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Echinococcus granulosus pig strain (G7 genotype) protoscoleces did not develop secondary hydatid cysts in mice

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ABSTRACT

Echinococcus granulosus, the aetiological agent of cystic hydatid disease, exists as a series of strains or genotypes which differ in biological features. Pig strain (G7 genotype) has been shown to differ from sheep strain (G1 genotype) in phenotypical characters such as intermediate host range, geographical distribution and rate of development of the adult worm. Since *in vivo* studies of different parasite genotypes can provide insights into host–parasite relationship we analysed for the first time the behaviour of *E. granulosus* G7 genotype protoscoleces in the murine experimental model. Our results show that G7 protoscoleces were unable to establish a regular infection in mice in contrast to G1 protoscoleces which developed intraperitoneal hydatid cysts. This inability was observed in co-infection experiments, *i.e.* even in the presence of a controlled immune response that allows G1 genotype protoscoleces establishment. In addition, the implantation of *in vitro* obtained *E. granulosus* G7 genotype microcysts resulted in a low percentage of hydatid cysts establishment. These results show a difference in the biological ability of both *E. granulosus* strains to develop secondary hydatid cysts in mice. We suggest that the comparison of infective and non infective genotypes of *E. granulosus* in the experimental host can be regarded as a new model to study the mechanisms of infection of *Echinococcus* spp. This knowledge could provide helpful information for the development of therapies, drugs and/or vaccines against cystic hydatid disease.

1. Introduction

Echinococcus granulosus is a cestode parasite whose larval stage is responsible for cystic hydatid disease, a worldwide extended zoonosis of public health concern. Usually, dogs and other canids act as definitive hosts for this intestinal tapeworm, while domestic and wild ungulates, lagomorphs, marsupials and occasionally humans are involved as intermediate hosts for the metacestode larval stage. The metacestode or hydatid cyst is a fluid-filled, unilocular cyst that is covered by an acellular laminated layer and is internally lined with a cellular germinal layer from which protoscoleces are asexually produced. When ingested by the definitive host, protoscoleces develop into adult tapeworms in the intestine, but they are also able to develop into secondary hydatid cysts if rupture and content leakage from a primary cyst occur within the intermediate host.

E. granulosus exists as a complex of genetic variants or strains which differ in phenotypical characteristics such as intermediate host specificity, rate of development

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(McManus and Thompson, 2003), chemical composition and carbohydrate metabolism (McManus, 2009). Furthermore, after phylogenetic, transmission and epidemiological analyses of the genus *Echinococcus*, some of the strains were designated as species (reviewed in Thompson, 2008). According to nuclear and mitochondrial molecular markers each strain has been assigned a genotype (reviewed in Thompson, 2008 and references therein). Each strain or genotype can successfully establish and develop fertile cysts (with protoscoleces) in a specific intermediate host, although some of them can also infect additional hosts but producing mostly infertile cysts. For example, G1 genotype (sheep strain) can develop fertile cysts in sheep, but it can also infect cattle, pigs, goats, camelids, macropods and humans. On the other hand, G7 genotype (pig strain) has been mostly isolated from pigs and wild boars and less frequently from humans (Thompson, 2008; Schneider et al., 2010) and cattle (Bruzinskaite et al., 2009). Also, G1 and G7 genotypes display different geographic distribution and prevalence, being G1 the most prevalent and worldwide distributed (Thompson, 2008). Additionally, they have been shown to differ in the rate of development in the definitive host (Eckert et al., 1993). However, very little is known about the factors that determine host specificity or developmental differences among strains.

The current experimental model of *E. granulosus* secondary infection, introduced by Dévé (1934), is based on the development of hydatid cysts in mice peritoneal cavity, after the inoculation of viable protoscoleces. BALB/c is the most widely used strain for this model (Siles-Lucas and Hemphill, 2002; Dematteis et al., 2003; Baz et al., 2006). *E. granulosus* infection in BALB/c mice is well characterised and can be divided into two stages: an early stage (3–6 weeks post inoculation) during which the infection establishes, *i.e.* the protoscoleces differentiate into cysts and develop the laminated layer (Schwabe et al., 1959; Rogan and Richards, 1989), and a late stage during which the cysts grow in size and eventually develop new protoscoleces. The formation of the outer laminated layer is of main importance in the establishment of the infection, since it not only provides mechanical support for metacestode turgidity allowing its growth, but also protects the germinal layer from immunological attack (Díaz et al., 2011). The importance of the laminated layer for parasite establishment and development in the murine model has been experimentally shown for *Echinococcus multilocularis* (Gottstein et al., 2002) and *E. granulosus* (Casado et al., 1992; Zhang et al., 2005).

While trying to establish *E. granulosus* infections in BALB/c mice, we serendipitously found that G7 genotype protoscoleces were unable to develop into secondary hydatid cysts. This prompted us to perform additional experiments to compare the behaviour of G1 and G7 genotype protoscoleces in the experimental infection model. This comparison could be useful to provide a model for the systematical study of host specificity and development of *E. granulosus*.

2. Materials and methods

2.1. Parasites

Protoscoleces were collected by aseptic puncture of porcine and bovine hydatid cysts provided by local abattoirs from Buenos Aires province (Argentina) and Montevideo (Uruguay), respectively. Parasites were washed several times with sterile phosphate buffered saline (PBS) (Dulbecco's Phosphate-Buffered Saline w/o calcium chloride, w/o magnesium chloride, GIBCO) pH 7.2, supplemented with gentamicin (50 μg/ml) to remove cyst wall debris. Parasite viability was determined by the eosin exclusion test and flame cell activity. Samples showing more than 95% viability were stored at 4 °C for one to seven days until use. Prior inoculation, protoscoleces viability was again determined as previously described. Only samples with ≥90% viability were used.

2.2. Analysis of *E. granulosus* genotype

E. granulosus genotype was determined by sequencing a fragment of the gene coding for mitochondrial cytochrome c oxidase subunit 1 (CO1), as previously described (Cucher et al., 2011). To confirm genotyping results in the mixed infection experiment (see item 2.6), sequencing of a fragment of the mitochondrial gene ATP6 gene was performed as already reported (Yang et al., 2005).

2.3. Mice

Adult female BALB/c and C57BL/6 mice were obtained from DI.LA.VE. (Uruguay) or from "Instituto de Medicina Experimental" (IMEX) (Academia Nacional de Medicina, Argentina), and were housed at the animal facility of Instituto de Higiene (Montevideo, Uruguay) or Facultad de Medicina (Universidad de Buenos Aires, Argentina). Adult female CD1 mice were obtained from DI.LA.VE. (Uruguay) and housed at the animal facility of Instituto de Higiene (UdelaR, Uruguay).

Animal experiments were performed in compliance with Comisión Honoraria de Experimentación Animal (CHEA)-UdelaR according to National Uruguayan Legislation No. 18.611 (2009), Protocol Number: 030510, which was approved by the ethical committee of Facultad de Química-UdelaR (Uruguay), and according to the NIH Guide for the Care and Use of Laboratory Animals and approved by the ethical committee of IMEX (Argentina).

2.4. Standard experimental infections

BALB/c, C57BL/6 and CD1 mice were inoculated i.p. with a suspension of 2,000 G1 or G7 viable protoscoleces in 200 μl of sterile PBS with gentamicin (30 μg/ml). Each mouse strain was inoculated with either genotype in independent experiments. For each genotype, two different batches of protoscoleces from cysts belonging to different animals were used. The scheme of standard experimental infections is shown in Table 1. Mice were euthanized 5 or