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Toxoplasma gondii in wild felides in Poland

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Abstract

Background *Toxoplasma gondii* is a globally distributed protozoan parasite that infects a wide range of warm-blooded vertebrates, including humans. Felids, as definitive hosts, play a central role in its transmission through shedding of environmentally resistant oocysts. While numerous studies on domestic cats exist, less is known about the epidemiology of *T. gondii* in wild felids, particularly in Central Europe. In Poland, two strictly protected carnivore species—the Eurasian lynx (*Lynx lynx*) and the European wildcat (*Felis silvestris*)—may contribute to parasite circulation, yet no data on their infection status were previously available. This study aimed to investigate the prevalence of *T. gondii* in these species and to explore the genotypes present in Polish populations.

Results Samples (sera/tissue fluid and fragments of organs) from 29 wild felines (21 lynx, 8 wildcats) collected across three regions of Poland were analyzed using serological (ELISA) and molecular (nested and real-time PCR) methods. Specific IgG antibodies were detected in 14/25 (56%) individuals, while IgM was identified in two wildcats, suggesting recent infection. *T. gondii* DNA was confirmed in 11/27 (40.7%) animals by nested PCR and in 10/27 (37.0%) by real-time PCR, with concordant results (Cramer's V test, $p = 0.018$). Parasite DNA was recovered from multiple tissues, most frequently the heart, lungs, spleen, and brain. Genotyping of positive samples revealed predominance of type II lineages (82.6%), followed by type I (13.0%) and type II/III (4.3%).

Conclusions This study demonstrates that both Eurasian lynx and European wildcats in Poland are frequently exposed to and infected with *T. gondii*, confirming their role in the parasite's sylvatic cycle. The predominance of type II genotypes mirrors patterns in Central European domestic cats and livestock, suggesting shared transmission pathways. Given the conservation concerns for these endangered species and their ecological importance, systematic surveillance of *T. gondii* and other pathogens in wild felids, as well as in their prey, is recommended. These data expand the understanding of parasite circulation in Central Europe and highlight potential health risks for vulnerable carnivore populations.

Keywords *Felis silvestris*, Genotyping, *Lynx lynx*, Serology, Toxoplasmosis, Wildlife

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Background

Toxoplasma gondii is a widespread protozoan parasite able to infect warm-blooded vertebrates, including farm animals, wildlife and humans [9, 37]. Humans usually become infected by ingestion of undercooked meat containing tissue cysts, or by consumption of food or water contaminated with sporulated oocysts, excreted by cats. Felids, in which the sexual reproduction of the parasite occurs, serve as the definitive hosts of *T. gondii* [54]. The main inter-species transmission route in herbivores is the consumption of food contaminated with oocysts, while in carnivores and omnivores, it is eating infected prey with *T. gondii* tissue cysts [8]. Being definitive hosts, felids play a crucial role in the epidemiology of toxoplasmosis and are responsible for *T. gondii* outbreaks. Therefore, determining the prevalence of infection not only in domestic cats but also in wild felids is essential for developing effective wildlife control measures [48]. Shedding of *T. gondii* oocysts has been confirmed in several wild felid species [37, 48]. High *T. gondii* seroprevalence has been reported in most free-living felid species worldwide. Very high seroprevalence was observed in European wildcat (*Felis silvestris*) in Spain (85%, [27]). In the Eurasian lynx (*Lynx lynx*), seroprevalence reached 86.1% in Finland [20] and 75.6% in Sweden [35]. Three clonal lineages (I, II, III) of *T. gondii* are the most frequently isolated in Europe, of which type II was most often isolated from animals [1, 17, 34]. The genotyping studies in South America and Europe have also shown the presence of virulent *T. gondii* hybrid isolates or atypical strains [25].

In Poland, there are two species of wild felids: Eurasian lynx (*Lynx lynx*) and European wildcat (*Felis silvestris*). Despite the increasing trend in other countries [4], the Eurasian lynx population in Poland decreased between 1980 and 2001 [19] and has not yet recovered [38]. Both wild felid species are listed as strictly protected species of large carnivores in Poland. The Eurasian lynx occurs in Poland in two separate areas: north-eastern and south-eastern parts of the country [21]\, while the European wildcat inhabits only the eastern part of the Polish Carpathians [30]. Both species face major threats in Europe, including habitat fragmentation, human-related mortality, and infectious diseases [13, 22, 52]. Additionally, lynx populations suffer from low genetic diversity [39], while wildcats are impacted by hybridization with domestic cats [2]. Therefore, studies on the prevalence of pathogens and the susceptibility of these species to infections can provide important information to increase knowledge about the potential threats to their populations as well as their role in pathogen circulation.

