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Parasites in brains of wild rodents (Arvicolinae and Murinae) in the city of Leipzig, Germany

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ABSTRACT

Small rodents serve as intermediate or paratenic hosts for a variety of parasites and may participate in the transmission of these parasites into synanthropic cycles. Parasites with neuroinvasive stages, such as *Toxoplasma gondii* or *Toxocara canis*, can cause detrimental damage in the brain of intermediate or paratenic hosts. Therefore, the occurrence of neuroinvasive parasite stages was evaluated in brains of wild rodents captured in the city of Leipzig, Germany. In addition, a few specimens from the cities of Hanover, Germany, and Vienna, Austria were included, resulting in a total of 716 rodents collected between 2011 and 2016. Brains were investigated for parasitic stages by microscopic examination of native tissue, artificially digested tissue as well as Giemsa-stained digestion solution to verify positive results. Infective stages of zoonotic ascarids or other helminths were not detected in any sample, while coccidian cysts were found in 10.1% (95% CI: 7.9–12.5%; 72/716) of examined brains. The most abundant rodent species in the study was the bank vole (*Myodes glareolus*; Arvicolinae), showing an infection rate with cerebral cysts of 13.9% (95% CI: 11.0–17.8%; 62/445), while 2.7% (95% CI: 1.0–5.8%; 6/222) of yellow-necked mice (*Apodemus flavicollis*; Murinae) were infected. Generalized linear modelling revealed a statistically significant difference in prevalence between *M. glareolus* and *A. flavicollis*, significant local differences as well as an effect of increasing body mass on cyst prevalence. Coccidian cysts were differentiated by amplification of the *18S rRNA* gene and subsequent sequencing. The majority of identifiable cysts (97.9%) were determined as *Frenkelia glareoli*, a coccidian species mainly circulating between *M. glareolus* as intermediate and buzzards (*Buteo* spp.) as definitive hosts. The zoonotic pathogen *Toxoplasma gondii* was confirmed in one *M. glareolus* originating from the city of Leipzig. Overall, it can be concluded that neuroinvasion of zoonotic parasites seems to be rare in *M. glareolus* and *A. flavicollis*.

1. Introduction

The increasing urbanisation and the conversion of natural habitats to agricultural areas provoke the synurbanisation of wild animals (Mackenstedt et al., 2015). The resulting habitat overlap of wild animals with livestock, companion animals or stray dogs and cats entails the risk of pathogen spillover from sylvatic to domestic or even synanthropic cycles (Duscher et al., 2015). In Central Europe, rodents from the subfamilies Arvicolinae (e.g., *Myodes glareolus* and *Microtus agrestis*) and Murinae (e.g., *Apodemus agrarius*, *Apodemus flavicollis* and *Apodemus sylvaticus*) are widely distributed and highly abundant (Niedzialkowska et al., 2010), and therefore of interest as potential intermediate or paratenic hosts in research studies on zoonotic parasites. Even though various zoonotic parasites cannot be transmitted directly from rodents to humans or the risk of direct transmission is rather low, predation of rodents may cause infections in carnivore pets as definitive hosts, which may then contaminate households, gardens and further human-related environments. For instance, environmental contamination rates in public areas in Central Europe with eggs of *Toxocara* spp., the dog and cat roundworm, using amongst others

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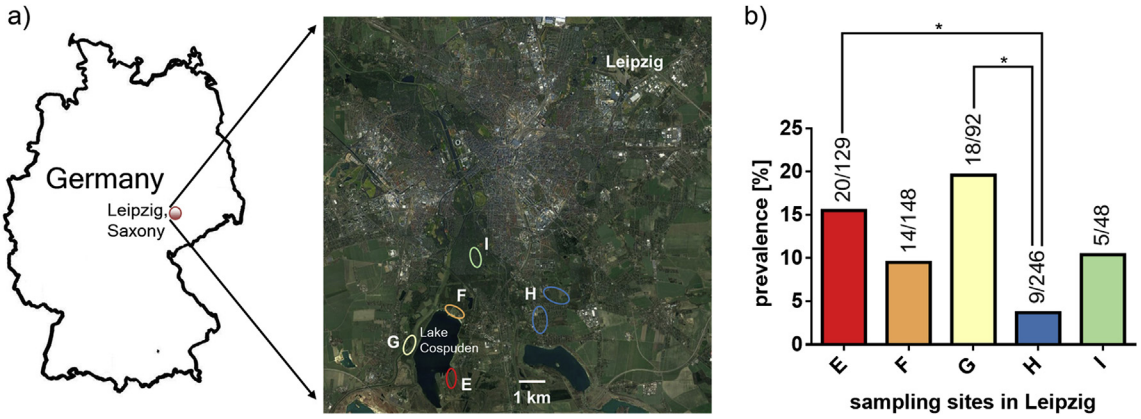

