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Feeding patterns and parasitofauna of wolves in southwestern Bohemia, Czech Republic

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Abstract

Feeding patterns and parasitofauna composition of wolves returning to human-dominated landscape of Central Europe are interconnected phenomena that reflect the dynamics of herbivore populations and parasite communities. This study aims to evaluate diet composition, prey selection and endoparasites of wolves in the newly recolonized area of southwestern Bohemia, where several wolf territories were established in the last decade. Analysis of 361 scats revealed that wild ungulates made up 92.8% of the total biomass consumed (Bio%). Significant alternative prey after wild ungulates was Eurasian beaver (3.9% of Bio). Despite small proportion in the local ungulate community, wolves showed a strong preference for roe deer (D = 0.76), while wild boar was totally avoided (D = –0.94). Coproscopic analysis of 369 scats showed that 81.6% of the scats were positive for fecal stages of at least one parasite species, including spurious parasites originating from predation, scavenging and/or coprophagia. Based on morphological and molecular analyses, nine groups of endoparasites were identified. The most frequently detected were *Sarcocystis* spp. (55.3%) followed by species of family Capillariidae (38.8%) and taeniid tapeworms (18.4%). This study represents the first examination of endoparasites of free-ranging wolves in the Czech Republic based on the examination of scats, and it reports the highest rate of parasite stages compared to similar studies from neighbouring countries.

Key words: wolf, diet composition, prey selection, endoparasites

Introduction

The dietary range of the grey wolf is as vast as its distribution (Peterson & Ciucci 2003, Newsome et al. 2016). Consequently, it is not unsurprising that there are intricate connections between prey species communities and the parasites they transmit (Poulin 2007, Leung & Koprivnikar 2019). These interrelationships are also evident in grey wolves, where the composition of diet is closely linked to the parasites they harbour and spread (Stronen et al. 2021).

One of the key factors in the wolf´s return to a human-dominated landscapes in Europe is the restoration of populations of natural prey apex predators – free ranging ungulates (CHAPRON et al. 2014). In the Czech Republic, wild ungulates populations have reached historical population maximum (CZECH STATISTICAL OFFICE 2023) providing a sufficient and diverse food base for the recovering wolf populations in the country (KUTAL et al. 2017, HULVA et al. 2018, BUFKA & ČERVENÝ 2021, HULVA et al. 2024, VOREL et al. 2024, VOREL et al. 2025). Based on current knowledge on wolf feeding habits from neighbouring countries with stable wolf populations (e.g. Germany, Poland or Slovakia; NOWAK et al. 2011, KUTAL et al. 2024, LIPPITSCH et al. 2024), a clear preference for wild ungulates is evident. However, wolf feeding patterns are also influenced by prey availability in specific areas (OKARMA 1995, JĘDRZEJEWSKI et al. 2000, ANSORGE et al. 2006, MERIGGI et al. 2011, WAGNER et al. 2012, ZLATANOVA et al. 2014).

The recent return of wolves to Central Europe, including southwestern Bohemia (BUFKA & ČERVENÝ 2021, HULVA et al. 2024, VOREL et al. 2024, VOREL et al. 2025), where they were previously extinct, offers a valuable opportunity to study the parasitofauna of these large mammals. Due to its diversity, the parasitofauna serves as an indirect indicator of the overall health of the ecosystem. Data on the parasite fauna of wild wolves are available from neighbouring countries such as Poland, Germany, and Slovakia.

Grey wolves can serve as definitive hosts of a range of endoparasites. In recent years, several parasitological studies utilizing faecal material from free-ranging wolves have been conducted in neighbouring countries such as Poland, Germany and Slovakia (KLOCH et al. 2005, ČABANOVÁ et al. 2017, BINDKE et al. 2019). The percentage of samples testing positive for at least one parasite species was similar across the studies, ranging from 56.2% to 66%. However, some studies focused only on the detection of helminths (POPIOLEK et al. 2007). While the spectrum of parasitic species detected varied significantly, the most commonly identified parasites were tapeworms from the family Taeniidae, and nematodes such as *Eucoleus aerophilus*, hookworms *Ancylostoma/Uncinaria* species, and ascarids *Toxocara canis* (KLOCH et al. 2005, ČABANOVÁ et al. 2017, BINDKE et al. 2019).

