Annals of Agricultural and Environmental Medicine 2019, Vol 26, No 4, 656–660
www.aaem.pl

ORIGINAL ARTICLE

Toxoplasma gondii infection in selected species of free-living animals in Poland

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A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of article

Sroka J, Karamon J, Wójcik-Fatla A, Dutkiewicz J, Bilska-Zając E, Zając V, Piotrowska W, Cencek T. *Toxoplasma gondii* infection in selected species of free-living animals in Poland. Ann Agric Environ Med. 2019; 26(4): 656–660. DOI: 10.26444/aaem/114930

Abstract

Introduction and objective. Free-living animals can play an important role as a reservoir of *Toxoplasma gondi;*, however, data concerning this issue in Poland are still limited.The aim of study was to assess the occurrence of *T. gondii* infection by using molecular methods in free-living animals in selected regions of Poland.

Materials and method. Tissues samples of 396 animals (foxes, muskrats, birds, martens, badgers, polecats, raccoons, minks, raccoon dogs, otters, small rodents and insectivores, and grass snakes were collected from various regions of Poland. After samples digestion, DNA was isolated using QIAmp DNA Mini Kit (Qiagen). DNA extraction from small rodents and insectivores samples was performed without digestion. Next, nested PCR (B1 gene) and, for a part of nested PCR positive amplicons, RFLP PCR, were performed according to the method by Grigg and Boothroyd (2001). The other part of nested PCR positive DNA isolates were genotyped using 5 genetic markers: SAG1, SAG2 (5'- and 3'), SAG3, BTUB and GRA6, based on the method by Dubey et al. (2006). These PCR products were sequenced and compared with the NCBI database using Blast.

Results. In total, in 50 of the 396 examined animals DNA of *T. gondii* was detected (12.6%). The highest percentages of positive results in PCR was obtained in martens (40.9%) and badgers (38.5%), lower in birds (27.3%) and the lowest in foxes (7.4%). The RFLP and multilocus PCR analysis showed the dominance of *T. gondii* clonal type II (or II/III).

Conclusions. The results of this study indicate the frequent *T. gondii* infection among free-living animals in Poland, especially martens and badgers, which may indirectly indicate that these animals contribute to the spread of the parasite in the sylvatic environment in Poland. The genotyping analysis showed the dominance of *T. gondii* clonal type II (or II/III).

Key words

Poland, Toxoplasma gondii, nested PCR, genotyping, free-living animals

INTRODUCTION

Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii*, is one of the most common parasitic infections of warm-blooded organisms, including humans [1]. Recently, toxoplasmosis has been ranked in the 4th position among foodborne parasites as disease causing the greatest global concern [2]. *T. gondii* infection can result in abortion in pregnant women or cerebral and ocular damage in newborns. An acquired infection of *T. gondii* can cause ocular abnormalities, and in immunocompromised individuals even leads to severe disease [3, 4].

T. gondii strains were classified into 3 major clonal lineages, types I, II, and III; however, other lineages, including atypical and recombinant genotypes, were also detected [5]. Type II is predominantly found in animals in Europe. However, recently, e.g. in Slovakia, Italy and Switzerland, type III and type I have also been detected [6–10]. Genotyping of isolated strains can improve knowledge about the circulation of the parasite, both on the global and a local geographical scale [11].

Free-living animals can play an important role as a reservoir of *T. gondi;* however, data concerning this issue in Poland are still limited. In the transmission of parasite in the sylvatic environment, an important role can play carnivores, typically infected by feeding on meat or carrion containing tissue cysts, but also herbivores who acquire an infection via food or water contaminated with oocysts excreted by definitive hosts – wild and domestic cats [1]. Small mammals are considered an important reservoir of *T. gondii* for foxes and raptorial birds, and their role has also been confirmed in the transmission of the parasite to pigs [12]. Rodents are considered to be important paratenic and intermediate hosts of this protozoan, and reservoirs for many bacterial and viral pathogens that can affect humans and animals. Companion animals (dogs and cats) feeding on small rodents contribute to the transmission of these pathogens from sylvatic to synanthropic environment, and to disease incidence in humans.

