# Molecular detection of *Toxoplasma gondii* from a naturally infected Alpine chamois (*Rupicapra r. rupicapra*) from Italian Alps

<u>Nicoletta Formenti<sup>1</sup></u>, Alessandra Gaffuri<sup>2a</sup>, Nadia Vicari<sup>2b</sup>, Tiziana Trogu<sup>1</sup>, Roberto Viganò<sup>1</sup>, Nicola Ferrari<sup>1</sup>, Franco Paterlini<sup>2a</sup>, Paolo Lanfranchi<sup>1</sup>

Corresponding author: Nicoletta Formenti, nicoletta.formenti@unimi.it

# **Background**

The protozoan *Toxoplasma gondii* affects many species of domestic (1; 2) and wild (3; 4; 5) warm-blooded animals, raising public health issues related to its zoonotic potential. In this sense wild ungulates may therefore be a source of *T. gondii* infection for consumers (raw, undercooked meat and fresh sausages) (6; 7; 8) and for hunters and slaughterers through manipulation, evisceration and handling of carcasses (9; 10; 11).

Alpine chamois (*Rupicapra r. rupicapra*) is the most hunted wild ungulate in the Italian Alps with a significant increase of density in the last decades (12); as positive results of serological testing for *T. gondii* have been reported in the population from the Italian Alps (13; 14) and in southern chamois (*Rupicapra pyrenaica*) from Spanish Pyrenees (6), we investigated the presence of the protozoan DNA in brain tissues in order to define the receptivity of this species to *T. gondii* infection and its role in the protozoan lifecycle.

#### Materials and methods

During the hunting season 2011, 11 samples of chamois brain tissues were collected in the Lepontine Alps (VB). DNA extraction was performed with the QIAamp DNA Mini Kit (Qiagen, Italy). All the samples were assayed by targeting a 529 bp non-coding region (15), then the positive one was confirmed by a PCR-RFLP assay targeting the 18S small-subunit ribosomal gene of *T. gondii*, using primers that identify also *Neospora caninum* and *Sarcocystis spp.* (16).

#### **Results and Discussion**

T. gondii DNA was detected in a six-year-old male chamois hunted at an altitude of 1700 m.a.m.s.l.. The subject was in a good body condition and its behaviour was normal; the post-mortem examination did not reveal any systemic macroscopic lesions.

The protozoan DNA was detected by both PCR protocols. The PCR-RFLP restriction enzyme analysis of the amplified product confirmed the presence of *Toxoplasma gondii*, excluding eventual cross-reactions with *N. caninum* and *Sarcocystis spp.*, closely related to *T. gondii*. As far as we know, this is the first detection of *T. gondii* DNA from Alpine chamois.

This result confirms the Alpine chamois as intermediate host of *T. gondii* and demonstrates the protozoan presence in the Alpine ecosystem, even in remote areas.

Considering the sporadic presence of linx in the Italian Alps, feral cats are the only definitive hosts of *T. gondii*, even if transplacental transmission can not be excluded. The impact on chamois population dynamics can not properly be evaluated without a better understanding of the

<sup>&</sup>lt;sup>1</sup>Università degli Studi di Milano, Department of Veterinary Sciences and Public Health, Milan, Italy, 20133. <sup>2a</sup>Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini" (IZSLER), Bergamo, Italy, 24100.

<sup>&</sup>lt;sup>2b</sup>Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini" (IZSLER), Pavia, Italy, 27100.

epidemiology of infection. In addition, the consumption of raw or undercooked chamois meat could be a possible source of *T. gondii* infection in humans. In particular, the fact that *T. gondii* usually affects the host without producing clinical signs (17) could increase the risk of human infection ascribed to the apparent healthiness of chamois meat.

### Perspectives and future research priorities

Further analysis are needed to define the epidemiology of *T.gondii*, in particular performing serological study of antibodies against the parasite and the genotyping of the present and future PCR positives samples in order to define (a) prevalence of *T. gondii* infection in Alpine chamois populations, (b) which parasite strains are circulating in this alpine ruminant, (c) its pathogenicity and the related zoonosis risk.

## 6) Acknowledgements

We wish to thank all the hunters of the Alpine hunting district (VCO2) in the province of Verbania for their help during research in the field, Maria Chiara Cerutti for her useful technical contribution, Donatella Ghidotti and Marzia Marchionni for their helpfulness and collaboration in the lab activities, Ilaria Marangi for her invaluable suggestions that improved the drafting of this manuscript.

