Circulating genotypes of Toxoplasma gondii in Northwestern Italy

CIRCULATING GENOTYPES OF TOXOPLASMA GONDII IN NORTHWEST ITALY

Elena Battisti[a], Stefania Zanet[a], Anna Trisciuoglio[b], Simona Bruno[a], Ezio Ferroglio[a,*]

[a] Department of Veterinary Science, University of Turin, Largo Braccini 2, 10095 Grugliasco (TO), Italy

[b] Department of Agricultural, Forest and Food Sciences, Largo Braccini 2, 10095 Grugliasco (TO), Italy

[*] Corresponding Author: ezio.ferroglio@unito.it

Abstract

Toxoplasma gondii is a zoonotic protozoan belonging to the phylum Apicomplexa, recently considered re-emergent like other food-borne parasites. The food-borne transmission of *T.gondii* by ingestion of meat with cysts seems to be one of the most common infection routes. *T.gondii* in Europe is genetically characterized by three main clonal genotypes, with a lesser prevalence of atypical genotypes. The aim of the study was to evaluate the distribution of *Toxoplasma gondii* genotypes circulating in domestic and wild animals in Northwest Italy, by using PCR-RFLP method. Out of 65 samples tested, we genotype 43 of them. Skeletal muscle samples derived from cattle (*Bos taurus*) (n=6), pig (*Sus scrofa domesticus*) (n=15), fox (*Vulpes vulpes*) (n=7), roe deer (*Capreolus capreolus*) (n=1) and wild boar (*Sus scrofa*) (n=14), and were processed by using a nested PCR of 6 RFLP markers: alt. SAG2, GRA6, 5'SAG2, BTUB, C22-8 and SAG1 [4]. PCR products were sequenced to perform in silico digestion by using the free online software NEBCutter. Thirty-one samples showed Type I alleles at least at one locus (p = 72.09%; CI95% 57.31% - 83.25%), while Type II and III alleles were present in two (p = 4.65%; CI95% 1.28% - 15.46%) and three (p = 6.98%; CI95% 2.40% - 18.61%) samples respectively. Seven samples (p = 16.28%; CI95% 8.12% - 29.97%) showed mixed Type alleles. Although genotypes prevalence did not differ statistically between species, wildlife showed more genetic variability than livestock with a higher prevalence of atypical genotypes (X2=4.10; p=0.04).

This is the first *T.gondii* genotyping study carried out in Northern Italy on both wild and domestic animals from the same area, showing unexpected high prevalence of genotype I and atypical genotypes. In other European countries, genotype II is the most common, with a prevalence ranging between 50% in Spain to

100% in France. Results show high prevalence of genotypes with a significant impact on human health, especially in immunocompromised patients and in newborns. It is likewise important to note the higher genetic variability of *T.gondii* in sylvatic species, and the potential role of wildlife as a source of atypical genotype of the parasite.

1. Introduction

Toxoplasma gondii is an obligate intracellular parasitic protozoan belonging to the phylum Apicomplexa, representing a potential hazard for public health. Human toxoplasmosis is spread worldwide with a seroprevalence that ranges between 22.5% and 75% (Montoya and Liesenfeld, 2004), and is considered the second leading cause of death among foodborne pathogens in United States (Jones et al., 2014). *Toxoplasma gondii* is a zoonotic parasite which has two types of hosts: the definitive host is a feline, while a wide range of warm blooded animals can act as intermediate hosts including men.

Humans can get infected by *Toxoplasma gondii* through different transmission routes: (i) food-borne transmission; (ii) vertical transmission; (iii) transplants and transfusions transmission. Food-borne transmission can occur by ingestion of water and vegetables contaminated by sporulated oocysts (Torrey and Yolken, 2013), by ingestion of raw or undercooked meat containing bradyzoites (Schluter et al., 2014) and finally by consuming unpasteurized milk or cheese made with raw milk containing tachyzoites (Mancianti et al., 2013). Primary infection of *Toxoplasma gondii* in pregnant women can lead to congenital toxoplasmosis in foetuses and newborns following the dissemination of tachyzoites through the placenta (McAuley, 2014).

While human toxoplasmosis in immunocompetent adults is mainly asymptomatic or characterized by mild symptoms like cervical lymphadenopathy, fever, malaise and myalgia (Weiss and Dubey, 2009), *Toxoplasma gondii* has been recently associated with different central nervous system disorders like epilepsy (Palmer, 2007) and schizophrenia (Torrey et al., 2007), and with an increased risk of car accidents (Flegr et al., 2002).

