HELMINTHOLOGY - SHORT COMMUNICATION

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Helminth infections of wild European gray wolves (*Canis lupus* Linnaeus, 1758) in Lower Saxony, Germany, and comparison to captive wolves

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Abstract

This study aimed to investigate the endoparasite fauna of wild European gray wolves, which are currently recolonizing Germany. In total, 69 fecal samples of wild wolves were collected in Lower Saxony, Germany, from 2013 to 2015, analyzed by the sedimentation-flotation and McMaster techniques and compared to previous results on captive European Gray wolves living in zoological gardens in Germany. In addition to coproscopy, taeniid-positive samples from wild as well as captive wolves were differentiated by amplification and sequencing of small subunit ribosomal RNA (*SSU rRNA*) and NADH dehydrogenase 1 (*nad1*) gene fragments. Missing *Taenia krabbei SSU rRNA* reference sequences were generated from two *T. krabbei* specimens. Overall, 60.87% (42/69) of wild wolve samples were microscopically positive for at least one of seven egg types. *Capillaria/ Eucoleus* spp. showed the highest frequency (31.88% [22/69]), followed by Taeniidae (21.74% [15/69]), Ancylostomatidae (20.29% [14/69]), *Alaria alata* (15.94% [11/69]), *Toxocara canis* (13.04% [9/69]), and *Toxascaris leonina* and *Trichuris vulpis* (each 5.80% [4/69]). Amplification of *SSU rRNA* was successful for 7/15 Taeniidae-positive samples from wild and 20/39 samples from captive wolves, revealing *T. hydatigena* in two and 14 samples, respectively. *Taenia krabbei* was detected in two further samples of wild and three samples of captive wolves, while for the remaining samples, no differentiation between *T. serialis/T. krabbei* was possible. *Echinococcus* spp. were not detected. Sequence comparisons revealed that the *SSU rRNA* gene fragment was not suitable to differentiate between *T. serialis* and *T. krabbei*. Therefore, the use of this fragment alone cannot be recommended for species identification in future studies.

Keywords *Canis lupus lupus* · Coproscopy · Taeniids · Taeniidae · *Taenia* · *Echinococcus*

Introduction

The European gray wolf (*Canis lupus lupus*) was eradicated from Germany in the nineteenth century, but recolonization started in the 1990s (Contact Office "Wolves in Saxony" [*Kontaktbüro Wölfe in Sachsen*] 2018). In 2014/2015, the German wolf population was estimated at approximately 110 adult individuals. Thereof, five packs, three pairs, and two individual wolves were resident in the federal state of Lower Saxony (Federal Documentation and Advice Centre on the Wolf [*Dokumentations- und Beratungsstelle des Bundes zum Thema Wolf*] 2018).

Recolonization of top predators, such as wolves, may have a considerable impact on ecosystems via trophic cascades (Ripple and Beschta 2012). Furthermore, they represent final hosts of several parasites circulating between predators and their prey as intermediate hosts. Previous studies demonstrated that wolf recolonization may lead to an increase of wolf-associated parasites in the ecosystem (Lesniak et al. 2018). Among wolf parasites may be species of zoonotic relevance, such as *Echinococcus* spp. and *Toxocara canis* (e.g., Szafrańska et al. 2010; Lesniak et al. 2017). Furthermore, wolves and other carnivores represent final hosts of several *Taenia* spp., e.g., *T. hydatigena*, *T. serialis*, and *T. pisiformis* (Guerra et al. 2013; Poglayen et al. 2017), which may cause cysticercosis in domestic ruminants. Thus, overlap of wolf

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territories with agricultural areas may potentially put domestic animals and humans at risk of infection.

The aim of this study was to investigate the helminth fauna of free-ranging European gray wolves in Lower Saxony, Germany, by coproscopical examination and to compare the results to previously published data on captive European gray wolves in Germany (Bindke et al. 2017). Furthermore, to gain more insight into the zoonotic potential of taeniid infections in wolves, Taeniidae-positive samples from this study and from the study on captive wolves (Bindke et al. 2017) were subjected to PCR for species identification.

