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Toxoplasma gondii infection in meat-producing small ruminants: meat juice serology and genotyping.

Running title: *Toxoplasma gondii* in slaughtered sheep and goats

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Abstract

The consumption of ovine and caprine meat is considered one of the major transmission routes for *Toxoplasma gondii* infection in humans. The present study aimed at obtaining epidemiological and molecular data on *T. gondii* infection in small ruminants slaughtered or commercialized in Italy. Meat juices from 227 sheep and 51 goats were analyzed with a commercial ELISA and antibodies were detected in 28.6% sheep and 27.5% goats. A significant difference was highlighted between adult sheep and the other considered categories (young sheep, young and adult goats) concerning the detection of antibodies (94.1%; *p*-value=0.008). Muscles of positives samples were submitted to molecular analysis, and *T. gondii* DNA was detected in 15 sheep and three goats; sequencing of B1 gene showed that all belonged to Type II. The present study confirmed small ruminants' meat as a possible source of *T. gondii* infection for consumers eating raw or undercooked meat, particularly in those countries where the consumption of sheep and goats' meat products is a traditional gastronomic habit.

Keywords

Toxoplasmosis; sheep; goats; serology; genotyping; B1 gene

Toxoplasmosis is a significant public health issue worldwide, with data on human infection in Europe showing an average number of 1.48 cases of congenital infection per 100,000 live births in 2013–2016 [1]. Among the several ways of transmission, the consumption of products of animal origin containing cysts with bradyzoites or other food contaminated by oocysts is the most common [2]. Small ruminant products seem a major source of toxoplasmosis, mostly in some countries and among certain ethnic groups where the consumption of undercooked meat is a cultural and traditional habit [3]. Moreover, *T. gondii* is one of the most common abortifacient parasitic agents in small ruminants [4]; in Italy, seroprevalence data varying from 23.3% to 59.3% at individual level showed a widespread of *T. gondii* among small ruminants [5-6]. Considering the high seroprevalence values encountered in small ruminants in our country, the main aim of the present survey was the characterization of the risk for consumers represented by sheep and goat meat. In order to obtain epidemiological data focusing on sheep and goats destined to human consumption, a serosurvey based on the detection of antibodies anti-*T. gondii* in meat juice was planned. Furthermore, since the pathogenicity of *T. gondii* also depends on the involved parasite genotype [7], the molecular detection and genotyping of parasite DNA in meat samples were performed.

A convenience sampling was carried out from April 2013 throughout January 2014. Overall, 227 sheep and 51 goats were sampled from five slaughterhouses and a local retail meat store in Northern Italy (Lombardy). All sampled goats originated from herds raised in Italy, while a part of sheep (111 animals) were from other European countries (55 from Great Britain, 29 from Greece and 27 from Romania). Both young (≤ 12 months, 171 animal) and adult (> 12 months, 107 animals) animals were included in the sampling.

From each animal, heart or diaphragm tissue samples (approximately 50 grams per animal) were obtained according to availability. Samples were collected in individual plastic boxes and kept refrigerated during transportation to the laboratory, where were divided into two aliquots. The first aliquot (25 g) was frost in a plastic bag overnight, subsequently defrosted to extract meat juice [8],

and stored at -20 °C until analysis. The second one (25 g) was mechanically homogenized and then stored at -20°C until molecular analysis.

Meat juice samples were analyzed for *T. gondii* antibodies with a commercial ELISA kit (ID Screen® Toxoplasmosis Indirect Multi-Species, IDVET, Montpellier), using 1:2 dilution according to manufacturer's instruction.

Muscle samples of animals showing antibodies anti-*T. gondii* in meat juice were processed for molecular analysis. Only samples from seropositive animals were analyzed since negative results in serology are considered predictive of absence of infection in both sheep and goats, with the detection of parasitic DNA in a neglectable percentage (<5%) of seronegative animals [9]. DNA extraction was performed by using a commercial kit (Nucleospin tissue, Macherey-Nagel GmbH and Co., Germany), following manufacturer's instruction. Extracted DNA was stored at −20°C until analyzed.