The meat-borne transmission of *Toxoplasma gondii* seems to be one of the most frequent infection routes (Cook et al., 2000). The prevalence of this parasite in livestock differs according to the considered species

and management systems. Concerning species, cattle has been considered an unsuccessful host for *Toxoplasma gondii,* for its ability to control infection by reducing or eliminating the parasite (Dubey and Jones, 2008). However, a serological survey showed antibodies against *T.gondii* in up to 92% of cattle (Avezza et al., 1993), while a meta-analysis study showed an average prevalence of 2.6% in beef of (Belluco et al., 2016). Pig, sheep and goat are the most infected species (Tenter et al., 2000), with an average prevalence of 12.3%, 14.7% and up to 26% respectively (Belluco et al., 2016). Management system of livestock has been considered a risk factor for *Toxoplasma gondii* infection. Organically raised pigs, for instance, show a significant higher seroprevalence than conventionally raised pigs, because this "animal-friendly" management system allows animals to come in contact with source of *Toxoplasma gondii* like contaminated soil, grass, water and infected rodents and birds (Kijlstra and Jongert, 2008).

Recently, the population of wild animals has increased in some parts of Europe - especially red deer, roe deer and wild boar (Ramanzine et al., 2010) - and consequently also the popularity of game meat. Consumption of game meat has been associated with an increased risk of congenital toxoplasmosis in pregnant women (Cook et al., 2000) and was the cause of several outbreaks of toxoplasmosis (Ross et al., 2001). Furthermore, Ferroglio et al. (2014) have observed that the prevalence of *Toxoplasma gondii* in wild animals was different according to feeding habits (carnivores and omnivores showed higher prevalence than herbivores) and to habitat types (in chamois and red deer authors recorded a lower prevalence than in roe deer).

The aim of this study was to evaluate the distribution of *Toxoplasma gondii* genotypes circulating in domestic and wild animals in Northwest Italy.

2. Materials and methods

Sixty-five previously PCR positive skeletal muscle samples (Bosio et al., 2013; Ferroglio et al., 2014) were analysed. Samples derived from cattle (*Bos taurus*) (n= 11), pig (*Sus scrofa domesticus*) (n= 15), fox (*Vulpes vulpes*) (n= 18), roe deer (*Capreolus capreolus*) (n= 3) and wild boar (*Sus scrofa*) (n= 18). Wild animals derived from hunting activities or were found dead between October 2009 and December 2012.

Samples were processed for *T. gondii* genotyping using a nested PCR of 6 RFLP markers: alt.SAG2, GRA6, 5'SAG2, BTUB, C22-8 and SAG1. Internal and external primers were previously described by Su et al. (2010), together with the PCR protocols. PCR reaction for Step 1 was carried out in a final volume of 25 µl containing 12.5 µl of PCR Master Mix (Promega, Madison, WI), 4 pmol of each external primer and 4 µl of target DNA. A gradient of temperature (55.3°C, 55.9°C, 56.6°C, 57.5°C, 58.2°C) was used for the annealing step in order to assess which of them was the best for each sample. The thermal cycler protocol was 95°C for 5 minutes, 30 cycles of 94°C for 30 seconds, 55/59°C for 1 minute and 72°C for 2 minutes, followed by a step of 72°C for 10 minutes. The I step product was used as template for step 2 reaction. Briefly, 12.5 µl of PCR Master Mix (Promega, Madison, WI), 8 pmol of each internal primer and 3 µl of template were used. The thermal cycler protocol was 95°C for 5 minutes, 35 cycles of 94°C for 30 seconds, 60°C for 1 minutes and 72°C for 1.30 minutes, and finally 72°C for 10 minutes. DNA extracted from *Toxoplasma gondii* genotype I, II and III cell cultures was used as positive control and amplified during each PCR step, together with a negative control.

RFLP was carried out for alt.SAG2 and GRA6 markers, with the digestion protocol previously described by Su et al. (2010). At the same time, all the PCR products were sequenced (Macrogen Europe, The Netherlands) and in silico digested for all the markers by using the free online software NEBCutter (New England Biolabs Inc; http://tools.neb.com/NEBcutter2/index.php).