Material and methods

Fecal samples of wild wolves (diameter of > 3 cm, containing large pieces of bones, hooves, and hair) in Lower Saxony, Germany, were collected between June 2013 and June 2015 (Table 1). Samples were preserved at − 20 °C until coproscopical examination as described previously (Bindke et al. 2017; Becker et al. 2016). Briefly, 5 g feces was processed using the combined sedimentation-flotation method and in case of positivity subsequently examined with the McMaster method. In both methods, $ZnSO_4$ (specific gravity 1.30) was chosen as flotation medium. Parasite detection frequencies were compared to previously published data on captive European gray wolves (Bindke et al. 2017) using Fisher's exact test.

Taeniid eggs were picked from positive samples of wild wolves, as well as from samples of captive wolves originating from a previous study (Bindke et al. 2017). Eggs were homogenized using the Precellys® 24 Dual system (Peqlab Biotechnologie GmbH, Erlangen, Germany) at 2×5 s at 5000 rpm prior to DNA extraction with the NucleoSpin® Tissue Kit (Macherey-Nagel, Düren, Germany). To amplify a fragment of the NADH dehydrogenase subunit 1 (*nad1*) of *E. multilocularis*, as well as a fragment of the small subunit ribosomal RNA (*SSU rRNA*) gene of *E. granulosus* and *Taenia* species, a multiplex PCR as described by Trachsel et al. (2007) was carried out. In deviation to Trachsel et al. (2007), the reaction volumes of master and primer mix were halved, but the template increased to 5 μl. PCR products were sequenced (Seqlab Sequence Laboratories, Göttingen, Germany) and compared to publicly available sequences.

As no *T. krabbei SSU rRNA* sequence was publicly available, DNA isolated from two *T. krabbei* specimens (kindly provided by Ines Lesniak and Heribert Hofer, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany)

Table 1 Detection frequencies (no. of positive samples/no. of samples) of gastrointestinal helminths of wild European gray wolves in Lower Saxony, Germany

Sampling month	No. of samples	A. caninum/ U. stenocephala	T. canis	T. leonina	T. vulpis	Capillaria/ Eucoleus spp.	Taeniidae	A. alata
June 2013	2	1/2 (0)	0/2	0/2	0/2	1/2 (25)	0/2	0/2
July 2013–August 2013	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
September 2013	2	1/2 (25)	1/2 (25)	0/2	0/2	1/2 (825)	0/2	2/2 (0)
October 2013	1	1/1 (450)	1/1 (25)	0/1	1/1 (325)	1/1 (1275)	0/1	0/1
November 2013	1	1/1 (200)	1/1 (550)	0/1	1/1 (25)	1/1 (500)	0/1	0/1
December 2013–January 2014	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
February 2014	2	0/2	2/2 (0–25)	0/2	0/2	1/2 (100)	1/2 (25)	2/2 (0)
March 2014–July 2014	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
August 2014	1	0/1	0/1	0/1	1/1 (0)	1/1 (0)	0/1	0/1
September 2014	6	1/6 (0)	0/6	1/6 (0)	0/6	0/6	2/6 (0)	1/6 (0)
October 2014	8	2/8 (25–100)	0/8	0/8	0/8	0/8	3/8 (0–25)	0/8
November 2014	4	3/4 (0–50)	1/4 (50)	1/4 (25)	0/4	1/4 (0)	2/4 (0–100)	2/4 (0–25)
December 2014	5	1/5 (0)	1/5 (25)	0/5	0/5	1/5 (25)	1/5 (0)	3/5 (0)
January 2015	6	0/6	0/6	0/6	0/6	2/6 (0–75)	1/6 (175)	0/6
February 2015	11	2/11 (100–150)	2/11 (25)	1/11 (75)	1/11 (0)	6/11 (0–200)	3/11 (200–5625)	1/11 (50)
March 2015	5	0/5	0/5	0/5	0/5	1/5 (0)	1/5 (25)	0/5
April 2015	4	0/4	0/4	0/4	0/4	1/4 (0)	0/4	0/4
May 2015	7	1/7 (0)	0/7	0/7	0/7	3/7 (0)	0/7	0/7
June 2015	4	0/4	0/4	0/4	0/4	1/4 (0)	0/4	0/4

