Toxoplasma gondii infection in raptors from Italy: seroepidemiology and risk factors analysis

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Abstract

Toxoplasma gondii is a zoonotic parasite infecting a wide range of intermediate hosts, including birds. Nevertheless, scant information on the spread of infection in wild bird populations is available to date. With the aim of updating information on *T. gondii* infection in birds of prey and possible risk factors associated to the infection, a serosurvey was planned on both wild and captive raptors. An overall of 93 raptors from Northern Italy were tested for the presence of anti-*T. gondii* antibodies with a commercial modified agglutination test (MAT). A *T. gondii* prevalence of 10.7% was recorded; the highest seroprevalence was observed within the Family *Strigidae* (12.5%). Only wild animals tested positive; any statistical difference among species, taxonomic family, age, origin, use, migratory behavior and diet composition was not highlighted. Toxoplasmosis in birds of prey, sentinel species for the environmental spread of *T. gondii*, should be always considered within sanitary programs devoted to avian species protection.

Key words

Toxoplasma gondii, birds of prey, wild birds, MAT, serology

1. Introduction

Toxoplasma gondii is an Apicomplexa parasite able to infect a wide range of vertebrates, including birds [1]. *T. gondii* infection has been reported in numerous domestic and wild avian species by serologic and molecular tools [2, 3, 4, 5, 6, 7]. Nevertheless, clinical cases of toxoplasmosis were rarely reported in birds [5, 8, 9, 10] with the record of neurological signs including ataxia, head tilt, walking in circle and blindness [11]. Birds of prey seem to be resistant to clinical toxoplasmosis. In experimental studies performed on raptors (*Caracara plancus*, *Bubo virginianus*, *Strix varia*, *Asio otus* and *Buteo jamaicensis*), all the animals developed anti-*T. gondii* antibodies without clinical signs [12, 13, 14]. In free-ranging birds, a fatal case of toxoplasmosis was diagnosed by immunohistochemistry in a bald eagle (*Haliaeetus leucocephalus*) in the USA [15], whereas severe hepatitis caused by *T. gondii* was reported in a road-killed barred owl (*Strix varia*) from Canada [16]. Despite the limited clinical importance, birds of prey are good sentinel species for the evaluation of the environmental circulation of *T. gondii* and indirect indicators of the risk of acquire the infection mainly for humans and livestock [17]. Indeed, carnivore birds are exposed to the risk of acquiring the infection horizontally, through the consumption of infected preys harboring tissue cysts or through the ingestion of water or food contaminated with sporulated oocysts [5].

Data on the spread of *T. gondii* among raptors in Europe are scarce, with seroprevalence values varying according to the investigated species [2, 3, 6]. In France, a serosurvey recorded a prevalence of *T. gondii* infection of 35.8% [2]; similarly, in Portugal a seroprevalence of 50% was detected in surveyed wild birds, including Accipitridae (64.9%) and Strigidae (25%) [6]. Finally, in a large-scale serosurvey carried out in Spain, Falconidae (31.8%), Accipitridae (24.1%), Strigidae (50.2%) and Tytonidae (13.3%) showed antibodies anti-*T. gondii* [3].

Besides wild birds, *T. gondii* is reported to infect also a wide range of avian domestic species and animals kept in captivity [11]. Nevertheless, few surveys have been carried out on captive birds of prey, mostly on animals kept in zoological gardens [18, 19], whereas *T. gondii* infection has not been investigated so far in falconry raptors. The holding of raptors in captivity in Europe is continuously

increasing: indeed, falconry has being gaining importance not only for hunting purposes, but also for its social function. Raptors are used for the "bird control" in airports, agricultural fields, farms, landfills, and public buildings [20, 21, 22], as well as for educational purposes in teaching farms, historical reenactment or flight exhibition.

The spread of falconry has lead in last years to an increase in the number of captive raptors, often in structures hosting a wide numbers of animals. The maintenance of birds of prey in captivity may raise several critical points: from a sanitary viewpoint, the high density of animals hosted in the same aviary may lead to an increase of the risk of transmission of infective diseases [23]. Moreover, from an ethological point of view, the limited space available for each animal, smaller than those in nature, may be a source of stress for territorial animals. In captivity birds of prey may therefore be exposed to several sources of stress, possibly leading to immunosuppression with the consequent increase of susceptibility to certain diseases [24]. Particularly, toxoplasmosis may exacerbate in immunocompromised subjects [25], and therefore it should be considered among the infectious diseases possibly occurring in captive animals.

