Alveolar echinococcosis in the Zoological Garden Basle

P. Rehmann[1,2], A. Gröne[2], B. Gottstein[3], H. Sager[3], N. Müller[3], J. Völlm[4], L.N. Bacciarini[1,2]

[1]Zentrum für Fisch- und Wildtiermedizin, [2]Institut für Tierpathologie und [3]Institut für Parasitologie der Universität Bern, [4]Zoologischer Garten Basel

Abstract

Alveolar echinococcosis (AE) is a rare, but potentially severe zoonotic disease caused by *Echinococcus (E.) multilocularis.* Recent findings indicated an increasing importance of AE for non-human primates living in regions endemic for *E. multilocularis*. The death of five cynomolgus monkeys *(Macaca fascicularis)* and a lowland gorilla *(Gorilla g. gorilla)* due to AE raised concern about the incidence of this parasite in the Basle Zoo. Consequently, a project was initiated to investigate the prevalence amongst an affected group of cynomolgus monkeys, as well as in foxes and mice. Three out of 46 monkeys were seropositive for *E. multilocularis* antigen. In two of these monkeys and in another three animals, which were seronegative, structures compatible with metacestodal cysts were observed using ultrasonography. Seven out of 35 free roaming foxes caught at the zoo were positive for an intestinal *E. multilocularis* copro-antigen ELISA, four of them shed taeniid eggs simultaneously. No lesions compatible with AE were present in 50 necropsied mice from the zoo area. These results indicate that the fox population is a potential source to introduce *E. multilocularis* and may thus represent a risk for the zoo animals.

Keywords: Echinococcus multilocularis, zoo, alveolar echinococcosis, epidemiology

Alveoläre Echinokokkose im Zoo Basel

Die alveoläre Echinokokkose (AE) gilt als seltene, jedoch schwere zoonotische Erkrankung. Sie erlangt zunehmend Bedeutung bei Affen, die in endemischen Gebieten des kleinen Fuchsbandwurms *(Echinococcus multilocularis)* leben. Im Zoo Basel warf der Tod von fünf Javaneraffen *(Macaca fascicularis)* und einem Gorilla *(Gorilla g. gorilla)* durch AE die Frage auf, wie weit dieser Parasit im Zoo verbreitet ist. Daraufhin wurde im Rahmen eines Projekts abgeklärt, wie hoch die Prävalenz in verschiedenen Spezies ist. In die Untersuchungen wurden die Gruppe der Javaneraffen sowie Füchse und Mäuse einbezogen. Drei der 46 untersuchten Affen waren seropositiv für *E. multilocularis*-Antigen. Bei zwei dieser Affen sowie bei drei weiteren Tieren, die seronegativ waren, fanden sich bei der Ultraschalluntersuchung Strukturen, die mit Metacestodenzysten vereinbar waren. Sieben von 35 frei lebenden, im Zoo gefangenen Füchsen, waren positiv im *E. multilocularis* copro-antigen ELISA, gleichzeitig schieden vier dieser Tiere Taeniideneier aus. Bei 50 sezierten Mäusen im Zooareal konnten keine histopathologischen Veränderungen gefunden werden, die einer AE entsprachen. Diese Resultate weisen darauf hin, dass die Fuchspopulation eine potentielle Quelle zur Einschleppung von *E. multilocularis* und folglich ein Risiko für die Zootiere darstellt.

Schlüsselwörter: Fuchsbandwurm, Zoo, alveoläre Echinokokkose, Epidemiologie

Introduction

For several reasons more attention was paid to alveolar echinococcosis (AE) in recent years (WHO/OIE). A drastic increase of the red fox population in Europe, a widening of the geographic range of *E. multilocularis* in Europe and North America (Eckert and Deplazes, 1999), and a high prevalence of *E. multilocularis* in the population of red foxes in certain areas resulted in a putatively high exposure risk for humans (Gottstein et al., 2001). Nevertheless, the incidence of AE in humans of about 10 new cases per year in Switzerland has so far not increased (Gottstein et al., 2001). Conversely, an increasing number of cases of AE in captive monkeys was reported over the past years (Rietschel and Kimmig, 1994; Kondo et al., 1996; Taniyama et al., 1996; Deplazes and Eckert, 2001). It is unclear, whether this is due to a raise in

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awareness or an actual increase in cases. Nevertheless, AE has to be considered as a threatening health problem for zoos in areas endemic for *E. multilocularis*.