The range of eggs per gram feces as determined by the McMaster method is indicated in parentheses

n.a. not available (no samples received)

was subjected to the multiplex PCR described above. For sequencing of the complete amplicon, a second sequencing primer (5′-AAGCAGCACATAGACTTGGC-3′) was designed in addition to Cest5Seq (Trachsel et al. 2007). However, obtained sequences (accession numbers MH843683 and MH843684) showed 100% identity to published *T. serialis* (e.g., MF495485, query cover [QC] 100%) and 99% identity to published *T. multiceps* sequences (e.g., LC271556, QC 100%). Therefore, a second PCR targeting a 507-bp fragment of the *nad1* gene was carried out (Armua-Fernandez et al. 2011). Again, reactions except the template amount volumes were halved. The sequenced PCR products were 99% identical to published *T. krabbei nad1* sequences (e.g., EU544625, query cover 100%), but only 94% and 90% identical to *T. multiceps* (e.g., KC794811, 100% QC) and *T. serialis* (e.g., AB731674, 99% QC), respectively. In consequence, all samples which could not be unambiguously identified based on *SSU rRNA* were subjected to the *nad1* PCR and subsequent sequencing.

Availability of data and materials All datasets supporting the conclusions of this article are included within the article.

Results and discussion

In total, 69 fecal samples were collected from three wolf packs (5, 6, and 58 samples per pack). The number of samples per month ranged from 0 to 13 (Table 1 and Fig. 1). *Capillaria/ Eucoleus* spp. showed the highest frequency (31.88%, 22/69 samples), followed by Taeniidae (21.74%, 15/69),

Ancylostomatidae (20.29%, 14/69), *Alaria alata* (15.94%, 11/69), *Toxocara canis* (13.04%, 9/69), and *Toxascaris leonina* and *Trichuris vulpis* (5.80%, 4/69, respectively). All of these parasites have been identified in wild wolves in Europe before (e.g., Guerra et al. 2013; Lesniak et al. 2017). In 60.87% (42/69) of samples, at least one of these egg types was detected. Eggs of *Ascaris suum* (5.80%, 4/69) and *Fasciola hepatica* (2.90%, 2/69) were also found, which most likely (*F. hepatica*) or certainly (*A. suum*) represent gastrointestinal passengers.

When comparing parasite detection frequencies between wild and captive wolves (Bindke et al. 2017), most parasites with an indirect life cycle and/or paratenic hosts, namely, taeniids, *A. alata*, *Capillaria/Eucoleus* spp., *T. canis*, and *T. leonina*, occurred significantly more often in samples of wild wolves (Table 2). This was expected due to different diet composition between wild and captive wolves (Wagner et al. 2012). Additionally, captive wolves were treated with anthelmintics up to four times a year (Bindke et al. 2017). Nevertheless, hookworm as well as *Trichuris* spp. detection frequencies were similar between captive and wild wolves. Anthelminthic treatment frequency in zoological gardens may have been too low to significantly affect these parasites. In addition, some commonly used anthelmintic formulations are insufficiently effective against *Trichuris* spp. (e.g., Prelezov and Bauer 2003). In wild wolves, *Capillaria/ Eucoleus* spp. (31.88%) represented the most frequently detected egg type, whereas in captive wolves, strongyle eggs (30.84%) occurred most frequently (Bindke et al. 2017). Both of these could be gastrointestinal passengers; thus, the differences might reflect differences in diet rather than
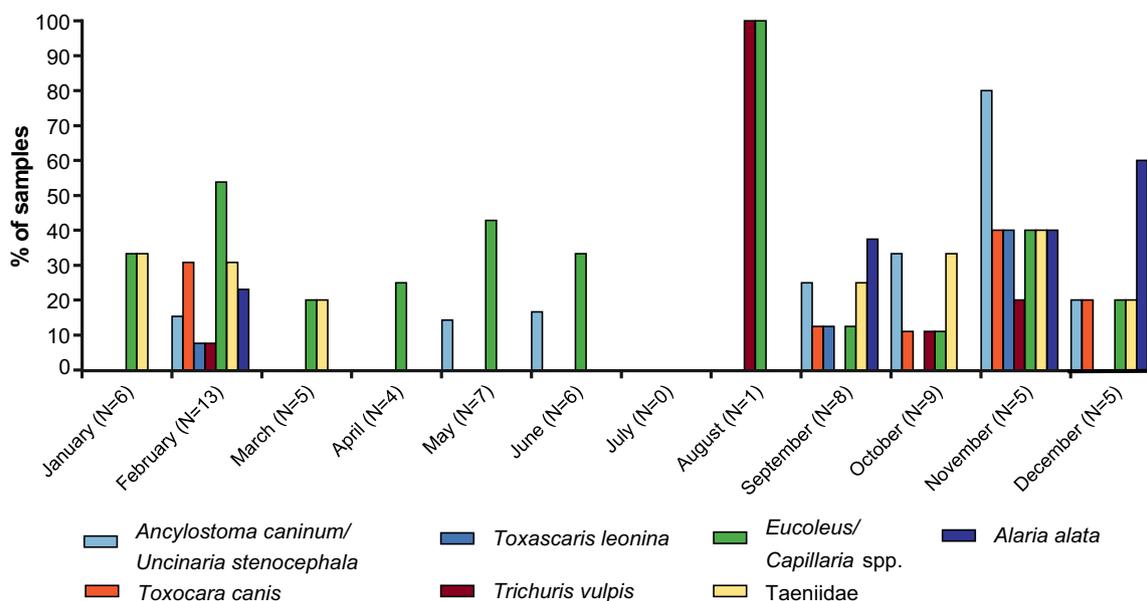