Considering the importance of *T. gondii* in avian species and the scarce available information, a seroepidemiological survey on the infection in both wild and captive raptor species was planned, with the aim of updating epidemiological data and evaluating a selection of possible risk factors associated to the infection.

2. Materials and methods

2.1 Study population and sample collection

In the period comprised between July 2015 and July 2016, an overall of 93 raptors were recruited for the study. Seventy-six wild birds were sampled during first medical examination prior to their hospitalization in a Wildlife Recovery Centre in Northern Italy. In addition, 17 raptors kept in captivity were included in the study population and sampled during routine medical examinations; particularly, four animals were housed and exhibited in a zoological garden, whereas 13 belonged to private owners using animals for educational purposes or for falconry (hunting, breeding, or bird

control). Seventeen different species belonging to the Families Accipitridae (8), Falconidae (2), Strigidae (6) and Tytonidae (1) were represented. All species included in the study are listed on the IUCN Red List of Threatened Species as "least concern" [26].

Individual data of each animal were collected; age was recorded or estimated on the basis of size, weight, and plumage features, according to the species [27], classifying animals as young or adult. Animals from Wildlife Recovery Centre were categorized as "wild"; in addition, wild birds were classified as migrant or sedentary (non-migrant), according to migratory behavior [28]. Moreover, for wild animals the diet composition was recorded [27]. Captive birds were classified according to their use: exhibition, education, and falconry.

Prior an adequate containment, from each animal approximately 0.5 ml of blood was collected from the jugular or the ulnar subcutaneous vein in tubes containing anticoagulant (lithium heparin). Once clotted, sera were separated by centrifugation (15 min, 2120 g) and then stored at -20°C until analysis.

2.2 Serological examination

Sera samples were tested for the presence of anti-*T. gondii* Immunoglobulin-G using a commercial modified agglutination test (MAT) (Toxo-Screen DA, bioMérieux, Marcy-l'Étoile, France). The assay was performed according to manufacturer's instructions, testing sera at 1:40 and 1:4000 dilution. Positive and negative controls provided in the kit were added in each testing plate.

2.3 Statistical analysis

Seroprevalence was calculated for each of the considered categories [29]. Chi-square test was used to verify the possible association between *T. gondii* infection and the following variables: species; taxonomic Family; age (young, adult); origin (wild, captive); if wild, migratory behavior (migratory, sedentary) and diet composition (mainly mammals, mainly birds); if captive, their use: exhibition, education, and falconry. Fisher's exact test was used when the number of observations per category was below 5. The level of significance for independent variables was set to 0.05. Statistical analysis was performed by SPSS (version 19.0; SPSS, Chicago, IL).

3. Results

An overall prevalence of 10.7% (95% CI: 5.9-18.7) was recorded, with ten birds out of 93 tested showing anti-*T. gondii* antibodies (MAT≥1:40). Particularly, one Eurasian sparrowhawk, one Eurasian buzzard, one Western Marsh-harrier (*Circus aeruginosus*), one Eurasian hobby (*Falco subbuteo*), four little owls (*Athene noctua*) and one tawny owl were positive to serological assay with low antibody titre (1:40); only one common kestrel (*Falco tinnunculus*) showed an antibody titre of 1:4000. Considering the taxonomic Family, three Accipitridae (11.5%), two Falconidae (8.7%) and two Strigidae (12.5%) scored positive, whereas none of the four barn-owl, Family Tytonidae, included in the study showed antibodies anti-*T. gondii* (Table 1).

All positive birds proceeded from the Wildlife Recovery Centre, whereas none of the captive animals showed anti-*T. gondii* antibodies. Considering age, the prevalence of infection in adult and young animals was calculated at 11.1% and 10.3%, respectively. Statistical analysis did not show any differences among considered categories (species, taxonomic Family, age, origin, use, migratory behavior, and diet composition) (Table 2).

4. Discussion

The present survey reported data of epidemiological relevance, contributing to the knowledge on the diffusion of *T. gondii* infection in birds of prey populations. *T. gondii* seroprevalence was investigated in 17 species of birds of prey, and a new species was added to the list of wild birds potentially representing intermediate hosts of *T. gondii*. Indeed, to the best of our knowledge, this is the first report of anti-*T. gondii* antibodies in an Eurasian hobby. The serological survey, the first on raptors in Italy, was carried out using a MAT: this serological test, not requiring a species-specific conjugate, is considered the technique of choice in wild animals and particularly in wild birds (Dubey, 2002). The use the same technique in several surveys [2, 3, 6, 30] allows a direct comparison of the obtained data.