Since *T. gondii* is transmitted to a considerable extent by free-living carnivores, their high population densities (e.g. foxes, raccoons), pose increased health risks for humans and animals [13]. In the last decades, in Poland, the density of foxes has dramatically increased. Foxes often considerably exceed their natural habitats and occupy urban and peri-urban areas [14]. In the epidemiology of toxoplasmosis, an important role also seems to be played by birds of prey, which can indicate infections in local rodent populations. Birds also represent a source of infection for cats [15].

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Received: 17.10.2019; accepted: 29.11.2019; *first published: 19.12.2019*

Identification and genetic characteristics of *T. gondii* isolated from free-living animals can help in understanding the ways of circulating of the parasite in the environment. Thus, the aim of this study was to assess the occurrence of *T. gondii* infection in free-living animals in selected regions of Poland using molecular methods.

MATERIALS AND METHOD

Samples collection. Tissues samples from 396 animals: 148 red foxes (*Vulpes vulpes*), 16 muskrats (*Ondatra zibethicus*), 11 birds (species listed in Tab. 1), 22 European pine martens (*Martes martes*), 13 European badgers (*Meles meles*), 3 European polecats (*Mustela putorius*), 61 raccoons (*Procyon lotor*), 16 American minks (*Neovison vison*), 13 raccoon dogs (*Nyctereutes procyonoides*), 7 otters (*Lutra lutra*), 70 small rodents and insectivores (species listed in Tab. 1) and 16 grass snakes (*Natrix natrix*), were collected from various regions of Poland in 2013 – 2017 (Tab. 1).

Samples from birds and small carnivores (liver, muscles, heart, brain), as well as samples from snakes (heart, muscles), were collected in cooperation with the Polish Hunting Association; these animals had been shot by hunters or found dead on roads.

Samples foxes (muscles, heart, liver, tongue) were collected from animals shot during an official survey concerning the efficacy of an anti-rabies vaccination in different parts of Poland.

Samples from small rodents and insectivores (liver, kidney, lung) were collected from dead animals found in the vicinity of human settlements in Lublin province.

Sample processing and DNA extraction. Tissue samples (10–50 g) from birds, carnivores, and snakes were digested according to the method described by Dubey and Beattie (1988) [16]. Samples were cut and homogenized in 25–125 mL of 0.9% NaCl, followed by mixing with 50–250 mL of acid-pepsin solution (2.6 g pepsin, 7 mL HCl, and 0.9% NaCl filled-up to 500 mL, pH 1.1–1.2). After incubation at 37°C for 90 min in a shaker bath, the solution was poured out through the gauze, and centrifuged at 1,200 g for 10 min. The pellets were collected, resuspended in 4–20 mL of phosphate buffered saline (PBS, pH 7.4), neutralized with 3–15 mL of freshly prepared 1.2% sodium bicarbonate (pH 8.3) and centrifuged (1,200 g × 10 min). Supernatant was removed and the pellet resuspended in 1–5 mL of 0.9% NaCl.

Each pellet sample was divided into 2 sub-samples (100 mL) from which DNA was extracted using a commercial kit (QIAmp DNA Mini Kit, Qiagen), according to the manufacturer's instructions, except for an overnight incubation with proteinase K. Brain samples and samples from small rodents and insectivores (liver, kidney, lung) were homogenized, and approximately 25 mg of each sample (without digestion step) was destinated for DNA extraction, as described above. All DNA samples were stored at –20 °C until examination.

Polymerase chain reaction (PCR). *T. gondii* DNA was detected by amplification of 35-fold-repetitive B1 fragment gene in nested polymerase chain reaction (PCR), according to the method by Grigg and Boothroyd (2001) [17]. PCR were carried out in a C1000 Thermal Cycler (Bio-Rad).

Genetic characterization. For a part of PCR positive amplicons, restriction fragment length polymorphism (RFLP) PCR was performed according to the method by Grigg and Boothroyd (2001) [17], where an amplicons were digested with restriction enzymes Eco 721 (PmII) and XhoI (Thermo Scientific). For the other part of B1 positive DNA isolates the genotyping was performed using 5 genotyping markers (SAG1, SAG2 (5'- and 3'), SAG3, BTUB and GRA6), based on the method by Dubey et al. (2006) [18].

