



Seroprevalence of *Trichinella* spp. infection in bank voles (*Myodes glareolus*) – A long term study

Maciej Grzybek^{a,*}, Aleksandra Cybulska^b, Katarzyna Tołkacz^c, Mohammed Alsarraf^c, Jolanta Behnke-Borowczyk^d, Klaudiusz Szczepaniak^e, Aneta Strachecka^f, Jerzy Paleolog^g, Bożena Moskwa^b, Jerzy M. Behnke^{h,1}, Anna Bajer^{c,1}

^a Department of Tropical Parasitology, Medical University of Gdansk, Poland

^b Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland

^c Department of Parasitology, University of Warsaw, Warsaw, Poland

^d Department of Forest Pathology, Poznan University of Life Sciences, Poznan, Poland

^e Department of Parasitology and Invasive Diseases, University of Life Sciences in Lublin, Poland

^f Laboratory of Environmental Biology and Apidologie, University of Life Sciences in Lublin, Lublin, Poland

^g Department of Zoology, Ecology and Wildlife Management, University of Life Science in Lublin, Lublin, Poland

^h School of Life Sciences, University of Nottingham, Nottingham, UK

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ABSTRACT

Rodents play an important role as reservoir hosts of zoonotic diseases. As a component of our long-term programme of monitoring parasitic infections in bank vole populations in three ecologically similar sites in NE Poland, we screened blood samples for signs of a serological response to the presence of *Trichinella* spp. The overall seroprevalence of *Trichinella* spp. was 1.52%, but prevalence was largely concentrated in one of our three study sites and confined to the oldest individuals in the study. Seroprevalence of *Trichinella* spp. did not differ between the sexes. Although a local prevalence of 1.52% may seem low, when this is extrapolated to the national population of bank voles in peak years, perhaps numbering hundreds of millions of animals, the number of infected bank voles on a country wide scale is likely to be huge. Our results suggest that bank voles may be reservoirs of *Trichinella* spp. However, on the basis of our results we consider their importance as epidemiologically significant hosts for *Trichinella* spp. to be moderate and their role in this context to require further investigation.

1. Introduction

Trichinella spp., the causative agents of human and animal trichinellosis, are widespread in domestic animals and in wildlife (Pozio, 2000). Although a range of susceptible wildlife species may be available in a particular habitat, not all are likely to be equally important for the maintenance of the parasite's life cycle (Schmitt et al., 1978). Distinguishing between host species that help to maintain the life cycle and others that merely contract spill-over infections that serve no further role in transmission, is important for the purpose of implementing monitoring strategies and designing targeted control measures (Haydon et al., 2002) to limit trichinosis in domestic animals and in the human population.

Sylvatic *Trichinella* infections have been frequently reported from

Central European (Chmurzyńska et al., 2013) and in Polish wild carnivores (Cybulska et al., 2016), among which red foxes (*Vulpes vulpes*) are known to play a crucial role as reservoirs for these nematodes (Cabaj et al., 2000; Cabaj, 2006; Chmurzyńska et al., 2013; Cybulska et al., 2016; Moskwa et al., 2013; Pozio, 2005). A major portion of the diet of red foxes and other local carnivores (Dell'Arte et al., 2007) is comprised of bank voles (*Myodes glareolus*), one of the most wide spread rodent species in European forests (Hutterer et al., 2016; Tenseth, 1985; Wilson and Reeder, 2005). Bank voles are generally considered to be omnivorous, however they occasionally scavenge on and consume the tissues of encountered dead vertebrates (Gębczyńska, 1976; Watts, 1968). Therefore potentially, bank voles may play a role in the maintenance of the sylvatic life cycle of *Trichinella* spp. if infected animal tissue is available for them to feed on.

* Corresponding author. Department of Tropical Parasitology, Medical University of Gdansk, Powstania Styczniowego 9B, 81-519 Gdynia, Poland.
 E-mail address: maciej.grzybek@gumed.edu.pl (M. Grzybek).

¹ These authors contributed equally to this work.

