First detection and low prevalence of *Pearsonema* spp. in wild raccoons (*Procyon lotor*) from Central Europe

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**ABSTRACT**

The urinary bladder and lower urinary tract of domestic and wild carnivores can be parasitised by filamentous nematodes from the genus *Pearsonema* (syn. *Capillaria*). Infestations are often asymptomatic, but severe courses in dogs and cats have been described. Hosts are infested through the ingestion of earthworms (*Lumbricidae*) which act as intermediate hosts. Epidemiological studies of *Pearsonema* in raccoons (*Procyon lotor*) in North America are scarce and previous studies of urinary bladder parasites of European raccoons did not provide evidence of infection. We examined urine sediment or rinse water from urinary bladders of 499 wild raccoons from Luxembourg, Poland and five study sites in Germany. *Pearsonema* eggs were found in the urine sediment of 31 (6.2%) raccoons. Infested animals were found in all study areas with prevalence values ranging from 3.7% to 8.7%. No significant difference in prevalence was found either between animals in urban and rural areas or between sexes and age classes. Based on their morphology, the eggs were likely to be *P. feliscati*. Considering their increasing density in Central Europe, raccoons may play a previously overlooked role in environmental contamination with *Personema* eggs.

**1. Introduction**

In Europe, the urinary bladder and the lower urinary tract of domestic and wild carnivores can be parasitised by three species of filamentous nematodes from the genus *Pearsonema* (syn. *Capillaria*; family Capillariidae). The cosmopolitan bladderworm *Pearsonema plica* can be found in Canidae - especially red foxes (*Vulpes vulpes*) and dogs (*Canis lupus familiaris*) - and sometimes Felidae and Mustelidae (Moravec et al., 1987; Basso et al., 2013). *P. mucronata* infests several species of mustelids (Moravec et al., 1987; Varodi et al., 2017), while the presence of *P. feliscati* has been reported from domestic cats (*Felis catus*; Beugnet and Knaus, 2015) and sometimes from wild felids (Bagrade et al., 2003; Krone et al., 2008). Infestations with *Pearsonema* nematodes are usually asymptomatic, but severe cases of ‘urinary capillariosis’, characterised among others by dysuria, fever and urinary incontinence, have been described in both domestic dogs and cats (Beugnet and Knaus, 2015; Ilić et al., 2021).

The life cycles of *P. plica* and *P. mucronata* are relatively similar. The carnivores act as final hosts and excrete the eggs of the nematodes via their urine. In the environment, *Pearsonema* eggs are ingested by earthworms (*Lumbricidae*), which act as obligate intermediate hosts and where the larvae develop to the L1 infective stage (Moravec et al., 1987). Final hosts become infested after ingestion of infested earthworms. In case of *P. plica*, the second stage larvae (L2) develop in the wall of the small intestine of the final host and remain there for 8–10 days. The third stage larvae (L3) migrate to the urinary bladder and develop into the mature nematode within 60 days of infestation (Low, 1999). The life cycle of *P. feliscati* has not been investigated, but is believed to be...
similar. Since cats eat earthworms only rarely, they are likely to become infested via paratenic hosts, i.e. their prey species that do feed on earthworms (Beugnet and Knaus, 2015).

Even though the *Pearsonema* nematodes and their pathogenic potential have been known for some time, prevalence studies in Central Europe have been conducted almost exclusively on the red fox, which is considered the main reservoir of *P. plica* in the region (Bork-Mimm and Rinder, 2011). The raccoon (*Procyon lotor*), a medium-sized carnivore that originated in North and Central America (Kaufmann, 1982), has been accidentally or intentionally introduced into several Asian and European countries during the last century (Okuyama et al., 2020; Stubbe, 1999). Within Europe, the distributional focus of the raccoon is in Central Europe (Fischer et al., 2017). The species is particularly abundant and widespread in Germany and adjacent countries where the population can be traced back to several separate introduction events (Siedrzycka et al., 2014; Fischer et al., 2015; Maas et al., 2022). In recent decades, raccoons have greatly increased their density and geographical distribution in Central Europe (Fischer et al., 2011) and are spreading rapidly into urban areas, where they live in close proximity to humans and domestic animals (Hohmann and Bartussek, 2011; Heddergott et al., 2020b).