Due to the protected status of Eurasian lynx and European wildcat in Poland, each pathogen analysis gives a unique opportunity to increase the knowledge of potential threats to their population as well as their role in the circulation of pathogens. This study aimed to investigate if free-living wild felids (Eurasian lynx and European wildcat) are involved in the *T. gondii* cycle in wildlife in Poland, as well as to address knowledge gap about *T. gondii* genotypes circulating in Polish wild felid population.

Methods

Sample collection

Samples were collected from 29 wild felids (21 Eurasian lynx and eight European wildcats) from three voivodeships of Poland: Podkarpackie ($n = 14$), Podlaskie ($n = 14$) and Lubelskie ($n = 1$) (Fig. 1). When possible, the age of the animals was determined based on dentition, dimensions, body condition, and fur. Individuals were classified into three age groups: juveniles (< 1 year old), subadults (1–2 years old), and adults (> 2 years old).

Samples from 28 animals were collected post-mortem (from individuals found dead, mostly due to vehicle collisions), while one serum sample from a wildcat was obtained ante-mortem during a veterinary intervention. No animal was sacrificed for this study. Consent to collect and store the material from protected animal species was issued by the Polish General Director for Environmental Protection (DZP-WG.6401.1.2022.EB).

During necropsy, depending on the possibilities and condition of the corpse, about 200 g fragments of organs (lungs, liver, spleen, brain, skeletal muscle, heart, tongue) and tissue fluid from the chest cavity were collected. Samples were transported to the laboratory immediately, and serum was separated after blood centrifugation. Serum and tissue samples were frozen at −20 °C until further analysis. Before testing, samples were brought to room temperature. Tissues were used for molecular analysis to determine the presence of *T. gondii* DNA, while serum samples or tissue fluids were used for serological analysis to detect specific antibodies [3].

Serological tests

Due to sample availability (as tissue fluid or serum could not be obtained from all animals), the presence of antibodies was tested in 25 of the 29 animals (20 Eurasian lynx and 5 European wildcats; see details in Table 1). IgG antibodies to *T. gondii* were detected using a commercial ELISA kit (NovaTec VetLine Toxoplasma ELISA, Gold Standard Diagnostic, Frankfurt, Germany) designed for the quantitative determination of anti-*T. gondii* antibodies in serum/tissue fluid samples. The sensitivity and specificity of this ELISA for feline samples is over 98% according to the producer. The test was performed according to the manufacturer's instructions.

The VetLine *T. gondii* IgM ELISA (Gold Standard Diagnostic, Frankfurt, Germany) was used for the quantitative determination of specific IgM antibodies to *T. gondii.*