The aim of the current study was to assess the prevalence of antibodies against *Trichinella* spp. in *M. glareolus* in three geographically separated but ecologically similar forest sites in Poland and to identify both intrinsic and extrinsic factors that primarily contribute to variation in seroprevalence of *Trichinella* spp. in this rodent species.

2. Material and methods

2.1. Ethical approval

This study was carried out in strict accordance with the recommendations in the Guidelines for the Care and Use of Laboratory Animals of the Polish National Ethics Committee for Animal Experimentation. Our project was approved by the First Warsaw Local Ethics Committee for Animal Experimentation which also has overarching responsibility for field work involving the trapping and culling of wild vertebrates for scientific purposes (protocol no 73/2010).

2.2. Study sites

Our study sites (Urwitałt, Tały, Pilchy) are located in the Mazury Lake District region in the northeastern corner of Poland approximately 10 km from one another in a NW-SE transect. Site 1 is referred to as Urwitałt (N 53°48'153, EO 21°39'784), Site 2 as Tały (N 53°53'644, EO 21°33'049) and Site 3 as Pilchy (N 53°42'228, EO 21°48'499) after nearby settlements. These forest sites are separated by natural barriers, although genetic studies have revealed some gene flow between the three populations (Kloch et al., 2010). The sites have been described comprehensively in our earlier papers (Behnke et al., 2008a, 2001; Grzybek et al., 2015a).

2.3. Collection of bank voles and the sampling protocols

The study sites were sampled at the same time of year (mid-August to mid-September) in 2002, 2006 and 2010. To confirm seroprevalence in the oldest group of forest rodents and survival of infected individuals, an additional group of overwintered individuals ($n = 12$) was collected in May 2018 in Urwitałt.

Rodents were caught live in wooden traps. Trapping was carried out for 3–4 consecutive days at a time at each site. The methods used for trapping rodents, and for sampling and processing trapped animals have all been thoroughly described (Behnke et al., 2001). Age classes were established as described earlier (Behnke et al., 2001; Grzybek et al., 2014). Age class 1 voles were immature juveniles, age class 2 voles were mostly young adults and age class 3 were breeding older animals. All overwintered rodents were classified as age class 3, as they had originated from previous late-summer or early-autumn cohorts, and were thus at least 7–8 months old in May at the time of sampling.

Blood samples were collected directly from the heart using a sterile 1.5 ml syringe immediately after death from over-exposure to Isoflurane (Baxter, USA), and blood was allowed to clot at room temperature. After separation of the blood clot, samples were centrifuged at 5000 rpm for 10 min using a MPW High-Speed Brushless Centrifuge. Serum was collected and stored at -80°C until samples were analysed on completion of the fieldwork.

2.4. Serological screening of *Trichinella* spp

Bank vole serum samples were analysed using ID Screen® *Trichinella* Indirect Multi-species ELISA kit for the detection of anti-*Trichinella* antibodies (targeting the ES stages of muscle larvae of *T. spiralis*, *T. pseudospiralis*, *T. britovi* and *T. nativa*) in animal serum, according to the manufacturer's instructions. Because limited volumes of serum were available, 5 μl of serum were used from each sampled animal. Optical density was measured at a wave length of 450 nm (0.1s) using an ELx800™ Absorbance Microplate Reader (Bio-Tek). Calculation of the

concentration of anti-*Trichinella* antibodies was carried out using KCJr software (Bio-Tek).