Fig. 1 Monthly detection frequencies of helminth parasites in wild European gray wolves in Lower Saxony, Germany. Results were pooled for the study years 2013–2015

Table 2 Helminth detection frequencies among 69 samples of wild European gray wolves compared to detection frequencies among 1041 samples of captive European gray wolves (Bindke et al. 2017) by Fisher's exact test

Helminth species	Frequency in wild wolves (%)	Frequency in captive wolves (%)	*P* value	Odds ratio	Confidence interval (95%)
A. caninum/U. stenocephala	20.29	30.84	0.078	0.57	0.29–1.06
T. canis	13.04	5.19	**0.013**	2.74	1.13–5.95
T. leonina	5.80	1.25	**0.018**	4.85	1.12–16.29
T. vulpis	5.80	3.65	0.326	1.62	0.41–4.72
Capillaria/Eucoleus spp.	31.88	19.88	**0.021**	1.89	1.06–3.27
Taeniidae	21.74	3.75	**< 0.001**	6.93	3.34–13.79
A. alata	15.94	3.65	**< 0.001**	4.99	2.19–10.62

Significant *P* values are shown in bold

infection pressure. However, most zoos fed only partial carcasses, excluding the gastrointestinal system (unpublished data). Therefore, the (non-significantly) higher strongyle (hookworm) detection frequency in captive wolves may result from a stronger buildup of infection pressure in contaminated enclosures as compared to free-ranging wolves.

Taeniid eggs were the second most common egg type in wild wolves, with a detection frequency (21.74%) similar to previous reports from Portugal (23.5%) (Guerra et al. 2013). In contrast, higher detection rates of taeniid eggs were reported from Italy (42.1%; Poglayen et al. 2017), while lower prevalences were detected in Croatia (1.5%; Hermosilla et al. 2017) and in captive wolves (3.75%; Bindke et al. 2017). Differences in prey spectrum as well as taeniid prevalence in intermediate hosts may explain these variations. Additionally, necropsy is more sensitive than coproscopy because parasite eggs may be shed discontinuously. Furthermore, the freeze-thaw process may have affected detection of parasite eggs in the present study.

Regarding differentiation of Taeniidae, PCR and *SSU rRNA* sequencing were successful for 7/15 positive samples from wild wolves (Table 3). *T. hydatigena* was identified twice based on *SSU rRNA* sequences (98–100% identity, 96–98% QC). The remaining two samples were identified as *T. krabbei* based on their *nad1* sequences (99% identity, 97% QC). Three further samples yielded a *SSU rRNA* sequence 97–100% identical to *T. serialis* (97–100% QC), but no *nad1* sequence could be generated for final species identification. In a necropsy-based study, Lesniak et al. (2017) reported *T. krabbei* as the most prevalent parasite of German wolves, followed by *T. hydatigena*. In wild ungulates in Germany, *T. krabbei* occurs most frequently in roe deer, whereas *T. hydatigena* is more prevalent in red deer (Lesniak et al. 2017). Prey of wolves in north-eastern Germany consists mainly of roe deer, followed by red deer, wild boar, leporids, and domestic animals (Wagner et al. 2012). Thus, a higher prevalence of *T. krabbei* may be expected in Germany. In contrast, in Spanish, Italian, and Portuguese wolves, *T. hydatigena* was detected more often

(Guerra et al. 2013; Poglayen et al. 2017; Segovia et al. 2001). Unfortunately, in the present study the taeniid species of many samples could not be identified, either because no PCR product could be generated, or because a *SSU rRNA*, but no *nad1* sequence was obtained. Sequencing of the *SSU rRNA* fragment of two *T. krabbei* specimens obtained with the multiplex PCR by Trachsel et al. (2017) revealed 100% identity to *T. serialis*. Thus, this fragment is not suitable to differentiate between these two species. While it is possible that *T. serialis* occurred in our samples, this could not be definitely confirmed. *T. serialis* mainly infects lagomorphs and rodents as intermediate hosts, and was detected at low frequencies in wolves from Portugal (Guerra et al. 2013) and Spain (Segovia et al. 2001).