Parasitological examinations offer valuable insights into the feeding ecology of host species by revealing the presence of heteroxenous parasites or the passage of parasites from prey animals. A recent study from Germany found that the prevalence of *Sarcocystis* spp. in red deer (*Cervus elaphus*) was significantly higher in areas inhabited by wolves compared to control areas, though no such difference was observed in roe deer (*Capreolus capreolus*) or wild boar (*Sus scrofa*). Since *Sarcocystis* spp. are typically host-specific in their intermediate hosts, this suggests that species of Sarcocystis associated with red deer are more commonly spread, implying that red deer are the primary prey of wolves in these areas (LESNIAK et al. 2018).

Studying the feeding patterns of wolves returning to areas within their former range is a crucial step in addressing human-carnivore conflicts and establishing effective conservation and management strategies. The aim of this study is, therefore, to assess the diet composition and prey selection of wolves in the newly recolonized region of southwestern Bohemia, specifically in the Šumava National Park and adjacent protected area.

Given that diet is directly linked to parasite transmission, another key objective of this study is to determine the spectrum of gastrointestinal and pulmonary parasites in wild wolves in the Šumava National Park. This includes the evaluation of the frequency of individual parasite species and the assessment of the role of wolves in the life cycles of selected multi-host parasites.

Material and methods

Collection of scat samples and diet analyses

Scats were systematically collected in Šumava region, primarily within the Šumava National Park and Protected Landscape, from January 2020 to September 2023 (Fig. 1). This area is home to a stable wolf population and recently established wolf territories (Bufka & Červený 2021, Vorel et al. 2024, Vorel et al. 2025). Scat samples were detected through the winter months by tracking wolves in areas with sufficient snow cover as well as by walking along forest roads, trails, paths and within wolf territories during the warmer seasons. Additionally, telemetry data from GPS tracked individuals were used to identify potential scat locations (Vorel et al. 2024, Vorel et al. 2025).

A total of 361 scats were analysed using standard methods, which included washing the samples through 0.5 mesh sieve, oven drying at 60°C, and weighing. Prey items were identified through macroscopic examination of bones, hooves, hind-hooves, claws, feathers and microscopic analysis of hairs, following identification keys (Dziurdzik 1973, Teerink 1991, Tóth 2017). Additionally, comparisons were made with our own reference collection of ungulate body parts, including various ages and seasonal variations The diet composition was expressed in terms of frequency of occurrence (%Fo) and the percentage of total biomass consumed (%Bio). When multiple food items were present in a single scat, each component was weighed individually or its proportion estimated visually. The biomass of each diet

