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An epidemiological updating on cystic echinococcosis in cattle and sheep in Sicily, Italy

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Cystic echinococcosis (CE) is a zoonotic parasitic disease caused by larval stage of dog tapeworm *Echinococcus granulosus*. Transmission occurs predominantly in synanthropic cycles, involving sheep, goats, cattle and pigs as intermediate hosts. In Italy, according to sheep breeding, the infection rate increases from north to south regions including islands. Previous CE prevalence recorded in Sicily in cattle and sheep were 11.1% and 15.6%, respectively. The cyst viability, studied in sheep only, was 1,9% (Magliarditi D, Niutta PP, 1995, Atti Associazione Siciliana di Sanità Veterinaria: 165-167; Scala A *et al.*, 2001, 20th International Congress of Hydatidology, Kusadasi, Turkey: 303). This paper reports preliminary results on prevalence and viability of CE in cattle and sheep in Sicily and is part of a larger research focused on the epidemiological updating of *E. granulosus* infection.

Between May 2004 and October 2004, a total of 393 cattle and 411 sheep from northeast and midwest of the island were examined for CE at local abattoirs. Cysts from positive organs were examined for protoscoleces presence and classified as fertile (with viable protoscoleces) or sterile (without or with not viable protoscoleces). Aliquots of viable cysts (germinal layer and protoscoleces), were stored at –20°C in glass tubes. From these isolates and with the strain typing purpose, DNA was extracted with a commercial kit (High pure PCR template preparation kit, Roche) and PCR (Dinkel A *et al.*, 2004, Int J Parasitol, 34: 645-653) were carried out to discriminate the various strains (Eckert J *et al.*, 2001, WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. World Health Organization, Paris, France: 265). The strain obtained by PCR was confirmed by sequencing COI and NADH mithocondrial genes (Bowles J, McManus DP, 1993, Int J Parasitol, 23: 969-972; Bowles J *et al.*, 1994, Parasitology, 109: 215-221).

Cyst frequencies from the different organs and infection frequency in the different host age classes were compared by chi-squared test. The variances of CE prevalence within the host age were analyzed using logistic regression model. Statistical analyses were performed with the software SPSS 12.0 for Windows (Apache Software Foundation, Chicago).

The overall CE prevalence was 67.1% (264/393) in cattle and 57.6% (237/411) in sheep. In Table 1 are showed the infection rates by age classes of cattle and sheep. The logistic regression model for general trend of CE age-prevalence showed a positive correlation with an odds ratio per year of 1.23 (1.16-1.30) and 1.45 (1.19-1.76) for cattle and sheep, respectively. Additionally, sheep were found to be 2.2 time (1.4-3.3) more at risk than cattle for CE infection. Distribution of cysts in the internal organs (Table 2) showed different patterns in the 2 animal species.

Table 1. Age prevalence of *E. granulosus* infection in cattle and sheep.

Age (years)	Cattle rate (infected/examined)	Sheep rate (infected/examined)
0-1	0[a] (0/24)	0[a] (0/6)
1-2	0.33[b] (3/9)	0.42[b] (5/12)
2-3	0.38[b] (5/13)	0.43[b] (12/28)
3-4	0.23[b] (3/13)	0.69[c] (49/71)
4-5	0.59[c] (10/17)	0.56[c] (89/159)
5-6	0.77[c] (10/13)	0.82[d] (27/33)
6-7	0.58[c] (7/12)	0.71[c] (5/7)
7-8	0.65[c] (11/17)	0.86[c] (6/7)
8-9	0.78[c] (21/27)	–
9-10	0.72[c] (23/32)	1 (4/4)
>10	0.79[c] (171/216)	–
χ^2	91.64*	29.16*
Total	67.1 (264/393)	60.2 (197**/411)

* The differences in age groups with different letter in the same column are statistically significant ($p < 0.05$).
** Out of 411 sampled sheep only for 197 was possible the age determination.

Table 2. Distribution of *E. granulosus* cysts in cattle and sheep.