As screening, samples were assayed by a single tube nested-PCR (N-PCR) targeting a region of 227 bp of the internal transcribed spacer 1 (ITS1), as described [10].

For genotyping purposes, samples scored positive to N-PCR were submitted to a Real-Time PCR (RT-PCR) targeting a region of about 129 bp within the 35-fold repetitive B1 gene, as described [11].

Positive amplicons obtained by RT-PCR were purified with a commercial kit (NucleoSpin® Gel and PCR Clean-up kit) and sent for bidirectional sequencing to a commercial service (Eurofins MWG Operon, Ebersberg, Germany). Electropherograms were checked, and consensus sequences were manually assembled. Subsequently, sequences were aligned using the ClustalW program (BioEdit software v.7.2.5) and compared to nucleotide sequences available in GenBank using BLASTn software (https://www.ncbi.nlm.nih.gov/blast/).

A general linear model (GLM) with a binomial distribution and logit link function was performed to determine factors that could be considered predictors of seropositivity to *T. gondii*. Results obtained in ELISA (presence/absence, dichotomous variable) were used as dependent variables. The

variables species (dichotomous variable: sheep, goat) and age (dichotomous variable: young ≤12 months, adult >12 months), and their interaction were entered in the model with a pairwise comparison of the estimated means of the interaction. The variable "origin" was not considered since only a part of lambs was imported from abroad. The model was developed using a backward procedure until retained variables were significant (p-value < 0.05). Statistical analysis was performed using SPSS (version 19.0; SPSS, Chicago, Illinois).

Antibodies anti-*T. gondii* were found in 28.4% of examined animals (79 out of 278), with similar prevalence values in sheep (28.6%) and goats (27.5%). In both species, young animals were found to be less infected than adults (Table 1).

T. gondii DNA was detected by N-PCR in 18 muscle samples of 15 sheep and three goats, corresponding to 23.1% and 21.4%, respectively, of seropositive animals examined. The prevalence value calculated on the overall sampling (seropositive plus seronegative animals) was equal to 6.5% (18/278). While in goats similar values were obtained both in young and adult animals (6.9% and 4.5%, respectively), a greater difference was registered between adult sheep and lambs (38.2% and 1%, respectively) (Table 1). Sequencing of B1 real-time PCR amplicons showed that all belonged to Type II, with a homology of 99-100% with sequences deposited in GenBank.

Statistical analysis showed that seropositivity to ELISA was associated with the age and the interaction between species and age (Table 2). Mainly, adults resulted in being at higher risk of infection than young animals (OR=77.576, p-value=0.0001), also considering the interaction with the variable "species". Indeed, pairwise comparisons showed statistically significant differences between adult sheep and the other categories (young sheep, adult goats and young goats, p-value=0.0001).

The present survey, aimed at estimating the presence of *T. gondii* in small ruminants' meats consumed in Italy, revealed seroprevalence values lower than those previously reported in sheep

(59.3%) and goats (41.7%) bred in Northern Italy [6]. In the study area, *T. gondii* showed to be widely spread also in other domestic [11, 12] and wild animals [13-15]. Moreover, compared to the previous epidemiological survey [6] mostly focusing on adult meat producing sheep and dairy goats, in the present study both adult and young sheep and goats were sampled since a large proportion of lambs and kids are slaughtered in Italy (respectively 90.3% and 86%) (data obtained from the National Institute of Statistic, ISTAT, http://dati-censimentoagricoltura.istat.it/). Young animals showed low seroprevalence values, both in sheep and goats, with a statistically significant difference between adults and young sheep less than one-year-old. Indeed, age is one of the risk factors associated with *T. gondii* infection, increasing the risk of horizontal transmission with the increasing age, as previously reported [6, 16-20]. Concerning sheep, since in Italy more than 1 million sheep and about 25 thousand tons of ovine meat are imported from abroad (http://dati-censimentoagricoltura.istat.it/), both national and imported types of meat were sampled. In particular, imported animals were mostly lambs older than two months, while those bred in Italy were mostly slaughtered at two months of age. The highest seropositivity values found in Italian lambs (34.1%, 28/82) than those imported from abroad (4.5%, 5/111) could, therefore, be attributed to the persistence of maternal immunity: in fact, maternal antibodies were found in sheep up to three months age [21].

