

Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in red deer from Central Italy

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Abstract

Neospora caninum and *Toxoplasma gondii* are cosmopolite protozoan parasites impacting on human and animal health. In particular, *T. gondii* commonly infects human beings and all warm-blooded animals, while *N. caninum* is responsible for bovine abortion and neuromuscular disease in dogs. The aim of the presented survey was to evaluate the occurrence and prevalence of these parasites in the most numerous Italian red deer population. The sera of 60 red deer (*Cervus elaphus*) inhabiting Central Italy (43°56'N 10°55'E) and killed by selective hunting were examined using an indirect fluorescent antibody test (IFAT) for both *N. caninum* and *T. gondii* antibodies. White blood cells (buffy coat) were also checked by PCR and *T. gondii* DNA was genotyped. Thirteen out of 60 sera (22%) scored positive for *Toxoplasma*, 17 samples (28%) were *Neospora* positive. Coinfection was recorded in 5 cases (8%). *T. gondii* (genotype II) and *N. caninum* DNA was detected in one and 3 samples of buffy coat, respectively. The presented study is the first to examine the occurrence of these parasites in the most numerous red deer Italian population, confirming this animal species as carrier of the investigated pathogens. These animals spread near human settlements, co-inhabiting with final hosts of *T. gondii* and *N. caninum* and could contribute to their transmission to domestic ruminants and humans. In particular, the seroprevalence value for *N. caninum* was the highest among European records.

Key words

Cervus elaphus, tissue apicomplexa, IFAT, PCR, genotyping

INTRODUCTION

The expansion of red deer (*Cervus elaphus*) in the last decades of the 20th century, with a tenfold growth in abundance between 1970 – 1998, fits into a more general phenomenon of recovery experienced by the species in Europe in the same period [1]. In the Northern and Central Apennines the present occurrence of red deer is exclusively due to reintroduction. These wild ruminants have been consistently present in the province of Pistoia (43°56'N 10°55'E) since at least 1995, with a gradual spread across these areas, and becoming the most abundant deer population in Central Italy. Hence, since 2000, the population has been the target of selective hunting, also in response to the increasing request for a careful planning of sustainable densities to minimize the impact of these populations on human activities [2]. These animals, in fact, show a high density near human settlements. The presence of red deer is especially proven 5 km from Pistoia city centre. For these reasons, they would be exposed to some parasite infection, transmitted by domestic carnivores, such as toxoplasmosis and neosporosis. The life cycle of the etiological agents involves cats and dogs as definitive hosts, respectively. Herbivores infection occurs by ingestion of oocysts on vegetation or in feed [3]. Infections with *Toxoplasma gondii* are widely prevalent in humans and animals worldwide, especially food animals. *Neospora caninum* can cause neuromuscular disease in dogs and high rates of abortion in cattle [4], and the occurrence of specific

antibodies has been reported in healthy humans [5]. Such infections impact on human and animal health. In particular, *T. gondii* commonly infects many domesticated and wild animals used for food production and the European Food Safety Authority (EFSA) has recommended the surveillance and monitoring of toxoplasmosis in humans, animals and foodstuffs [6].

Data from literature on red deer from the Italian Alps report the occurrence of antibodies to *T. gondii* with prevalences from 0% [7] – 40% [8] and to *N. caninum* in 2% [9]. Furthermore, other records from Europe refer to the seroprevalence of *T. gondii* in a 0% of animals from Belgium [3], 45% in animals from the Czech Republic, Slovakia and Austria [10]. The reports about the occurrence of *N. caninum* in red deer in Europe refer 0% from Belgium [3], 6% from Czech Republic [10], 12% from Spain [11] and 13% from Poland [12].

However, to the best of our knowledge, there are no data about the presence of such infections in the population of *C. elaphus* from Central Italy. Therefore, the aim of the current study was to evaluate the occurrence of antibodies to *T. gondii* and *N. caninum* in red deer killed by selective hunting during the 2014–2015 hunting season.

MATERIALS AND METHOD

Blood samples were collected from the hearts of 60 red deer (43 females and 17 males), killed between January – February 2015 during selective hunting, to which was added ethylenediaminetetraacetic acid (EDTA). Age classes were attributed by histology from the incisors and animals were

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grouped as yearlings (6–24 months old, 14 males and 13 females), sub-adult (2–4 years old, 3 males) and adult (>2 years, 30 females). All the animals were shot in an area comprising the North-Eastern Pistoia province.

Plasma was used to perform indirect immunofluorescent antibody test (IFAT) and kept frozen at -20°C until processed. IFAT for *T. gondii* was performed using 10-well slides (ToxoSpot IF, BioMerieux Roma, Italy) and an antiovine-IgG fluorescein isothiocyanate antibody produced in rabbit (Sigma-Aldrich, USA). Specimens were screened with a threshold dilution of 1:40 and positive samples were end-titrated using 2-fold dilution. Antibodies versus *N. caninum* were determined using 12-well slides (Fullerton Lab, Fullerton CA., USA). Plasma samples were screened with a threshold dilution of 1:50 and positive samples were then end-titrated using 2-fold dilution [10]. Positive and negative reference sera were used.