Fig. 1. a) Map of the city of Leipzig, Germany, depicting the five sampling sites analysed in the present study (Google earth, V 7.1.8.3036 [04 September 2018] Leipzig, Germany, 51° 19′ 50.00″N 12° 23′ 19.33″O, eye alt 18.0 km), b) prevalence of parasitic stages in brains of examined wild rodent species originating from the five sampling sites between the years 2012 and 2014. Asterisks indicate statistically significant differences ($P \leq 0.05$).

rodents as paratenic hosts, varied between 14.0% and 20.4% (Dubná et al., 2007; Vanhee et al., 2015). In the city of Hanover, Germany, up to 41.3% of inspected sandpits on playgrounds were contaminated with *Toxocara* eggs, with 23.9% containing embryonated and thus potentially infective eggs (Kleine et al., 2017).

Regarding zoonotic cestodes, many rodent species may act as suitable intermediate hosts for *Echinococcus multilocularis* (Deplazes et al., 2004; Hegglin et al., 2015; Vuitton et al., 2003), using particularly foxes, but also other wild and domestic carnivores as definitive hosts, while humans can be infected as intermediate hosts (Deplazes et al., 2004).

An important zoonotic coccidia infecting rodents is *Toxoplasma gondii*, using felids as definitive hosts. Hunting small prey constitutes a risk factor for cats to acquire *T. gondii* infection; however, the risk is related to the general prey availability and the composition of prey species (Afonso et al., 2007). Kijlstra et al. (2008) demonstrated the impact of the rodent subfamilies Arvicolinae and Murinae as well as the shrew subfamily Crocidurinae in the transmission of *T. gondii* to pigs and the relevance of pest control in the production of *T. gondii*-free pork. Furthermore, *Toxoplasma gondii* can be transmitted to domestic animals and humans by ingesting infective oocysts from the environment or through the consumption of cysts in raw or undercooked meat (Petersen, 2007; Tenter et al., 2000). Even though *T. gondii*-infected cats may exhibit clinical signs such as fever, anorexia, lethargy, and neurologic abnormalities, feline toxoplasmosis is commonly clinically inapparent (Vollaire et al., 2005; Elmore et al., 2010). In humans, toxoplasmosis is one of the most frequently reported parasitic zoonosis globally (Montoya and Liesenfeld, 2004; Pappas et al., 2009; Petersen, 2007; Tenter et al., 2000).

Neuroinvasion of *Toxocara* spp. in humans may result in meningitis, encephalitis, myelitis or cerebral vasculitis as well as neuropsychological disturbances like dementia or depression (Eberhardt et al., 2005; Fan et al., 2015; Macpherson, 2013; Moreira-Silva et al., 2004). Furthermore, human *Toxocara* spp. as well as *T. gondii* infection may result in reduced cognitive functions and behavioural alterations (Fan et al., 2015; Flegr, 2007, 2013; Walsh and Haseeb, 2012). As both parasitoses may have severe consequences for human health, the US-American Centers for Disease Control and Prevention (CDC) assigned them to the five neglected human parasitic infections with priority for public health action (CDC, 2018). Experimental infections of C57BL/6 mice with *T. canis* and *T. cati*, respectively, resulted in abnormal behaviour and impaired memory functions (Janecek et al., 2017). *Toxoplasma gondii*-infected mice lose their aversion against feline odors and exhibit more risky behaviours (Afonso et al., 2012; Vyas et al., 2007). Such experimental data lead to the hypothesis that those parasite-mediated changes in host behaviour enhance the chance of transmission to the specific definitive host (Klein, 2005; Thomas et al., 2005). However, information about the occurrence of cerebral parasite stages in wild rodents is scarce. Krücken et al. (2017) examined arvicolid and murid rodents including cerebral tissues in the city of Berlin, Germany, by using ELISA and PCR assays. However, these techniques do not necessarily target current infections. Therefore, the present study aimed to evaluate the presence of neuroinvasive parasites in brains of wild-caught rodents by microscopic examination.