Considering adult animals, while sheep showed very high values of seroprevalence (94.1%), with 32 positive sheep out of 34, in adult goats the seroprevalence amounted to 36.4% (8 positive animals out of 22). The difference, also reported in previous studies carried out on sheep and goats reared together or in the same study areas, could be affected by several factors [19, 22-24]. A different feeding behavior, being goats mostly browsers and sheep grazers, could explain a greater exposure of sheep to the risk of ingesting parasitic elements, including environmental *T. gondii* oocysts, as already suggested for other parasites [25]. Moreover, differences in rearing system, identified as a risk factor associated to *T. gondii* infection in small ruminants [6, 19], could account for this discrepancy. In fact, in Northern Italy, goats are mainly dedicated to milk production, with

intensive or semi-intensive breeding. On the contrary, sheep are usually meat-producing animals, breed extensively, with the possibility of grazing, or they are transhumant.

Muscle samples of seropositive animals were subjected to molecular analysis to determine the presence of *T. gondii* DNA in the edible parts destined to human consumption and to characterize the involved genotype.

Compared to the total number of seropositive samples, only a part (18 out of 79, 22.8%) was found to be positive in N-PCR, as previously reported [17]. The low DNA detection may be due to the small size of the processed tissue sample in relation to the size of the host, to the inhomogeneity in the distribution of tissue cysts within the sample and to the different distribution of cysts in the organs [26]. The diaphragm for sheep and the cardiac muscle for both sheep and goats, used in the present study for the research of *T. gondii* DNA, were however among the preferential sites for the research of *T. gondii* DNA, compared to other muscle tissues [9, 27-28]. In any case, the values obtained both in goats and in sheep are in line with what was previously reported in Europe, varying the proportion of animals having cysts in the edible parts from 0-6% in young and 3.3-3.6% in adult animals [17, 29-30].

Particularly, a higher proportion of adult sheep showed meat containing *T. gondii* DNA (38.2%, 13 out of 34), if compared to the other considered categories. A similar value was previously reported in adult sheep reared in France (42%) [31]. Adult sheep are therefore the category at higher risk of *T. gondii* infection; however, lamb and kid meat, showing low seroprevalence values and fewer animals with tissue cysts detected by the N-PCR, are preferably consumed in Italy.

The risk for consumers also varies depending on the genotype of *T. gondii* involved, given that different genotypes can lead to different clinical outcomes [4]. In the present study, we characterized the genotype by sequencing a target region of the B1 gene: all 18 positive samples were found to belong to Type II, which is primarily involved in human infections both in congenital cases and in immunocompromised patients [7]. These results are in agreement with those previously

reported in Europe in sheep and goats, being Type II the most frequently found in slaughtered animals [29, 31, 32-33].

Data obtained in the present study confirmed small ruminants' meat as a potential source of *T. gondii* human infection, both for the high circulation of the pathogen and for the identified genotype involved. Referring to Italy, the preparation of traditional dishes based on sheep meat usually requires very long cooking times (i.e., "Abbacchio Romano", "Lamb of Central Italy" and "Lamb of Sardinia", Protected Geographical Indication) or an exposure to high temperatures as in the case of grilled preparations (i.e., "Arrosticini", grilled lamb skewers) able to inactivate *T. gondii* tissue cysts in meat. Nevertheless, the risk given by the consumption of traditional products based on cured meat (i.e., meat sausages) should not be neglected, especially in some geographical areas. Furthermore, the risk of contracting the infection during the slaughtering process and subsequent handling of meat during the preparation of the dishes should be taken into account, particularly for specific categories of workers (farmers, abattoir workers, butchers, cooks) more exposed to the risk of contracting the infection [34-35].

