SoIPa

SOCIETÀ ITALIANA DI PARASSITOLOGIA

XXVIII CONGRESSO NAZIONALE

ROMA, 24-27 Giugno 2014

LETTURE
RELAZIONI DEI SIMPOSI
COMUNICAZIONI SCIENTIFICHE

Casa dell'Aviatore, Viale dell'Università, 20 - Roma

SESSIONE PARALLELA O-10

PARASSITI DELLA FAUNA SELVATICA

O10.10

COMPARISON BETWEEN SEROLOGICAL AND MOLECULAR DIAGNOSTIC APPROACH TO TOXOPLASMA GONDII INFECTION IN ALPINE RED DEER (CERVUS ELAPHUS)

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INTRODUCTION:
The widespread Toxoplasma gondii affects several domestic and wild animal species raising public health issues related to its zoonotic value. As wild ungulates can be source of human infection, risk should be evaluated in relation to the amount of game meat available from harvest plans and besides a reliable diagnostic trial to monitoring T. gondii in free-ranging populations should be defined. Focusing on red deer (Cervus elaphus), because of intense hunting activity in many Alpine areas and since this species is consumed even raw or undercooked, we performed a serological and molecular investigations of Toxoplasma gondii to evaluate (i) the reliability of cardiac tissue fluids as an alternative to sera in ELISA test and (ii) the applicability of three PCR protocols.

MATERIALS AND METHODS:
Overall 78 sera and cardiac tissue fluids and 159 brain tissue samples were collected for respectively serological and molecular investigations from Lepontine Alps (VB) during the hunting season 2011 and during the culling management plan scheduled by the Stelvio National Park in 2012.
A commercial ELISA kit (IDVET, Montpellier, France), validates for ruminants' sera and tissue fluids, was used and the agreement between analytical approach was assessed calculating the Kappa (K) value.
DNA was extracted with the QIAamp DNA Mini Kit (Qiagen, Italy) and the whole samples were assayed by targeting a 529 bp non-coding region (Homan et al., 2000, Int. J. Parasitol., 30:69-75) (protocol 1). Positive and doubtful results were then submitted to a single tube nested PCR (Hurtado et al., 2001, Vet. Parasitol., 102:17-27) (protocol 2) and to a PCR-RFLP using primers that identify also Neospora caninum and Sarcocystis spp. (Magnino et al., 1998, Proc. IX ICOPA:1269-1272) (protocol 3).

RESULTS:
A T. gondii prevalence of 29% and 19% was recorded respectively in sera and cardiac fluids showing a "fair agreement" (K value = 0.38) between the two matrices and a loss in sensitivity and specificity using cardiac fluids.
The whole analysed samples resulted negative for T. gondii DNA. In 15 samples doubtful PCR products with no-specific bands resulted from the protocol 1 and thus they were submitted to the second and the third PCRs. The other two protocols cleaned PCR amplified products confirming the T. gondii negativity but Sarcocystis spp. DNA was detected by protocol 3 in a 6-month-old male and in a 1-year-old female. Sequencing analysis identified Sarcocystis hjorti DNA from the calf brain tissue.

CONCLUSIONS:
The study highlights a few diagnostic difficulties in both T. gondii serological and molecular investigation in red deer. Although working with wildlife it could be easier sampling an heart than a serum sample, cardiac tissue fluids cannot be considered as an alternative of serum in this species using this ELISA test.
The doubtful results emerged using PCR protocol 1 suggest that this molecular technique can have a lower specificity than two others with red deer samples and besides protocol 3 was useful to detect the cross reaction with Sarcocystis spp.. As application of diagnostic tests developed from livestock or human to wildlife could compromise their performances, the need is to set up diagnostic methods specific for wild species mainly for pathogens of public health significance.

Keywords:
Toxoplasma gondii, red deer, diagnostic approach