Seroprevalence of *Toxoplasma gondii* among sylvatic rodents in Poland

Maciej Grzybek* email: maciej.grzybek@gumed.edu.pl

Medical University of Gdansk, Gdańsk, Poland

ORCID: 0000-0002-8780-0304

Daniela Antolová email: antolova@saske.sk

Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia

ORCID: 0000-0002-9358-2163

Katarzyna Tolkacz email: k.h.tolkacz@ibb.waw.pl

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

University of Warsaw, Warsaw, Poland

ORCID: 0000-0002-8578-3870

Mohammed Alsarraf email: muha@biol.uw.edu.pl

University of Warsaw, Warsaw, Poland

ORCID: 0000-0002-6330-0719

Jolanta Behnke-Borowczyk email: jbehnke@up.poznan.pl

Poznan University of Life Sciences, Poznan, Poland

ORCID: 0000-0003-2085-038X

Joanna Nowicka email: joanna.nowicka@gumed.edu.pl

Medical University of Gdansk, Gdansk, Poland

ORCID: 0000-0002-5313-3232

Jerzy Paleolog email: jerzy.paleolog@up.lublin.pl

University of Life Sciences in Lublin, Poland

ORCID: 0000-0002-4447-4318

25 **Beata Biernat** email: beata.biernat@gumed.edu.pl

26 Medical University of Gdansk, Gdansk, Poland

27 ORCID: 0000-0003-4938-3113

28 **Jerzy M. Behnke**¹ email: jerzy.behnke@nottingham.ac.uk

29 University of Nottingham, Nottingham, UK

30 ORCID: 0000-0001-9396-2572

31 **Anna Bajer**¹ email: anabena@biol.uw.edu.pl

32 University of Warsaw, Warsaw, Poland

33 ORCID: 0000-0001-6199-8458

34

35 ¹These authors contributed equally to this work.

36

37

38

39 ***ADDRESS FOR CORRESPONDENCE:** Maciej Grzybek, PhD; Department of Tropical
40 Parasitology, Institute of Maritime and Tropical Medicine, Medical University of Gdansk,
41 Powstania Styczniowego 9B, 81-519 Gdynia, Poland. Email: maciej.grzybek@gumed.edu.pl
42 Tel: +48 58 3491941

43

44 **KEYWORDS:** *Toxoplasma gondii*; *Myodes glareolus*; *Microtus*; *Alexandromys*; intrinsic
45 factors; extrinsic factors; seromonitoring; rodent-borne diseases; prevention

46

47 **ABSTRACT**

48 *Toxoplasma gondii* is a significant pathogen affecting humans and animals. We conducted
49 seromonitoring for *T. gondii* in four sylvatic rodent species in Poland. We report an overall
50 seroprevalence of 5.5% (3.6% for *Myodes glareolus* and 20% for other vole species).
51 Seroprevalence in bank voles varied significantly between host age and sex.

52 INTRODUCTION

53 There is currently considerable interest in understanding not only the transmission of
 54 pathogens but also the range of different variables that influence infection dynamics. Among
 55 these, both extrinsic factors (e.g. geographic location) and intrinsic factors, (e.g. host sex) are
 56 known to play varying but crucial roles in the exposure of hosts and their susceptibility to
 57 infection (1,2). Analyzing pathogen dynamics in their wildlife reservoirs is essential in
 58 providing a good understanding of their epidemiology and facilitating informed decisions on
 59 appropriate measures for their control. Wild rodents pose a particular threat for human
 60 communities because they constitute the most abundant and diversified group of all living
 61 mammals (3). Although some zoonotic parasites are not transmissible directly from rodents to
 62 humans, or pose just a low risk of direct transmission, predation of rodents by carnivore pets
 63 may cause infections in these animals, leading to subsequent contamination of households and
 64 other human-related environments (4).

65 *T. gondii* is an intracellular Apicomplexan parasite with a broad range of intermediate
 66 hosts, including humans and rodents (5). The parasite is present first in the tachyzoite stage
 67 and later as bradyzoites, also called tissue cysts (6). These can pass from host to host via the
 68 food chain and across the placenta resulting in congenitally acquired infections. Rodents are
 69 considered to be reservoirs of infection for their predators that include cats, pigs and dogs.
 70 Felid species are the definitive hosts of *T. gondii* and the only hosts that can
 71 shed *T. gondii* oocysts into the environment

72 In 2017, 194 confirmed human congenital toxoplasmosis cases were reported from 22 EU
 73 countries. France accounted for 79% of all cases. The number of congenital infections per
 74 100 000 newborns was 5.3 in the EU/EEA. The highest incidence was reported in France
 75 (19.9), followed by Slovenia (9.98), Poland (4.48) and Bulgaria (3.13) (7). In 2018 only 25
 76 cases of congenital toxoplasmosis were reported in Poland, and in 2019 even fewer with only
 77 14 confirmed cases. (8).