In all PCR reactions as positive controls RH (type I), ME49 (type II), and C56 (type III) DNA isolates of *T. gondii* strains, and as a negative control nuclease-free water, were used. Nested PCR of B1 products after electrophoresis were identified on an agarose gel under ultraviolet light. The amplificons of PCRs with 5 genetic markers were sequenced by an external company (Genomed S.A.), and sequences analyzed using Geneious v. 11.1.4. software (Biomatters) and compared with the sequences deposited in NCBI database using Blast.

Statistical analysis. The differences between the prevalence of *T. gondii* were assessed by chi-square ($\chi 2$) test with Yates correction, and Student's t-test. The differences in all analyses were considered statistically significant when $p < 0.05$. Statistical analyses were performed using Statistica v.9.1 (StatSoft Inc.).

RESULTS

PCR and genotyping results. In total, in 50 of the 396 examined animals DNA of *T. gondii* was detected (12.6%). Among wild birds, the positive results in PCR was found in 3 of 11 birds (27.3%), including: one kestrel, one seagull and one buzzard. The percentage of positive results in rodents and insectivores was 8.1%: in small rodents and insectivores 8.6%, and in muskrats 6.3%. Among carnivores, the percentage of positive results was 14.1%; the highest *T. gondii* prevalence was found in martens (40.9%) and badgers (38.5%), lower in minks (25%) and the lowest in red foxes (7.4%) ($p<0.05$). No positive results were found among grass snakes.

RFLP PCR results for selected B1 positive samples (birds, foxes, raccoons) showed pre-dominance II and/or III genotype(s).

Genotyping with 5 genetic markers performed for selected B1 positive DNA isolates also showed pre-dominance of type II (including indistinguishable at SAG1 marker type(s) II/III) (78.6%) and less extent type III (21.4%) (Tab. 1).

DISCUSSION

In the presented study, the molecular screening of selected species of wild animals from various regions of Poland showed 12.6% *T. gondii* infection. Studies regarding *T. gondii* prevalence in wildlife in Poland are limited and mostly based on serology. To-date, *T. gondii* DNA has been detected in Poland in humans [19], raw meat products [20], goat milk [21], and in environmental matrices [22, 23, 24]. However, little is known about an importance of small, free-living animals in the epidemiological context of *T. gondii* infection.

Among free-living animals, wild birds are regarded as important reservoir of *T. gondii* for carnivores. Moreover, because of migration behaviour, wild birds may spread the

Table 1. Results of nested and RFLP PCR, and multilocus genotyping of *Toxoplasma gondii*.

Group of animals	Species	Locality (Province)	Results of nested PCR N positive/ N examined (%)	Results of genotyping by RFLP PCR (B1 gene) or PCRs with 5 genetic markers Type (N of samples) (marker)
Birds (Class: Aves)	Common kestrel (*Falco tinnunculus*)	Mazowieckie	1/3	II/III (1) (B1)
	Black-headed gull (*Chroicocephalus ridibundus*)		1/2	II/III (1) (B1)
	Long-eared owl (*Asio otus*)		0/1	-
	Northern goshawk (*Accipiter gentilis*)		0/1	-
	Peregrine falcon (*Falco peregrinus*)		0/1	-
	White stork (*Ciconia ciconia*)		0/1	-
	Common buzzard (*Buteo buteo*)		1/2	II/III (1) (B1)
Subtotal			**3/11 (27.3%)**	
Mammals (Class: Mammalia) Rodents (Order: Rodentia) and Insectivores (Order: Eulipotyphla)	Small rodents: Mice (N of examined animals): (*Apodemus agrarius*) (43), (*A. flavicollis*) (1), (*A. sylvaticus*) (5); Voles (*Microtus arvalis*) (2), (*Myodes glareolus*) (4) Insectivores: Common shrew (*Sorex araneus*) (8), European mole (*Talpa europaea*) (7)	Lubelskie	6/70 (8.6%)	II/III (4) (SAG1); II (1) (SAG2, GRA6, BTUB, SAG3); III (1) (GRA6, SAG3)
	Muskrat (*Ondatra zibethicus*)	Lubelskie	1/16 (6.3%)	N/a
Subtotal			**7/86 (8.1%)**	
Mammals (Class: Mammalia) Carnivores (Order: Carnivora)	American mink (*Neovison vison*)	Lubuskie	4/16 (25%)	N/a
	European otter (*Lutra lutra*)	Lubuskie	1/7 (14.3%)	N/a
	European polecat (*Mustela putorius*)	Pomorskie	1/3	N/a
	European marten (*Martes martes*)	Lubelskie, Lubuskie Mazowieckie	9/22 (40.9%)**	II (1) (SAG3, 3'SAG-2); II (1) (GRA6); III (1) (GRA6)
	Red fox (*Vulpes vulpes*)	Podlaskie, Warmińsko-Mazurskie, Kujawsko-Pomorskie	11/148 (7.4%)*	II/III (1) (B1); I and II/III (mixed) (3) (B1)
	Raccoon (*Procyon lotor*)	Lubuskie	8/61 (13.1%)	II/III (1) (B1)
	Raccoon dog (*Nyctereutes procyonoides*)	Lubuskie	1/13 (7.7%)	N/a
	European badger (*Meles meles*)	Lubuskie, Mazowieckie Pomorskie	5/13 (38.5%)**	N/a
Subtotal			40/283 (14.1%)	
Class: Reptilia	Grass snake (*Natrix natrix*)		0/16	-
Subtotal			**0.16**	
Total			**50/396 (12.6%)**	