Both in North America (Hamilton, 1936; Dexter, 1951) and Europe (Lutz, 1980; Engelmann et al., 2011; Michler, 2020), raccoons have been shown to consume earthworms. Consequently, while urinary bladder parasites are usually neglected in the numerous parasitological studies of raccoons in North America, *Pearsonema* spp. or *P. plica* infestations have been reported occasionally (Johnsen, 1970; Butterworth and Beverley-Burton, 1980; Cole and Shoop, 1987; Hamir and Rupprecht, 1998; Hamir and Snyder, 1999). Outside their original range, only two German studies have investigated urinary bladder parasites and both failed to find evidence for the presence of *Pearsonema* spp. (Lux and Priemer, 1995; Gey, 1998). The aim of the present study was to determine the prevalence of *Pearsonema* spp. in wild raccoons in their core distribution areas in Central Europe in order to determine its role as a possible reservoir in view of the increasing contact with domestic dogs and cats.

2. Material and methods

2.1. Ethics statement

As an invasive species, raccoons are not legally protected in Luxembourg, Poland and Germany and can be harvested by licensed hunters outside the closed season without special permission. No animal was killed with the aim of providing samples for this study. All hunted individuals were legally shot and made available to the authors.

2.2. Sample collection

Between 2015 and 2019, urinary bladders were collected from 499 Central European raccoons that were legally hunted and part of a zoonotic pathogen control study (Heddergott et al., 2017; Heddergott et al., 2020a; Heddergott et al., 2020b; Solarczyk et al., 2021; Frantz et al., 2021). The selected study sites were characterised by a high raccoon density (≥0.5 individuals per 100 ha; Fischer et al., 2016). We collected carcasses from northern Luxembourg (49° 45’N/06° 08’E; study area 1500 km²), from Zgorzelec County in Poland (51° 09’N/15° 00’E; 830 km²), as well as from five different German study areas: Prignitz (53° 7’N/11° 44’ E; 600 km²); Nördliches Harzvorland (51° 52’N/11° 19’ E; 50 km²); Hakel Wald; Eichsfeld (51° 20’N/10° 10’ E; 800 km²); Bautzen (51° 11’N/14° 26’ E; 800 km²); Göppingen (48° 42’N/9° 39’E; 300 km²) (Fig. 1). Raccoons were stored at −20°C and the sex and geographic origin of each animal was recorded. We determined the age-class of each raccoon based on the incremental growth lines in the cementum of a mandibular canine. Animals were classified either as juveniles (no growth line) or adults (one or more growth lines) (Heddergott et al., 2016). The dataset consisted of 237 males and 262 females, or of 275 adults and 224 juveniles. Altogether 429 raccoons were
collected in rural areas and 70 in built-up areas with more than 10,000 people.

2.3. Urinary sediment examination

We identified infestation with *Pearsonema* sp. by detecting *Pearsonema* eggs through microscopic analysis of the urinary sediment. *Pearsonema* eggs are barrel-shaped and have a plug at both poles, with smooth or sculptured shells depending on the species (Fig. 2; Butterworth and Beverley-Burton, 1980). To minimize the risk posed by most infectious parasites, all raccoons were stored at −80 °C for ≥5 days prior to necropsy (Bork-Mimm and Rinder, 2011; Shafir et al., 2007). During necropsy, the urinary bladders were removed, ligated and stored in a tight plastic bags. We collected 10 ml of urine from the ligature, or washed empty bladders (n = 95) with milliQ water (Merck KGaA, Darmstadt, Germany) (Petersen et al., 2018). The urine or collected water was centrifuged at 400 × g for 5 min using an EBA 200 (Hettich, Tuttlelingen, Germany). Subsequently, 0.1 ml of the sediment/water was pipetted onto a slide. We examined the slides and counted the *Pearsonema* eggs using a Nikon E600 light microscope (NikonInc., Melville, New York, USA) (Studzińska et al., 2015).

2.4. Morphometric analyses of eggs

Measurements of *Pearsonema* eggs were made using a digital image analysis system (Microimage 4, Microsoft Corp.) at 400 × magnification following Borba et al. (2021). The measurements were performed on 25 undamaged eggs, selected regardless of their study area of origin. The following measurements were obtained: the length and width (diameter) of the eggs, the width and height of the plugs, and the thickness of the eggshell (see also Borba et al., 2021).

2.5. Statistical analyses

We used a χ² test to test for differences in prevalence between study areas and habitat categories (urban vs. rural). We performed a logistic regression to test for an effect of sex and age on the presence of *Pearsonema* eggs. Given the relatively small amount of positive cases (see Results) we performed the regression without considering interactions. The statistical analyses were performed in R v.4.2.0 (R Core Team, 2022). Furthermore, we calculated the 95% confidence interval (CI) for the prevalence estimated for the entire data set and the different study areas using the “Wilson” Score interval method (Brown and Cat, 2011).