78 We conducted a multi-site, long-term study on *T. gondii* in northeastern Poland. Our
 79 objectives were to monitor seroprevalence of *T. gondii* in the four abundant vole species
 80 found in the region (*Myodes glareolus*, *Microtus arvalis*, *Microtus agrestis*, *Alexandromys*
 81 *oeconomus*) and to assess variation in seroprevalence attributable to both intrinsic and
 82 extrinsic factors that were quantified.

83

84 MATERIAL AND METHODS

85 Our study sites are located in the Mazury Lake District region in north-eastern Poland.
86 The sites, methods used for trapping rodents, and for sampling and processing trapped
87 animals have all been thoroughly described (9–11). Bank voles were sampled in 2002, 2006
88 and 2010 in three local but disparate forest sites. Other species of voles were sampled only in
89 2013 from fallow meadows close to one of the forest sites. A bespoke ELISA test was used to
90 detect antibodies against *T. gondii* using commercially available *T. gondii* antigen (CD
91 Creative Diagnostics, New York, USA) prepared from *T. gondii* RH strain (12). The statistical
92 approach has been documented comprehensively in our earlier publications (1,13). Prevalence
93 values are given with 95% confidence limits in square brackets. All of the procedures were
94 conducted with the approval of the First Warsaw Local Ethics Committee for Animal
95 Experimentation in Poland.

96 RESULTS

97 We examined 577 rodent individuals, and found *T.gondii* antibodies in the sera of all four
98 rodent species, with an overall seroprevalence of 5.5% (Table 1). There was a significant
99 difference in seroprevalence between vole species ($\chi^2_3=22.58$; $P=0.001$) with *Microtus* and
100 *Alexandromys* spp. showing 5.6-fold higher seroprevalence (20% [12-30.9]) than *M. glareolus*
101 (3.6% [2.6-4.9]). In a log-linear model restricted to *Microtus* and *Alexandromys* spp. there
102 was no difference between these three species ($\chi^2_2=0.8$, $P=0.7$), nor between the sexes ($\chi^2_1=0.37$; $P=0.55$) or age classes ($\chi^2_2=4.67$; $P=0.097$), although the latter was close to
103 significance and reflected the peak seroprevalence in age class 2 (35.7 [15.28-62.89]) in
104 comparison to zero seroprevalence among the youngest voles and 18.0% [9.46-30.87] among
105 the oldest (Figure 1). We therefore confined further analyses to bank voles (*M. glareolus*).
106

107 In a log-linear model confined to bank voles, and with year of sampling (3 years) and
108 site (3 sites) taken into account, *T.gondii* seroprevalence differed significantly between the
109 sexes of *M. glareolus* ($\chi^2_1=4.34$; $P=0.037$) and was 3.5 times higher for female compared
110 with male bank voles (Figure 1). *T. gondii* seroprevalence increased significantly with host
111 age ($\chi^2_2=11.57$; $P=0.003$), peaking in the oldest bank voles (6.1% [2.77-12.47]) but was lower
112 in bank voles from age class 2 (mostly young adults; 3.5% [1.22-9.50]). No immature bank
113 voles were found to be seropositive. The data in Figure 1 also indicate that seroprevalence
114 increased with host age faster among female bank voles compared with males, although the
115 interaction between age and sex was not significant ($\chi^2_2=0.28$; $P=0.87$).

Table 1. Seroprevalence of *T. gondii* in four rodent species in Poland. Seroprevalence is given in percentages with 95% CL in brackets.

Species	Negative	Positive	Total	Seroprevalence (%) $\pm$ CL ₉₅
<i>Microtus agrestis</i>	7	3	10	30.0 (8.7-61.9)
<i>Microtus arvalis</i>	37	9	46	19.6 (9.1-36.8)
<i>Alexandromys oeconomus</i>	12	2	14	14.3 (2.6-42.6)
<i>Myodes glareolus</i>	489	18	507	3.6 (2.6-4.9)
Overall	545	32	577	5.5 (4.2-7.3)

Figure 1. Seroprevalence of *T. gondii* in bank voles in Poland by host sex and by host age class (class 1—immature juvenile voles; class 2—young adult voles; and class 3—breeding older animals). Error bars indicate 95% CL.