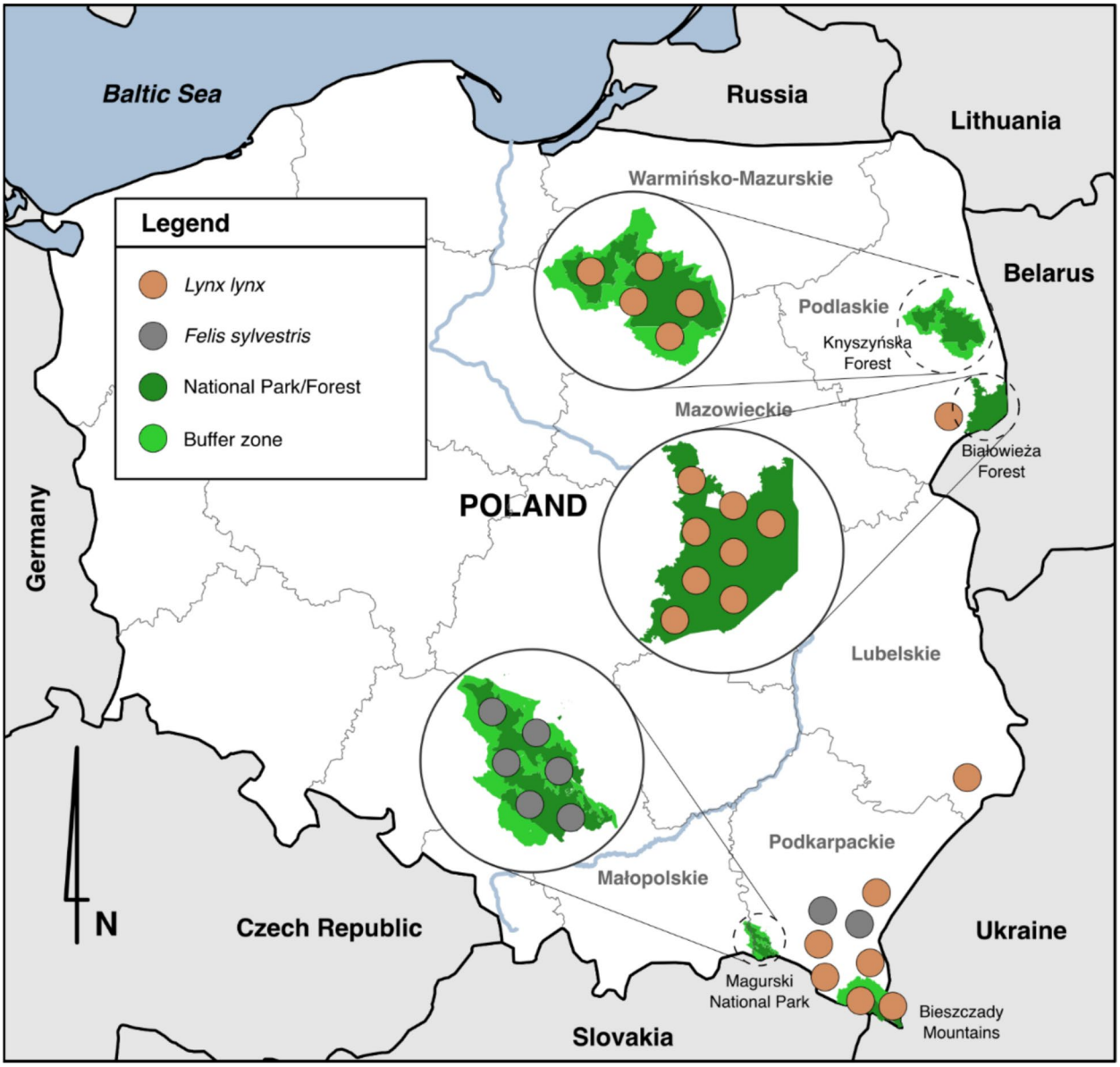

Fig. 1 Geographical distribution of wild felid samples collected in Poland. The map shows the sampling locations of Eurasian lynx (*Lynx lynx*, brown) and European wildcats (*Felis silvestris*, grey) across three voivodeships/states (Podkarpackie, Podlaskie, and Lubelskie). Green areas represent National and Landscape Parks, with lighter green indicating buffer zones

The test was performed according to the manufacturer's instructions.

The absorbances of both ELISAs were read at a wavelength of 450 nm with an EPOCH spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA). The results were calculated and interpreted according to the manufacturer's instructions.

Molecular examination

Sample processing and DNA extraction

Tissue samples of free-living wild felids (depending on the availability: lungs, liver, brain, skeletal muscles, heart, spleen and tongue were individually tested from each animal) digested with pepsin solution based on the method described by Dubey and Beattie [7]. Ten to thirty grams of samples were cut and homogenized in 25–75 ml of 0.9% NaCl. Next, the homogenates were mixed with 50–150 ml of acid-pepsin solution (2.6 g of pepsin, 7 ml of HCl, and 0.9% NaCl filled up to 500 ml, pH 1.1–1.2) and digested in a shaking water bath at 37 °C for 90 min. The digested material was filtered through gauze and centrifuged at 1200 × g for 10 min. The pellets were collected, resuspended in 7–21 ml of phosphate-buffered saline (PBS, pH 7.4), and centrifuged (1200 × g for 10 min). The supernatant was removed, and the pellet was resuspended in 1–3 ml of 0.9% NaCl [40, 45].

