Apicomplexa diffusion in tissue samples from slaughtered sheep in Sardinia (Italy)

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Sheep breeding plays in Sardinia a major role for the economy of the island with over than 3 millions of animals raised mostly with extensive methods. On the other hand, the food market requires more than in the past sanitary health targets of animal derived products. To evaluate this aspect and also to understand the diffusion of some abort agents related to the Phylum Apicomplexa, like *Toxoplasma gondii, Neospora caninum* and *Sarcocystis* spp. an investigation on butchered sheep in various abattoirs of Sardinia was performed. While the role of *Toxoplasma* seems to be clear and *N. caninum* presence not so diffused in sheep farms, microscopic *Sarcocystis* like *S. ovicanis* and *S. arieticanis*, are not yet well investigated in the isle, as their role in abortion and death of the animals in acute forms as reported by Heckeroth AR and Tenter AM (1999, Int J Parasitol, 29: 1331-1349). Two hundred slaughtered sheep (169 Sarda breed sheep and 31 crossed Merino breed Spanish sheep) were investigated in the period from 2003 to 2005 in slaughterhouses of north Sardinia. A total of 323 tissue samples including heart (141), diaphragm (58), oesophagus (32), tongue (37), masseter muscles (38), liver (13) and spleen (4) were first homogenized and than DNA extracted with a commercial kit (Roche). Samples were analyzed by a single tube nested PCR assay for the detection of *T. gondii* and *N. caninum*, according to the protocols respectively of Hurtado A *et al* (2001, Vet Parasitol, 102: 17-27) and Ellis JT *et al* (1999, Int J Parasitol, 29: 1589-1596). Genomic DNA of *S. arieticanis* and *S. ovicanis* was investigate applying the methodology of Heckeroth AR and Tenter AM (1999): this nested PCR allows to discriminate the two species. PCR products were stained with ethidium bromide after gel electrophoresis: the expected size of the amplified DNA fragment was 227 bp for *T. gondii*, 146 bp for *N. caninum*, 530 bp for *S. tenella* and 375 for *S. arieticanis*. Two sheep respectively from Sardinia and Spain were positive for *T. gondii* (0.59% and 3.22% respectively), both from heart tissues (χ^2 Yates corrected 0.14; P = 0.70). All examined samples did not show any positive to *N. caninum* infection. *S. ovicanis* was found in 96% of the examined sheep while *S. arieticanis* in 29.5%. Prevalence for each examined organ are reported the following table:

Table 1.

Parasite	Heart (141)	Diaphragm (58)	Oesophagus (32)	Tongue (37)	Masseter (38)	Liver (13)	Spleen (4)
T. gondii	3.3% (2)	0	0	0	0	0	0
S. ovicanis	94.3% (133)	93.1% (54)	81.2% (26)	83.8% (31)	78.9% (30)	7.7% (1)	25% (1)
S. arieticanis	26.2% (37)	43.1% (25)	9.4% (3)	18.91% (7)	31.6% (12)	0	25% (1)

The results of the present work allow us to conclude that *N. caninum* did not constitute a health problem for the sheep coming from the monitored districts. On the other hand *S. ovicanis* seems to be widespread in sheep flocks of Sardinia and also in animals coming from Spain (96.4% from Sardinia and 93.5% from Spain; χ^2 Yates corrected = 0.07; P = 0.79). Spanish animals have shown high prevalence for *S. arieticanis* (74.2%) that in Sardinia seems to be a lower diffusion (21.3%) (χ^2 Yates corrected = 32.74; P < 0.001). Our investigation lead us to light up the presence of *T. gondii* in few sheep coming form Sardinia and Spain, even if the PCR technique seems to be not so effective in showing the real diffusion of the parasite, as the results of previous investigations with other techniques indicate (Pereira-Bueno J *et al*, 2004, Vet Parasitol, 121: 33-43; Piergili-Fioretti D, 2004, Parassitologia, 46: 177-181). This could be related to the modalities of sampling (that for DNA extraction are limited to few grams) and also to the not so widespread diffusion in the above mentioned organs of bradizoites of *Toxoplasma*. We can conclude that the sanitary level of sheep meat of Sardinian animals was good, anyway other deepenings are necessary to investigate the role of endemic presence of *Sarcocystis* as abort cause and also to set up new screening methods for the research of *T. gondii* in meat products for human consumption.

Acknowledgements: Research financed by Sassari University (ex 60% - 2006).