N/a – not amplified
* prevalence significantly smaller compared to other species of this group of animals ($p<0.05$)
** prevalence significantly greater compared to other species of this group of animals ($p<0.05$)

parasite worldwide. In the current study, DNA of parasite was detected in 3 of 11 wild birds; mostly in birds of prey (common kestrel and common buzzard).

To the best of the authors' knowledge, this is the first study on *T. gondii* prevalence in wild birds in Poland. In other countries, detection of DNA or live *T. gondii* in birds of prey have been recorded, e.g. in the Czech Republic [25] viable *T. gondii* was isolated from 8% of common buzzards, while in the USA, Lindsay et al. [26] isolated live *T. gondii* from 26.7% of examined raptors, including 11 of 27 red tailed hawks and 1 of 3 kestrels. Dubey et al. (2010) [15], also in the USA, isolated viable *T. gondii* from 6 of 51 raptors or carnivorous birds. In Slovakia, a total of 10 birds from wildlife were examined and the prevalence of *T. gondii* DNA was 40% (types II and III strains were detected) [27]. However, the highest *T. gondii* infection rate (up to 92%) was found in raptors in Turkey [11].

Small rodents and insectivores, being the main prey for many species of carnivores (including cats) and omnivores, are probably the most important *T. gondii* reservoir and may potentially contribute to the transmission of this parasite in both synantropic and sylvatic fauna. In Poland, there are no current data concerning *T. gondii* prevalence in wild, small rodents, thus the results of the present study can have new epidemiological value. *T. gondii* prevalence among rodents and insectivores (8.6% in mice, voles, common shrew and moles, and 6.3% in muskrats) in the current study showed moderate values; however, this is higher than the prevalence found in free-living rodents in the Czech Republic (1%) [28], and in yellow-necked mice in Germany (2.7%) [29]. The prevalence found in the current study in muskrats (6.3%) is lower than in the Czech Republic, where it ranged between 9–47% [30].

Carnivores are another group of free-living animals important in epidemiology of toxoplasmosis. Carnivores can be infected with tissue cysts when feeding on infected animals, or by ingestion of oocysts from the environment. High population densities of carnivores, in particular of foxes and raccoons, certainly pose increased health risks, not only for companion animals, but also for humans since important parasites of carnivores, such as *T. gondii*, *Toxocara* spp. and *Echinococcus multilocularis,* are also causative agents of zoonoses [13, 31]. Raccoon dogs (*Nyctereutes procyonoides*) are a neozoan species that has rapidly expanded its geographical range and shares many parasites [32]. In Poland, raccoon dogs are observed more often in the western regions of the country (Lubuskie and Zachodniopomorskie Provinces) [33, 34], hence these areas were chosen for the current research.