AE, caused by the larval stage of *E. multilocularis,* affects primarily the liver in most cases. The natural intermediate hosts are usually small rodents. They acquire the infection by oral ingestion of eggs, which develop into metacestodes. The initially hepatic lesions may spread to other locations, especially in advanced cases (Williams et al., 1987; Kondo et al., 1996). The larvae form a cluster of multiple small vesiculated cysts, in which protoscoleces may develop. The cyst mass grows slowly and infiltratively and causes a chronically progressive host tissue damage. Humans as well as non-human primates and several other species such as feral pigs, dogs, nutria *(Myocastor coypus)* and horses among others may be aberrant hosts (Pfister et al., 1993, Eckert, 1997). An increasing number of cases of AE in domestic pigs has been observed in connection with augmentation of alternative free-range husbandry (Kamiya et al., 1987; Sydler et al., 1998). In human AE the slow proliferation of metacestodes leads to a long incubation period of about 5–15 years. In the past, mortality ranged around 94% within 10 years after primary diagnosis, if cases were left without treatment (Ammann and Eckert, 1995). In more recent times, the mortality rate has considerably decreased to 10 to 14%, most likely because of marked improvements in diagnosis and therapy (Gottstein, 1992).

The source of infection for intermediate and aberrant hosts are *E. multilocularis* eggs shed in the faeces of infected final hosts, including mainly foxes, dogs, and occasionally some other carnivores. These animals are usually asymptomatic carriers of the intestinal form (adult stage) of this parasite. About 30 days after ingestion of an infective intermediate host, the protoscoleces have developed and maturated into adult stages including the, shedding of *E. multilocularis* eggs.

Northern Switzerland is known as a high endemic area. The average prevalence in rural red foxes was shown to be about 30% in 21/26 cantons with a range of percentage from 3–53% (Ewald et al., 1991). Urbanization of fox populations has imported the parasite into cities, thus similar prevalences may be temporarily encountered in urban areas as usually detected in rural areas (Hofer et al., 2000; Tsukada et al., 2000). Such findings may help to explain the importation of the *E. multilocularis* problem into peri-urban recreational places such as zoos. In the Basle Zoo, a potential problem of AE had to be considered after the death of five cynomolgus monkeys *(Macaca fascicularis)* and one lowland gorilla *(Gorilla g. gorilla)*. Therefore, a project was initiated to evaluate the prevalence of *E. multilocularis* in some potentially affected zoo animal groups.

Animals, Materials and Methods

To evaluate the prevalence of AE in the group of cynomolgus monkeys *(Macaca fascicularis)*, 46 out of 55 animals were examined ultrasonographically. Changes were assessed according to criteria described for cystic structures: ovoid structures with well-defined thin walls, hypoechoic or anechoic edge shadows and distal acoustic enhancement (Nyland et al., 2002). Simultaneously, sera were analyzed for specific antibodies against the *E. multilocularis*-specific Em^2-antigen as described, with one modification that included the replacement of the anti-human alkaline-phosphatase conjugate with an anti-monkey IgG conjugate which recognizes cynomolgus monkey IgG (A140–102AP, Bethyl Laboratories, Montgomery, USA) as described (Rehmann et al., 2005). Selected animals were again examined a second time serologically and by imaging procedures 5 and 9 months later.

Potential final hosts including foxes, domestic cats and the carnivore collection of the zoo were examined coprologically for *E. multilocularis*. Thirtyfive foxes were trapped within or at the close border of the zoo from December 1999 to March 2002 and subsequently euthanized. Faecal samples were enriched by the use of a conventional flotation technique (density 1.3) and examined microscopically for the presence of taeniid eggs as described (Eckert, 2000). Additionally, a copro-antigen sandwich-enzyme-linked immunosorbent assay (Checkit Echinotest ELISA test kit, Bommeli Diagnostics, Liebefeld, Switzerland) was done for the detection of intestinal *E. multilocularis* antigens (Deplazes et al., 1992). Faecal samples of 5 roaming domestic cats, caught occasionally in the area of the zoo, were analysed in an identical manner.

