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

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A B S T R A C T

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 ascards 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%; 6/445), 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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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 inapparent (Vollaire et al., 2005; Elmore et al., 2010). In humans, toxoplasmosis is one of the most frequently reported parasitic zoonosis globally (Montoya and Lienfeld, 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; 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 CO2 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 10/04/2015 and certificate MA 22-364.620/30/6/2) 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 were “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 preys upon 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.

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), 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 addition, 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 squashes 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 genera were presented.
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 coccidial 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 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 | none identified | 2/2 | 6/222 |
| A. agrarius | 0.7 | none identified | none identified | 0.0% [0.0–41.0%] |
| Myodes glareolus | 62/445 | none identified | none identified | 13.9% [11.0–17.0%] |
| Microtus arvalis | 0.9 | none identified | none identified | 0.9 |
| Unknown/other rodent species | 0/1 | 1/21 | 3/11 | 4/33 |
| Total | 66/682 | 1/21 | 5/13 | 72/716 |

* Note that only parasite positive specimens were subjected to rodent species identification.

† Identified as Microtus agrestis.

### 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, \( df = 1 \), \( \chi^2 = 29.92, P < 0.001 \)). 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         | $-4.71$  | $1.00$     | $-4.73$ | $< 0.001$ | Increased probability with higher body mass |
| Body mass                     | NA         | $0.06$   | $0.03$     | $2.04$  | $0.042$ |                                     |
| (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                          |           |          |           |         |         |                                     |
|-------------------------------|-----------|----------|-----------|---------|---------|-------------------------------------|
| 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                       |           |          |           |         |         |                                     |
|-------------------------------|-----------|----------|-----------|---------|---------|-------------------------------------|
| 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. summer             | $0.71$    | $-0.34$  | $0.91$    | $-0.38$ | $0.980$ |                                     |
| Winter vs. summer             | $1.75$    | $0.56$   | $0.86$    | $0.65$  | $0.909$ |                                     |

| Sampling site                |           |          |           |         |         |                                     |
|-------------------------------|-----------|----------|-----------|---------|---------|-------------------------------------|
| Site F vs. E                  | $0.74$    | $-0.30$  | $0.39$    | $-0.77$ | $0.937$ |                                     |
| Site G vs. E                  | $1.18$    | $0.52$   | $0.40$    | $0.81$  | $0.927$ |                                     |
| 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                  | $3.29$    | $1.19$   | $0.63$    | $1.90$  | $0.310$ |                                     |

* 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 neurotocoarcosis (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 Microtus) 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, so-called neurocycticercosis. Cycisticerci 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. Grikenienė 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; Machaňová et al., 2016; Meerbürg 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 parasitism of wild rodents. Additionally, in the field of human neurocysticercosis, the use of Urea-based cerebral perfusion (UCP) for the diagnosis of neurocysticercosis has been proposed (Peng et al., 2006). However, further studies are needed to confirm the effectiveness of this method.
cysts in wild Arvicolinae and Muriniae (Krücke 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 (Juráňková et al., 2015). Such host species-specific predilection sites might explain the low cerebral prevalences in the examined Arvicolinae and Muriniae 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 Lippman-Hand (1983), resulted in similar confidence intervals. Furthermore, Arvicolinae and Muriniae have relatively small home range sizes and the obtained data on M. glareolus and A. flaviolens 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; Hutchinson et al., 1989), 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; Jánecck 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 immunogenity 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 (Krücke 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. flaviolens. 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. flaviolens 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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