DISCUSSION

Our results confirm that both *M. glareolus* and *Microtus/Alexandromys* spp. have contact with *T. gondii* and become infected. Therefore, they may potentially play a role as reservoirs of this parasite in the sylvatic environment. According to the meta-analysis carried

out by Galeh and colleagues, the overall seroprevalence of anti-*Toxoplasma* IgG antibodies in rodents in Europe is 1% (14). Here, we report an overall seroprevalence of *T. gondii* in voles of 5.5%, with *Microtus* and *Alexandromys* spp. showing a considerably higher seroprevalence at 20%. Although the reason for this discrepancy between the rodent genera is not clear, we have noted that cats from local farms are often observed in the meadows where we trapped *Microtus/ Alexandromys* spp., but seldom enter the forests to which bank voles are confined. This is in line with the results obtained with radio-collared farm cats in Switzerland, which kept a short distance from the house and preferred meadows over forests (15).

We found that female bank voles were 3.5 times more likely to be infected with *T. gondii* than males, and in this respect, our data contrast with reports in the literature in which *T. gondii* seropositivity in male rodents has been recorded generally to be higher than in females (14). It was not unexpected to find in our study that older animals were more likely to have experienced infection than juveniles, since the current work was based on the presence/absence of specific antibody against *T. gondii*, reflecting the history of previous infections and not necessarily a current infection. Older animals will have had a longer time frame over which to encounter the infective stages of *T. gondii*, and hence greater likelihood of infection than younger individuals.

Transmission of the parasite from wildlife to human habitats may occur especially in rural areas where cats escape human settlements and forage on wild rodents. Further studies are necessary therefore, to reveal comprehensively the status of toxoplasmosis in wildlife and to assess the risk of infection for local inhabitants, as well as for visitors to the region.

ACKNOWLEDGEMENTS:

We thank Dr Ewa Mierzejewska for her help in rodent trapping and the fieldwork. We also thank the University of Nottingham, University of Warsaw, Institute of Parasitology, Slovak Academy of Sciences and the Medical University of Gdańsk for financial support. JMB was supported by the Royal Society, the British Ecological Society and the Grabowski Fund. AB was supported by the Polish State Committee for Scientific Research and the British Council's Young Scientist Programme). This research was funded through the 2018-2019 BiodivERsA joint call for research proposals, under the BiodivERsA3 ERA-Net COFOUND programme. MG was supported by the National Science Centre, Poland under BiodivERsA3 programme (2019/31/Z/NZ8/04028). MG thanks Alicja Rost and Ewa Zieliniewicz for their assistance in the laboratory.

163

164 **AUTHORSHIP:**

165 The study was conceived and designed by MG, DA and BB. Supervision of the long-term
166 monitoring of bank vole populations in the region was by JMB and AB. Samples were
167 collected in the field by JMB, AB, MA, MG, KT& JBB. The immunological analysis and
168 laboratory work was conducted by DA, KT, JN, JMB, AB & MG. Data handling – MG.
169 Statistical analysis was carried by JMB & MG. The manuscript was written by MG, BB, and
170 JMB in consultation with all co-authors. MG, DA, AB & JMB revised the manuscript. All
171 authors accepted the final manuscript version.

172 **AUTHOR BIO:**

173 Dr Grzybek is a parasitologist holding a position of associate professor at the Department of
174 Tropical Parasitology, Institute of Maritime and Tropical Medicine, Medical University of
175 Gdansk, Poland. His research interests include epidemiology and ecology of macro- and
176 microparasites in rodents, especially in bank voles. He is also interested in host-parasite
177 interactions and intrinsic and extrinsic factors that influence these relationships.

178 **REFERENCES:**

- 179 1. Grzybek M, Bajer A, Bednarska MM, Al-Sarraf M, Behnke-Borowczyk J, Harris PD,
180 et al. Long-term spatiotemporal stability and dynamic changes in helminth
181 infracommunities of bank voles (*Myodes glareolus*) in NE Poland. *Parasitology*
182 [Internet]. 2015 Dec 7;142(14):1722–43. Available from:
183 http://www.journals.cambridge.org/abstract_S0031182015001225
- 184 2. Behnke JM, Bajer A, Harris PD, Newington L, Pidgeon E, Rowlands G, et al.
185 Temporal and between-site variation in helminth communities of bank voles (*Myodes*
186 *glareolus*) from N.E. Poland. 2. The infracommunity level. *Parasitology* [Internet].
187 2008 Jul;135(8):999–1018. Available from:
188 <http://www.ncbi.nlm.nih.gov/pubmed/18513460>
- 189 3. Wilson DE, Reeder DM. *Mammal Species of the World*. Vol. 1, A Taxonomic and
190 Geographic Reference. 2005. 2000 p.
- 191 4. Waindok P, Özbakiş-Beceriklisoy G, Janecek-Erfurth E, Springer A, Pfeffer M,
192 Leschnik M, et al. Parasites in brains of wild rodents (*Arvicolinae* and *Murinae*) in the