Meat juice serology and molecular analysis demonstrated both the circulation of *T. gondii* in sheep and goats intended for human consumption and the presence of the pathogen in the musculature. The consumption of raw or undercooked ovine and caprine meat and derived products (e.g., sausages, salami) is thus confirmed as a possible source of *T. gondii* infection in humans, especially in countries or geographic areas where the consumption of sheep and goat meat is part of the gastronomic and traditional culture [3].

Conflict of interest

None.

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References

[1] EFSA-ECDC, The European Union summary report on trends and sources of zoonoses, zoonotic agents and food- borne outbreaks in 2017, EFSA J. 16 (2018). doi:10.2903/j.efsa.2018.5500.

[2] O. Djurković-Djaković, J. Dupouy-Camet, J. Van der Giessen, J.P. Dubey, Toxoplasmosis: Overview from a One Health perspective, Food Waterborne Parasitol. 15 (2019) 12–15. doi:10.1016/j.fawpar.2019.e00054.

[3] A. Kijlstra, E. Jongert, Control of the risk of human toxoplasmosis transmitted by meat, Int. J. Parasitol. 38 (2008) 1359–1370. doi:10.1016/j.ijpara.2008.06.002.

[4] J. P. Dubey, Toxoplasmosis of Animals and Humans., second edition, CRC Press, Boca Raton, Florida, 2010.

[5] B.T. Cenci-Goga, A. Ciampelli, P. Sechi, F. Veronesi, I. Moretta, V. Cambiotti, P.N. Thompson, Seroprevalence and risk factors for *Toxoplasma gondii* in sheep in Grosseto district, Tuscany, Italy, BMC Vet. Res. 9 (2013) 1. doi:10.1186/1746-6148-9-25.

[6] A.L. Gazzonis, F. Veronesi, A.R. Di Cerbo, S.A. Zanzani, G. Molineri, I. Moretta, A. Moretti, D.P. Fioretti, A. Invernizzi, M.T. Manfredi, *Toxoplasma gondii* in small ruminants in Northern Italy - prevalence and risk factors., Ann. Agric. Environ. Med. 22 (2015) 62–68. doi:10.5604/12321966.1141370.

[7] D. Maubon, D. Ajzenberg, M.P. Brenier-Pinchart, M.L. Dardé, H. Pelloux, What are the respective host and parasite contributions to toxoplasmosis?, Trends Parasitol. 24 (2008) 299–303. doi:10.1016/j.pt.2008.03.012.

[8] K. Nöckler, F.J. Serrano, P. Boireau, C.M.O. Kapel, E. Pozio, Experimental studies in pigs on *Trichinella* detection in different diagnostic matrices, Vet. Parasitol. 132 (2005) 85–90. doi:10.1016/j.vetpar.2005.05.033.

[9] M. Opsteegh, M. Maas, G. Schares, J. van der Giessen, Relationship between seroprevalence in the main livestock species and presence of *Toxoplasma gondii* in meat (GP/EFSA/BIOHAZ/2013/01) An extensive literature review. Final report, EFSA Support. Publ. 13 (2017). doi:10.2903/sp.efsa.2016.en-996.

[10] A. Hurtado, G. Aduriz, B. Moreno, J. Barandika, A.L. García-Pérez, Single tube nested PCR for the detection of *Toxoplasma gondii* in fetal tissues from naturally aborted ewes, Vet. Parasitol. 102 (2001) 17–27. doi:10.1016/S0304-4017(01)00526-X.

[11] A.L. Gazzonis, M. Marangi, L. Villa, M.E. Ragona, E. Olivieri, S.A. Zanzani, A. Giangaspero, M.T. Manfredi, *Toxoplasma gondii* infection and biosecurity levels in fattening pigs and sows: serological and molecular epidemiology in the intensive pig industry (Lombardy, Northern Italy), Parasitol. Res. 117 (2018) 539–546. doi:10.1007/s00436-017-5736-z.

