

Biomolecular analysis of *Echinococcus granulosus* cysts from Sardinian human patient

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Key words: Cystic Echinococcosis, *Echinococcus granulosus*, Biomolecular analysis.

The purpose of our study was to characterize the larval form of *Echinococcus granulosus* by biomolecular analysis.

Immunofluorescence investigations were performed on 34 patients suspected to be affected by Cystic Echinococcosis (CE) from Sassari and Nuoro Hospital Wards. All sera were screened by “ELISA-Echinococcus IgG” (DRG-Germany) and “Echinococcus-Western blot (WB) IgG” (LDBIO-France).

Hydatids were excised from 7 patients (HU1 -> HU7). Cysts were inspected, then parasite material was observed by microscope, finally, analysed by biomolecular tests. PCR *E.g.s.s.* [primers for Calreticulin (Cal) gene-fragment of 1001bp (F5':CAATTTACGGTAAAGCAT-3' R5':CCTCATCTCCACTCTCT-3')], was performed on DNA extracted using DNeasy Blood and Tissue Kit (Qiagen, Germany). Afterwards, amplicons generated by PCR [primers for Cytochrome Oxidase 1 (COX1) fragment of 800bp (F5'-TTTTTTGGCCATCCTGAGGTTTAT-3' R5'-TAACGACATAACATAATGAAAATG-3')], were sequenced for genotype and haplotype determination. A *Maximum Likelihood Phylogenetic Tree* was built on a dataset comprising 4 human DNA isolates (HU1 -> HU4) and other reference sequences of *E. granulosus* s.l. retrieved from GenBank. Moreover, a *Haplotype Network* was calculated on the 4 human and the 83 DNA sequences of *E. granulosus* isolated from different animal species in Sardinia.

Ultrasound techniques revealed one or more cysts attributable to CE on 23 patients, of whose 21 were localized in the liver and 2 in the lungs. According to WHO classification 2 (HU1, HU2) were active, 2 (HU3, HU4) transitional, and 3 (HU5 -> HU7) inactive. ELISA-DRG and WB-LDBIO evidenced 14 CE positive sera. The microscopy evidenced non-viable protoscolices in 4 cysts (HU1 -> HU4): 2 active and 2 transitional, of the 7 analysed. The remaining 3 presented necrotic materials.

Biomolecular analysis identified *E. granulosus* s.s., G1 genotype for 2 cysts (HU2, HU4) and G3 genotype for the others 2 (HU1, HU3). *Haplotype Network* displayed evidence that 2 human DNA sequences were common to other animal species, while 2 were found only in human.