Table 1 Detailed data of wild felid individuals tested for *Toxoplasma gondii* by species, sex, age, test type, and results of analyzes

ID	Species	Sex	Age	Serology IgG	Serology IgM	Nested PCR	Real-time PCR
1	*Felis silvestris*	nd	nd	1	0	LS—0	LS—0
						SN—0	SN—0
						LR- 0	LR—0
2	*Felis silvestris*	nd	nd	0	0	MS – 1 (N)	MS– 1 (N)
						SN—0	SN—0
3	*Felis silvestris*	nd	nd	0	0	MS –0	MS –0
						SN – 0	SN – 0
						LR—0	LR—0
4	*Felis silvestris*	nd	nd	nt	nt	BN – 1 (D)	BN – 1 (D/N)
						MS – 1(D)	MS – 1 (D/N)
						SN – 0	SN – 0
						LR—0	LR—0
5	*Felis silvestris*	nd	nd	nt	nt	TE – 1 (D/N)	TE – 1 (D/N)
						MS—0	MS—0
6	*Lynx lynx*	M	nd	1	0	MS –0	MS –0
						SN – 0	SN – 0
						LR—0	LR—0
7	*Felis silvestris*	F	nd	nt	nt	MS – 0	MS – 0
						BN—0	BN—0
8	*Lynx lynx*	nd	nd	nt	nt	MS – 1 (D)	MS – 1 (D)
9	*Lynx lynx*	F	nd	0	0	MS – 0	MS – 0
						SN—0	SN—0
10	*Lynx lynx*	F	ad	0	0	MS – 0	MS – 0
						BN—0	BN—0
11	*Lynx lynx*	F	subad	0	0	MS – 0	MS – 0
						BN – 0	BN – 0
						HT – 0	HT – 0
						SN—0	SN—0
12	*Felis slivestris*	nd	nd	1	0	BN – 0	BN – 1 (D)
						HT – 0	HT – 0
						SN – 0	SN – 0
						LS—0	LS—0
13	*Felis silvestris*	M	juv	1	1	nt	nt
14	*Lynx lynx*	F	juv	1	0	LS – 0	LS – 0
						HT – 0	HT – 0
						SN – 0	SN – 0
						LR—0	LR—0
15	*Lynx lynx*	M	juv	0	0	LS – 0	LS – 0
						HT – 0	HT – 0
						SN – 0	SN – 0
						LR—0	LR—0
16	*Lynx lynx*	F	ad	1	0	LS – 0	LS – 1 (N)
						HT – 1 (N)	HT – 1 (D/N)
						SN – 0	SN – 1 (N)
						LR—0	LR – 1 (N)
17	*Lynx lynx*	nd	juv	0	0	nt	nt
18	*Lynx lynx*	M	juv	1	0	HT- 1 (N)	HT-1 (D)
19	*Lynx lynx*	M	juv	0	0	LS – 0	LS – 0
						HT – 0	HT – 0
						SN – 0	SN – 0
						LR—0	LR—0

Table 1 (continued)

ID	Species	Sex	Age	Serology IgG	Serology IgM	Nested PCR	Real-time PCR
20	*Lynx lynx*	M	ad	0	0	MS – 0	MS – 0
						BN—0	BN—0
						HT – 0	HT – 0
						SN—0	SN—0
21	*Lynx lynx*	F	ad	1	0	LS – 0	LS – 0
						HT – 0	HT – 0
						SN – 0	SN – 0
						LR—0	LR—0
22	*Lynx lynx*	F	ad	1	0	LS – 0	LS – 0
						HT – 0	HT – 1 (D)
						SN – 0	SN – 0
						LR—0	LR—0
23	*Lynx lynx*	F	ad	1	0	LS – 0	LS – 0
						HT – 1 (N)	HT – 1 (D/N)
						SN – 0	SN – 0
						LR—0	LR—0
24	*Lynx lynx*	M	ad	1	0	LS – 0	LS – 0
						HT – 1 (D/N)	HT – 0
						SN – 0	SN – 0
						LR—0	LR—0
25	*Lynx lynx*	M	subad	1	0	LS – 1 (D/N)	LS – 0
						SN – 1 (D)	SN – 0
						LR – 1 (D/N)	LR—0
26	*Lynx lynx*	M	ad	0	0	HT – 1 (N)	HT – 0
						LS – 1 (D/N)	LS – 0
						SN – 1 (D)	SN—0
27	*Lynx lynx*	M	juv	1	0	LS – 0	LS – 0
						HT – 1 (N)	HT – 0
						SN – 0	SN – 0
						LR—0	LR—0
28	*Lynx lynx*	F	ad	1	0	LS – 0	LS – 0
						HT – 0	HT – 1 (D)
						SN – 0	SN – 0
						LR—0	LR—0
29	*Lynx lynx*	M	ad	0	0	LS – 0	LS – 0
						HT – 0	HT – 0
						SN – 0	SN – 0
						LR—0	LR—0

ad adult, *juv* juvenile, *subad* subadult, *nd* no data, *nt* not tested, *0* negative result, *1* positive result, *0/1* doubtful result, *MS* muscles, *LS* lungs, *SN* spleen, *LR* liver, *BN* brain, *TE* tongue, *HT* heart, *D* digested sample, *N* non-digested sample