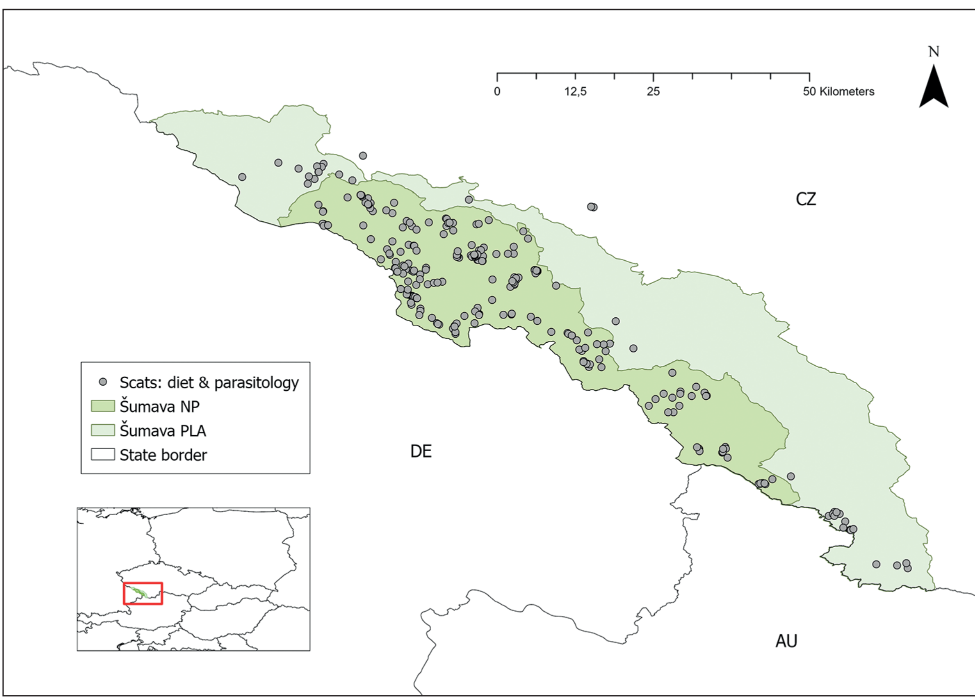

Fig. 1. Map of the study area and positions of collected samples for diet analyses and parasitology examination.

component was calculated by multiplying dry matter by coefficients of digestibility: ungulates (118), medium-sized mammals (50), small rodents, insectivores and birds (23; GOSZCZYŃSKI 1974, LOCKIE 1961, JEDRZEJEWSKI & JEDRZEJEWSKA 1992). Plant material was excluded from biomass calculations.

Calculation of prey availability and prey selection

The species structure, abundance and biomass of the ungulate community in study area were based on annual game census data obtained from hunter inventories, which involved systematic game counts within hunting grounds. These data were provided by the Administration of Šumava NP. The abundance of each species – roe deer, red deer and wild boar – was assessed by calculating the five-year average (2019–2023) across two age categories (juveniles, adults).

To estimate the total biomass contribution of each ungulate species (percentage), we used average body mass values (in kg) for each species and age category, based on data from NOWAK et al. (2005) and ANSORGE et al. (2006). The following average weights were applied: roe deer – adult 19 kg, fawn 4 kg; red deer – adult 100 kg, calf 25 kg; wild boar – adult 40 kg, piglet 10 kg.

Species preferences were calculated using the selectivity index *D* (JACOBS 1974), with the formula $D = (r - p)/(r + p - 2rp)$, where r represents the percentage of species in the ungulate composition found in wolf scats (biomass consumed), and p represents the percentage of the species in the overall ungulate community in the study area (estimated biomass). The index *D* ranges from –1 (total avoidance of a species) to 0 (selection proportional to its occurrence) to 1 (maximum positive selection). For the undetermined cervid category, the proportions of specific species (red deer and roe deer) were estimated based on the proportion of these species in accurately identified samples.

Parasitology

A total of 369 scats were analysed using three standard coproscopic methods – faecal flotation, Baermann larvoscopy and faecal sedimentation. Prior to analysis, all samples were first frozen at –70°C for one week to inactivate any potential eggs of *Echinococcus* spp.

For the faecal flotation method, approximately 1–4 g of scat faecal material was mixed with 20–30 ml of water in a mortar and homogenized. The resulting mixture was sieved into a 10 ml centrifugation tube and centrifuged at 875× g for 2.5 minutes, while the remaining homogenised sieved mixture was used for the faecal sedimantation. The supernatant was then discarded, and the sediment was mixed with modified Sheather's sugar flotation solution (specific gravity 1.30). The sample was centrifuged again at 875× g for 2.5 minutes. Using a wire loop, material of the surface film including parasite stages, were transferred to a glass slide, covered with a cover slip and examined under a microscope.