[122] L. Villa, A.L. Gazzonis, G. Álvarez-García, C. Diezma-Díaz, S.A. Zanzani, M.T. Manfredi, First detection of anti-*Besnoitia* spp. specific antibodies in horses and donkeys in Italy, Parasitol. Int. 67 (2018) 640–643. doi:10.1016/j.parint.2018.06.008.

[13] A.L. Gazzonis, L. Villa, K. Riehn, A. Hamedy, S. Minazzi, E. Olivieri, S.A. Zanzani, M.T. Manfredi, Occurrence of selected zoonotic food-borne parasites and first molecular identification of *Alaria alata* in wild boars (*Sus scrofa*) in Italy, Parasitol. Res. 117 (2018). doi:10.1007/s00436-018-5908-5.

[14] S.A. Zanzani, A. Di Cerbo, A.L. Gazzonis, S. Epis, A. Invernizzi, S. Tagliabue, M.T. Manfredi, Parasitic and Bacterial Infections of *Myocastor coypus* in a Metropolitan Area of Northwestern Italy, J. Wildl. Dis. 52 (2016) 126–130. doi:10.7589/2015-01-010.

[15] A.L. Gazzonis, S.A. Zanzani, A. Santoro, F. Veronesi, E. Olivieri, L. Villa, E. Lubian, S. Lovati, F. Bottura, S. Epis, M.T. Manfredi, *Toxoplasma gondii* infection in raptors from Italy: Seroepidemiology and risk factors analysis, Comp. Immunol. Microbiol. Infect. Dis. (2018). doi:10.1016/j.cimid.2018.10.002.

[16] A.E. Berger-Schoch, D. Bernet, M.G. Doherr, B. Gottstein, C.F. Frey, *Toxoplasma gondii* in Switzerland: A Serosurvey Based on Meat Juice Analysis of Slaughtered Pigs, Wild Boar, Sheep and Cattle, Zoonoses Public Health. 58 (2011) 472–478. doi:10.1111/j.1863-2378.2011.01395.x.

[17] D. Halová, G. Mulcahy, P. Rafter, L. Turčeková, T. Grant, T. de Waal, *Toxoplasma gondii* in Ireland: Seroprevalence and Novel Molecular Detection Method in Sheep, Pigs, Deer and Chickens, Zoonoses Public Health. 60 (2013) 168–173. doi:10.1111/j.1863-2378.2012.01514.x.

[18] A. Iovu, A. Györke, V. Mircean, R. Gavrea, V. Cozma, Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in dairy goats from Romania, Vet. Parasitol. 186 (2012) 470–474. doi:10.1016/j.vetpar.2011.11.062.

[19] A.P. Lopes, J.P. Dubey, F. Neto, A. Rodrigues, T. Martins, M. Rodrigues, L. Cardoso, Seroprevalence of *Toxoplasma gondii* infection in cattle, sheep, goats and pigs from the North of Portugal for human consumption, Vet. Parasitol. 193 (2013) 266–269. doi:10.1016/j.vetpar.2012.12.001.

[20] F. Spišák, L. Turčceková, K. Reiterová, S. Špilovská, P. Dubinský, Prevalence estimation and genotypization of *Toxoplasma gondii* in goats, Biologia (Bratisl). 65 (2010) 670–674. doi:10.2478/s11756-010-0070-2.

[21] H. Waldeland, Toxoplasmosis in sheep. I. Long-term epidemiological studies in four breeding flocks. II. Influence of various factors on the antibody contents. III. Hematological, serological and parasitological studies, Acta Vet. Scand. 18 (1977) 227-256

[22] I. García-Bocanegra, O. Cabezón, E. Hernández, M.S. Martínez-Cruz, Á. Martínez-Moreno, J. Martínez-Moreno, *Toxoplasma gondii* in Ruminant Species (Cattle, Sheep, and Goats) from Southern Spain, J. Parasitol. 99 (2013) 438–440. doi:10.1645/12-27.1.

