

SUMMARY

“Molecular characterization of *Toxoplasma gondii* in Central Italy: new insights to the risk of consumption of raw meat.”

Toxoplasmosis is a cosmopolitan zoonotic parasitic infection of warm-blooded animals, including humans. The aetiological agent (*Toxoplasma gondii*) lives in the tissues of herbivorous preys and intermediate host carnivorous predators and concludes its life cycle in the final host represented by felids in which intestinal sexual reproduction occur.

As an intermediate host, humans may acquire infection or by accidental ingestion of oocysts eliminated by feline faeces and present on the soil, vegetables, water or by ingestion of raw meat (produced by zootechnical animals) containing bradyzoites or for vertical transmission by tachyzoites.

Raw meat represents one of the main sources of *T. gondii* infections in Europe, and as show by a survey conducted in Europe, about 30-63% of infections in pregnant women have been attributed to consumption of raw meat, while 6 to 17% seem to originate from soil contamination (Cook et al., 2000).

In order to obtain a distinct scenario about the role of meat as a source of human infection with *T. gondii*, it is important to have detailed data on the presence of infectious tissue cysts in the animal hosts raised for meat production. These are generally obtained using serological assays, and seroprevalence of infection *T. gondii* in outdoor breeding cattle (eg sheep and cattle) so far described is generally higher, whereas seroprevalence in indoor livestock (for example, indoor pigs) is lower (Kijlstra and jongert, 2008). However, the detection of *T. gondii* antibodies in animals does not necessarily represent a reliable indication of the presence of infectious tissue cysts and the risk of human infection. Although theoretically there should be a strong correlation, as both antibodies and tissue cysts are presumed to persist for a long time in sheep and pigs (Dubey, 2009).

Biomolecular methods should be considered as reference methods, but in the case of toxoplasmosis show the weakness of being less sensitive because tissue cysts may be scant, even below a cyst for 50 grams of tissue, and thus provide falsely negative outcomes. To overcome this critical issue, the immune concentration of *T. gondii* cysts is useful, described by Opsteegh M et al, (2010), which would appear to be more sensitive than that of laboratory mice.

With this study, we aimed to analyse the role of edible food producing animals, all intermediate hosts of toxoplasmosis, in the circulation of the parasite instead of the final host as cats and other felids. In detail the aims of this study are to analyse the occurrence and prevalence of *T. gondii* in domestic animals raised, in a non-intensive way, for the meat production in selected areas of the Lazio Region, to assess the role of each animal species considered as a source of infection for humans, to study the variations of target genes between *T. gondii* isolated from edible domestic animals and humans, in order to assess the risk of infection and identification of *Toxoplasma* genotypes circulating in the Region.

The muscular tissues of N=92 horses regularly slaughtered in slaughterhouses in the Province of Rieti for serological and biomolecular research have been collected and analysed.

In addition, N=268 serum samples of sheep from a flock with a history of abortion have been submitted to serological screening ELISA method; after the serological examination, the residual clots were immediately frozen for the biomolecular analyses.

Organ samples were collected from 72 animals belonging to meat-producing species sent for cause of death, (brain, heart and lung) for histological research on *T. gondii* cysts.

Concerning human samples, a total of 97 HIV-infected patients and 98 healthy subjects blood donors paired by gender and age, admitted at the Academic hospital Policlinico Umberto I, were included in the study. Both groups were subjected to serological and biomolecular investigations. The detection of *T. gondii* specific antibodies with indirect ELISA in meat extracts from 92 horses slaughtered in Rieti province showed the following prevalence: 19% positive and 6.7% doubtful.

As regards the results obtained from the 268 sheep sera, the results were as follows: 7.6% positive and 3.2% positive.

The results of histology and molecular tests from the animal matrices analysed were all negative.

Instead N=2 HIV-positive patients resulted positive with the PCR for toxoplasmosis.

Overall, with the results obtained, the risk of toxoplasmosis is confirmed following consumption of raw or undercooked meat for the detection of serological positivity observed from both raw horse meat and goats' serums with a history of toxoplasmosis abortions. The negative results in all animal matrices considered, which have already been positive for serological testing, confirmed the needing to analyse such samples using the magnetic-capture polymerase chain reaction (MC-PCR), today considered the reference technique for the correct detection of *T. gondii* cysts in the tissues, as described by Opsteegh M et al (2010). Failure to find positive results in animal matrices did not allow comparison with circulating genotypes in human population.