

## First molecular isolation of *Echinococcus granulosus* horse strain (G4) in Sardinia (Italy)

Scala A., Varcasia A., Pipia A.P., Pilo C., Garippa G.

Dipartimento di Biologia Animale, Sezione di Parassitologia e Malattie Parassitarie, Università degli Studi di Sassari, Italy.

*Echinococcus granulosus* (*Eg*) is one of the smallest tapeworms that in its larval stage (hydatid cyst) develops in several species of wild and domestic mammals and in humans, causing hydatidosis, a zoonosis of great veterinary and medical importance. This organism shows a great intraspecific variation in relation to host specificity, epidemiology, morphology, developmental biology, biochemistry, physiology and genetics. This variability led to the identification of subspecific variants or strains, and some authors have proposed a revision of the taxonomy of the genus *Echinococcus*. Actually, the subspecific variants are identified as 10 different genotypes of the parasite (G1-G10) (Thompson RCA, McManus DP, 2002, Trends Parasitol, 18: 452-457).

Several papers have been published in Sardinia on epidemiology and genetic characterization of *Eg*, although data on hydatidosis in horse are scarce and dated (Tanda S, 1960, Parassitologia, 2: 315-320). In this work we investigated the situation of Cystic Echinococcosis in horses of Sardinia through a retrospective study compared with new cases recently examined in our laboratory.

According to official data provided by the Government of the Regione Autonoma della Sardegna, 8383 horses during the years 2003 and 2004 were slaughtered. In thirty seven animals were recognized cystic forms in the liver identified as *Eg* hydatids, with a total prevalence of 0.4%.

At the same time, liver and lungs of 81 Equids (60 horses and 21 donkeys) were collected from various abattoirs of Sardinia Isle, between February 2002 and February 2006. The above-mentioned organs were examined by visual inspection and palpation. When cysts were found, they were examined in the laboratory to determine their number, location, type and viability. In fertile cysts DNA was extracted from laminar layers and protoscoleces using a commercial kit (Roche DNA template extraction kit). Sequencing reactions were then performed in order to strain typing PCR products as described by Bowles J and McManus DP (1993, Acta Trop, 53: 291-305) for NADH and COI mitochondrial genes.

Cysts were found in three horses out of the 81 examined (3.7%). In two subjects sterile cysts were recovered only in the liver. In the third horse, three fertile cysts were recovered (2 in lungs and 1 in liver), with viable protoscoleces and white solid lamellar layer. The results of strain typing showed 100% of identity with the G4 strain (horse strain) of *Eg* of the hydatid material coming from the fertile cysts. This allow us to describe for the first time with molecular tools the presence of the *Eg* horse strain in Italy, 39 years after its first (and probably unique) morpho-biological description in Sicily by Macchioni G and Gallo C (1967, Ann Fac Med Vet Pisa, 20: 58-77). Horse in which fertile cysts were found was a male of Argentina breed of 17 years old, and was from Piedmont region where lived more than 15 years. This animal was imported in Sardinia and slaughtered after 18 months, so it could be possible that the horse became infected outside the island. On the other hand, our data on prevalence, morphology (acephalocysts, sterile) and localization (liver) of the hydatids cysts, lead us to hypothesize a G1 strain infection in Sardinia horses. This *Eg* strain, in fact does not recognize horse as good intermediate host. Anyway, the isolation of the G4 in Sardinia seems to be of an epidemiological significance, being a further marker of the Cestode infection in Sardinia, after the isolation of G1 and G7 strains (Varcasia A *et al*, 2006, Parasitol Res, 98: 273-277) and existing the possibility of its spread in the isle, considering the high number of Equids breed in Sardinia.