- city of Leipzig, Germany. Int J Parasitol Parasites Wildl [Internet]. 2019 Dec;10:211–7.
Available from: <https://linkinghub.elsevier.com/retrieve/pii/S2213224419301476>
5. Johnson HJ, Koshy AA. Latent Toxoplasmosis Effects on Rodents and Humans: How
Much is Real and How Much is Media Hype? Garsin DA, editor. MBio [Internet].
2020 Mar 17;11(2). Available from: <https://mbio.asm.org/content/11/2/e02164-19>
6. Hill D, Dubey JP. Toxoplasma gondii: transmission, diagnosis and prevention. Clin
Microbiol Infect [Internet]. 2002 Oct;8(10):634–40. Available from:
<https://linkinghub.elsevier.com/retrieve/pii/S1198743X1462509X>
7. ECDC. Annual epidemiological report for 2017 [Internet]. Stockholm; 2019. Available
from: [https://www.ecdc.europa.eu/sites/default/files/documents/congenital-
toxoplasmosis-annual-epidemiological-report-2016.pdf](https://www.ecdc.europa.eu/sites/default/files/documents/congenital-toxoplasmosis-annual-epidemiological-report-2016.pdf)
8. NIPH – NIH. National Institute of Public Health – National Institute of Hygiene in
Poland [Internet]. 2021 [cited 2020 Dec 20]. Available from:
http://www.wold.pzh.gov.pl/oldpage/epimeld/index_p.html
9. Tołkacz K, Alsarraf M, Kowalec M, Dwużnik D, Grzybek M, Behnke JM, et al.
Bartonella infections in three species of Microtus: prevalence and genetic diversity,
vertical transmission and the effect of concurrent Babesia microti infection on its
success. Parasit Vectors [Internet]. 2018 Dec 30;11(1):491. Available from:
<https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-018-3047-6>
10. Behnke JM, Barnard CJ, Bajer A, Bray D, Dinmore J, Frake K, et al. Variation in the
helminth community structure in bank voles (Clethrionomys glareolus) from three
comparable localities in the mazury lake istrict region of Poland. Parasitology.
2001;123(4):401–14.
11. Grzybek M, Bajer A, Behnke-Borowczyk J, Al-Sarraf M, Behnke JM. Female host sex-
biased parasitism with the rodent stomach nematode Mastophorus muris in wild bank
voles (Myodes glareolus). Parasitol Res [Internet]. 2014;114(2):523–33. Available
from: <http://link.springer.com/article/10.1007/s00436-014-4214-0/fulltext.html>
12. Hughes HP, Van Knapen F, Atkinson HJ, Balfour AH, Lee DL. A new soluble antigen
preparation of Toxoplasma gondii and its use in serological diagnosis. Clin Exp
Immunol. 1982;49(1):239–246.

- 223 13. Grzybek M, Alsarraf M, Tołkacz K, Behnke-Borowczyk J, Biernat B, Stańczak J, et al.
224 Seroprevalence of TBEV in bank voles from Poland-a long-term approach. Emerg
225 Microbes Infect [Internet]. 2018 Aug 15;7(1):145. Available from:
226 <http://www.ncbi.nlm.nih.gov/pubmed/30108201>
 - 227 14. Galeh TM, Sarvi S, Montazeri M, Moosazadeh M, Nakhaei M, Shariatzadeh SA, et al.
228 Global Status of Toxoplasma gondii Seroprevalence in Rodents: A Systematic Review
229 and Meta-Analysis. Front Vet Sci [Internet]. 2020 Jul 31;7(461). Available from:
230 <https://www.frontiersin.org/article/10.3389/fvets.2020.00461/full>
 - 231 15. Weber J-M, Dailly L. Food habits and ranging behaviour of a group of farm cats (Felis
232 catus) in a Swiss mountainous area. J Zool [Internet]. 1998
233 Jun;245(2):S0952836998286092. Available from:
234 <http://doi.wiley.com/10.1017/S0952836998286092>
- 235