2.5. Statistical analysis

Prevalence values (percentage of animals infected) are given with 95% confidence limits in parenthesis (CL_{95}) or error bars on figures, calculated by bespoke software "PERCENTAGE CONFIDENCE LIMITS VS 13" (courtesy of Dr. F.S. Gilbert and Prof. J.M. Behnke, University of Nottingham) based on the tables of Sokal and Rohlf (1995). The statistical approach has been documented comprehensively in our earlier publications (Bajer et al., 2005; Behnke et al., 2008b, 2001, Grzybek et al., 2018, 2015a). For analysis of seroprevalence in bank voles collected between 2002 and 2010, we used maximum likelihood techniques based on log-linear analysis of contingency tables in the software package IBM SPSS Statistics Version 21 (IBM Corporation). Initially, full factorial models were fitted, incorporating as factors SEX (2 levels, males and females), AGE (3 levels), YEAR (3 levels, 2002, 2006, 2010), and SITE (3 levels, Urwitałt, Tały, Pilchy). The presence or absence of antibodies against *Trichinella* spp. (SEROPREVALENCE) was considered as a binary factor. The importance of each term in interactions involving SEROPREVALENCE in the final model was assessed by the probability that its exclusion would alter the model significantly and these values are given in the text. Since the number of overwintered bank voles was low and animals were collected from one site only, we did not include them in statistical analysis.

3. Results

The overall seroprevalence of *Trichinella* spp. for all investigated animals (2002–2010 and 2018; $n = 668$) was 1.52% (0.9–2.7). Based on animals sampled in 2002, 2006 and 2010, the lowest seroprevalence of *Trichinella* spp. was recorded in 2002 and the highest in 2006 but the difference between years was not significant and hence seroprevalence was relatively stable over this period (Fig. 1). However there was significant variation between study sites (SITE x SEROPREVALENCE, $\chi^2_2 = 10.15$; $P = 0.006$) and the difference was maintained in all three surveys (Fig. 1; the SITE x YEAR x SEROPREVALENCE interaction was not significant). Individuals sampled from Urwitałt showed 3.3-fold higher seroprevalence than those from Tały and prevalence at Urwitałt varied in the range 1.6–4.3%. No bank voles from Pilchy were found to be *Trichinella* spp. seropositive at any time point.

Trichinella spp. seroprevalence differed significantly between host age classes (AGE x SEROPREVALENCE, $\chi^2_2 = 13.58$; $P = 0.001$) with 0.0% (0.0–3.5), 0.4% (0.2–1.6) and 3.4% (2.0–5.5) voles in 1, 2 and 3 age classes, respectively showing seropositivity. Of nine seropositive individuals, eight were mature bank voles and one was classified as a young adult. Seroprevalence was essentially identical in both sexes (for males 1.5% [0.6–3.6] and females 1.35% [0.5–3.2]) (NS). Since most individuals that were positive with anti-*Trichinella* spp. antibodies were recovered from Urwitałt, we performed also a *post-hoc* analysis that included individuals from Urwitałt only. This confirmed a significant effect of host age on seroprevalence of *Trichinella* spp. (AGE x SEROPREVALENCE, $\chi^2_2 = 16.67$; $P < 0.001$) with only the oldest individuals being infected (Table 1).

The presence of anti-*Trichinella* spp. antibodies was confirmed also in one female vole out of the 12 (8.3% [0.4–37.0]) overwintered individuals that were sampled in May 2018.

4. Discussion

In this paper, we have reported the results of an extensive serological monitoring of bank voles from the Mazury Lake District region of Poland for evidence of presence/absence of antibodies against *Trichinella* spp. and our analysis has revealed that seropositivity was dependent primarily on the study site and on host age, but not on host