3. Results

Out of 65 samples tested, we obtain sequences that allow us to genotype 43 of them (7 bovines, 15 pigs, 7 foxes, 1 roe deer and 14 wild boars). Only for one sample it was possible to obtain the amplification for all the inquired markers, while most of the samples were genotyped at less than 6 alleles because PCR amplifications failed. Results are summarized in Table 1. Thirty-one samples out of 43 showed Type I alleles at least at one locus (p = 72.09%; CI95% 57.31% - 83.25%), while Type II and III alleles were present in two (p = 4.65%; CI95% 1.28% - 15.46%) and three (p = 6.98%; CI95% 2.40% - 18.61%) samples respectively. Seven samples (p = 16.28%; CI95% 8.12% - 29.97%) showed mixed Type alleles. Although genotype prevalence did

not differ statistically between species, wildlife showed more genetic variability than livestock with a higher prevalence of atypical genotypes (X2=4.10, p=0.04, Fisher's exact test) (Fig.1).

4. Discussion

To the authors knowledge, this is the first *Toxoplasma gondii* genotyping study carried out in Northern Italy on both wild and domestic animals from the same area. In fact, previous studies conducted in Italy (Dubey et al., 2008; Mancianti et al., 2013; Verin et al., 2013; Mancianti et al., 2014; Bacci et al., 2015; Formenti et al., 2016) have not investigated both wildlife and livestock at the same time.

Particularly in Europe and North America, genetic structure of the parasite has been considered mainly clonal, with 3 distinct genotypes (type I, II and III) (Boothroyd and Grigg, 2002). Recently, a fourth clonal genotype has been observed in USA (Khan et al., 2011). In South America, however, the genetic structure of the parasite is more heterogeneous with the presence of many non-clonal atypical genotypes (Shwab et al., 2014). Although amplification for some markers failed, genotype I seems to be the most prevalent in the analysed area followed by atypical genotypes, while Type II and III show a significantly lower prevalence. These results are in contrast with other European countries, in which the prevalence of genotype II is high and ranges between 50% in Spain (Calero-Bernal et al., 2015) to 100% in France (Richomme et al., 2009; Aubert et al., 2010).

Interestingly, there were no differences between wildlife and livestock in the genotypes prevalence, except for atypical strains that show higher prevalence in wild animals. Such results could suggest an overlap of domestic and sylvatic cycle of the parasite. As a matter of fact, the points of contact between livestock and wildlife have increased in last decades. The enhanced number of wild animals on one hand, together with the moving of the farming systems from intensive to more extensive ones due to consumer requirements, could have increased the risk of pathogens transmission at wild-domestic interface (Gortazar et al., 2007). Although definitive sylvatic hosts are not present in the wild, atypical genotypes seem to be more prevalent in wild hosts than in livestock. In particular, a higher prevalence was recorded in foxes and wild boars. This finding can be explained by the scavenging habits of these species; for them, the meat-transmission of

Toxoplasma gondii is possible, and through this way they could come in contact with different genotypes infected-preys. For instance, they could feed on migrating birds which act as biological carriers and reservoir hosts of the parasite (Yan et al., 2016). Furthermore, the infection may reduce the motility of birds and hence increasing the susceptibility to predation (Hubalek, 2004).

5. Conclusion

Despite the disease burden even greater to that of salmonellosis, campylobacteriosis and other food-borne pathogens (Scharff, 2012), human toxoplasmosis is still considered a neglected disease. *Toxoplasma gondii* is frequently underreported as a food safety concern; however, like other parasites, it has recently been considered a re-emerging pathogen (Dorny et al., 2009). In the Northwest Italy, toxoplasmosis is the second most frequent zoonosis, with 893 reported cases between 1999 and 2012 (Ferroglio et al., 2016).

Results of the study showed high prevalence of genotype I and atypical genotypes of the parasite both in livestock and in game species, with a significant impact on human health. This parasite can bring on a wide spectrum of symptoms, and the severity of the symptomatology could vary also based on the genotype. A difference in the mouse virulence of genotypes has been observed: genotype I have an LD_{100} of 1 tachyzoites while genotype II and III have an LD_{100} of more than 1000 tachyzoites (Sibley and Boothroyd, 1992). Also in human, genotype I and atypical genotypes have been associated to a more severe symptomatology in immunocompromised patients (Khan et al., 2005) and in newborns (Xiao and Yolken, 2015). It is likewise important to note the higher genetic variability of *Toxoplasma gondii* in sylvatic species, and the potential role of wildlife as a source of atypical genotype of the parasite.