2. Material and methods

2.1. Locations and sampling of rodents

Wild rodents were collected between 2011 and 2015 in the city of Leipzig, Germany. Additionally, a few specimens were included from the cities of Hanover, Germany, and Vienna, Austria. In Leipzig, rodents were caught in Sherman© live animal traps (H. B. Sherman Traps Inc., Tallahassee, FL, USA) set for two successive nights each month at each site at the same time. Apple slices were used as bait and hay as insulation material. The traps were controlled twice a day; captured animals were anesthetized on the spot with CO_2 and euthanized by cervical dislocation. The specimens were morphologically identified using a taxonomic key (Hauer et al., 2009), dissected in the laboratory and brain samples were stored at −80 °C until further processing. In case species identification was not possible by visual inspection, the partial *cytochrome b* gene was used to define the species as described below. Trapping in and around the city of Leipzig (permit numbers AZ 36.11–36.45.12/4/12-001-MH, AZ 364.620/2009-102-2 and AZ 364.620/30/6/2) was described in detail previously (Silaghi et al., 2011, 2016). In brief, five different sites were selected (Fig. 1a). Of these, site "G" (51°16′02.1″N, 12°19′00.3″E) was sampled only in 2012, while site "I" (51°18′02.6″N, 12°22′17.1″E) was used in 2012 and 2013. Both of these sites consisted of old alluvial forest. The three remaining sites "E" (51°15′36.5″N, 12°21′00.4″E), "F" (51°17′00.9″N, 12°21′02.8″E) and "H" with two close locations (H1: 51°18′14.6″N, 12°24′41.4″E, and H2: 51°17′35.5″N, 12°24′07.5″E) were sampled from March 2012 to April 2015. They are also recreational areas, but have been artificially created from a former brown coal mining area (site E & F) and a former waste disposal area (site H). Live trapping was also conducted in Vienna, Austria, where rodents were sampled in 2016 at the Schönbrunn Park using 8 traps (ethical committee permission ETK-10/04/2015 and certified by urban administration office MA 22-358324/2015). From the city of Hanover, Germany, wild rodents preyed upon by pet cats during regular outdoor access were collected in 2016. When brought to the owner, dead rodents were immediately frozen at −20 °C. Regarding rodents collected in Hannover and Vienna,

only animals that harboured cerebral parasite stages were subjected to molecular identification of the rodent species.

2.2. Detection of brain parasitic stages by microscopy

Brain samples were thawed on ice and divided into hemispheres. For analysis of native preparations, approximately 25 mg of cerebral tissue from one hemisphere (approx. 10% the total hemisphere weight) of each sample were squashed on glass slides under coverslips and examined microscopically (100-400x magnification) for parasitic stages.

To assure reliable detection of nematode larvae, the remaining brain tissue was cut into small pieces and digested in 6–8 ml of a solution containing 300 mM HCl (Carl Roth GmbH, Germany) and 1% pepsin (Merck, Germany) at 37 °C in a shaker for four to five hours. Afterwards, 0.8–1.0 ml digestion solution was retained for Giemsa staining (see below). The remaining digestion solution was microscopically examined (100-400x magnification).

If a sample was parasite-positive, additional Giemsa staining was applied. Thin smears of the digestion solution were prepared on glass slides, air dried, then fixed with methanol (99.9% v/v) for 3 min and stained with freshly prepared 10% Giemsa staining solution (in Weise buffer, pH 7.2) for 30 min. Afterwards, the staining solution was carefully completely removed with distilled water. Stained slides were air dried and examined microscopically (100x magnification).

2.3. DNA extraction from parasite-positive brains

Genomic DNA was extracted from positive brain samples using the NucleoSpin® Tissue Kit (Macherey Nagel, Germany) according to the manufacturer's instructions. The tissue used for squash preparation was gathered from the microscope slide with PBS and homogenised in 180 µl of supplied T1 buffer and 25 µl proteinase K (Macherey Nagel, Germany) using the Precellys® 24 (Peqlab Biotechnologie, Germany) by two cycles of 15 s with 5400 rpm with a break of 5 s to avoid overheating. The homogenate was incubated at 56 °C overnight. The following steps were conducted according to the manufacturer's instructions with the modification that DNA was eluted twice with each 50 µl bidistilled water.

2.4. Molecular identification of parasite species

For identification of detected coccidian cysts, a part of the *18S rRNA* gene was amplified in a 25 µl reaction volume containing 16 µl DEPC-treated water, 2.5 µl 10x *Taq* buffer, 0.5 µl dNTP mix (10 mM each), 1 µl each of primers COC1 and COC2 (10 µM) (Ho et al., 1996), 1 µl PerfectTaq DNA Polymerase (0.2 U/µl; 5 PRIME GmbH, Germany) and 3 µl DNA template. PCR cycling parameters comprised an initial denaturation step at 94 °C for 3 min, followed by 35 cycles at 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min and subsequent final elongation at 72 °C for 10 min. PCR products were run on 2% agarose stained with GelRed® (Biotium Inc., Fremont, CA, USA) and visualised under UV light. If an amplification product was visible, the band was cut out for custom Sanger-sequencing (Seqlab Sequence Laboratories, Germany and GATC Biotech, Germany). Obtained sequences were compared with published sequences in NCBI GenBank using BLAST.