A 100 µL of each suspension was used for DNA extraction using a commercial kit (QIAmp DNA Mini Kit; Qiagen, Hilden, Germany), according to the manufacturer's instructions. DNA was also extracted from 25 mg of each homogenized tissue sample without a digestion step. All DNA samples were stored at – 20 °C until further analysis.

Polymerase chain reaction (PCR)

DNA samples extracted separately from each type of sample from each animal were analyzed by nested polymerase chain reaction (PCR) for the presence of the B1 gene fragment of *T. gondii* using the method described by Grigg and Boothroyd [14]. DNA extracted from the RH and ME49 *T. gondii* strain was used as a positive controls and nuclease-free water was used as a negative control. The amplification products after electrophoresis were identified on an agarose gel under ultraviolet light. The PCR was carried out in a C1000 Thermal Cycler (Bio-Rad, Hercules, USA).

DNA extracted from tissue samples was also examined by real-time PCR for the presence of the B1 gene fragment of *T. gondii* according to the method by Lin et al. [26] using the commercial master mix IQ Supermix

(Bio-Rad). Positive and negative controls were included as described above.

To estimate the clonal type for selected B1 gene positive samples, Multiplex multilocus nested PCR-RFLP (Mn-PCR-RFLP) and Multilocus sequence typing (MLST) methods according to Su et al. [46] method were used with the using following markers: SAG1, SAG2 (5' and 3'), altSAG2, SAG3, GRA6, BTUB, C22–8, C29–2, L358, PK1 and APICO, as previously described [44].

Statistical analysis

Results were analyzed using basic descriptive statistics with the usage of Wilson score to calculate the 95% confidence interval (CI) (https://www.statskingdom.com/proportionconfidence-interval-calculator.html).

Statistical analysis was performed to assess the association between the results obtained from the nested PCR and real-time PCR assays. Cramer's V test was used, which compared positive and negative PCR results in the analyzed individuals, regardless of the organs in which the tests were performed. Comparison of the convergence of results between the analyzed organs was not possible due to the too small number of PCR and serology analyses in different organs of a given animal. No statistical analysis of the dependence of prevalence on area, sex or age was performed due to too few *T. gondii* detections for a given felide species.

Results

Serology

Overall, *T. gondii* IgG antibodies were detected in 14/25 (56%, 95% CI: 35.75–74.40) wild felids, while one sample yielded a doubtful (4%, 95% CI: 0.21–19.56). IgM antibodies were detected in one out of 25 (4%, 95% CI: 0.21–19.56) tested animals, one result was doubtful (4%, 95% CI: 0.21–19.56). Both cases concerned European wildcat. Antibodies to *T. gondii* were detected in 3/5 (60%, 95% CI: 18.93–92.35) of European wild cats and in 11/20 (55%, 95% CI: 32–75.57) of Eurasian lynx (Table 1, 2).

Table 2 Summary results of serology (IgG) and PCR depending on the species, origin, sex, and age of the tested felides

Variable		Serology	PCR
		N pos./N tested (% pos.)	N pos./N tested (% pos.)
Region	Podkarpackie	5/14 (35.7%)	5/14 (35.7%)
	Podlaskie	9/14 (64.3%)	8/14 (57.1%)
	Lubelskie	1/1	1/1
Sex	Female	7/9 (77.8%)	4/10 (40%)
	Male	6/11 (54.5%)	5/10 (50%)
Age	Juvenile	4/7 (57.1%)	2/6 (33.3%)
	Subadult	1/2 (50%)	1/2 (50%)
	Adult	7/10 (70%)	6/10 (60%)