In the presented study, the highest prevalence among carnivores was found in martens (40.9%), badgers (38.5%), and American minks (25%); also one of 3 European polecats was PCR positive. A lower prevalence was found in European otters (14.3%), raccoons (13.1%), raccoon dogs (7.7%) and red foxes (7.4%). It is noteworthy that the aggregated prevalence in the weasel-like animals belonging to family Mustelidae (badgers, martens, minks, otters, poles) equal to 32.8% was significantly greater ($p<0.001$), compared to aggregated values stated in other carnivores belonging to the families Canidae and Procyonidae (9.0%), as well as in rodents and insectivores (8.9%). This seems to indicate that Mustelidae form an important reservoir in the circulation of *T. gondii* in nature.

In another study in Poland, *T. gondii* DNA was found, depending on the methods used, in 13.8–19% of red foxes, 25–32% of badgers, 24–29% of raccoon dogs and 28–33% of martens [35]. In the UK, the presence of *T. gondii* DNA in brains collected from wild carnivores was detected in 19 of 65 Eurasian badgers (29.2%) [36]. In the Czech Republic, Turčeková et al. [27] showed a high *T. gondii* prevalence in martens (50%), in which revealed genotypes II and III. The results of another study in the Czech Republic showed a much lower *T. gondii* prevalence (5%) in martens than recorded in the presented study (40.9%) [37]. Turčeková et al. [27] found one *T. gondii* DNA sample (type II) out of 7 tested samples of badgers, whereas *T. gondii* DNA was not detected in the tissues of raccoon dogs.

In red foxes, a higher prevalence (18.8%) than that recorded in the current study (7.4%) was found in Belgium [38], similarly in Germany 13.4–18.4% foxes were identified as *T. gondii* positive [39]. In France, *T. gondii* was isolated from 9 of 14 seropositive red foxes [40], whereas in the UK, only 6.0% of foxes were PCR positive and type II lineage was demonstrated [36]. A study on *T. gondii* prevalence in minks in the UK [36] showed similar results to those obtained in the current study (20% and 25%, respectively), whereas in Chile, *T. gondii* DNA was demonstrated only in one of 73 American minks [41].

Little is known about the possibility of *T. gondii* infection in poikilothermic animals. According to the scientific literature, experimental transmission of *T. gondii* to reptiles failed under normal temperatures, but it has been shown that *T. gondii* can be successfully transferred to reptiles when kept at higher temperatures. The results of the presented study have not confirmed the presence of *T. gondii* DNA in grass snakes tissues.

Since a part of the research was carried out in the past, the method then in use and based on a single genetic marker (B1), it allowed only for preliminary genotype assessment. This method can distinguish type I contrary types II and/or III. Moreover, not all DNA samples, positive in B1 PCR, were successfully amplified by multilocus PCR used in subsequent years. No amplification with the use of other markers may be caused by the existence of a lower number of copies of particular markers in the genome, which resulted in lower sensitivity.

Summarized, 8 of 22 (36.4%) and 9 of 15 (60%) of positive samples (B1) were successfully genotyped by RFLP PCR and multilocus PCR methods, respectively. By multilocus PCR, successful amplifications enabled at one marker for 4 DNA isolates, at 2 markers for 2 isolates, and at 4 markers for one isolate. The obtained results showed a predominance of *T. gondii* clonal type(s) II and II/III, followed by type III; however, mixed types I and II/III was also found. These results obtained in wild animals seem to correspond with the results of genotyping of *T. gondii* DNA isolated from the raw meat of pigs and cattle in Poland (Sroka et al., article in press); however, another own study showed a high prevalence of type III among *T. gondii* DNA samples isolated from goat milk and raw meat products in Poland, while types I and II were less common [20, 21]

CONCLUSION

The results of this study indicate frequent *T. gondii* infection among free-living animals in Poland, especially martens and badgers, which may indirectly indicate that these animals contribute to the spread of the parasite in the sylvatic environment in Poland. RFLP and multilocus PCR analysis showed the dominance of *T. gondii* clonal type II (or II/III). Although the presented results may correspond with the results of research in other European countries, further research is needed on the prevalence, distribution and virulence of *T. gondii* in free-living animals in Poland, as well as genetic characterization of the isolated parasite.