Faecal samples from the zoo carnivore collection have been systematically examined since the beginning of the nineteen seventies. Samples were analysed using a microscope after conventional flotation enrichment as described (Eckert, 2000). The following species were included: grey wolf *(Canis lupus)*, maned wolf *(Chrysocyon brachyurus)*, African hunting dog *(Lycaon pictus)*, cheetah *(Acinonyx jubatus),* snow leopard *(Uncia uncia)*, leopard *(Panthera pardus)*, tiger *(Panthera tigris)*, lion *(Panthera leo)*, brown bear *(Ursus arctos)*, polar bear *(Thalarctos maritimus)*, malayan sun bear *(Helarctos malayanus)*, spectacled bear *(Tremarctos ornatus)*, fossa *(Cryptoprocta ferox)*, red meerkat *(Cynictis penicillata)*, dwarf mongoose *(Helogale*

parvula), slender-tailed mierkat *(Suricata suricatta)*, raccoon-dog *(Nyctereutes procyonoides)*, common otter *(Lutra lutra)* and river otter *(Lutra canadensis)*. From September 2000 till June 2001, 50 mice *(Mus musculus)* were trapped in the area of the zoo and post-mortem examinations were performed in standard manner. Tissue samples, especially the whole liver, were fixed in 4% buffered formalin, paraffin-embedded and cut. Sections were stained with hematoxylin and eosin (HE) and periodic acid schiff (PAS) stain.

Results

Three cynomolgus monkeys had antibodies against the *E.multilocularis* Em2-antigen. Two of these monkeys and another three animals had cystic structures within the abdominal organs, mainly the liver, compatible with metacestodal cysts. AE was confirmed at post-mortem examination of one animal, which was positive serologically and ultrasonographically (Fig. 1). In contrast, in another animal, that was seronegative, the ultrasonographically observed cysts were identified as bile duct cysts at post-mortem. The other monkeys with results suspicious of AE are still alive. Since this study had been conducted, one monkey, that had been negative in both tests, died due to AE with rapid development of metacestodal cysts and severe lesions (Bacciarini et al., 2005). This animal was much younger (<5 years) compared to the average age of the other monkeys, which was about 20 years.

Of the examined carnivores, only foxes were found to be positive for *E. multilocularis* (Tab. 1). In 20% of the free ranging foxes (7/35), the copro-antigen-ELISA was positive, and taeniid eggs were found additionally in 4 from these 7 foxes. In the faeces of 5 cats, neither taeniid eggs nor *E. multilocularis* copro-antigens were detected. In none of the carnivores kept at the zoo, the routine coprology revealed the presence of any

Figure 1: Ultrasonographic image of the liver from a cynomolgus monkey demonstrating four hypoechoic structures with well-defined margins interpreted as cysts.

Table 1: Examination of fox faeces by microscopy and copro-antigen-ELISA

		Copro-Ag-ELISA	
		+	-Total
Taeniid-eggs detected	+	4	26
	–	3	2629
Total		7	2835

+ = positive, – = negative

taeniid eggs during the last years. The post mortem examination of 50 mice caught at the area of the Basle Zoo did not show any morphological changes in the liver consistent with AE.

Discussion

Several cases of AE in nonhuman primates had raised concern about a possible increase of the respective infectiological problem in the Basle Zoo. This concern was reinforced by reports about a significant growth of the fox population all over Switzerland and the persistence of a high prevalence of *E. multilocularis* in foxes in certain areas of Central Europe (Ewald et al., 1991; Eckert and Deplazes, 1999; WHO/O.I.E., 2001; Tackmann et al., 2001). As a consequence of these findings an increased contamination of the environment with *E. multilocularis* eggs could be assumed.

In the case of the group of cynomolgus monkeys addressed in this study, the exposure risk by *E. multilocularis* eggs originally seemed to have been only temporary (as a single episode), as AE appeared then not to be a problem in this group anymore. However, the death of a young animal due to AE suggests that an infection source is at least intermittently present. Such a source could either be infected and shedding foxes, as 20% of the trapped foxes were positive for *E. multilocularis*, thus could have shed infectious eggs in the vicinity of the zoo. In future, we will have to address this point more precisely. The availability of specific PCR for *E. multilocularis*-eggs (Mathis et al., 1996) will prompt a more detailed investigation, aim at drawing up a definitive origin of the taeniid eggs detected. Nevertheless, for an anticipated prevalence of approximately 20%, the positive predictive value of the ELISA would be about 75% (WHO/O.I.E., 2001), thus 3 out of the 4 foxes found positive for copro-antigen and taeniid eggs should at least have shed *E. multilocularis* eggs in larger quantities.