Together with the increase in the number of susceptible persons globally due to the rise of cancer, ageing, malnutrition and HIV infection (WHO, 2014; Dorny et al., 2009), these results should put the focus on *Toxoplasma gondii* and its consequence for human health, to act accordingly. In particular, it is of fundamental importance to increase the prevention of the infection through the spread of *Toxoplasma gondii*-knowledge in the society. It is of the same importance also to progress in the genotyping method, in

order to increase the sensitivity and the simplicity of the technique, and in the spectrum of analysed species, to assess the real diffusion of the parasite and its genetic structure.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgement

The authors would like to acknowledge Prof. Laura Kramer who kindly provide positive controls for this study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Ajzenberg, D., Banuls, A.L., Dumetre, A., Demar, M., Carme, B., Dardè, M.L., **2004.** Genetic diversity, clonality and sexuality in *Toxoplasma gondii*. Int. J. Parasitol. 34(10), 1185-1196

Aubert, D., Ajzenberg, D., Richomme, C., Gilot-Fromont, E., Terrier, M.E., de Gevigney, C., Game, Y., Maillard, D., Gibert, P., Dardé, M.L., Villena, I., **2010.** Molecular and biological characteristics of *Toxoplasma gondii* isolates from wildlife in France. Vet. Parasitol. 171, 346-349

177

178 Avezza, F., Greppi, G., Agosti, M., Belloli, A., Faverzani, S.,**1993.** Bovine Toxoplasmosis: the Results of Sero-
179 Epidemiologic Study (in French). Att. soc. ital. buiatria. 25: 621-624.

180 Bacci, C., Vismarra, A., Mangia, C., Bonardi, S., Bruini, I., Genchi, M., Kramer, L., Brindani, F., **2015.** Detection
181 of *Toxoplasma gondii* in free-range, organic pigs in Italy using serological and molecular methods. Int. J. Food
182 Microbiol. 202, 54-56

183

184 Bellucco, S., Mancin, M., Conficoni, D., Simonato, G., Pietrobelli, M., Ricci, A., **2016.** Investigating the
185 determinants of *Toxoplasma gondii* prevalence in meat: a systematic review and meta-regression. PLoS One.
186 11(4): e0153856

187

188 Boothroyd, J.C., Grigg, M.E., **2002**. Population biology of *Toxoplasma gondii* and its relevance to
189 human infection: do different strains cause different disease? Curr. Opin. Microbiol. 5, 438-442

190

191 Bosio, F., Zanet, S., Gennero, M.S., Bergagna, S., Canton, C., Trisciuoglio, A., Grande, D., Ferroglio, E., **2013.**
192 *Toxoplasma gondii* in piedmont: epidemiology in wildlife and livestock. LXVII Congress SISVet Società Italiana
193 Scienze Veterinarie, Brescia (Italy), September 17-19

194

195 Calero-Bernal, R., Saugar, J.M., Frontera, E., Perez-Martin, J.E., Habela, M.A., Serrano, F.J., Reina, D., Fuentes,
196 I., **2015.** Prevalence and Genotype Identification of *Toxoplasma gondii* in Wild Animals from Southwestern
197 Spain. J. Wildl. Dis. 51(1), 233-238

198

199 Cook, A.J.C., Gilbert, R.E., Buffolano, W., Zufferey, J., Petersen, E., Jenum, P.A., Foulon, W., Semprini, A.E.,
200 Dunn, D.T., **2000.** Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control
201 study. BJM. 321, 142-147

Dorny, P., Praet, N., Deckers, N., Gabriel, S., **2009.** Emerging food-borne parasites. Vet. Parasitol. 163, 196-206

Dubey, J.P., Jones, J.L., **2008.** *Toxoplasma gondii* infection in humans and animals in the United States. Int. J. Parasitol. 38(11), 1257-1278

Dubey, J.P., Huong, L.T., Lawson, B.W., Subekti, D.T., Tassi, P., Sundar, N., Velmurugan, G.V., Kwock, O.C., Su, C., **2008.** Seroprevalence and isolation of *Toxoplasma gondii* from free-range chickens in Ghana, Indonesia, Italy, Poland, and Vietnam. J. Parasitol. 94(1), 68-71