REFERENCES

1. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: From animals to humans. Int J Parasitol. 2000; 30: 1217–1258.
2. Torgerson PR, Devleesschauwer B, Praet N, Speybroeck N, Willingham AL, Kasuga F, Rokni MB, Zhou XN, Fèvre EM, Sripa B, Gargouri N, Fürst T, Budke CM, Carabin H, Kirk MD, Angulo FJ, Havelaar A, de Silva N. World Health Organization estimates of the global and regional disease burden of 11 foodborne parasitic diseases, 2010: a data synthesis PLoS Med. 2015; 12, Article e1001920
3. FAO, WHO, 2014. Multicriteria-based ranking for risk management of food-borne parasites. Microbiol Risk Assess Series. 23, 287.
4. Bouwknegt M, Devleesschauwer B, Graham H, Robertson L, van der Giessen JWB. The Euro-FBP Workshop Participants, 2018. Prioritisation of food-borne parasites in Europe, 2016. Eurosurveillance. 2018; 23: 17–00161.
5. Shwab EK, Zhu XQ, Majumdar D, Pena HF, Gennari SM, Dubey JP, Su C. Geographical patterns of *Toxoplasma gondii* genetic diversity revealed by multilocus PCR-RFLP genotyping. Parasitology. 2014; 141(4): 453–461. doi.org/10.1017/S0031182013001844
6. Turčeková Ľ, Antolová D, Reiterová K, Spišák F. Occurrence and genetic characterization of *Toxoplasma gondii* in naturally infected pigs. Acta Parasitol. 2013; 58: 361–366.
7. Verin R, Mugnaini L, Nardoni S, Papini R, Ariti G, Poli A, Mancianti F. Serologic, molecular and pathologic survey of *Toxoplasma gondii*

