

An epidemiological and biomolecular survey of cystic echinococcosis in cattle in Sardinia

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Although Cystic Echinococcosis (CE) is still a health, economic and social problem of great importance in Sardinia today, not all aspects of it have been studied, and in particular its epidemiology in cattle. This note updates the epidemiological data on Bovine CE in Sardinia, and with the help of advances made in biomolecular taxonomy identifies the strains of *Echinococcus granulosus* in cattle.

Between January and June 2004, 392 cattle of various breeds were examined. These were mainly raised extensively and were slaughtered in Sardinia. Their ages were determined from slaughterhouse data. The number, locality and types of hydatids found were then classified as fertile, acephalocysts, caseous and calcified.

Samples were taken of the proligerous membranes and the cystic liquid (0.1 g) and the DNA was extracted with a commercial kit (Roche, DNA Template extraction kit).

A fragment of the mitochondrial gene codified for NADH dehydrogenase (ND1) was then amplified with PCR using the primers suggested by J. Bowles and D.P. McManus (1993, Int J Parasitol 23(7): 969-972).

The amplicons obtained were then sequenced with capillary sequencers (Applied Bio-systems). The sequences were then compared with those in the web data banks to determine the level of agreement of each sequenced sample, using BLAST (NCBI) and Bioedit software.

CE was found in 19.6% of the samples, although only 0.76% of the cattle had fertile hydatids. CE was found in 17% of cases in the liver and in 12.5% of cases in the lungs ($\chi^2=3.28$; $P=0.070$). A greater percentage of viable hydatids were found in the lungs (2.5% against 0.25% in the liver - $\chi^2=10.09$; $P=0.0014$).

With respect to the typology of the cysts, caseous/purulent hydatids were found more frequently in the liver (50%) and then calcified cysts (45.4%), while acephalocysts were the most frequent type in the lungs (40.8%).

Abundance (number of hydatids/animals sampled) was 2.2, while average intensity (number of hydatids/positive animals) was 11.2.

9.9% of the animals examined were massively infested (>10 hydatids). In these cases both the liver and lungs contained hydatid cysts.

The animals were divided into 5 age groups. This allowed us to highlight the following prevalence and the respective Odds Ratio values. These are shown in Table 1.

Table 1. Prevalence and Odds Ratio for the cattle divided by age.

Age	No. of examined animals	No. of positive animals	Prevalence	Odds Ratio
≤2 years	229	6	2.6%	1.00
>2-≤ 4 years	109	35	32.1%	17.58
>4-≤ 6 years	8	3	37.5%	22.30
>6-≤ 8 years	20	12	60%	55.75
>8 years	26	21	80.8%	156.10

The differences in prevalence by age were statistically significant (χ^2 trend=131.03; $P<0.0001$).

Sequence analysis determined that the strain of *E. granulosus* in the processed isolates was exclusively G1 (sheep strain).

The prevalence rate was 19.6%. This confirms that there is still strong parasitic pressure from the G1 strain CE in Sardinia. This is not the specific strain for cattle (G5 or bovine strain), which could possibly explain the low fertility values found (0,25%).

The sheep strain (G1) is the genetic variant which has been found most often in Sardinia in the most recent works on genotyping the parasite and thus can be considered endemic on the island (Idini G *et al.*, 2000, Atti SIPAOC 14: 33-36; Varcasia A *et al.*, 2004, Parassitologia 46 Suppl 1: 193), as it is also in Spain, Tunisia and Eastern Europe (Gonzales LM *et al.*, 2002, Exp Parasitol 102(1): 46-56; Thompson RCA, McManus DP, 2002, Trends Parasitol 18(10): 452-457).

It should be reiterated that the G1 strain of *E. granulosus* in cattle behaves in exactly the same way as it does in sheep. In fact in Sardinia it was found in cattle more frequently in the liver and with higher fertility in the pulmonary cysts (Scala A *et al.*, 2001, XX International Congress of Hydatidology 34: 303).

The prevalence and level of fertility in cattle confirms the results of a similar survey conducted in

Sardinia 20 years ago by Nieddu *et al.* (1980, Atti Tavola Rotonda Echinococcosi/Idatidosi, Alghero 22/5/1980, 33-35). However this previous survey was conducted in a pre-biotech period and the low fertility level (0.99%) was attributed to the parasite, not being or being less suitable to develop in a host which was found less widespread in the island. It may be that in this intermediate host there is a strong immunitary response to a strain of CE (G1) not species-specific for cattle, that inhibits the hydatids which are generally found in the liver from developing a caseous-purulent reaction and then calcifying (50% and 45% respectively of the cysts found). In the lungs this phenomenon was observed less often and acephalocysts were found more frequently.

CE tended to progressively accumulate in adult cattle in Sardinia.

In conclusion we can state that cattle, although they are not considered an important host for the persistence of CE in Sardinia, may be considered a further indicator of infestation and that even though sometimes the parasitosis could be limited to a single calcified hydatid, it damages the organs and thus reduces the value of the animal when it is slaughtered, especially in the case of older animals.

Acknowledgements

This study could be performed thanks to the invaluable collaboration of Mr Mario Salvatore Nieddu and financed by MIUR PRIN 2003 prot. n. 2003070410_001 and Finalized Sanitary Search IZS Sardinia (prot. n. DGRSVE/CRS/RF-2003/90).