Ferroglio, E., Bosio, F., Trisciuoglio, A., Zanet, S., **2014.** *Toxoplasma gondii* in sympatric wild herbivores and carnivores: epidemiology of infection in the Western Alps. Parasites & Vectors. 7:196

Ferroglio, E., Tiberti, D., Guiso, P., Durbiano, N., Zanet, S., De Micheli, S., **2016.** Epidemiology of zoonotic disease in Piedmont- Northwestern Italy: a retrospective analysis of hospital discharge records. LXX Congress SISVet Società Italiana Scienze Veterinarie, Palermo (Italy), June 13-16

Flegr, J., Havlicek, J., Kodym, P., Maly, M., Smahel, Z., **2002.** Increased risk of traffic accidents in subjects with latent toxoplasmosis: a retrospective case-control study. BMC Infect.Dis. 2, 11

Formenti, N., Gaffuri, A., Trogu, T., Viganò, R., Ferrari, N., Lanfranchi, P., **2016.** Spread and genotype of *Toxoplasma gondii* in naturally infected alpine chamois (*Rupicapra r. rupicapra*). Parasitol.Res. 115(5), 2115-2120

225 Gortazar, C., Ferroglio, E., Höfle, U., Frolich, K., Vicente, J., **2007.** Diseases shared between wildlife and
226 livestock: a European perspective. Eur.J.Wildl.Res. 53, 241-256
227
228 Hubalek, Z., **2004.** An annotated checklist of pathogenic microorganisms associated with migrating birds.
229 J.Wild.Dis. 40(4), 639-659
230
231 Jones, J.L., Parise, M.E., Fiore, A.E., **2014**. Neglected parasitic infection in the United States: toxoplasmosis.
232 Am. J. Trop. Med. Hyg., 90(5), 794–799
233
234 Khan, A., Su, C., German, M., Storch, G.A., Clifford, D.B., Sibley, L.D., **2005.** Genotyping of *Toxoplasma gondii*
235 strains from immunocompromised patients reveals high prevalence of type I strains. J. Clin. Microbiol. 43(12),
236 5881-5887
237
238 Khan, A., Dubey, J.P., Su, C., Ajioka, J.W., Rosenthal, B.M., Sibley, L.D., **2011.** Genetic analyses of atypical
239 *Toxoplasma gondii* strains reveal a fourth clonal lineage in North America. Int. J. Parasitol. 41, 645-655
240
241 Kijlstra, A., Jongert, E., **2008.** Toxoplasma-safe meat: close to reality? Trends Parasitol. 25(1), 18-22
242
243 *Mancianti, F., Nardoni, S., D'Ascenzi, C., Pedonese, F., Mugnaini, L., Franco, F., Papini, R., **2013.*** Seroprevalence,
244 detection of DNA in blood and milk, and genotyping of *Toxoplasma gondii* in a goat population in Italy. Biomed.
245 Res. Int. ID:905326
246
247 Mancianti, F., Nardoni, S., Papini, R., Mugnaini, L., Martini, M., Altomonte, I., Salari, F., D'Ascenzi, C., Dubey,
248 J.P., **2014.** Detection and genotyping of *Toxoplasma gondii* DNA in the blood and milk of naturally infected
249 donkeys (*Equus asinus*). Parasites & Vectors. 7:165
250

McAuley, J.B., **2014.** Congenital toxoplasmosis. J. Pediatric Infect. Dis. Soc. 3(1), 30-35

Montoya, J.G., Liesenfeld, O., **2004.** Toxoplasmosis. Lancet. 363, 1965–1976

Palmer, B.S., **2007.** Meta-analysis of three case controlled studies and an ecological study into the link between cryptogenic epilepsy and chronic toxoplasmosis infection. Seizure. 16(8), 657-663

Ramanzin, M., Amici, A., Casoli, C., Esposito, L., Lupi, P., Marsico, G., Mattiello, S., Olivieri, O., Ponzetta, M.P., Russo, C., Trabalza Marinucci, M., **2010.** Meat from wild ungulates: ensuring quality and hygiene of an increasing resource. Italian J Animal Science. 9:e61