Infected organs	Cattle (n=264) Number	%	Sheep (n=237) Number	%
Liver	32	12.1[a]	88	37.1[a]
Lung	40	15.1[a]	27	11.3[b]
Liver+lung	190	72[b]	121	51[c]
Liver+lung+other	2	0.7[c]	1	0.4[d]
χ^2	430.38*		204.17*	

* The CE percentages among internal organs with different letter in the same column are statistically significant ($p<0.05$).

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Table 3. *E. granulosus* strains found from cattle and sheep.

Species (organ)	Number of isolates	G1 PCR (Dinkel 2004)	G5/G6/G7 PCR (Dinkel 2004)	NADH sequence	COI sequence
Sheep (lungs)	13	Positive	Negative	G1	G1
Sheep (liver)	5	Positive	Negative	G1	G1
Cattle (lungs)	5	Positive	Negative	G1	G1
Cattle (liver)	6	Positive	Negative	G1	G1

While in cattle most infections were characterized by the presence of cysts both in the liver and the lung followed by lung only and liver only, in sheep cysts were found mostly in liver. Overall cysts fertility rate was 4% (16/393) in cattle and 9.2% (38/411) in sheep. A positive correlation between cysts viability and sheep ages was found with rates ranging from 2% to 28% in 1-2 years and 8-9 years age classes, respectively. Furthermore, concerning the site of fertile cysts, significant differences were found in sheep where most viable cysts were found in lung (28/148; in liver 23/209). Strain typing by PCR and mithocondrial genes NADH and COI sequencing showed the presence of G1 or Sheep Strain (Table 3) from both sheep and cattle isolates.

Although in this paper are presented the preliminary results, the data have important implications. The positive trend recorded for CE age-prevalence, in agreement with other studies, shows that there is no evidence of parasite-induced host immunity or at least that the natural immunity response in cattle and sheep has no efficacy in CE infection control (Roberts MG *et al.*, 1986, Parasitology, 92: 621-641; Cabrera PA *et al.*, 1996, Int J Parasitol, 25: 807-813; Dueger EL, Gilman RH, 2001, Trans R SocTrop Med Hyg, 95: 379-383; Torgerson PR *et al.*, 2003, Vet Parasitol, 114: 143-153). Furthermore, the results show that sheep tend to be more at risk than cattle for CE infection, confirming their role as most important intermediate host in Sicily. The high number of positive animals and the cysts viability rates recorded, especially in old cattle, showed that these ruminants could act as active intermediate hosts in *E. granulosus* infection maintenance in the island. Additionally, although the high number of viable cysts found in sampled cattle no G5 or cattle strain (Thompson RCA, MacManus DP, 2002, Parasitol Today, 18: 452) was found. The finding of the G1 strain, only. It suggests that factors due to the strain type might influence the cyst viability in cattle. Compared to previous surveys in Sicily, our data show higher prevalence values. This could be as a consequence of several factors such as the ages of the sampled animals, the differences of the geographical prevalence and the methods of study. Thus, concerning the age, in the last survey carried out on sheep CE (Scala A *et al.*, 2001, 20th International Congress of Hydatidology, Kusadasi, Turkey: 303), overall prevalence and viability were 15.5% and 1.9% respectively, but all the sampled sheep were aged 2-3 year-old. Regarding the differences in geographic prevalence, Poglayen G *et al.* (2003, WAAVP 19th International Conference, New Orleans, USA: 164) well described the importance of climatic and environmental factors that could influence the CE prevalence in Sicily. About the method of study we preferred to personally check animals at abattoirs. In fact, it is important to note that surveys based on records from slaughterhouses usually showed lower prevalence respect to the real infection rate (Umur S, 2003, J Vet Med, 50: 247-252). In conclusion, according with Scala *et al.* (2001, 20th International Congress of Hydatidology, Kusadasi, Turkey: 303) "echinococcosis-hydatidosis continues to be a public health problem in the 2 biggest Italian islands" and these results, at least for the Italian endemic regions, should be used to stimulate the requirement of a continuous control program of this important parasitic zoonotic disease.

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