infection in free ranging red foxes (*Vulpes vulpes*) in Central Italy. J Wildl Dis. 2013; 49: 545–551.
8. Bacci C, Vismarra A, Mangia C, Bonardi S, Bruini I, Genchi M, Kramer L, Brindani F. Detection of *Toxoplasma gondii* in free-range, organic pigs in Italy using serological and molecular methods. Int J Food Microbiol. 2015; 202: 54–56.
9. Battisti E, Zanet S, Trisciuoglio A, Bruno S, Ferroglio E. Circulating genotypes of *Toxoplasma gondii* in Northwestern Italy. Vet Parasitol. 2018; 253: 43–47.
10. Berger-Schoch AE, Herrmann DC, Schares G, Müller N, Bernet D, Gottstein B, Frey CF. Prevalence and genotypes of *Toxoplasma gondii* in feline faeces (oocysts) and meat from sheep, cattle and pigs in Switzerland. Vet Parasitol. 2011; 177: 290–297.
11. Karakavuk M, Aldemir D, Mercier A, Atalay Şahar E, Can H, Murat J-B, Döndüren Ö, Can Ş, Özdemir HG, Değirmenci Döşkaya A, Pektaş B, Dardé ML, Gürüz AY, Döşkaya M. Prevalence of toxoplasmosis and genetic characterization of *Toxoplasma gondii* strains isolated in wild birds of prey and their relation with previously isolated strains from Turkey. PLoS ONE. 2018; 13(4): e0196159. https://doi.org/10.1371/journal.pone.0196159
12. Dubey JP, Weigel RM, Siegel AM, Thulliez P, Kitron UD, Mitchell MA, Mannelli A, Mateus-Pinilla NE, Shen SK, Kwok OCH. Todd KS. Sources and reservoirs of *Toxoplasma gondii* infection on 47 swine farms in Illinois. J Parasitol. 1995; 81: 723–729.
13. Duscher GG, Leschnik M, Fuehrer HP, Joachim A. Wildlife reservoirs for vector-borne canine, feline and zoonotic infections in Austria. Int J Parasitol Parasites Wildl. 2015; 4: 88–96. doi: 10.1016/j.ijppaw.2014.12.001 PMID: 25830102
14. Karamon J, Kochanowski M, Sroka J, Cencek T, Różycki M, Chmurzyńska E, Bilska-Zając E. The prevalence of *Echinococcus multilocularis* in red foxes in Poland – current results (2009–2013). Parasitol Res. 2014; 113: 317–22. https://doi:10.1007/s00436-013-3657-z.
15. Dubey JP, Felix TA, Kwok OC. Serological and parasitological prevalence of *Toxoplasma gondii* in wild birds from Colorado. J Parasitol. 2010; 96: 937–939, https://doi.org/10.1645/GE-2501.1
16. Dubey JP, Beattie CP. Toxoplasmosis of Animals and Man. Boca Raton, CRC Press Inc, 1988.
17. Grigg ME, Boothroyd JC. Rapid identification of virulent type I strains of the protozoan pathogen *Toxoplasma gondii* by PCR restriction fragment length polymorphism analysis at the B1 gene. J Clin Microbiol. 2001; 39: 398–400.
18. Dubey JP, Patitucci AN, Su C, Sundar N, Kwok OC, Shen SK.: Characterization of *Toxoplasma gondii* isolates in free-range chickens from Chile, South America. Vet Parasitol. 2006; 140: 76–82.
19. Nowakowska D1, Colón I, Remington JS, Grigg M, Golab E, Wilczynski J, Sibley LD. Genotyping of *Toxoplasma gondii* by multiplex PCR and peptide-based serological testing of samples from infants in Poland diagnosed with congenital toxoplasmosis. J Clin Microbiol. 2006; 44: 1382–1389.
20. Sroka J, Bilska-Zając E, Wójcik-Fatla A, Zając V, Dutkiewicz J, Karamon J, Piotrowska W, Cencek T. Detection and molecular characteristics of *Toxoplasma gondii* DNA in retail raw meat products in Poland. Foodborne Pathog Dis. 2019; 16: 195–204; DOI: 10.1089/fpd.2018.2537
21. Sroka J, Kusyk P, Bilska-Zając E, Karamon J, Dutkiewicz J, Wójcik-Fatla A, Zając V, Stojecki K, Różycki M, Cencek T. Seroprevalence of *Toxoplasma gondii* infection in goats from the south-west region of Poland and the detection of *T. gondii* DNA in goat milk. Folia Parasitol. 2017; 64: 023. doi: 10.14411/fp.2017.023
22. Sroka J, Wójcik-Fatla A, Szymańska J, Dutkiewicz J, Zając V, Zwoliński J. The occurrence of *Toxoplasma gondii* in people and animals from rural environment of Lublin region – estimate of potential role of water as a source of infection. Ann Agric Environ Med. 2010; 17: 125–132.
23. Sroka J, Wójcik-Fatla A, Zwoliński J, Zając V, Sawczuk M, Dutkiewicz J. Preliminary study on the occurrence of *Toxoplasma gondii* in *Ixodes ricinus* ticks from north-western Poland with the use of PCR. Ann Agric Environ Med. 2008; 15: 333–338.
24. Lass A, Pietkiewicz H, Szostakowska B, Myjak P. The first detection of *Toxoplasma gondii* DNA in environmental fruits and vegetables samples. Eur J Clin Microbiol Infect Dis. 2012; 31: 1101–1108.