The Baermann larvoscopy was performed using the standard Baermann apparatus, which consists of a glass funnel connected to a rubber tube at the bottom with a metal clamp attached to the tube. The funnel was filled with warm tap water and approx. 5 g of faeces wrapped in gauze was added. After approx. 24 hours, the sediment was examined under a microscope in a Petri dish. Metastrongyloid first-stage larvae (L1) detected during the examination were collected for molecular analysis.

For the faecal sedimentation, the remaining homogenised and sieved mixture was transferred to a 100 ml beaker which was filled up with tap water and left to settle at room temperature for approx. 5 minutes. The supernatant was then carefully poured off and tap water added again. The process was repeated until the sediment was cleared, typically 3 to 5 times, depending on the sample's nature.

All three methods were complemented by microscopy, where individual parasite stages were identified and subjectively quantified for each representative. The quantification was based on "cross system" scored by 1 to 4 crosses (+), where + means less than 1 parasite stage/view field; ++ means approx. 1–5 parasite stages/view field; +++ means approx. 5–10 parasite stages/view field and ++++ is represented by more than 10 parasite stages/view field. This method is semiquantitative and meant to roughly indicate the intensity of infection. The identification of the parasite stages was based on reference manual (FOREYT 2001).

As the morphological speciation of some parasite fecal stages is either unreliable (e.g. lungworms) or impossible (e.g. taeniid tapeworms), molecular techniques were used to identify the detected larvae and a subset of samples positive for taeniid eggs, to classify them at the genus or species level. Genomic DNA was extracted from L1 larvae using the NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany) and from eggs using DNeasy Blood & Tissue Kit (Qiagen, Germany) for lungworms and taeniid tapeworms, respectively. For lungworms, PCR targeting the partial internal transcribed spacer 2 (ITS2) was performed using primers NC1 and NC2 according to GASSER et al. (1993). To differentiate taeniid eggs, multiplex PCR targeting the partial small subunit ribosomal RNA (12S) and partial NADH dehydrogenase subunit 1 (NAD1) was used, following the protocol by TRACHSEL et al. (2007). The resulting amplicons were separated via electrophoresis on a 1.5% agarose gel stained with Midori Green Advance (Nippon Genetics Europe, Germany) and visualised under UV light. All PCR products of the expected size were excised from the gels, purified using Gel/PCR DNA Fragments Kit (Geneaid, Taiwan), and sequenced in both directions with the amplification primers. Sequence analysis was carried out using the Macrogen Capillary Electrophoresis Sequencing services (Macrogen Europe, the Netherlands). The obtained sequences were processed with Geneious Prime software and compared to available sequences in the GenBank® database using the Basic Local Alignment Search Tool (BLAST).

RESULTS

Composition of diet and prey selection

We identified 16 different food items from 361 wolf scats. Wild ungulates made up 92.8% of the total biomass (Bio%) consumed by wolves (Table 1). Red deer compromised the largest proportion, representing 42.0% of Bio, followed by roe deer (28.5% of Bio). Wild boar compromised only 0.3% of the total biomass consumed. Among medium-sized mammals, the Eurasian beaver and brown hare were the most significant prey items, accounting for 3.9% and 1.1% of the biomass, respectively. Sheep remains, were the most frequently found livestock remains in the wolf scats, making up 1.7% of Bio (Table 1). Other prey items (e.g. small mammals) contributed only a negligible partion to the wolf diet (Table 1). Roe deer was the most positively selected prey species (D = 0.76), despite

Table 1. Diet composition of wolves in Šumava region expressed as frequency of occurrence (%Fo) and total consumed biomass (%Bio) based on 361 scats collected between 2020 and 2023.