PCR

Out of 27 tested wild felids (no tissue samples available from two wildcats tested *ante-mortem*), *T. gondii* DNA was proved by nested PCR in 11 of them (40.7%, 95% CI: 23.71–59.81). Real-time PCR allowed detection of *T. gondii* DNA in 10/27 felids (37.0%, 95% CI: 20.18–57.04). Positive samples were obtained from 3/15 lungs, 2/16 liver, 3/22 spleen, 2/6 brain, 3/11 skeletal muscle, 8/16 heart, and 1/1 tongue tissues. One European wild cat was tested positive in two tissues (brain and skeletal muscle), one Eurasian lynx was tested positive in four tissues (lungs, heart, spleen, and liver), two Eurasian lynx were tested positive in three tissues (lungs, spleen, liver and lungs, heart, spleen, respectively) (Table 1). Real-time PCR and nested PCR results were significantly, but moderately associated with each other, as indicated by the Cramer's V test ($V = 0.457$, approximate significance: $p = 0.018$).

DNA extracted from tissues (brain, heart and skeletal muscle) of 5 animals was successfully genotyped (Table 3); one sample (E. wildcat, no. 4, brain) was able to genotype with 10 markers (SAG1, 5′SAG2, altSAG2, SAG3, GRA6, BTUB, C22–8, L358, PK1 and APICO) and according ToxoDB, this pattern may correspond to the ToxoDB #3 genotype. In the case of another sample (E. lynx, no. 16, heart) genotyping was successfully performed with 7 markers (SAG3, GRA6, BTUB, L358, PK1, C29-2, and APICO). Other samples of heart (E. lynx, no. 23) and skeletal muscle (E. wildcat, no. 2) were able to genotype with only 3 markers (5′SAG2, SAG3, GRA6 and BTUB, L358, APICO) By using 12 markers, a total of 23 amplicons were obtained. In total, type II, type I and type II/III of *T. gondii* lineages were determined for 19 (82.6%), 3 (13.0%) and 4.3% amplicons, respectively (Table 3).

Discussion

This study provided data on prevalence of *T. gondii* in two species of rare felids, the Eurasian lynx and European wildcat in their Central European populations. Since both species are considered endangered in the area, the results may contribute to a better understanding of the limits to their population development.

In the present study, total *T. gondii* seropositivity in both species was 56% which is a similar or lower result than in other European countries [20, 27, 35, 37]. In Poland, seroprevalence in domestic cats *T. gondii* was 50% [47], while taking into account only the south-eastern part of the country it was higher (69%) [41].

Even though *T. gondii* was not tested before in wild felids in Poland, it has been investigated and confirmed in other wildlife species, such as: European bison (*Bison bonasus*) [5, 24], red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and wild boar (*Sus scrofa*) [23, 33, 53], American mink (*Neogale vison*) [16], Eurasian badger

Table 3 The results of genotyping B1 positive samples using additional markers

No of animals	Species	Type of sample	SAG1	5'+3' SAG2	SAG3	GRA6	BTUB	Alt SAG2	C22-8	C29-2	L358	PK1	APICO
23	E. lynx	Heart	n/a	II	II	II	n/a	n/a	n/a	n/a	n/a	n/a	n/a
4	E. wildcat	Brain	II/III	II	II	II	II	II	II	n/a	II	II	I
2	E. wildcat	Skeletal muscle	n/a	n/a	n/a	n/a	II	n/a	n/a	n/a	II	n/a	I
16	E. lynx	Heart	n/a	n/a	II	II	II	n/a	n/a	II	II	II	I

*At SAG1 locus, types II and III are indistinguishable, *n/a* product not amplified

(*Meles meles*), martens (*Marten* sp.) [40, 45] and several rodent species [15]. However, most of the samples were collected in regions where wild felids are not present, so studies of other wild mammals should be extended to these areas.

Most epidemiological studies in felid populations are based on determining seroprevalence by detecting specific IgG antibodies to *T. gondii* (e.g. [6, 51]). In the present study, occurrence of IgM antibodies was also assessed. Interestingly, not only IgG antibodies but also IgM antibodies were found in two European wildcats, which can indicate that they were in the acute phase of the *T. gondii* infection. However, as demonstrated in experimental studies, in some cases of *T. gondii* infection in cats, IgM levels may be undetectable. The opposite situation was also observed, where persistent (> 16 weeks) IgM was detected, e.g. in cats infected with Feline Immunodeficiency Virus (FIV). Therefore, IgM titers cannot be used to predict when a cat will shed oocysts.

