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Short Communication

Echinococcus canadensis (G7) and *Echinococcus granulosus sensu stricto* (G1) in swine of southern Brazil

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ABSTRACT

The cystic echinococcosis (CE) is an important zoonotic disease caused by the parasite *Echinococcus* spp. In Brazil, this parasite is present in Rio Grande do Sul (RS) state, border with Argentina and Uruguay, causing several damages to human and animal health. This study aimed to identify *Echinococcus* spp. in hydatid cysts of swine and evaluate the similarity of the genotypes through the phylogenetic analysis. A total of 3,101,992 swine were slaughtered in the central/northern region of RS/Brazil, during 2008–2012. Five isolates were characterized as hydatid cyst by molecular analysis, based on the mitochondrial gene cytochrome c oxidase subunit I (cox-I). The genotypes *E. granulosus sensu stricto* (G1) ($n = 2$) and *E. canadensis* (G7) ($n = 3$) were identified in the hydatid cysts. The swine represents a potential intermediate host for different genotypes of *Echinococcus* spp., besides it can contribute to the perpetuation of the parasite's life cycle in rural areas.

1. Introduction

The cystic echinococcosis (CE) is a disease caused by parasites belonging to the genus *Echinococcus*, which causes many health problems in different regions of the world, and it is currently considered as a neglected parasitic disease in many countries. In Brazil, the highest prevalence occurs in the of Rio Grande do Sul state (RS), mainly in regions bordering with Argentina and Uruguay, countries that are considered potentially endemic for this parasite. Rural areas are the most affected, since livestock production is one of the main economic activities in RS (de la Rue et al., 2006; Moro and Schantz, 2009).

The genotypes reported for *E. granulosus sensu lato* in southern Brazil include: *E. granulosus sensu stricto* (G3 and G1); *E. ortleppi* (G5), and *E. canadensis* (G7), usually affecting sheep, cattle, dogs and humans (Badaraco et al., 2008; de la Rue et al., 2006, 2011). G1 genotype has been described in several countries as the main cause of CE in humans, and it is widespread in Peru, Chile, Argentina and Brazil (de la Rue et al., 2011; Grosso et al., 2012).

Genotypes G1 and G7 are morphological and genetic different in some aspects, as well as in their cycle in the host (Thompson and McManus, 2002). However, both are able to develop the parasitism in humans and pigs, causing damage to the public health, as well as to the meat industry. Based on the presented aspects, this study aimed to characterize molecularly the isolates of *Echinococcus* spp. obtained from viscera of swine containing cysts, as well as

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to identify genetic variants present in a potentially endemic area in southern Brazil.

2. Materials and methods

2.1. Biological samples

Biological samples consisted of cysts obtained from viscera of swine slaughtered in the central/northern region of RS, from January 2008 to December 2011. The cysts were stored in 70% ethanol and subsequently characterized by molecular analysis.

2.2. Molecular analysis

the total DNA was extracted from a fragment of hydatid cyst using a commercial kit (QIAamp tissue) (QIAGEN Inc. Chatsworth, CA) according to manufacturer's instructions. PCR was performed using a pair of primers to amplify a fragment of *cox-I* gene (Bowles et al., 1992), with modifications. The reactions were carried out in a thermocycler model PTC 100 (MJ Research, Inc.). To detect the pattern of bands, the electrophoresis in 1.2% agarose gel was performed, stained with ethidium bromide and visualized under UV light.

2.3. Phylogenetic analysis

The similarity of DNA sequencing samples of gene cox-I was carried out with using BLAST program (*Basic Local Alignment Search Tool* – http://www.ncbi.nlm.nih.gov). The phylogenetic analysis was performed using the Gap4 software/Staden package (Staden, 1996). The sequences used for comparison with studied samples were obtained from GenBank (http://www.ncbi.nlm.-nih.gov/GenBank) and their access numbers were: M84661 (G1) (Bowles et al., 1992); GU980906 (G1) (Soriano et al., 2010); GU233854 (G1) (Sánchez et al., 2010); EU048826 (G1) (Maillard et al., 2009); JF828337 (G1) (Sánchez et al., 2012); EU048821 (G7) (Maillard et al., 2009); JF828340 (G7) (Sanchez et al., 2012); M84667 (G7) (Bowles et al., 1992); G7: GU980914 (G7) (Soriano et al., 2010) and for M84665 (G5) (Bowles et al., 1992). The AB243755 (*Taenia solium* – Sato et al., 2005) was used as outgroup. The positive samples obtained in this study were deposited in Genbank, with the following access numbers: KC878433 (G7), KC878432 (G7), KC878431 (G7), KC853748 (G1), and KC660075 (G1). A phylogenetic tree was constructed through neighbor-joining analysis (NJ), where the data sets alignments were performed using the Clustal W algorithm in the software MEGA 5 (Tamura and Nei, 1993). The bootstrap value was based on 1000 replicates.

3. Results

A total of 3,101,992 swine were slaughtered during the period in which this study was executed and 5 samples from five different animals were positive for hydatid cyst.

The fertility of the cysts were not entirely evaluated due to storage of the sample, which allows the degradation of protoscoleces; however, it was observed in the analyzed liquid the presence of hooks and debris of hydatid sand.

PCR analysis of the five samples obtained in this study amplified a fragment of approximately 366pb, corresponding to the mitochondrial gene *cox-I*. DNA sequences generated were compared with sequences in Genbank obtaining two samples with similarity to *E. granulosus sensu stricto* (G1) and three with *E. canadensis* (G7).

The phylogenetic tree revealed two distinct groups (Fig. 1), where samples with genotype G7 were grouped to compose the E1 group. E2 group was composed by the grouping sequences of G1 genotype. The samples were compared with reported isolates from Argentina by Soriano et al. (2010), where the sample G1 came from sheep and the G7 from swine. Interesting, the Brazilian swine and Soriano et al. (2010) samples when compared with Bowles et al. (1992) sequences were very similar (Fig. 1).

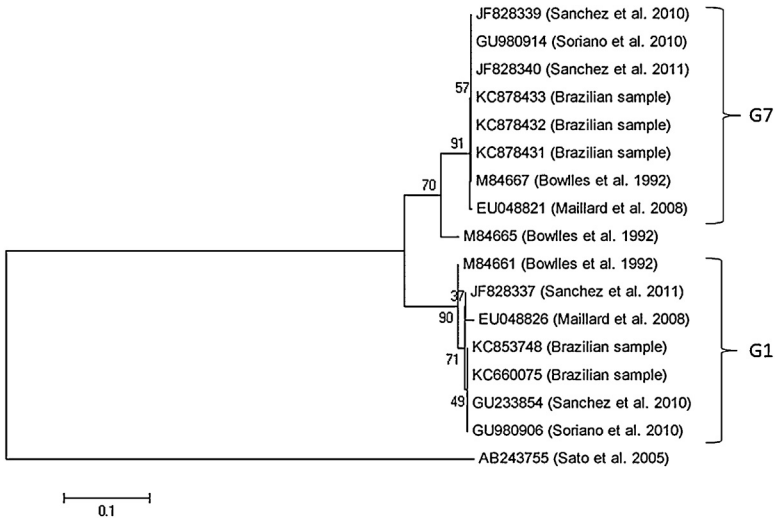

Fig. 1. Neighbor joining (NJ) tree based on cytochrome c oxidase I gene (cox-I), for *Echinococcus* spp. isolates/genotypes. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown. Phylogenetic analyses were conducted in MEGA5.