Prey item	%Fo	%Bio	Bio (g)
Undetermined Cervids	32.69	21.87	78248.63
Red deer *Cervus elaphus*	32.96	42.00	150280.75
Roe deer *Capreolus capreolus*	26.04	28.54	102114.66
Wild boar *Sus scrofa*	1.38	0.34	1227.79
Wild ungulates total	**92.52**	**92.75**	**331871.83**
Sheep *Ovis aries*	1.66	1.68	59940.00
Dog *Canis familiaris*	0.28	0.16	563.50
Livestock total	**1.93**	**1.83**	**6557.90**
Eurasian beaver *Castor fiber*	4.99	3.93	14061.00
Red fox *Vulpes vulpes*	1.11	0.33	1183.00
Brown hare *Lepus europaeus*	2.22	1.13	4042.75
Medium mammals total	**8.31**	**5.39**	**19286.75**
Short-tailed field vole *Microtus agrestis*	0.28	≤0.05	16.56
Bank vole *Myodes glareolus*	0.28	≤0.05	3.45
Undetermined vole *Microtus* sp.	1.38	≤0.05	26.79
Undetermined mouse *Apodemus* sp.	0.28	≤0.05	24.15
Undetermined weasel *Mustela* sp.	0.28	≤0.05	6.90
Small mammals total	**2.49**	**≤0.05**	**77.85**
Birds	**0.28**	**≤0.05**	**0.13**
Insects	**0.28**	**≤0.05**	**4.45**
Plant material	**14.13**	—	—
Number of scats		**361**	

its relatively small share in the ungulate community (8.4%; Fig. 2). In contrast, red deer was negatively selected (D = –0.49) and wild boar was completely avoided by wolves in Šumava NP (Fig. 2).

Parasitology

Results of coproscopic analysis of the scat samples are shown in Table 2 and examples of detected parasite stages in Fig. 3. Using the flotation method 301 samples (81.57%) tested positive for the presence of one or more faecal stage of parasites. Of these, 154 samples (41.74%) had one type, 124 samples (33.60%) had two types, 20 samples (5.42%) had three types, and three samples were positive for four types of parasite stages (1.08%). These include also the stages of parasites originating from prey.

Regarding the frequency of the parasite stages, the most frequently detected parasite stages were sporocysts of coccidia from the genus *Sarcocystis*, found in 204 samples (55.28%).

Among helminths, eggs of nematodes of the Capillariidae family were detected in 143 samples (38.75%). Eggs of taeniid tapeworms were detected in 68 samples (18.42%). Molecular analyses of a subset of taeniid egg samples revealed the presence of three tapeworm species – *Taenia hydatigena*, *T. krabbei* and *T. lynciscapreoli*. Additionally, *Toxocara* eggs were found in 18 samples (4.87%), while *Toxascaris leonina* eggs were detected in two samples (0.54%). Eggs of representatives of the order Strongylida were observed in seven samples (1.89%), and Trichuris eggs were found in the same proportion (1.89%). Cryptosporidium oocysts were detected in only one sample (0.27%). The intensity of infections varied from low to medium (1 to 2 crosses) in most cases. However, in some instances, higher intensities of secreted oocysts and anecdotal records of eggs were observed, particularly for *Sarcocystis* spp., eggs of Capillariidae nematodes and eggs of taeniid tapeworms.