In the present study, prevalence value resulted higher in Strigidae (12.5%) and Accipitridae (11.5%) if compared to Falconidae (8.7%), although the difference was not significant. Considering the species highly spread in the study area, including Eurasian buzzard, common kestrel, little owl and tawny owl, a consistent number of samples was collected, allowing the comparison with previous published data on *T. gondii* seroprevalence in these species. For other species, i.e. Eurasian sparrowhawk, western marsh-harrier, and Eurasian hobby, only few individuals were sampled in the present survey, not allowing to obtain representative data on the spread of the infection within the population, however the results confirmed the potential of such species to act as intermediate host for *T. gondii*. Only one Eurasian buzzard tested positive to MAT, resulting in a much lower prevalence (8.3%) if compared to those reported in France (79%) [2], Portugal (69.2%) [6] and Spain (51%) [3]. Similarly, anti-*T. gondii* antibodies were detected in one common kestrel out of 18 examined (5.5%); in analogy, Cabezon et al. [3] found only four positive common kestrels out of 13 tested (30.8%) while those examined by Aubert et al. [2] resulted all seronegative. Considering Strigidae, in the present survey a higher prevalence was recorded in little owls (23.5%) than tawny owls (8.3%); similar prevalence values ranging from 15.8% in little owls [3] and from 13.2% to 20% in tawny owls [3, 6] were reported, while in France a higher prevalence of 50% was reported in tawny owls [2].

Different levels of seroprevalence in the sampled species may be due to differences in diet composition, reflecting variable infection values in prey species [2]; indeed, Cabezon et al. [3] demonstrated higher *T. gondii* prevalence values in carnivorous or scavenger birds if compared to piscivorous birds. The present study included species preying on small rodents and other small mammals (i.e. Eurasian buzzard, northern long-eared owl, tawny owl, barn-owl), species preying principally on other birds, such as Eurasian sparrowhawk and Eurasian hobby, and species preying on both small mammals and other birds or even small amphibians and reptiles (i.e. western marsh-harrier, Eurasian eagle-owl) [27]. Both small mammals and predated birds, showing prevalence values varying according to the species [31, 32], have been demonstrated to act as reservoir of *T.*

gondii for other wild and domestic animals [32, 33]. The main way of *T. gondii* infection in raptors is therefore represented by the ingestion of preys containing tissue cysts.

The feeding habits could explain also the lack of antibodies anti-*T. gondii* in captive birds included in the survey; indeed, their diet is based on raw, de-frost meat, and the procedure of freezing the meat has been demonstrated to inactivate *T. gondii* tissue cysts [34]. However, *T. gondii* infection was recorded also in raptors kept in zoological gardens [18]; it could be hypothesized the possibility for captive raptors to acquire the infection preying on infected small mammals or birds entering into the exhibit and thus acting as a source of infection. Since the infection is not to be excluded also in controlled environment, *T. gondii* should be always considered among raptors pathogens, both in wild and in captivity.

Birds of prey, as predators at the top of trophic chains, may thus have an important role in both domestic and sylvatic cycle of *T. gondii*. As predators, they represent sentinel species: prevalence value recorded in these animals may be considered an indicator of the spread of *T. gondii* in the environment and in the species sharing the same ecological habitats and thropic chains [17]. Indeed, considering the study area, *T. gondii* infection has been previously reported in other domestic and wild animals, with seroprevalence values variable according to considered species [35, 36, 37, 38, 39, 40].

Particularly, sedentary species, such as little owl and tawny owl, could be used as indicators of the environmental spread of *T. gondii*. On the contrary, migrant species (western marsh-harrier, common kestrel, and Eurasian hobby) [28] may acquire the infection during the migration and thus may be used as indicators of the genetic variability of *T. gondii* [41, 42]. In wild birds, besides the report of clonal types [7, 43], atypical strains of *T. gondii* have been reported [42, 44]. The sexual recombination is indeed an event occurring mostly in the sylvatic cycle of *T. gondii*, with the consequent possibility to generate new strains with unpredictable biological properties and virulence [45], suggesting the need to investigate on the circulation of *T. gondii* genotypes among avian populations.