[23] G. Masala, R. Porcu, L. Madau, A. Tanda, B. Ibba, G. Satta, S. Tola, Survey of ovine and caprine toxoplasmosis by IFAT and PCR assays in Sardinia, Italy, Vet. Parasitol. 117 (2003) 15–21. doi:10.1016/j.vetpar.2003.07.012.

[24] N. Tzanidakis, P. Maksimov, F.J. Conraths, E. Kiossis, C. Brozos, S. Sotiraki, G. Schares, *Toxoplasma gondii* in sheep and goats: Seroprevalence and potential risk factors under dairy husbandry practices, Vet. Parasitol. 190 (2012) 340–348. doi:10.1016/j.vetpar.2012.07.020.

[25] H. Hoste, S. Sotiraki, S.Y. Landau, F. Jackson, I. Beveridge, Goat-Nematode interactions: Think differently, Trends Parasitol. 26 (2010) 376–381. doi:10.1016/j.pt.2010.04.007.

[26] D.E. Hill, S. Chirukandoth, J.P. Dubey, J.K. Lunney, H.R. Gamble, Comparison of detection methods for *Toxoplasma gondii* in naturally and experimentally infected swine, Vet. Parasitol. 141 (2006) 9–17. doi:10.1016/j.vetpar.2006.05.008.

[27] J. Juránková, M. Opsteegh, H. Neumayerová, K. Kovařčík, A. Frencová, V.V. Baláž, J. Volf, B. Koudela, W. Basso, H. Neumayerová, A. Frencová, V.V. Baláž, P. Deplazes, B. Koudela, Predilection sites for *Toxoplasma gondii* in sheep tissues revealed by magnetic capture and real-time PCR detection, Food Microbiol. 52 (2015) 150–153. doi:10.1016/j.fm.2015.07.005.

[28] J. Juránková, M. Opsteegh, H. Neumayerová, K. Kovařčík, A. Frencová, V. Baláž, J. Volf, B. Koudela, Quantification of *Toxoplasma gondii* in tissue samples of experimentally infected goats

by magnetic capture and real-time PCR, Vet. Parasitol. 193 (2013) 95–99. doi:10.1016/j.vetpar.2012.11.016.

[29] A.E. Berger-Schoch, D.C. Herrmann, G. Schares, N. Müller, D. Bernet, B. Gottstein, C.F. Frey, Prevalence and genotypes of *Toxoplasma gondii* in feline faeces (oocysts) and meat from sheep, cattle and pigs in Switzerland, Vet. Parasitol. 177 (2011) 290–297. doi:10.1016/j.vetpar.2010.11.046.

[30] A.I. Paştiu, D. Ajzenberg, A. Györke, O. Şuteu, A. Balea, B.M. Rosenthal, Z. Kalmár, C. Domşa, V. Cozma, Traditional Goat Husbandry May Substantially Contribute to Human Toxoplasmosis Exposure, J. Parasitol. 101 (2014) 45–49. doi:10.1645/13-483.1.

[31] L. Halos, A. Thébault, D. Aubert, M. Thomas, C. Perret, R. Geers, A. Alliot, S. Escotte-Binet, D. Ajzenberg, M.L. Dardé, B. Durand, F. Boireau, I. Villena, An innovative survey underlining the significant level of contamination by *Toxoplasma gondii* of ovine meat consumed in France, Int. J. Parasitol. 40 (2010) 193–200. doi:10.1016/j.ijpara.2009.06.009.

[32] M. Opsteegh, M. Langelaar, H. Sprong, L. den Hartog, S. De Craeye, G. Bokken, D. Ajzenberg, A. Kijlstra, J. van der Giessen, Direct detection and genotyping of *Toxoplasma gondii* in meat samples using magnetic capture and PCR, Int. J. Food Microbiol. 139 (2010) 193–201. doi:10.1016/j.ijfoodmicro.2010.02.027.

[33] A. Vismarra, E. Barilli, M. Miceli, C. Mangia, M. Genchi, F. Brindani, L. Kramer, C. Bacci, *Toxoplasma gondii* in the Cornigliese sheep breed in Italy: Meat juice serology, in vitro isolation and genotyping, Vet. Parasitol. 243 (2017) 125–129. doi:10.1016/j.vetpar.2017.06.013.