2.5. Molecular identification of captured rodents

For molecular discrimination of captured rodents, a part of the *cytochrome b* gene was amplified using genomic DNA isolated from the brain (rodents from the cities of Hanover and Vienna) or skin (rodents from the city of Leipzig) as template. Amplification was carried out in a 25 µl reaction volume containing 17.5 µl DEPC-treated water, 2.5 µl of 10x DreamTaq buffer, 0.5 µl of DreamTaq DNA Polymerase (0.1 U/µl; Thermo Fisher Scientific, USA), 0.5 µl dNTP mix (10 mM each), 1 µl each of primers cytb n f: 5′-CAT CCA ACA TCT CAT CAT GAT GAA A-3′ and cytb n r: 3′-CCT CAR AAT GAY ATT TGT CCT CA-5’ (10 µM each) (Parson et al., 2000) and 3 µl template DNA. Cycling conditions comprised an initial denaturation at 95 °C for 3 min, followed by 45 cycles of 94 °C for 30 s, 49 °C for 30 s, 72 °C for 60 s and subsequent final elongation at 72 °C for 10 min. Amplification products were custom Sanger-sequenced (Seqlab Sequence Laboratories, Germany and GATC Biotech, Germany) and compared against sequences available in NCBI GenBank using the Blast algorithm.

2.6. Statistical analyses

Statistical analyses were conducted in R. v. 3.5.1 (R Core Team, 2018). To analyse differences in the prevalence of coccidian cysts between *M. glareolus* and *A. flavicollis*, a general linear mixed model (GLMM) with binomial error structure and logit link function was implemented. Other or unidentified specimens were not considered in this analysis. Rodent species was included as a fixed factor, while the city of sampling was included as a random factor.

Furthermore, data on body mass and sex were available for rodents collected in Leipzig from 2012 to 2014. Thus, we fitted a general linear model (GLM) with binomial error structure and logit link function to test the effect of these variables on coccidian cyst prevalence. Sampling year, sampling site, season of sampling (summer/autumn/winter/spring) and rodent species were included as additional fixed factors. Only data from *M. glareolus* and *A. flavicollis* were included in this analysis, as sample sizes of the other species were low. Interactions between predictor variables were removed if not significant. Multiple comparison between the levels of sampling year, sampling season and sampling site were conducted using Tukey HSD contrasts with single-step *P*-value adjustment (package “multcomp”, Hothorn et al., 2008). Final models were compared to null models containing only the random factor (GLMM) or an intercept term (GLM) in a likelihood ratio test.

3. Results

3.1. Captured rodents at the different sampling sites

In total, 716 rodents were captured and included in the study. Of these, 682 specimens originated from Leipzig, Germany, comprising bank voles (*Myodes glareolus*; n = 445), yellow-necked mice (*Apodemus flavicollis*; n = 220), common voles (*Microtus arvalis*; n = 9), striped field mice (*Apodemus agrarius;* n = 7), and a field vole (*Microtus agrestis*; n = 1). Morphological species identification was possible for 657 specimens from Leipzig, molecular species identification was successfully applied for the 25 remaining specimens. From the city of Hanover, Germany, 21 rodents were available. Molecular species discrimination of the parasite-positive rodent failed. Thirteen rodents originated from the city of Vienna, Austria. Molecular species determination was successful for two of five parasite-positive rodents from Vienna; both were identified as *A. flavicollis* (Table 1).

3.2. Occurrence of neuroinvasive parasites

In the analysed brains, only coccidia were found, ascarid larvae or other helminths were not detected in any of the samples. Coccidian cysts were detected in 10.1% (95% CI:7.9–12.2%; 72/716) of the brain tissue preparations. In additional Giemsa-stained smears of the digestion solution, cysts were detected in 31 (43.1%) of the 72 positive samples. The number of coccidian cysts per positive sample in the squash preparations varied between 1 and 58 (mean: 6.88 cysts). Cysts were grouped in two different size classes. Big cysts (average diameter of about 180 µm) were found in 84.7% (61/72) of the brains (1-58 cysts, mean cyst number: 7.23), small cysts (average diameter of about 30 µm) in 13.9% (10/72; 1-3 cysts, mean cyst number: 2.00), and only one rodent harboured both size classes (1.4%, 1/72; 3 big cysts and 1 small cyst). Detailed results on the prevalence in the different host

Table 1
Examined rodent species at the different sampling sites (individuals harbouring neuroinvasive parasites/total number) and percentage of neuroinvasive parasite-positive rodents [95% confidence interval].