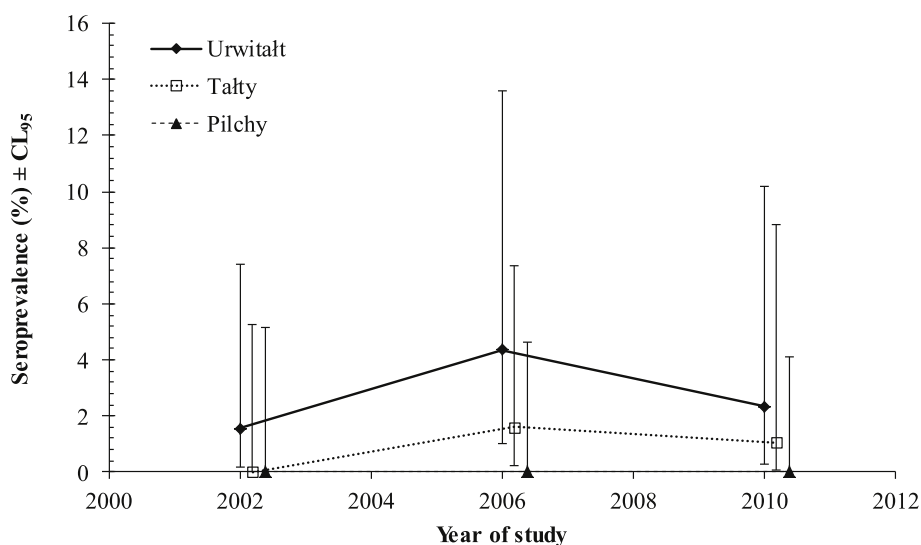


Fig. 1. Variation in *Trichinella* spp. seroprevalence between years (2002–2010) and sites of surveys.

Table 1

Seroprevalence of *Trichinella* spp. between 2002 and 2010 by study site and by host age. *N* – number of animals tested. CL₉₅ – confidence limits.

| Study site | Host age | <i>N</i> tested | Seropositive | Seroprevalence % (± CL ₉₅) |
|--------------------------|----------|-----------------|--------------|---|
| Urwitalt | 1 | 55 | 0 | 0.0 (0.0–4.1) |
| | 2 | 111 | 0 | 0.0 (0.0–2.1) |
| | 3 | 76 | 7 | 10.1 (4.9–19.2) |
| By site | | 242 | 7 | 2.9 (1.6–5.0) |
| Tałty | 1 | 72 | 0 | 0.0 (0.0–5.4) |
| | 2 | 69 | 1 | 1.5 (0.2–7.5) |
| | 3 | 87 | 1 | 1.2 (0.1–8.3) |
| By site | | 228 | 2 | 0.9 (0.3–2.3) |
| Pilchy | 1 | 60 | 0 | 0.0 (0.0–4.5) |
| | 2 | 51 | 0 | 0.0 (0.0–3.8) |
| | 3 | 75 | 0 | 0.0 (0.0–5.6) |
| By site | | 186 | 0 | 0.0 (0.0–3.5) |
| Overall 2002–2010 | | 656 | 9 | 1.37 (0.8–2.5) |

sex and did not vary significantly with time. Although rodents of the genus *Myodes* have been found previously to be infected with *Trichinella* spp. in the USA (Holliman and Meade, 1980), reports from Europe are few, based entirely on occurrence in animal samples from just a single site and often just one survey over a limited period of time. In contrast to our findings, most earlier European studies had failed to detect *Trichinella* spp. in bank voles (Pozio et al., 1996; Välimaa et al., 2010), although there is a single report of the presence of *Trichinella* antibodies in two out of 13 examined bank voles in Poland (Dvoroznakova et al., 2016).

All three of the study sites utilised in the current work are woodlands with a very similar habitat structure (Behnke et al., 2008b, 2001) and all are visited regularly by hunters, forestry workers and by tourists to the region. Seroprevalence was highest among bank voles from the Urwitalt site, whereas no positive voles were detected from Pilchy, a difference that was significant suggesting local differences in intensity of transmission to bank vole populations and possibly even local foci of infection. Our finding that seroprevalence was highest among the oldest voles is in agreement with our expectation and consistent with the results of our previous studies describing the influence of host age on the prevalence of various micro- and macroparasites (Bajer et al., 2014,

2005; Behnke et al., 2008a; Grzybek et al., 2018, 2015b). Seropositivity in bank voles was confirmed also by our finding of a positive individual among a relatively small sample of overwintered rodents examined in May 2018. Small rodents are generally short-lived animals (the mean life span for bank vole is about three months (Bujalska, 1990; Crespin et al., 2002)), and this short life span creates a limitation for serological studies because antibody responses require time to develop to their maxima. Thus, total prevalence based on serology is likely to be underestimated due to some false-negatives among individuals that have not had enough time following infection to develop a sufficiently intense specific antibody response to enable detection.