#### References

- 1. Masala G., Porcu R., Madau L., Tanda A., Ibba B., Satta G., Tola S. 2003. Survey of ovine and caprine toxoplasmosis by IFAT and PCR assays in Sardinia, Italy. Veterinary Parasitology, 117: 15-21.
- 2. Dubey J.P. 1992. Isolation of *Toxoplasma gondii* from a Naturally Infected Beef Cow. The Journal of Parasitology, 78: 151-153.
- 3. Jokelainen P., Isomursu M., Näreaho A., Oksanen A. 2011. Natural *Toxoplasma gondii* infections in european brown hares and mountain hares in Finland: proportional mortality rate, antibody prevalence, and genetic characterization. Journal of Wildlife Diseases, 47: 154-163.
- 4. Sobrino R., Cabezón O., Millán J., Pabón M., Arnal M.C., Luco D.F., Gortázar C., Dubey J.P., Almeria S. 2007. Seroprevalence of *Toxoplasma gondii* antibodies in wild carnivores from Spain. Veterinary Parasitology, 148: 187-192.
- 5. Bártová E., Sedlák K., Literák I. 2006. Prevalence of *Toxoplasma gondii* and *Neospora caninum* antibodies in wild boars in the Czech Republic. Veterinary Parasitology, 142: 150-153
- 6. Gauss C.B.L., Dubey J.P., Vidal D., Cabezón O., Ruiz-Fons F., Vicente J., Marco I., Lavin S., Gortázar C., Almería S. 2006. Prevalence of *Toxoplasma gondii* antibodies in red deer (*Cervus elaphus*) and other wild ruminants from Spain. Veterinary parasitology, 136: 193-200.
- 7. Ross R.D., Stec L.A., Werner J.C., Blumenkranz M.S., Glazer L., Williams G.A. 2001. Presumed acquired ocular Toxoplasmosis in deer hunters. Retina, The Journal of Retinal and Vitreous Diseases, 3: 226-229.
- 8. Dubey J.P. & Beattie C.P. 1988. Toxoplasmosis of Animals and Man. CRC Press, Boca Raton, Florida. 220 pp.
- 9. Kapperud G., Jenum P.A., Stray-Pedersen B., Melby K.K., Eskild A., Eng J. 1996. Risk Factors for *Toxoplasma gondii* Infection in Pregnancy Results of a Prospective Case-Control Study in Norway. American Journal of Epidemiology, 144: 405-412.
- 10. Dubey J.P. 1994. Toxoplasmosis. Journal of the American Veterinary Medical Association, 205: 1593-1598.

- 11. McDonald J.C., Gyorkos T.W., Alberton B., MacLean J.D., Richer G., Juranek D. 1990. An Outbreak of Toxoplasmosis in Pregnant Women in Northern Québec. Journal of Infectious Disease, 161: 769-774.
- 12. Pedrotti L., Duprè E., Preatoni D., Toso S. 2001. Banca Dati Ungulati. Status, distribuzione, consistenza, gestione, prelievo venatorio e potenzialità delle popolazioni di Ungulati in Italia. Istituto Nazionale per la Fauna Selvatica "Alessandro Ghigi", 115-129 pp.
- 13. Gaffuri A., Giacometti M., Tranquillo V.M., Magnino S., Cordioli P., Lanfranchi P. 2006. Serosurvey of Roe Deer, Chamois and Domestic Sheep in the Central Italian Alps. Journal of Wildlife Diseases, 42: 685-690.
- 14. Gennero M.S., Meneguz P.G., Mandola M., Masoero L., De Meneghi D., Rossi L. 1993. Indagini sierologiche su ruminanti selvatici in Piemonte. Atti Societa` Italiana delle Scienze Veterinarie, 47: 979-983.
- 15. Homan W.L., Vercammen M., De Braekeleer J., Verschueren H. 2000. Identification of a 200-to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. International Journal for Parasitology, 30: 69-75.
- 16. Magnino S., Vigo P.G., Bandi C., Colombo M., De Giuli L., Fabbi M., Genchi C. 1998. PCR diagnosis for *Neospora caninum* infection in aborted bovine foetuses and for *Toxoplasma gondii* infection in hares and goats in Italy. Pp. 1269-1272. In: *Proceedings of the IX International Congress of Parasitology*, Makuhari Messe, Chiba, Japan, 24-28 August 1998.
- 17. Marco I., Velarde R., López-Olvera J.R., Cabezón O., Pumarola M., Lavín S. 2009. Systemic toxoplasmosis and Gram-negative sepsis in a southern chamois (*Rupicapra pyrenaica*) from the Pyrenees in northeast Spain. Journal of Veterinary Diagnostic Investigation, 21: 244-247.