Richomme, C., Aubert, D., Gilot-Fromont, E., Ajzenberg, D., Mercier, A., Ducrot, C., Ferté, H., Delorme, D., Villena, I., **2009.** Genetic characterization of *Toxoplasma gondii* from wild boar (Sus scrofa) in France. Vet. Parasitol. 164(2-4), 296-300

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. 21(3), 226-229

Scharff, R.L., **2012.** Economic burden from health losses due to foodborne illness in the United States. J. Food Prot. 75(1), 123-131

Schluter, D., Daubener, W., Schares, G., Groß, U., Pleyer, U., Luder, C., **2014.** Animal are key to human toxoplasmosis. Int. J. Med. Microbiol. 304, 917-929

Shwab, E.K., Zhu, X.Q., Majumdar, D., Pena, H.F.J., Gennari, S.M., Dubey, J.P., Su, C., **2014.** Geographical patterns of *Toxoplasma gondii* genetic diversity revealed by multilocus PCR-RFLP genotyping. Parasitol. 141, 453-461

Sibley, L.D., Boothroyd, J.C., **1992.** Virulent strains of *Toxoplasma gondii* comprise a single clonal lineage. Nature 359, 82–85

Su, C., Shwab, E.K., Zhou, P., Zhu, X.Q., Dubey, J.P., **2010.** Moving towards an integrated approach to molecular detection and identification of *Toxoplasma gondii*. Parasitol. 137, 1-11

Tenter, A.M., Heckeroth, A.R., Weiss, L.M., **2000.** *Toxoplasma gondii:* from animals to humans. Int.J.Parasitol. 30, 1217-1258

Torrey, E.F., Bartko, J.J., Lun, Z.R., Yolken, R.H., **2007.** Antibodies to *Toxoplasma gondii* in patients with schizophrenia: a meta-analysis. Schizophr.Bull. 33(3), 729-736

Torrey, E.F., Yolken, R.H., **2013.** *Toxoplasma* oocyst has a public health problem. Trends Parasitol. 29(8), 380-384

Verin, R., Mugnaini, L., Nardoni, S., Papini, R., Ariti, G., Poli, A., Mancianti, F., **2013.** Serologic, molecular and pathologic survey of *Toxoplasma gondii* infection in free-ranging red foxes (*Vulpes vulpes*) in Central Italy. J. Wildl. Dis. 49(3), 545-551

Weiss, L.M., Dubey, J.P., **2009.** Toxoplasmosis: a history of clinical observation. Int.J.Parasitol. 39, 895-901

WHO, **2014.** Global status report on noncommunicable diseases. (http://apps.who.int/iris/bitstream/10665/148114/1/9789241564854_eng.pdf?ua=1)

Xiao, J., Yolken, R.H., **2015.** Strain hypothesis of *Toxoplasma gondii* infection on the outcome of human disease. Acta Physiol. 213, 828-845

Yan, C., Liang, L.J., Zheng, K.Y., Zhu, X.Q., **2016.** Impact of environmental factors on the emergence, transmission and distribution of *Toxoplasma gondii*. Parasites & Vectors. 9:137

Sample ID		PCR-RFLP markers					
		alt.SAG2	GRA6	SAG1	BTUB	5'SAG2	C22-8
Roe deer	12/11	I	I	na	na	na	na
Fox	116/11	I	I	na	na	na	na
Fox	324/11	na	I	na	na	na	na
Fox	335/11	na	III	na	na	na	II
Fox	339/11	I	I	na	na	na	na
Wild boar	697/11	III	na	na	na	na	na
Wild boar	698/11	I	III	na	na	na	na
Wild boar	699/11	I	III	na	na	na	na
Wild boar	700/11	I	I	na	na	na	na
Wild boar	723/11	na	I	na	na	na	na
Wild boar	730/11	I	I	na	na	na	na
Wild boar	766/11	III	na	na	na	na	na
Wild boar	780/11	I	I	na	na	na	na
Wild boar	783/11	na	I	na	na	na	na