Rodent species	Sampling site			Total
	Leipzig	Hanover	Vienna	
Apodemus flavicollis	4/220 1.4% [0.3–4.0%]	none identified –	2/2[a] 100.0% [15.8–100.0%][a]	6/222 2.7% [1.0–5.8%]
A. agrarius	0/7 0.0% [0.0–41.0%]	none identified –	none identified –	0/7 0.0% [0.0–41.0%]
Myodes glareolus	62/445 13.9% [10.9–17.5%]	none identified –	none identified –	62/445 13.9% [11.0–17.8%]
Microtus arvalis	0/9 0.0% [0.0–36.9%]	none identified –	none identified –	0/9 0.0% [0.0–36.9%]
Unknown/other rodent species	0/1[b] 0.0% [0.0–97.5%]	1/21 4.8% [0.1–23.8%]	3/11 27.3% [6.0–61.0%]	4/33 12.1% [3.4–28.2%]
Total	66/682 9.7% [7.5–12.1%]	1/21 4.8% [0.1–23.8%]	5/13 38.5% [13.9–68.4%]	72/716 10.1% [7.9–12.5%]

[a] note that only parasite positive specimens were subjected to rodent species identification.
[b] identified as *Microtus agrestis*.

species are provided in Table 1.

Considering data on *M. glareolus* and *A. flavicollis* from all study years, the prevalence of coccidian cysts was significantly higher in *M. glareolus* than in *A. flavicollis* (GLMM, $P < 0.001$, Table 2). In fact, the odds of being cyst-positive were 8.29 times higher for *M. glareolus* than *A. flavicollis*. Protozoan cysts were not detected in *M. arvalis*, *M. agrestis* and *A. agrarius* specimens.

The overall prevalence of neuroinvasive coccidia in specimens derived from Leipzig was 9.7% (95% CI: 7.5–12.1%; 66/682). Among the *A. flavicollis* and *M. glareolus* specimens collected in Leipzig from 2012 to 2014 (n = 646), a significant relationship of cyst prevalence with increasing body mass was found (GLM, $P = 0.042$, Table 3) in addition to a significant species difference ($P < 0.001$). Furthermore, differences in prevalence between local sampling sites were detected. The local prevalence was significantly lower at site H (3.7%, 95% CI: 1.7–6.8%; 9/246) than at site E (15.5%, 95% CI: 9.7–22.9%; 20/129; $P = 0.047$) and at site G (19.6%, 95% CI: 12.0–29.1%; 18/92; $P = 0.012$, Fig. 1b). Moreover, a significantly lower prevalence was found in summer as compared to spring ($P = 0.037$). Host sex and sampling year had no significant influence.

Among rodents derived from Hanover, the prevalence of coccidian cysts was 4.8% (95% CI: 0.1–23.8%; 1/21), while rodents from Vienna showed a prevalence of 38.5% (95% CI: 13.9–68.4%; 5/13). However, due to the low sample sizes in both cities results should be treated with caution.

3.3. Species discrimination of coccidian cysts

Species differentiation by analysis of a part of the *18S rRNA* gene was successful for 48 (68.6%) of the 72 microscopically positive samples. One of the 48 obtained sequences (2.1%; 95% CI: 0.5–11.1%) was 100% identical to *T. gondii* (GenBank accession no. L49390). The *T. gondii*-positive specimen was a *M. glareolus* originating from the city of Leipzig, harbouring small cysts. Sequences of the remaining 47 samples (95% CI: 88.9–99.9%) were identified as *Frenkelia glareoli* (98–100% nucleotide identity to GenBank acc. no. AF009245). Forty-six *F. glareoli*-positive samples were from *M. glareolus* originating from Leipzig, the remaining one was an *A. flavicollis* specimen from Vienna. In the remaining 24 cyst-positive cases (15 samples from *M. glareolus*/Leipzig, four from *A. flavicollis*/Leipzig, one from *A. flavicollis*/Vienna and four from unknown species [one from Hanover; three from Vienna]) molecular species discrimination of cysts failed due to lacking amplification products or unsatisfactory sequencing results. Regarding cyst size, sequencing of samples harbouring big cysts resulted in *F. glareoli* in 46 cases, while in 15 cases PCR and/or sequencing were unsuccessful. Regarding small cysts, sequencing revealed *T. gondii* in one case and *F. glareoli* in another, while the coccidian species in the remaining 8 samples could not be identified. The molecular species discrimination for the sample harbouring cysts in both size classes identified an infection with *F. glareoli*.