The animals seropositive for IgG were probably in the chronic phase of infection (with tissue cysts), which was confirmed in most cases by detecting *T. gondii* DNA in tissue samples (in those where DNA was not detected, cysts might have been in other, not tested organs). Failure to extract DNA from all potentially infected tissues may have been due to the limited sample size or number of parasites, as well as the random distribution of tissue cysts or poor-quality DNA. The efficiency of *T. gondii* DNA detection can be increased by multiplying the parasites using a bioassay or cell culture before DNA extraction, which was not performed in this study.

In the present study, *T. gondii* DNA has been detected in 14 (51.9%) wild felids using the nested and/or real-time PCR (Tables 1, 2). Genotyping selected nested PCR samples showed predominance of genotype II *T. gondii* (82.6%). The knowledge about the genetic diversity of *T. gondii* in Poland is limited. In the studies on detection of *T. gondii* in retailed raw meat products from slaughter animals in Poland, type II was the most prevalent followed by type III [40, 42, 45]. However, another study from Poland showed a high prevalence of type III, detected in goat milk, while type II was less common [43]. Genotyping of *T. gondii* from domestic cats in Poland revealed type II alleles at almost all loci corresponds to the ToxoDB#3 genotype, commonly identified amongst cats in Central Europe [11, 18, 44]. Similarly, in the present study, genotyping of *T. gondii* DNA detected in the brain of one E. wildcat (No. 4) indicated the genotype ToxoDB#3. The predominance of genotype II in cats was also reported in Germany [36], Portugal [50] and Spain [29].

In Poland, molecular characterization of *T. gondii* in wild felids has not been reported so far. Presented results in wild felids partly correspond to a study in Switzerland,

where two different *T. gondii* genotypes in Eurasian lynx (*Lynx lynx*): a lineage II variant (ToxoDB #3) and a II x III recombinant strain were revealed [37]. In another study from Switzerland, the type II variant was also detected in small wild mammals (wild cats prey) [32].

Although toxoplasmosis is usually asymptomatic in domestic felids, infection in definitive hosts can alter the behaviour of intermediate hosts, increasing predation risk in rodents [32] and promoting bolder behaviour in infected wolves, such as higher dispersal and leadership rates [28]. Additionally, in domestic cats many co-infections with *T. gondii* occur, which may also be a phenomenon in wild felids, which needs further studies [10, 12, 49].

This study has some limitations. The relatively small sample size restricts the generalizability of the findings; however, obtaining sufficient material from endangered wildlife is inherently difficult. Moreover, all samples were frozen before testing, which might have caused potential degradation of antibodies and nucleic acids may have led to false negatives, suggesting that detection rates could have been higher in freshly collected specimens. This can also explain the positive result of molecular tests with the negative result of serological tests in this study. It should also be noted that the vast majority of samples used for serological testing consisted of tissue fluids rather than serum. This may have affected the test sensitivity and could also have led to the occurrence of false-negative results.

Results of the present study show that it is necessary to establish surveillance and control programs for wild felids, but also for the prey species that are part of their food chain and parasite circulation in the environment. This will result not only in the health protection of the rare felid species but also of other wild animal species. Another important aspect is to compare the sensitivity of different diagnostic techniques, as well as the best tissues to be analyzed to validate methods for *T. gondii* infection detection in the wild. Finding tissues with higher tropism in host species might be helpful for future monitoring studies.

In the present study, it has been confirmed that wild felids in Poland play a role in the transmission of *T. gondii* in the areas of their existence. Considering felid susceptibility to infection and protection of Eurasian lynx and European wildcat, it is recommended to monitor programs to assess the health status of wild felids.

Abbreviations

CI	Confidence interval
PCR	Polymerase chain reaction

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Authors' contributions

Conceptualization, A.D., J.K. and J.Z.; methodology, A.D., M.K. and J.S.; sample collection, M.K-S., K.S. and S.K.; investigation, A.D., D.K., M.K.-S., P.K. and J.Z.; resources, A.D., E.K. and J.S.; data curation, A.D., M.K-S., J.S., and P.K.; writing—original draft preparation, A.D. and J.S.; writing—review and editing, A.D., J.S., E.K., K.S. and M.K-S..; supervision, J.S. All authors listed have significantly contributed to this article's development and writing. All authors had read, edited, and approved the final manuscript.

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Data availability

All data are available from the corresponding authors.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare no competing interests.

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