Fox	806/11	I	I	na	na	na	na
Wild boar	807/11	na	I	na	na	na	na
Wild boar	817/11	II	II	II/III	II	II	II
Wild boar	846/11	III	I	na	na	na	na
Wild boar	847/11	I	III	na	na	na	na
Fox	869/11	na	I	na	na	na	na
Fox	876/11	II	I	II/III	na	III	II
Wild boar	890/11	I	na	na	na	na	na
Pig	A4	na	I	na	na	na	na
Pig	A7	na	I	na	na	na	na
Pig	B8	na	I	na	na	na	na
Pig	C5	na	I	na	na	na	na
Pig	P51	na	I	na	na	na	na
Pig	P54	na	I	na	na	na	na
Pig	P55	I	I	na	na	na	na
Pig	P60	I	I	na	na	na	na
Pig	Q64	III	II	na	na	na	na
Pig	Q67	I	I	na	na	na	na
Pig	Q68	na	I	na	na	na	na
Pig	Q69	na	I	na	na	na	na
Pig	Q70	na	I	na	na	na	na
Pig	Q72	I	I	na	na	na	na
Pig	Q73	I	I	na	na	na	na
Cattle	6	I	I	na	na	na	na
Cattle	41	I	I	na	na	na	na

Cattle	60	I	I	na	na	na	na
Cattle	63	III	na	na	na	III	na
Cattle	64	na	II	na	na	na	na
Cattle	91	na	I	na	na	na	na

Table 1

Genotypes of *T.gondii* obtained in this study. The first two columns show the animal species and the ID of samples. Remaining columns show the results of RFLP for each marker (na = marker region could not be PCR-amplified).

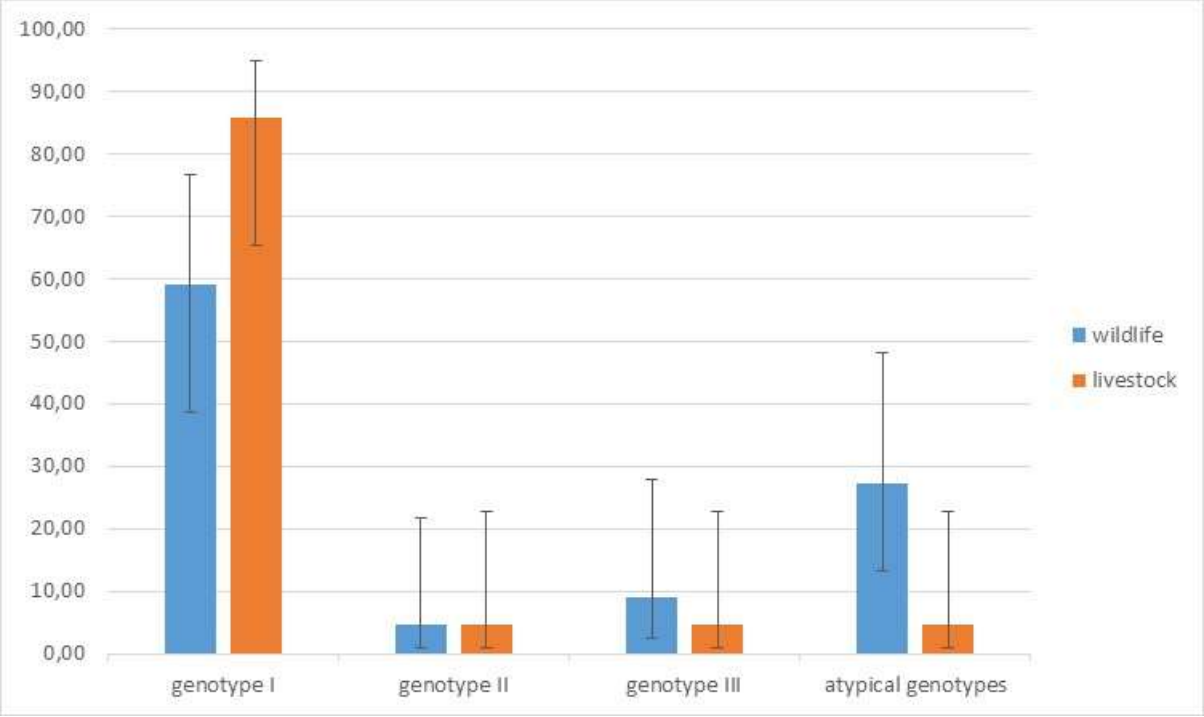

Fig. 1

Prevalence of *T.gondii* genotypes within wildlife and domestic populations. Significant differences ($p<0.05$; Fisher's exact test) were observed in the prevalence of atypical genotypes between wildlife and domestic populations. Whiskers represent 95% confidence intervals.