HYDATIDOSIS IN PORTUGAL – A MITOCHONDRIAL PHYLOGENY APPROACH

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INTRODUCTION AND OBJECTIVES

Echinococcus granulosus is the aetiological agent of hydatidosis, which is an important cause of morbility and mortality (Breyer *et al*., 2004). This disease is one of the most important helminthic zoonoses, and up to the present date a significant public health problem worldwide (Thompson, 2008). A number of well characterized strains are now recognized, all of them seemingly adapted to particular life cycle patterns and host assemblages (McManus & Thompson, 2003). The taxonomy and phylogeny of the genus *Echinococcus* has remained a controversial issue for several years (Thompson, 2008). Nevertheless, the use of molecular tools have proved particularly useful for the characterization of *Echinococcus*, based on which a new classification has been proposed, one that is now widely accepted (Thompson, 2008). The aim of this work is to determine the range of genetic variability within and between Portuguese *E. granulosus* isolates.

MATERIALS AND METHODS

The Portuguese isolates (n=26) from liver and lungs of sheep and goat with *E. granulosus* cysts were collected in slaughterhouses. DNA was extracted from all of them, followed by the partial amplification of the mitochondrial COI gene with the JB3 and JB4.5 primers (Bowles & McManus, 1993). These amplification products were sequenced, and the obtained nucleotide sequences compared with those present in GenBank. In addition, phylogenetic analysis of *E. granulosus* isolates was performed using neighbour-joining (genetic distances corrected with Kimura 2-parameter correction) or Bayesian analyses.

RESULTS

Our analyses revealed the presence of G1 (Common sheep) and G3 (Indian Buffalo) genotype of *E. granulosus* in the Portuguese isolates. It also showed some that our isolates are all included, with some samples from other parts of the world, in the G1-G3 cluster of *E. granulosus*.

Fig. 1 – Phylogenetic analysis, based on COI gene of the genotypes of *E. granulosus* in Portuguese isolates made by Bayesian analysis. Posterior probabilities >0.8 are indicated.

Genotype / Place	G1	G2	G3
Ponte Sor	18	-	2
Castelo Branco	3	-	-
Idanha-a-Nova	2	-	-
Elvas	1	-	-
TOTAL	24	-	2

Table 1 – Presence of two different genotypes of *E. granulosus* in Portuguese isolates

DISCUSSION AND CONCLUSIONS

This preliminary study showed that at least two different genotypes of *E. granulosus* , the G1 (Common sheep strain) and the G3 (buffalo strain) genotype are circulating in Portugal, as showed in table 1.

It was found some degree of diversity within single isolates, from two different intermediate hosts (Sheep and goat) (Thompson, 2008) and a significant degree of variability between the cluster G1-G3 (Thompson, 2008 & Saarma, 2009), where Portuguese isolates were, and the other *Echinococcus sp.* (Fig. 1). Despite the diversity found among Portuguese isolates, they were all localized within one single robust cluster. The phylogenetic analysis made by two different methods (Kimura 2-parameter correction and Bayesian analysis) gave similar results.

This study also showed the first evidence of the presence of G3 genotype in Portugal.

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