[34] M. Seuri, P. Koskela, Contact with pigs and cats associated with high prevalence of *Toxoplasma* antibodies among farmers., Occup. Environ. Med. (2008). doi:10.1136/oem.49.12.845.

[35] B. Lassen, M. Janson, A. Viltrop, K. Neare, P. Hütt, I. Golovljova, L. Tummeleht, P. Jokelainen, Serological evidence of exposure to globally relevant zoonotic parasites in the Estonian population, PLoS One. (2016). doi:10.1371/journal.pone.0164142.

Table 1 – Serological and molecular results analysis carried out on meat juice (ELISA) and muscle samples (N-PCR) of sheep and goats intended for human consumption in northern Italy.

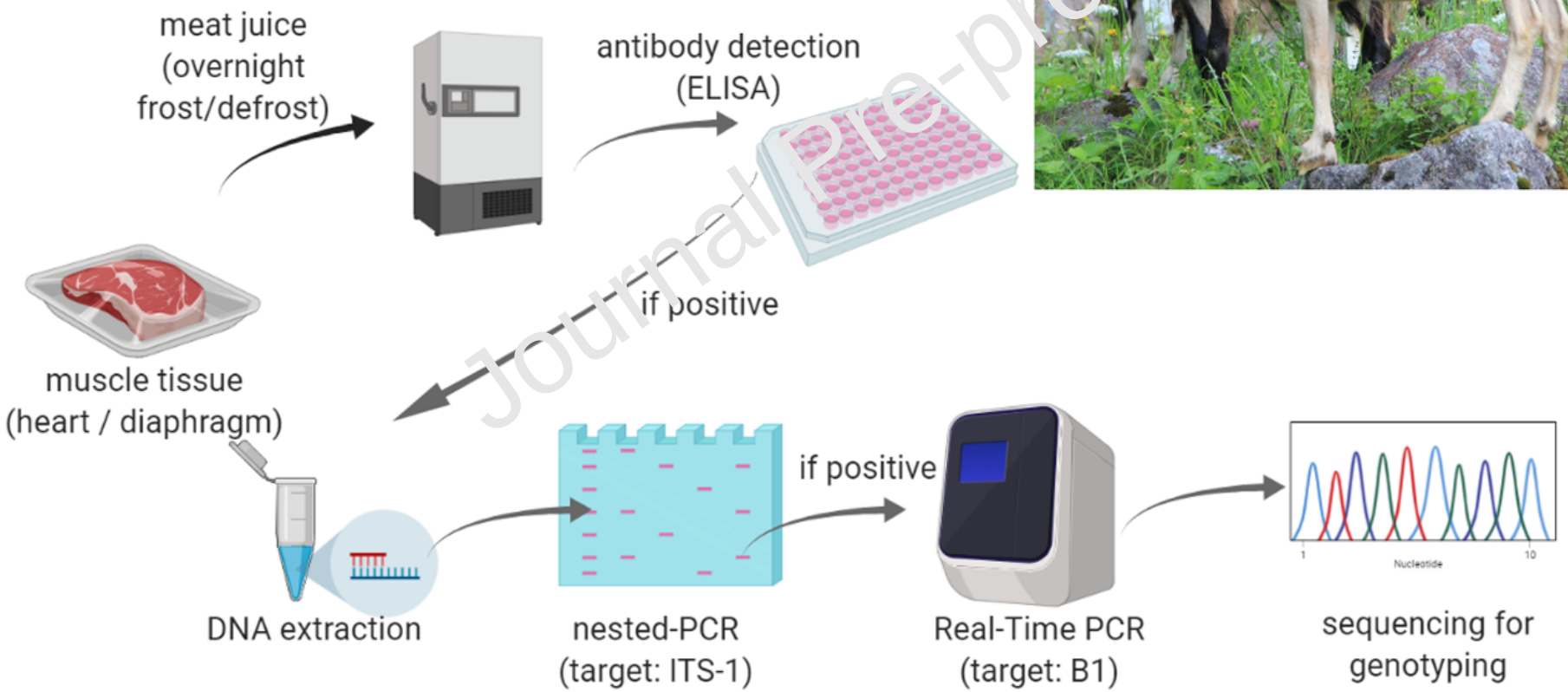
		ELISA			N-PCR			
variable	**category**	**positive /examined**	**P%[*] (95% CI[#])**	**S/P%[§] mean (S.D.[£])**	**positive/seropositive examined**	**P%[*] (95% CI[#])**	**positive /examined**	**P%[*] (95% CI[#])**
sheep		65/227	28.6 (23.14-34.83)	43.09 (52.17)	15/65	23.1 (14.52-34.65)	15/227	6.6 (4.05-10.62)
age	young (≤12 months)	33/193	17.1 (12.44-23.04)	29.65 (39.42)	2/33	6.1 (1.68-19.61)	2/193	1 (0.29-3.7)
	adult (>12 months)	32/34	94.1 (80.91-98.37)	119.39 (50.47)	13/32	40.6 (25.52-57.74)	13/34	38.2 (23.9-54.96)
origin	imported	5/111	4.5 (1.94-10.11)	15.65 (14.09)	0/5	0 (0-43.45)	0/111	0 (0-3.34)
	national	60/116	51.7 (42.72-60.61)	69.35 (61.14)	15/60	25 (15.78-37.23)	15/116	12.9 (7.99-20.24)
goat		14/51	27.5 (17.11-40.95)	42.9 (56.51)	3/14	21.4 (7.57-47.59)	3/51	5.9 (2.02-15.92)
age	young (≤12 months)	6/29	20.7 (9.85-38.39)	37.09 (61.67)	2/6	33.3 (9.68-70)	2/29	6.9 (1.91-21.97)
	adult (>12 months)	8/22	36.4 (19.73-57.01)	50.55 (49.26)	1/8	12.5 (2.24-47.09)	1/22	4.5 (0.81-2.18)
origin	imported	-	-	-	-	-	-	-
	national	14/51	27.5 (17.11-40.95)	42.9 (56.51)	3/14	21.4 (7.57-47.59)	3/51	5.9 (2.02-15.92)
overall		79/278	28.4 (23.44-33.99)	43.06 (52.89)	18/79	22.8 (5.22-18.73)	18/278	6.5 (4.13-10)