DNA from white blood cells (buffy coat) was extracted for molecular studies using Blood /cultured cell genomic DNA extraction mini kit (Fisher molecular biology, USA), using 200 µl of specimen, as recommended by the manufacturer. A nested PCR was performed, as described by Jones et al. [13], using the B1 gene as target. Genotypic characterization of *T. gondii* DNA was performed by PCR amplification of 12 genetic markers (SAG1, 3-SAG2, 5-SAG2, SAG2 new, SAG3, BTUB, GRA6, C22–8, C29–2, L358, PK1) [14].

PCR was carried out, as described by Müller et al. [15], using primers that amplify a 337 bp fragment of the Nc 5 region.

RESULTS AND DISCUSSION

Thirteen of 60 sera (22%) scored positive for anti-*Toxoplasma* antibodies, with titers ranging from 1:40 – 1:2560, while 17 samples (28%) were *N. caninum* positive, with antibody titers ranging from 1:50 – 1:1600. Co-infection was recorded in 5 cases (8%). Positive and negative animals seemed to be quite homogeneously spread in the sampled area.

T. gondii DNA (genotype II) was detected in one *Toxoplasma* serologically negative sample. Three of 60 samples were found to be *N. caninum* PCR positive. Among them, 2 specimens were from seropositive animals (titers 1:50 and 1:400), while the other was from a seronegative red deer (Tab. 1).

This study is the first report of *T. gondii* and *N. caninum* seroprevalence in red deer in Italy associated with molecular analysis aimed at demonstrating parasitic DNA. A molecular survey dealing with the detection of both parasites was carried out in red deer from Belgium [3] with no positive findings. In the presented study, buffy coat was employed to perform molecular examinations, since blood was the unique sample available.

The seroprevalence for *T. gondii* (22%) is in agreement with European data, showing an intermediate value. The occurrence of antibody titers ≥ 100 in 10/13 subjects, along with parasite DNA in the blood of a yearling male, would suggest a horizontal transmission pattern as hypothesized by Formenti et al., [8]. The authors are aware of the fact that blood is not the most sensitive specimen. Nevertheless, the occurrence of parasites' DNA in such a sample would be indicative of an active infection.

The striking finding was the high seroprevalence value versus *N. caninum*. This result is higher than in both Italian

Table 1. Gender, age class and parasitological data of positive red deer.

Number	Gender	Age class	Toxoplasma gondii IFAT titre	Neospora caninum IFAT titre	PCR results
1	female	Y	1:80	negative	negative
2	female	Y	1:80	negative	negative
3	female	A	1:80	negative	negative
4	female	Y	negative	1:200	negative
5	female	Y	1:2560	negative	negative
6	female	A	1:40	1:400	negative
7	male	Y	negative	1:50	negative
8	male	Y	negative	negative	<i>Toxoplasma gondii</i> positive
9	female	Y	1:320	negative	negative
10	male	Y	negative	negative	negative
11	female	Y	1:640	1:200	negative
12	male	Y	1:80	1:400	negative
13	female	A	1:80	1:800	negative
14	female	Y	negative	1:400	<i>Neospora caninum</i> positive
15	female	Y	1:40	negative	negative
16	male	SA	negative	1:50	<i>Neospora caninum</i> positive
17	female	A	negative	1:200	negative
18	female	A	negative	1:50	negative
19	male	Y	negative	1:200	negative
20	female	Y	negative	negative	<i>Neospora caninum</i> positive
21	male	Y	1:80	1:50	negative
22	male	Y	1:40	negative	negative
23	female	A	negative	1:400	negative
24	male	SA	negative	1:50	negative
25	female	Y	1:40	negative	negative
26	male	SA	negative	1:100	negative
27	female	A	negative	1:800	negative
28	female	A	negative	1:1600	negative

Y – yearling; A – adult; SA – sub-adult

and European records. High titers were also observed in this case, as well as the occurrence of 3 PCR positive animals.

Reservoir definitive hosts, both cats and dogs, are widely present in this area, in particular dogs for wild boar hunting. Furthermore, the Pistoiese Apennine region is densely frequented by several free-ranging dogs and cats cohabiting with 'Popolo degli Elfi' (Elfi -people), a large community movement (about 200 individuals in over 15 groups of families) who reject the consumer society and follow an alternative rural way of life, that occupy abandoned and isolated houses far from urban areas. The animals are not registered nor checked for health status, and feed mainly on live prey.

The results obtained in the current study reveal that *T. gondii* infection occurs in red deer in Central Italy, and the detection of *T. gondii* genotype II is in agreement with recent findings in small mammals from the Czech Republic [16]. These data suggest a potential risk for a growing group of consumers when meat is administered raw or undercooked, considering that genotype II is the main cause of human infections [17]. Compared with beef, in fact, venison is considered superior in nutrient composition, thus offering potential benefits for human consumption, and it received higher scores for most of the sensory attributes examined [18].

The presented results confirm red deer as the most important wild ruminant acting as intermediate host for *N. caninum* [4]. Furthermore, this species may be a carrier of pathogens, which can be transmitted to domestic ruminants and humans.

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