For captive wolves, 17/39 taeniid-positive fecal samples were successfully identified to species level. *Taenia hydatigena* was identified in 14/39 samples based on ≥ 99% identity in the *SSU rRNA* gene (97–100% QC), followed by *T. krabbei* in three samples (99% *nad1* sequence identity, 97% QC). Three further samples yielded a *SSU rRNA* sequence 97–100% identical to *T. serialis* (100% QC), but no *nad1* sequence could be generated. Infection of captive wolves with *T. krabbei* reflects that these animals are sometimes fed with carcasses of wild ungulates. Among taeniids, especially *Echinococcus* spp. are of zoonotic concern. Here, neither *E. granulosus*, which occurs sporadically in Germany, nor *E. multilocularis*, which is endemic to Germany, were detected. In the study by Lesniak et al. (2017), *E. multilocularis* occurred in only 2% of German wolves. Thus, the absence

Table 3 Results of species determination of Taeniidae-positive samples from wild and captive European gray wolves in Germany

Species	Free-ranging wolves (percent of samples)	Captive wolves (percent of samples)
Taenia hydatigena	2/15 (13.33%)	14/39 (35.89%)
T. krabbei	2/15 (13.33%)	3/39 (7.69%)
T. serialis or *T. krabbei*	3/15 (20.00%)	3/39 (7.69%)
Not determined	8/15 (53.33%)	19/39 (48.72%)

of *E. multilocularis* in the present study may be due to the small sample size.

Concerning *A. alata*, a frequency of 15.94% was found in the present study. In Poland, *A. alata* prevalences of up to 80.1% were described in wild wolves (Szafrańska et al. 2010), and a prevalence of 53% was found by Lesniak et al. (2017). *A. alata* may infect humans after consumption of raw or undercooked meat of paratenic hosts. An increase in *A. alata* prevalence in German wild boar has been described (Riehn et al. 2012), and it remains to be elucidated whether wolves play a role in the emergence of this trematode. The relatively low detection rates of *T. canis* and *T. leonina* are comparable to results from Hermosilla et al. (2017). As these parasites usually occur in juveniles, this could indicate that the samples represented no or only few pups. Furthermore, this finding might reflect the good health condition of the studied packs. Lesniak et al. (2017) reported higher *T. canis* and *T. leonina* prevalences; however, their study was based on necropsies, possibly including a higher proportion of young animals, which are more prone to road accidents, as well as sick or malnourished individuals. Furthermore, first-stage larvae of *Angiostrongylus vasorum* (1.45%, 1/69) were detected. As the sedimentation-flotation method is not well suited for detection of lungworm larvae, the actual prevalence may be substantially higher. The Baermann technique, which is the gold standard for this parasite, could not be used here because of the freezing process. However, Lesniak et al. (2017) did not report any *A. vasorum* infections in German wolves.

Coccidian oocysts were noted with a frequency of 8.70% (6/69). However, many oocysts were unsporulated or degraded because of the freeze-thaw process. Therefore, no differentiation was possible and it remains unclear whether they represented gastrointestinal passengers, or true wolf parasites. Lesniak et al. (2017) did not report coccidian infections in German wolves, whereas Hermosilla et al. (2017) detected *Cystoisospora* spp. in wild Croatian wolves. In other studies on wild wolves, further protozoan parasites such as *Cryptosporidium* spp., *Giardia* spp., and *Sarcocystis* spp. were identified (Bryan et al. 2012; Hermosilla et al. 2017); however, the methods applied here are limited with regard to protozoa.

In conclusion, the presented data offer useful information on the general occurrence of wolf parasites. Further studies are necessary to elucidate whether the presence of wolves influences the epidemiology of the detected parasites in wildlife, livestock, and companion animals. Based on this study, wolves do not contribute significantly to an increased risk of zoonotic parasite species in Germany.

Compliance with ethical standards

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