[*] P%: prevalence. [#] 95% CI: 95% confidence interval. [§] S/P% : sample to positive ratio. [£] S.D.: standard deviation.
Sample with S/P% ≥50 was considered positive. N-PCR was performed only on animals with positive ELISA results.

Table 2 - Risk factors associated with anti-*T. gondii* antibody detection in ELISA in sheep and goats intended for human consumption according to the generalized linear model.

Variable	Category	β+S.E.[a]	Wald's Chi-square	Odds ratio (95% CI)	*p*-value[b]
Species	sheep (reference)	0		1	
	goats	0.235±0.4967	0.224	1.265 (0.478-3.348)	0.636
Age	young (≤12 months) (reference)	0		1	
	adult (>12 months)	4.351±0.7535	33.345	77.576 (17.714-339.726)	**0.0001**
Species * age	goat * adult	-3.567±0.9871	13.059	0.028 (0.004-0.195)	**0.0001**
	goat * young				
	sheep * adult				
	sheep * young				

[a] Coefficient ± standard error; [b] Statistically significant variables are indicated by bold typing



Highlights

- *Toxoplasma gondii* infection in 278 slaughtered small ruminants was investigated in Italy
- *T. gondii* seroprevalence in sheep and goats was 28.6% and 27.5%, respectively
- *T. gondii* DNA was detected in muscle samples of 15 sheep and 3 goats
- Sequencing of a region within B1 gene revealed genotype II in all examined samples
- Small ruminants' meat was confirmed as an important source of human *T. gondii* infection