells, palisading epithelioid cells, macrophages and lymphocytes. Multifocally, there were crumpled fragments of the outer lamellar membrane enclosed by a particularly severe inflammatory infiltrate. The lung tissue showed also scattered peribronchial lymphocytic nodules (Fig. 2).

Molecular identification was carried out by PCR by amplifying fragments within 2 mitochondrial gene cytochrome C oxidase 1 (*cox1*) and NADH dehydrogenase 1 (*ND1*) using DNA extracted from the cysts. PCR products were purified using the High Pure PCR product purification kit (Roche Diagnostics, Mannheim, Germany) and commercially sequenced by MWG-Biotech (Ebersberg, Germany) using the PCR primers. Nucleotide sequences were compared to those available in GenBank® through the use of the basic local alignment search tool (BLAST) (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Molecular analyses of *cox 1* and *ND1* mitochondrial genes respectively shown an homology of 100% with *Echinococcus granulosus* s.s. sequences deposited in GenBank (accession numbers: GQ502212.1; GQ502217.1) revealing an involvement of *E. granulosus* s.s. as cause of CE in lemur examined in the present survey. The *Cox1* sequence was deposited in GenBank archive, accession number KT282119.

DISCUSSION

This, to our knowledge, is the first report of *E. granulosus* “*sensu stricto*” in a Ring-tailed lemur. Previously, case of hydatidosis in a captive *Lemur catta* has been reported from Zoological Center of Tel-Aviv (Shahar *et al.*, 1995), however, organism was not genotyped. Contrary to our finding, cysts observed in this case were, fertile, that may be ascribed to involvement of different strain. Other reports in the literature of echinococcosis in *Lemur catta* have been referred caused only by *E. multilocularis* (Palotay and Uno, 1975; Kondo *et al.*, 1996; Deplazes and Eckert, 2001, Umhang *et al.*, 2013). The last three reports were from captive animals in zoo located in endemic zone of Japan, Switzerland and France, respectively, and as infectious route, even if not clear, was suspected eggs-contaminated food or free-roaming foxes. The two cases described by Palotay and Uno (1975) were from a zoo in Oregon (USA) and was supposed that this primates were infected in the wild. Moreover, *E. granulosus* (G1) and *E. equinus* (formally G4), have been documented in other genus of lemur, *Cercopithecus ascanius* and *Varecia rubra*, respectively (Boufana *et al.*, 2012). In the latter animal, the presence of free-floating hydatids was also described similarly in our case. These primates were from a zoo of UK, where the *E. granulosus* (G1) and *E. equinus* are known to be endemic however the source of infection was uncertain.

In the present report, no suitable definitive hosts were present in the zoo and the lemurs lived in a status of permanent isolation from other animals. The food-water-borne infection (i.e. with contaminated vegetables/water) was excluded as the lemur were feed with vegetables and fruits produced industrially in the same area and tap water. The zoo is located in an area where CE has very low prevalence, so the most probable way of infection could be ascribed to the fact that infected lemur belonged to a colony that was previously hosted in another zoo in southern Italy, where CE is hyperendemic.

All these consideration allow to reassure staff and visitors of the zoo in respect of this important zoonosis agent but *E. granulosus* s.s. must be taken into account in exotic captive animals, for the possibility to administer to their contaminated food/water from endemic areas.

Author’s contribution: GP, BM and RG collected sample, analyzed the data, conceived and designed the paper, AV performed molecular identification; GB performed histological analysis; MG performed necropsy. All authors interpreted the data and approved the final version

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