4. Discussion

As rodents acting as intermediate or paratenic hosts for zoonotic parasites may serve as bridges between wildlife communities and human or domestic animal populations (Bordes et al., 2015; Meerburg et al., 2009; Reperant et al., 2009), surveillance of zoonotic parasites in rodents might be a useful tool in the risk assessment of human infections. Indeed, arvicolid and murid rodents are regarded as shared indicators for zoonotic parasites of carnivores, such as *T. gondii* or *Toxocara* spp., in urban environments (Reperant et al., 2009). Hildebrand et al. (2009) detected larvae of *Toxocara* spp. in 12.9% of arvicolid and murid rodents trapped at recreation grounds in Wroclaw (Poland). In one case, representing 3.2% of the study population, larvae were found in the brain (Hildebrand et al., 2009). In arvicolid and murid rodents captured in the city of Berlin, Germany, 3.1% were positive for *T. canis* DNA (Krücken et al., 2017). Of these, 2.3% were DNA-positive in the muscle tissues and 0.8% in cerebral tissues. Furthermore, 1.6% of the specimens were positive for *T. cati* DNA, which was detected only in muscle tissues. Even though detection of DNA does not necessarily

Table 2
Results of General Linear Mixed Model (GLMM) testing the effect of species (*M. glareolus vs. A. flavicollis*) in captured rodents (n = 667). The final model was significantly different from a null model containing only an intercept term (Likelihood ratio test, df = 1, $\chi = 29.92$, $P < 0.001$). Significant *P*-values are printed in bold.

Variable	Odds ratio	Estimate	Std. error	*z*-value	*P*-value	Interpretation
Intercept	NA	0.20	4.50	0.04	0.965	
Species (*M. glareolus vs. A. flavicollis*)	8.29	2.11	0.51	4.13	< **0.001**	*M. glareolus* > *A. flavicollis*

Table 3
Results of General Linear Model (GLM) testing the effect of different predictor variables on the occurrence of protozoan cysts in brains of *M. glareolus* and *A. flavicollis* collected in Leipzig, Germany, from 2012 to 2014 (n = 646). The final model was significantly different from a null model containing only an intercept term (Likelihood ratio test, df = 12, $\chi^2 = 62.29$, $P < 0.001$). Significant *P*-values are printed in bold.

Variable	Odds ratio	Estimate	Std. error	*z*-value	*P*-value	Interpretation
Intercept	NA	−4.71	1.00	−4.73	< **0.001**	
Body mass	NA	0.06	0.03	2.04	**0.042**	Increased probability with higher body mass
Sex (females *vs.* males)	0.62	−0.47	0.29	−1.62	0.105	
Rodent species (*M. glareolus vs. A. flavicollis*)	12.55	2.53	0.62	4.08	< **0.001**	*M. glareolus* > *A. flavicollis*
Year[a]						
2013 *vs.* 2012	1.21	0.19	0.52	0.37	0.927	
2014 *vs.* 2012	0.88	−0.12	0.45	−0.27	0.960	
2014 *vs.* 2013	0.73	−0.31	0.60	−0.53	0.857	
Season[a]						
Spring *vs.* autumn	1.16	0.15	0.44	0.34	0.986	
Summer *vs.* autumn	0.47	−0.75	0.40	−1.87	0.224	
Winter *vs.* autumn	0.82	−0.19	0.90	−0.22	0.996	
Summer *vs.* spring	0.41	−0.90	0.34	−2.64	**0.037**	Summer < spring
Winter *vs.* spring	0.71	−0.34	0.91	−0.38	0.980	
Winter *vs.* summer	1.75	0.56	0.86	0.65	0.909	
Sampling site[a]						
Site F *vs.* E	0.74	−0.30	0.39	−0.77	0.937	
Site G *vs.* E	1.18	0.32	0.40	0.81	0.927	
Site H *vs.* E	0.30	−1.21	0.44	−2.74	**0.047**	H < E
Site I *vs.* E	0.98	−0.02	0.58	−0.03	1.000	
Site G *vs.* F	1.87	0.63	0.41	1.52	0.545	
Site H *vs.* F	0.41	−0.90	0.46	−1.94	0.287	
Site I *vs.* F	1.33	0.29	0.58	0.49	0.988	
Site H *vs.* G	0.22	−1.53	0.48	−3.19	**0.012**	H < G
Site I *vs.* G	0.71	−0.34	0.57	−0.60	0.974	
Site I *vs.* H	3.29	1.19	0.63	1.90	0.310	

[a] Multiple comparisons between the levels of the factors “year”, “season” and “sampling site” were calculated using Tukey contrasts with single-step *P*-value adjustment.

indicate current infections with live pathogens, the low cerebral detection rates are in line with the present study, which did not detect ascarid larvae in any of the analysed brains. This is in contrast to high neurosusceptibility in laboratory inbred mice, serving as a model for human neurotoxocarosis (Epe et al., 1994; Hamilton et al., 2006; Janecek et al., 2014).