In the same way, but retrospectively, foxes may either have directly contributed to the infection sources for the primates within the zoo, or they may have

contaminated areas around the zoo where fresh grass, vegetables or fruits have been collected for the primates. The population control of both foxes and small rodents, as regularly practised within the zoo, may also lead to fluctuations in the numbers of animals and thus to the contamination intensity. In any case, the *E. multilocularis* prevalence found in foxes in this study does not contrast prevalences found in other Swiss cities studied so far (Deplazes and Eckert, 2001; Hofer et al., 2000).

Control strategies to decrease infection risk need to aim at these potential sources. Complete eradication of the final hosts like foxes from the zoo grounds appears impossible, therefore the treatment of such animals may be a suitable measurement to decrease contamination. Such treatment using drug containing baits is conducted in Switzerland and Germany (Schelling et al., 1997; Tackmann et al., 2001). A possible strategy to minimize exposure of zoo animals to *E.multilocularis* is to reduce the probability of food contamination with cestode eggs and to lessen the number of infected foxes by the use of drug containing baits.

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Echinococcose alvéolaire au Zoo de Bâle

L'échinococcose alvéolaire (AE) est une zoonose rare, mais néanmoins sévère. L'AE représente un problème d'importance croissante dans les zones de présence endémique du parasite *Echinococcus (E.) multilocularis*. Après la mort de cinq macaques de Java *(Macaca fascicularis)* et d'un gorille de plaine occidental *(Gorilla g. gorilla)* causée par l'AE au Zoo da Bâle, nous avons élaboré un projet visant à déterminer la prévalence de ce cestode. Dans le cadre de ce projet la colonie des macaques de Java, les renards et les souris ont été examinés.
Trois des 46 macaques étaient positives pour l'antigène de *E. multilocularis.* Deux de ces animaux et trois autres animaux qui étaient négatives à la sérologie on montrés des lésions à l'échographie propres à l'AE. Sept renards sur un total de 35 animaux piégés étaient positives à l'analyse ELISA pour le copro-antigène de *E. multilocularis*, quatre de ces animaux éliminaient des oeufs de ténia avec les selles. Les 50 souris piégées examinées n'ont montré aucune lésion compatible avec une infection due aux échinocoques. Ces résultats montrent que les renards sont une source potentielle d'AE et peuvent représenter un risque pour les animaux de la collection du zoo.

Echinococcosi alveolare allo Zoo di Basilea

L'echinococcosi alveolare (AE) è una zoonosi rara ma severa che assume un'importanza crescente nelle zone con presenza endemica del parassita *Echinococcus (E.) multilocularis*. Dopo la morte di cinque macachi di Giava *(Macaca fascicularis)* e di un gorilla di pianura occidentale *(Gorilla g. gorilla)* causata da AE allo Zoo di Basilea, si è iniziato un progetto tendente ad appurare l'incidenza di questo cestode negli animali della collezione. Nell'ambito di questo studio sono stati esaminati oltre alla colonia di macachi di Giava, le volpi e i topi che circolano liberi nello zoo. Tre dei 46 macachi esaminati sono risultati positivi all'antigene dell' *E. multilocularis*. In due di questi animali e in ulteriori 3 macachi, risultati negativi all'esame sierologico, l'esame ecografico ha rivelato lesioni riconducibili all'AE. Sette delle 35 volpi catturate all'interno dello zoo sono risultate positive all'ELISA per il copro-antigene dell'*E. multilocularis*, quattro di questi animali eliminavano uova di tenia con le feci. L'esame post-mortem di 50 topi catturati nell'area nello zoo non ha rivelato lesioni riconducibili ad una infezione da echinococchi. Questi risultati mostrano come le volpi che si introducono nello zoo rappresentano una fonte potenziale d'infezione e potrebbero rappresentare un rischio per la collezione.

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Corresponding author

Luca N. Bacciarini, Alla Motta, CH-6516 Cugnasco. Tel: +41 91 814 41 02, Fax: +41 91 840 99 46
E-Mail: exopath@bluemail.ch

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