Although, the diet of bank voles is composed mainly of herbs, roots, bark, seeds and some invertebrates (Hansson, 1979; Hansson, 1985a,b), it is known also that bank voles can enrich their diet with the tissues of mammals if these are available for consumption, usually as carrion (Butet and Delettre, 2011; Gębczyńska, 1976; Lennart Hansson, 1985a,b; Larsson and Hansson, 1977; Watts, 1968). Since the prevalence of *Trichinella* spp. in Polish red foxes is high (10%) (Cybulska et al., 2016), leaving the skinned carcasses of foxes and other carnivores after a hunt in the forest creates a risk factor for spreading of *Trichinella* infections to both wild rodents and other susceptible wild animals (Pozio et al., 2001). Furthermore, cannibalism is well recorded among rodent species (Reperant and Deplazes, 2005), especially in association with aggressive behavior (Marchlewska-Koj et al., 1989) linked to dominance hierarchies (Gustafsson et al., 1980), protection of nests and competition for mates (Horne and Ylönen, 1996; Marchlewska-Koj et al., 1989; Ylönen and Horne, 2002) and this may contribute also to dissemination of *Trichinella* spp. infections if the parasite is enzootic in a rodent population.

An additional possibility is transmission from wild boars, which are also known to be intensively infected with *T. spiralis* and *T. britovi* (Bilska-Zajac et al., 2013; Moskwa et al., 2015). Różycki et al. (2016) reported that prevalence was as high as 5.2% between 2009 and 2013 in wild boars that had been shot by hunters in the Warmińsko-Mazurskie voivodship (the region within which our study sites are located). The diet of wild boars can comprise various rodents including *Myodes* spp. (Schley and Roper, 2003), so transmission from bank voles and *vice versa* is theoretically possible, although to-date there are no reports focused on this route of transmission.

We detected anti-*Trichinella* spp. antibodies in just 1.52% of the animals that we sampled, and although at first sight this may indicate that *Trichinella* spp. infections are relatively rare in wild bank vole populations in NE Poland, suggesting a moderate/minor role for this species as a reservoir of *Trichinella* spp. in the region, when extrapolated

to a national scale, the picture is likely to be quite different. Although the actual number of bank voles likely to be carrying infection is difficult to estimate accurately, a simple calculation can give some idea of the potential numbers involved. Polish forests cover approximately 29.4% of Polish territory (9177.2 thousand hectares; [State Forests Information Center, 2014](#)), and bank vole populations probably number in thousands/hectare at peak time of the year ([Hansson and Henttonen, 1985](#); [Henttonen, 2000](#); [Mazurkiewicz, 1991](#); [Verhagen et al., 2000](#)), and many millions on a national scale. If our data reflect country wide trends the number of bank voles that are actually infected in Poland is likely to be huge. Clearly, further long-term monitoring in other regions of the country and throughout Europe are required to clarify the situation. World Organization for Animal Health's recommends the assessment of wild rodents to reduce exposure of domestic animals and humans to *Trichinella* infections ([Dupouy-Camet and Murrell, 2007](#)). However, this should be conducted with care and respect for the environment. Therefore, our findings are not only of considerable relevance to veterinary and public health services in Poland, but also have relevance for other European regions where *M. glareolus* populations peak at high densities in particular years and where *Trichinella* spp. are known to be enzootic in local sylvatic wildlife.

Authorship

The study was conceived and designed by MG. Supervision of the long-term monitoring of bank vole populations in the region was by JMB and AB. Samples were collected in the field by JMB, AB, MA, KT, JBB & MG. The immunological analysis and laboratory work was conducted by MG, AC, BM, KS and AS. Data analysis and statistic approach was carried by MG and JMB. The ms was written by MG, AB, JP and JMB in consultation with all co-authors. MG and JMB revised the manuscript. All authors approved the final version of the manuscript. The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2019.03.005>.

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