With 92.9% of the total sample size, *M. glareolus* and *A. flavicollis* were the major rodent species represented in this study. They mainly occur in forests, but also on agricultural land and rural areas. While the yellow-necked mouse, *A. flavicollis*, predominantly feeds on seeds, the diet of the bank vole, *M. glareolus*, is more versatile, containing seeds, fruits as well as leaves and grasses. Furthermore, both species occasionally feed on invertebrates (Stenseth et al., 2002). Antolová et al. (2004, 2013) found a significant effect of rodent diet on *Toxocara*-seropositivity, with higher exposure rates in granivores (genera *Apodemus*, *Mus* and *Micromys*) than herbivores (genera *Myodes* and *Microtus*), probably because the former are more likely to ingest contaminated substrates (Antolová et al., 2013).

Tapeworm cysts were also not detected in any of the specimens. Different *Taenia* species are known to cause cerebral infections, the so-called neurocysticercosis. Cysticerci of the mustelid tapeworm *Taenia martis*, a frequent cause of cysticercosis in rodents in Central Europe, normally settles in the pleural and peritoneal cavities (Brunet et al., 2015; Loos-Frank, 2000). However, there are reports of *T. martis* as the causative agent of human neurocysticercosis (Brunet et al., 2015; Eberwein et al., 2013). Interestingly, one case report refers to the removal of a *T. martis*-cysticercus from the eye of a woman living in a rural area near the city of Hanover (Koch et al., 2016).

All detected cerebral parasite stages in the presented study were determined as coccidian cysts, which were found in 10.1% of the rodent brains. The majority of identified cysts, 97.9%, was determined as *F. glareoli*, while *T. gondii* was also detected in one sample. Unfortunately, of the 72 samples tested positive in microscopy, molecular identification of the coccidian species was successful in only 68.6% of cases. One reason might be the relatively low cyst number in the utilized 25 mg brain tissue, and the extracted DNA that may not have contained enough cyst template for successful amplification and sequencing.

Frenkelia glareoli was identified in all successfully sequenced specimens harbouring big cysts in the brain. Furthermore, this species was also detected in one sample containing small cysts. It remains unclear whether these represented young *F. glareoli* cysts, or if the *F. glareoli* sequence originated from an undetected big cyst. The life cycle of *F. glareoli* is obligatory heteroxenous with buzzards as definitive hosts (Mugridge et al., 1999), while small rodents serve as intermediate hosts. In these, the cysts are exclusively located in cerebral tissues (Geisel et al., 1978, 1979; Mugridge et al., 1999). Of potential intermediate hosts, *M. glareolus* is the most important one. Grikienienė et al. (2003) monitored *F. glareoli* in small rodent species in Lithuania and found *F. glareoli* mainly in brains of *M. glareolus*, but not in brains of *Apodemus* species. Krücken et al. (2017) detected a comparable distribution of *F. glareoli* in brains of investigated arvicolid and murid rodents, as cysts were mainly detected in *M. glareolus*, but only in a few *A. flavicollis* and *M. agrestis*. These results are confirmed by the present study, which identified a significantly higher overall prevalence of coccidian cysts in *M. glareolus* than *A. flavicollis*. The majority of *F. glareoli* cysts identified by sequencing occurred in *M. glareolus*, with only one *A. flavicollis* also carrying these cysts. Furthermore, a small, but significant relationship of coccidian infection and body mass was detected, possibly reflecting cumulative parasite exposure as the animals mature and gain in body mass. A spillover of *F. glareoli* to domestic mammals or even a zoonotic potential has not been described so far.