3. Results

*Pearsonema* eggs were found in 6.2% (31/499; 95% CI: 4.4–8.7) of the examined raccoons. Infested raccoons were found in all study areas in Germany, Poland and Luxembourg (Fig. 1; Table 1). While the prevalence of *Pearsonema* eggs varied from 3.7% (Bautzen) and 8.7% (in Eichsfeld), there was no significant difference in prevalence between the study areas (χ² = 2.976, df = 6, p = 0.704). Three of the 70 raccoons (4.3%) originating from built-up areas were infested with a *Pearsonema* species, compared to 28 of 429 (6.5%) animals sampled in rural areas. This differences was not significant (χ² = 2.976, df = 1, p = 0.610). Males (18/219; 8.2%) were more frequently infested than females 13/219 (6.5%). However, neither sex nor age class had a statistically significant influence on the probable presence of *Pearsonema* eggs (Table 2). Well over half of the *Pearsonema*-positive raccoons (n = 23; 74.2%) had a low egg load of ≤20 eggs per ml (Table 1).

The 25 examined eggs had large pits that were consistent with the shell type I reticulate surface structure. The eggs had a length of (mean ± s.d.) 62.76 ± 1.23 μm and average width of 27.02 ± 0.57 μm. The plugs were 9.12 ± 0.66 μm wide and 4.46 ± 0.50 μm high. The thickness of the eggshell was on 2.09 ± 0.20 μm (Table 2).

4. Discussion

Even though the *Pearsonema* nematodes and the pathogenic risk they potentially pose to domestic cats and dogs have been known for some time, reliable reports on the prevalence of the parasites in Central European wild carnivores are relatively rare (Bork-Mimm and Rinder, 2011). A few studies on *P. plica* have focussed on prevalence in the red fox (Bork-Mimm and Rinder, 2011; but see Varodi et al., 2017; Petersen et al., 2018). There is very limited information on the prevalences of *P. mucronata* (but see Varodi et al., 2017) and *P. feliscati* in wildlife (but see Bagrade et al., 2003; Krone et al., 2008).

Certain identification of *Pearsonema* nematodes usually requires adult worms (Butterworth and Beverley-Burton, 1980; Moravec, 1982). Studies that diagnose infestation based on the presence of *Pearsonema* eggs in urinary sediment thus often report infestation without determination at the species level (e.g., Pelligrá et al., 2020; Eleni et al., 2021). However, the differing morphological characteristics of their eggs ought to allow distinction between *P. plica* and *P. feliscati* (Enzie, 1951; Butterworth and Beverley-Burton, 1980). *P. plica* eggs have a reticulated shell characterised by large depressions and ridges, referred to as shell type I by Borba et al. (2021). Depending on the focus, light microscopy either shows eggshells of *P. feliscati* to be striated (Freitas et al., 1936; Enzie, 1951) or to have a pitted surface (Zając and Conboy, 2012), with depressions that are not as large as those observed in *P. plica* (Borba et al., 2021). The sizes of the eggs of the different species partially overlap. *P. plica* eggs can be between 58 and 74 μm long and 23–31 μm wide (Enzie, 1951; Butterworth and Beverley-Burton, 1980; Borba et al., 2021). The eggs of *P. feliscati* appear to be somewhat shorter on average, ranging from 51 to 62 μm in length and 24–32 μm in width (Freitas et al., 1936). According to Pfeiffer et al. (1989), *P. mucronata* eggs are between 65 and 68 μm long and 28–31 μm wide.

Based on egg morphology, the *Pearsonema* eggs that we recovered from Central European raccoons in all likelihood were *P. plica*. All our morphological measurements of the eggs fall exactly within the range of values reported by Borba et al. (2021) for the corresponding features in *P. plica* (Table 3). In contrast, *P. feliscati* eggs can be shorter (Freitas et al., 1936).
While we found a low prevalence of _P. plica_ in raccoons in North America (see below). While we cannot be categorical about the identity of the parasite, we thus believe that it is reasonable to presume that the nematode eggs we observed were indeed _P. plica_.

To our knowledge, the present Central European study is the first study outside North America to demonstrate infestation with _Pearsonema_ spp. in raccoons. Two previous parasitological studies from Germany examined urinary bladders of raccoons but did not find evidence for the presence of _Pearsonema_ spp. (Lux and Priemer, 1995; Gey, 1998). Considering their increasing density, raccoons play an important role in the epidemiology of _P. plica_. Further research may thus be necessary to assess whether raccoons become infested via paratenic hosts, i.e., by consuming prey species that become infested via paratenic hosts, i.e., by consuming prey species that may play an important role in the epidemiology of _Pearsonema_ spp.