25. Literák I, Hejlíček K, Nezval J, Folk Č. Incidence of *Toxoplasma gondii* in populations of wild birds in the Czech Republic, Avian Pathol. 1992; 21: 659–665. DOI:10.1080/03079459208418887
26. Lindsay DS, Smith PC, Hoerr FJ, Blagburn BL. Prevalence of encysted *Toxoplasma gondii* in raptors from Alabama. J Parasitol. 1993; 79: 870–873.
27. Turčeková Ľ, Hurníková Z, Spišák F, Miterpáková M, Chovancová B. *Toxoplasma gondii* in protected wildlife in the Tatra National Park (TANAP), Slovakia. Ann Agric Environ Med. 2014; 21: 235–238. https://doi.org/10.5604/1232-1966.1108582 PMID: 24959767
28. Hejlícek K, Literák I, Nezval J. Toxoplasmosis in wild mammals from the Czech Republic. J. Wildl. Dis. 1997; 33: 480–485.
29. Waindok P, Özbakış-Beceriklisoy G, Janecek-Erfurth E, Springer A, Pfeffer M, Leschnik M, Strube Ch. Parasites in brains of wild rodents (Arvicolinae and Murinae) in the city of Leipzig, Germany, Int J Parasitol Parasites Wildl. 2019, 10, 211–217. https://doi.org/10.1016/j.ijppaw.2019.09.004.
30. Nezval J, Literák I. *Toxoplasma gondii* in muskrat (*Ondatra zibethicus*). Vet Med (Praha). 1994; 39: 743–6.
31. Krücken J, Blümke J, Maaz D, Demeler J, Ramünke S, Antolová D, Schaper R, von Samson-Himmelstjerna G. Small rodents as paratenic or intermediate hosts of carnivore parasites in Berlin, Germany. PLoS ONE. 2017; 12: e0172829. doi:10.1371/journal.pone.0172829
32. Sutor A, Schwarz S, Conraths F. The biological potential of the raccoon dog (*Nyctereutes procyonoides*, Gray 1834) as an invasive species in Europe – new risks for disease spread? Acta Theriol. 2014; 59: 49–59.
33. Bartoszewicz M, Okarma H, Zalewski A, Szczęsna J. Ecology of raccoon (*Procyon lotor*) from western Poland. Ann Zool Fennici. 2008; 45: 291–298.
34. Karamon J, Kochanowski M, Cencek T, Bartoszewicz M, Kusyk P. Gastrointestinal helminths of raccoons (*Procyon lotor*) in western Poland (Lubuskie province) – with particular regard to *Baylisascaris procyonis*. Bull Vet Inst Pulawy. 2014; 58: 547–552. doi: 10.2478/bvip-2014-0084
35. Kornacka A, Cybulska A, Popiołek M, Kuśmierek N, Moskwa B. Survey of *Toxoplasma gondii* and *Neospora caninum* in raccoons (*Procyon lotor*) from the Czech Republic, Germany and Poland. Vet Parasitol. 2018, 262: 47–50. https://doi.org/10.1016/j.vetpar.2018.09.006
36. Burrells A, Bartley PM, Zimmer IA, Roy S, Kitchener AC, Meredith A, Wright SE, Innes EA, Katzer F. Evidence of the three main clonal *Toxoplasma gondii* lineages from wild mammalian carnivores in the UK. Parasitology. 2013; 140: 1768–1776. doi:10.1017/S0031182013001169.
37. Hůrková L, Modrý D. PCR detection of *Neospora caninum*, *Toxoplasma gondii* and *Encephalitozoon cuniculi* in brains of wild carnivores. Vet Parasitol. 2006; 137: 150–4.
38. De Craeye S, Speybroeck N, Ajzenberg D, Darde M L, Collinet F, Tavernier P, Van Gucht S, Dorny P, Dierick K. *Toxoplasma gondii* and *Neospora caninum* in wildlife: common parasites in Belgian foxes and Cervidae? Vet Parasitol. 2011; 178: 64–69. doi: 10.1016/j.vetpar.2010.12.016
39. Herrmann DC, Maksimov P, Maksimov A, Sutor A, Schwarz S, Jaschke W, Schliephake A, Denzin N, Conraths FJ, Schares G. *Toxoplasma gondii* in foxes and rodents from the German Federal States of Brandenburg and Saxony-Anhalt: seroprevalence and genotypes. Vet Parasitol. 2012; 185: 78–85. doi:10.1016/j.vetpar.2011.10.030
40. Aubert D, Ajzenberg D, Richomme C, Gilot-Fromont E, Terrier M E, de Gevigney C, Game Y, Maillard D, Gibert P, Darde M L, Villena I. Molecular and biological characteristics of *Toxoplasma gondii* isolates from wildlife in France. Vet Parasitol. 2010; 171, 346–349. doi: 10.1016/j.vetpar.2010.03.033
41. Barros M, Cabezón O, Dubey JP, Almería S, Ribas MP, Escobar LE, Ramos B, Medina-Vogel G. *Toxoplasma gondii* infection in wild mustelids and cats across an urban-rural gradient. PLoS One. 2018;13(6): e0199085. doi: 10.1371/journal.pone.0199085

Ministry of Science and Higher Education
Republic of Poland

Generation of the DOI (Digital Object Identifier) – task financed under the agreement No. 618/P-DUN/2019 by the Minister of Science and Higher Education