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Reduced egg production of *Echinococcus multilocularis* in experimentally infected and re-infected red foxes (*Vulpes vulpes*)

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Abstract

Ingestion of eggs of the small fox tapeworm, *Echinococcus multilocularis*, causes the severe human disease alveolar echinococcosis. Previously, the dynamics of the egg excretion from infected carnivores have been studied only where the host animals have been exposed to a single experimental infection. In nature, foxes are most likely repeatedly infected. To study the effect of repeated exposure, twenty-one foxes were inoculated with a high dose of *E. multilocularis* protoscoleces three times over a 1-month period. For comparative purposes, three groups of twenty-one foxes were respectively inoculated with low, medium, or high single dose of protoscoleces. For each group, worm number and morphology were analyzed after necropsy of seven foxes at 1, 2, and 4 months after last inoculation. The establishment of intestinal worms was very low in all foxes, and surprisingly, most of the worms did not produce eggs. Although most reproductive structures were detectable, the genital pore and the cirrus pouch often had abnormal enlargements that spread internally, most likely preventing the reproductive function. The reason for this abnormality could not be determined, but the preparation and storage conditions of the inoculated protoscoleces may have contributed to the stunted development. Physical stress of *E. multilocularis* at the larval stage in rodents may later adversely affect the reproductive success of the adult tapeworm in the carnivore definitive host; as in the present study where a worm establishment in the definitive host was only followed by a neglectable egg production.

Keywords: *Echinococcus multilocularis*; Tapeworm; Egg production; Repeated infection; Abnormal morphology; Reduced reproduction; *Vulpes vulpes*

1. Introduction

The life cycle of *Echinococcus multilocularis* in Europe predominantly involves carnivores as definitive hosts and several rodent species as intermediate hosts. Foxes are thought to be the main source of environmental contamination with eggs in most of the endemic areas (Eckert and Deplazes, 2004), even though other wild carnivores are potential definitive hosts (Kapel et al., 2006).

The morphological development of *E. multilocularis* and its related species *E. granulosus* has been studied previously both *in vitro* and in *vivo*. Due to safety reasons concerning the housing of animals shedding infective eggs, *in vitro* techniques were developed to overcome this problem (Smyth and Howkins, 1966;

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Thompson and Eckert, 1982). Most knowledge about the sequential development of *Echinococcus* spp. in definitive and intermediate hosts has been provided by *in vitro* studies. For example, the classical work of Smyth and his colleagues on the development of *Echinococcus in vitro* has shown that germination, growth, and segmentation take place independently (Smyth and Davis, 1974; Smyth, 1979; Thompson, 1995).

However, the major limitation of *in vitro* cultivation of adult *E. multilocularis* was the failure of fertilization and production of infective eggs (Smyth and Davis, 1974; Smyth, 1979). On the other hand, *in vivo* experimental infections helped in understanding egg production dynamics, biotic potential, and worm survival. In an experimental infection, Nonaka et al. (1996) recovered 11 and 91 *E. multilocularis* worms at 125 days post inoculation (dpi) from two foxes inoculated with 150,000 protoscoleces. In a comparative study by Kapel et al. (2006) a major difference was found in the worm establishment in different carnivore species. In foxes, 84% of 20,000 inoculated protoscoleces established as worms 35 dpi, but thereafter worm burdens decreased sharply to 2% at 60 dpi and 1% at 90 dpi. In dogs, the initial establishment of worms was lower (12%) but the worms appeared to have higher persistency (5%, 60 dpi; 8%, 90 dpi). By mathematical modeling, a mean biotic potential of 346,473 eggs of *E. multilocularis*/fox, 335,361 in raccoon dogs, and 279,910 in dogs was calculated (Kapel et al., 2006). Thus, although an overall egg excretion may be comparable for different hosts, the dynamics of the excretion may vary greatly in between species.

The previous experimental studies on the development of adult worms dealt with fox infections as a result of exposure to a single dose of metacestodes (Nonaka et al., 1996; Thompson et al., 2006). Despite the importance of these studies, information on the effect of worm burden on the development and survival of the parasite is still lacking. Also, it is reasonable to predict that some infected foxes in endemic areas may prey repeatedly on *E. multilocularis* infected rodents and consequently become super-infected with different parasite populations. However data on the possible effects of repeated infections on worm development and egg excretion are lacking.

It is generally accepted that host factors play a major role in the establishment and survival of the parasite (Smyth and Davis, 1974; Thompson, 1995). In naturally infected foxes, infections are both highly over-dispersed and age dependent (Hofer et al., 2000; Raoul et al., 2001; Yimam et al., 2002). Previous studies of other taeniids on definitive hosts suggested that crowding and reinfection affect parasite survival (Richard et al., 1977; Williams and Shearer, 1981), but to date this has not been studied for *E. multilocularis*.

Long-term experimental infections with repeated infections are essential to observe dynamics of egg production that might simulate the status in endemic areas. Together with findings from natural infections, such data are critical for better understanding of the epidemiology of this parasite. Thus, the aim of the present study was to describe egg production and developmental morphology of *E. multilocularis* in foxes at different infection levels.

2. Materials and methods

In total, 91 pubs of red foxes (*Vulpes vulpes*) were obtained from a Danish fur farm (Møldrup Minkfarm) where all foxes are kept in confinement without any contact to surrounding habitat and where all food is heat-treated. The fox pups, 10–12 weeks of age of both sexes were housed at the facilities of the National Veterinary Institute (DK) and experimentally infected with protoscoleces of *E. multilocularis*. The vixens of the foxes were treated with fenbendazole 5 weeks before birth according to Kapel et al. (2006). The parasite material was originally isolated from naturally infected water vole (*Arvicola terrestris*) from Switzerland and subsequently propagated in rodents (*Meriones unguiculatus* and *Microtus arvalis*) at the Institute of Parasitology in Zurich, Switzerland. Within 24 h isolation of metacestodes from the rodents, the material was transported in physiological saline on ice to the Danish Centre for Experimental Parasitology in Copenhagen, Denmark. Metacestode tissue was pooled, minced using scissors, homogenized by passing through a 1 mm sieve and the infective doses were prepared by dilution technique. The metacestode suspension containing protoscoleces was kept in Hank's solution overnight at 4 °C prior to inoculation which was undertaken by stomach intubation under general anaesthesia as described by Kapel et al. (2006).

Three groups of 21 foxes, namely: group 1, 2, and 3, were inoculated with a single dose of 2000, 10,000 and 20,000 protoscoleces, respectively (at this point referred to as day 0 post last infection (dpi)) (Table 1). A further group (group 4) of 21 foxes was prior inoculated twice with 20,000 protoscoleces 27 and 14 days before day 0 post infection. As controls for these two repeated infections, seven animals were examined for intestinal worms 0 dpi. The remaining 21 animals (group 4) were re-infected with 20,000 protoscoleces each simulta-