Molecular genotyping of *Echinococcus granulosus* hydatid cysts in Italy by DNA sequencing of the 12S mitochondrial gene confirms the genetic differentiation of the G1 and G3 genotypes

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Cystic hydatid disease (CHD) is a zoonotic parasitic disease caused by the cestode *Echinococcus granulosus* and it represents a major public health problem in many countries around the world, including Italy. A phenomenon demanding close attention in the biology of hydatid disease in endemic areas is the extensive genetic variation that is characteristic of *E. granulosus*. Numerous studies have provided evidence that *E. granulosus* exists as a complex of different strains, that differ in a wide variety of criteria that impact on the epidemiology, pathology, and control of CHD and, to date, ten distinct genotypes (G1-G10) have been identified. In Italy, sequence analysis of the mitochondrial CO1 and ND1 genes showed the occurrence of the G1 genotype, the common sheep strain, the G3 genotype, the buffalo strain, one isolate identified as G2 genotype, the Tasmanian sheep strain (Busi M *et al*, 2004, Parassitologia, 46: 164), and, in Sardinia, the G7 genotype, the pig strain (Varcasia A *et al*, 2006, Parasitol Res, 98: 273-277).

In the present work, we have improved the analysis on *E. granulosus* strains in Italy, by genotyping a larger sample of isolates collected in sheep, cattle, boar and human from Latium, Sardinia and Piedmont, and by checking out the genetic differentiation between the G1 and G3 genotypes using an additional mitochondrial gene as marker, the 12S rRNA gene.

Sequencing of the 12S gene revealed a significant genetic differentiation between isolates identified as belonging to the G1 and G3 genotypes, with fixed nucleotide substitutions at the position 166 (T in G1, G in G3) and at the position 205 (A in G1, G in G3), while the CO1 gene showed fixed nucleotide substitutions at the position 50 (C in G1, T in G3) and the position 241 (T in G1, C in G3), (corresponding to the diagnostic nucleotide substitutions described by Bowles J *et al*, 1992, Mol Biochem Parasitol, 54: 165-173). Interestingly an individual host (cattle) from Lazio was found to harbour cysts belonging to the two distinct G1 and G3 genotypes, indicating that the two forms co-live in the same environment.

This study provides further evidence of the genetic differentiation between the G1 and the G3 genotypes and of the significantly wide occurrence of the *E. granulosus* G3 buffalo strain in Italy, a strain previously confined to the Indian region. This strain has also been recently detected in water buffaloes from southern Italy (Capuano F *et al*, 2006, Vet Parasitol, 137: 262-268).

The occurrence of intra-strain variation and the low reliability of ND1 gene sequencing for the discrimination between the G2 and the G3 genotypes, that may jeopardize the correct identification of hydatid cysts, advocates the need of using a multiple gene approach in the strain determination of this species.