In contrast, the coccidia *T. gondii* is one of the most frequently reported zoonotic parasites worldwide, with a major impact on human health (Petersen, 2007; Tenter et al., 2000). The role of wild rodents in the epidemiology of toxoplasmosis has been investigated by several authors (e.g. Dubey and Jones, 2008; Herrmann et al., 2012; Machačová et al., 2016; Meerburg et al., 2012; Tenter et al., 2000; Vujanić et al., 2011). In the current study, one (2.1%) of the successfully sequenced samples contained *T. gondii*-cysts. This low prevalence is in accordance with findings of previous studies concerning cerebral

cysts in wild Arvicolinae and Murinae (Krücken et al., 2017; Vujanić et al., 2011), and also in Crocidurinae (Meerburg et al., 2012).

Even though neuroinvasion of *T. gondii* and other parasites in intermediate or paratenic hosts is a well-known phenomenon, the predilection sites of, e.g., *Toxocara*-larvae differ between different laboratory rodent strains (Burren, 1972; Strube et al., 2013). Similarly, tissue burdens and predilection sites of *T. gondii* varied among different food animal species (Juránková et al., 2015). Such host species-specific predilection sites might explain the low cerebral prevalences in the examined Arvicolinae and Murinae in the current study. Additionally, further parameters including the host's susceptibility to particular parasites may influence infection rates. However, the absence of cerebral parasitic stages in *M. arvalis* and *A. agrarius* does not necessarily imply that these rodent species are insusceptible to neuroinvasive parasites. Sample sizes for both species were low (9 and 7 specimens, respectively), hence a low percentage of positive individuals in the population may have remained undetected, as illustrated by the high upper limits of the 95% confidence intervals (36.9% and 41.0%, respectively). Applying the “rule of three” when interpreting zero numerators according to Hanley and Lippmanhand (1983), resulted in similar confidence intervals. Furthermore, Arvicolinae and Murinae have relatively small home range sizes and the obtained data on *M. glareolus* and *A. flavicollis* from Leipzig showed that the prevalence of neuroinvasive parasites varied significantly over small geographical distances (cf. Fig. 1). The small sample sizes for *M. arvalis* and *A. agrarius* precluded detecting such local differences.

During neuroinfection, behavioural alteration is a well described effect, with *T. gondii* as a paragon for such parasite-induced alterations. The impact of toxoplasmosis has been demonstrated in numerous studies with laboratory mice, resulting amongst others in impaired motor performance (Havlíček et al., 2001; Hay et al., 1983; Hutchison et al., 1980), deficits in learning capacity and memory (Witting, 1979) as well as more risky behaviour and reduced aversion against feline odors (Afonso et al., 2012; Vyas et al., 2007). Infections with *Toxocara* spp. also induce behavioural alterations, memory impairments and cognitive dysfunctions in the murine model (Cox and Holland, 2001; Janecek et al., 2017) suggesting that observed changes in host behaviour may increase the chance of parasite transmission to the final hosts by predation (Klein, 2005; Thomas et al., 2005). However, the immunogenicity of these particular laboratory mouse strains is optimised to serve as model for human diseases. Therefore, laboratory mouse strains are highly divergent in their immune response patterns compared to wild mice (Sellers et al., 2012). The rather low prevalences of *Toxocara* spp. and *T. gondii* in brains of wild mice observed in this and other studies (Krücken et al., 2017; Meerburg et al., 2012; Schmidt et al., 2014; Vujanić et al., 2011) possibly indicate that wild rodents are not particularly susceptible to neuroinvasion, and associated behavioural changes may only play a subordinate role in the natural life cycle of these parasites. This might be construed from the sample set from the city of Hanover. All analysed rodents were preyed upon by cats during regular outdoor access, reflecting the predator-prey life cycle and possible parasite transmission routes. However, only one of these rodents (4.8%) carried cyst-forming coccidia in the brain. Unfortunately, parasite species discrimination was not successful for this sample, thus, the causative agent of the infection remains unknown. It needs, however, to be considered that the sample size in Hanover as well as Vienna was fairly small. Thus, obtained results do not necessarily represent the local prevalence and have to be treated with caution.

In conclusion, neuroinvasion of zoonotic parasites seems to be rare in *M. glareolus* and *A. flavicollis*. Even though coccidian cysts were found in the rodent brains, only 2.1% of the successfully identified samples revealed *T. gondii* infection, while the remaining cysts represented *F. glareoli*, which is not relevant for human or domestic animal health. Furthermore, neither ascarid larvae nor other neuroinvasive helminth stages were detected in the rodents’ brains. However, further studies on rodent species other than *M. glareolus* and *A. flavicollis* are required to assess whether zoonotic parasites exhibit neuroinvasion more frequently or even show neurotropism in other wild rodent species, or if this phenomenon is restricted to certain laboratory rodent species or strains, respectively.

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Declarations of interest

All authors declare that they have no competing interests.

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