Another unexplored aspect of *T. gondii* infection in wild birds is its pathogenicity and its clinical relevance. Clinical signs surely ascribable to *T. gondii* infection were reported in very few cases in raptors [15, 16]. Moreover, a few authors demonstrated the association between *T. gondii* seropositivity and the presence of clinical abnormalities in raptors; particularly, the case of three individuals with clinical signs typical of/ascribable to the acute phases of toxoplasmosis was reported [30]. Besides, *T. gondii* has been demonstrated able to modify the behavior and the fitness of various intermediate hosts [46]: the association between *T. gondii* infection and causes of mortality or of admission to wildlife centers (i.e. collisions with cars or buildings, illegal hunting) should thus be further investigated. Therefore, *T. gondii* should be always included within sanitary programs devoted to wild avian species protection.

Concluding, the study of *T. gondii* infection in birds of prey should be deepened, particularly verifying through molecular detection or isolation the effective competence of these avian species as intermediate hosts of *T. gondii*, and investigating the genetic variability of *T. gondii* strains involved in the infection and their association with clinical aspects that still remains unclear.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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331 **Table 1: Seroprevalence of *Toxoplasma gondii* in raptors from Northern Italy using the modified agglutination test (MAT≥1:40)**

Family Common name (species)	No. examined	No. positive (MAT titre)	P%	Origin (if captive, use[b])	If wild, migratory behavior	If wild, diet composition [c]
Accipitridae	**26**	**3**	**11.5**			
Eurasian Sparrow hawk (*Accipiter nisus*)	2	1 (1:40)	50	wild	sedentary	B
African Hawk-eagle (*Aquila spilogaster*)	1	0	0	captive (F)	-	-
Eurasian Buzzard (*Buteo buteo*)	12	1 (1:40)	8.3	wild (11), captive (1, F)	sedentary	M
Red-tailed Hawk (*Buteo jamaicensis*)	1	0	0	captive (Ed)	-	-
Ferruginous Hawk (*Buteo regalis*)	1	0	0	captive (F)	-	-
Western Marsh-harrier (*Circus aeruginosus*)	2	1 (1:40)	50	wild	migrant	M, B, I
Bald Eagle (*Haliaeetus leucocephalus*)	1	0	0	captive (Ex)	-	-
Harris's Hawk (*Parabuteo unicinctus*)	6	0	0	captive (1 F, 3 Ed, 2 Ex)	-	-
Falconidae	**23**	**2**	**8.7**			
Eurasian Hobby (*Falco subbuteo*)	3	1 (1:40)	33.3	wild	migrant	B, I
Common Kestrel (*Falco tinnunculus*)	18	1 (1:4000)	5.5	wild	migrant	M, I
n. d. [a]	2	0	0	captive (1 Ed, 1 Ex)	-	-
Strigidae	**40**	**5**	**12.5**			
Northern Long-eared Owl (*Asio otus*)	8	0	0	wild	sedentary	M
Little Owl (*Athene noctua*)	17	4 (1:40)	23.5	wild	sedentary	B, I, A, R
Rock Eagle-owl (*Bubo bengalensis*)	1	0	0	captive (Ed)	-	-
Eurasian Eagle-owl (*Bubo bubo*)	1	0	0	wild	sedentary	M, B
Eurasian Scops-owl (*Otus scops*)	1	0	0	wild	migrant	I
Tawny Owl (*Strix aluco*)	12	1 (1:40)	8.3	wild	sedentary	M, I
Tytonidae	**4**	**0**	**0**			
Barn-owl (*Tyto alba*)	4	0	0	1 wild, 3 captive (Ed)	sedentary	M, A, I

[a] not determined; [b] F: falconry, Ed: education, Ex: exhibition; [c] B: birds, M: mammals, I: insects, A: amphibians, R: reptiles

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Variable	Category	Positive/examined	prevalence %	p-value
Age	young	4/39	10.3	0.587 [a]
	adult	6/54	11.1	
Origin	captive	0/17	0	0.118 [a]
	wild	10/76	13.2	
Migratory behavoir (if wild)	migrant	3/23	13.5	0.637 [b]
	sedentary	7/52	13	
Diet composition (if wild)	mainly mammals	5/56	8.9	0.054 [a]
	mainly birds	5/19	26.3	

[a] Pearson's Chi-square test; [b] Fisher's exact test

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