The following people contributed to the sampling effort: H Auer, S Asam, M. Heddergott, M Speck, AB Stytz, B Steidl, A Weißenberger, R Wildanger, BP Zimmer and M. Zimmermann. Part of this study was supported by the Luxembourg National Research Fund (AFR 7484378). For the production and provision of the photo of the _Pearsonema_ egg we thank Amer Alić.

### Table 1

Pearsonema egg load in sediment from washing of the bladder or urine sediment of raccoons (Procyon lotor) from Luxembourg, Poland and Germany.

| State | Region | No examined | No positive animals (in %) | 95% confidence interval | 1-10 eggs per ml (in %) | 11-20 eggs per ml (in %) | 21-30 eggs per ml (in %) | >30 eggs per ml (in %) |
|-------|--------|-------------|---------------------------|------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Luxembourg |   | 50          | 2 (4.0)                   | 1.1–13.4                | 1 (50.0)              | –                     | 1 (50.0)             | –                     |
| Poland | Zgorzelecki | 70          | 5 (7.1)                   | 3.1–15.7                | 2 (40.0)              | 2 (40.0)              | 1 (20.0)              | –                     |
| Germany | Northern Harzvorland | 42          | 2 (4.8)                   | 1.3–15.8                | –                     | 1 (50.0)              | –                     | 1 (50.0)              |
|         | Büttzen | 82          | 3 (3.7)                   | 1.3–10.3                | –                     | 2 (66.7)              | 1 (33.3)              | –                     |
|         | Prignitz | 48          | 2 (4.2)                   | 1.2–14.0                | –                     | 2 (100.0)             | –                     | –                     |
|         | Göttingen | 57          | 4 (7.0)                   | 2.8–16.7                | 2 (50.0)              | 1 (25.0)              | 1 (25.0)              | –                     |
|         | Eichsfeld | 150         | 13 (8.7)                  | 5.1–14.3                | 6 (46.2)              | 4 (30.8)              | 2 (15.4)              | 1 (7.6)               |
| Total  |   | 499         | 31 (6.2)                  | 4.4–8.7                 | 11 (35.5)             | 12 (38.7)             | 6 (19.4)              | 2 (6.4)               |

### Table 2

Results of logistic regression identifying predictors for the presence of _Pearsonema_ spp. in raccoons (Procyon lotor).

| Predictor                  | Estimate | s.e. | z value | p value |
|----------------------------|----------|------|---------|---------|
| (Intercept)                | -2.89    | 0.32 | -9.11   | <0.001  |
| Sex-male                   | 0.47     | 0.38 | 1.25    | 0.213   |
| Age class-juvenile         | -0.17    | 0.26 | -0.64   | 0.521   |

The results of logistic regression identified predictors for the presence of _Pearsonema_ spp. in raccoons. The presence of _Pearsonema_ spp. was significantly associated with sex and age class. The estimate for sex-male was 0.47 with a standard error of 0.38. The estimate for age class-juvenile was -0.17 with a standard error of 0.26.

### Table 3

Selected morphometric measurements of _Pearsonema plica_ eggs (Borba et al., 2021) and _Pearsonema_ eggs measured in the present study.

| Measurement characteristics | Borba et al. (2021), a | Present examination b |
|-----------------------------|------------------------|-----------------------|
| Mean | Min-max | Mean | Min-max | ± SD |
| Egg length (μm) | 62.62 | 60.30-65.32 | 62.76 | 60.18-65.02 | 1.23 |
| Egg width (μm) | 27.47 | 26.35-28.76 | 27.02 | 26.30-28.55 | 0.57 |
| Plug base width (μm) | 9.62 | 8.48-10.87 | 9.12 | 8.45-10.75 | 0.66 |
| Plug base height (μm) | 4.47 | 3.40-5.28 | 4.46 | 3.42-5.21 | 0.50 |
| Shell thickness (μm) | 2.24 | 1.17-2.55 | 2.09 | 1.74-2.49 | 0.20 |

±SD Standard deviation.

a Examined eggs n = 30.
b Examined eggs n = 25.

e et al., 1936) and _P. mucronata_ eggs wider (Pfeiffer et al., 1989) relative to the sizes measured for _Pearsonema_ eggs in the present study. In addition, our eggshells had the large pits that are characteristic of _P. plica_ (but not _P. feliscati_). Finally, adult _P. plica_ have been recovered from raccoons in North America (see below). While we cannot be categorical about the identity of the parasite, we thus believe that it is reasonable to presume that the nematode eggs we observed were indeed _P. plica_.

### Declaration of competing interest

There is not any conflict of Interest.

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