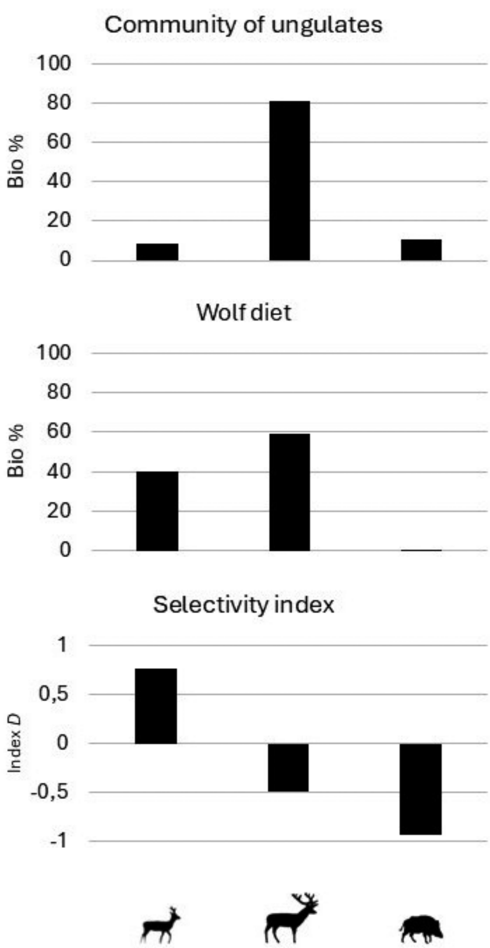

Fig. 2. Composition of wild ungulate community, wolf prey, and preference/avoidance of wolves based on selectivity index *D* index (Jacobs 1974) in the Šumava region.

Table 2. Results of faecal examination showing diversity and frequency of occurrence detected parasites.

Detected parasite stage	% of positive samples (n)	Method	Passage of parasites of prey
Alaria eggs	15.44 (57)	sedimentation	improbable
Anoplocephalid tapeworm eggs	0.54 (2)	flotation	yes
Capillariidae eggs	38.75	flotation	possible in some samples
Crenosoma vulpis larvae	2.45 (9)	larvoscopy + PCR	improbable
Cryptosporidium oocysts	0.27 (1)	flotation	possible
Eimeria oocysts	2.98 (11)	flotation	yes
Elaphostrongylus larvae	5.99 (22)	larvoscopy + PCR	yes
Metastrongylus eggs	2.17 (8)	flotation	yes
Nematodirus eggs	0.81 (3)	flotation	yes
Protostrongylus pulmonalis larvae	1.09 (4)	larvoscopy + PCR	yes
Sarcocystis sporocysts	55.28 (204)	flotation	improbable
Strongylid eggs	1.89 (7)	flotation	possible
Taeniid tapeworm eggs	18.42 (68)	flotation	improbable
Toxocara eggs	4.87 (18)	flotation	improbable
Toxascaris leonina eggs	0.54 (2)	flotation	improbable
Trichuris eggs	1.89 (7)	flotation	possible
Varestrongylus larvae	2.45 (9)	larvoscopy + PCR	yes
Total positives flotation	**81.57% (n = 301)**		
Total positives larvoscopy	**11.99% (n = 44)**		
Total positives sedimentation	**15.44% (n = 57)**		
Total positives coproscopy	**84.55% (n = 312)**		

The larvoscopic examination of 367 samples revealed the presence of L1 larvae from the pulmonary nematode *Crenosoma vulpi*s in nine cases (2.45%). The species was confirmed by PCR followed by sequencing. Sedimentation analyses were performed on 369 samples, and 57 samples (15.44%) were positive for *Alaria* sp. eggs.

In several samples, parasite stages typical of hosts other than wolves were detected, reflecting passage of parasite stages from prey species, scavenging and/or coprophagia which is a characteristic of free-living carnivores. Flotation analysis revealed the presence of oocysts of genus Eimeria, coccidia typical of herbivorous and omnivorous hosts, detected in 11 samples (2.98%). Additionally, eggs of *Metastrongylus* species, pulmonary nematodes typically found in wild boar, were detected, along with the eggs of *Nematodirus* spp. in three samples, i.e. 0.81% and eggs of anoplocephalid tapeworms in two samples (0.54%), both are parasites of prey (probably wild ruminants or lagomorphs). Due to the morphological similarity of the eggs of the various species of nematodes of the Capillariidae family, it is possible that a part of the detections represented eggs passed from prey, e.g. wild ruminants.

Larvoscopy combined with PCR revealed L1 larvae of pulmonary helminths, typically found in prey, such as those of the genera *Varestrongylus*, *Elaphostrongylus* and *Protostrongylus*, which parasitise wild ruminants or lagomorphs. These were detected in 35 samples (9.54%). In one case, L1 larvae of *Perostrongylus falciformis*, a lungworm of European badger, were identified, although the sample likely did not belong to wolf and originated from a European badger.

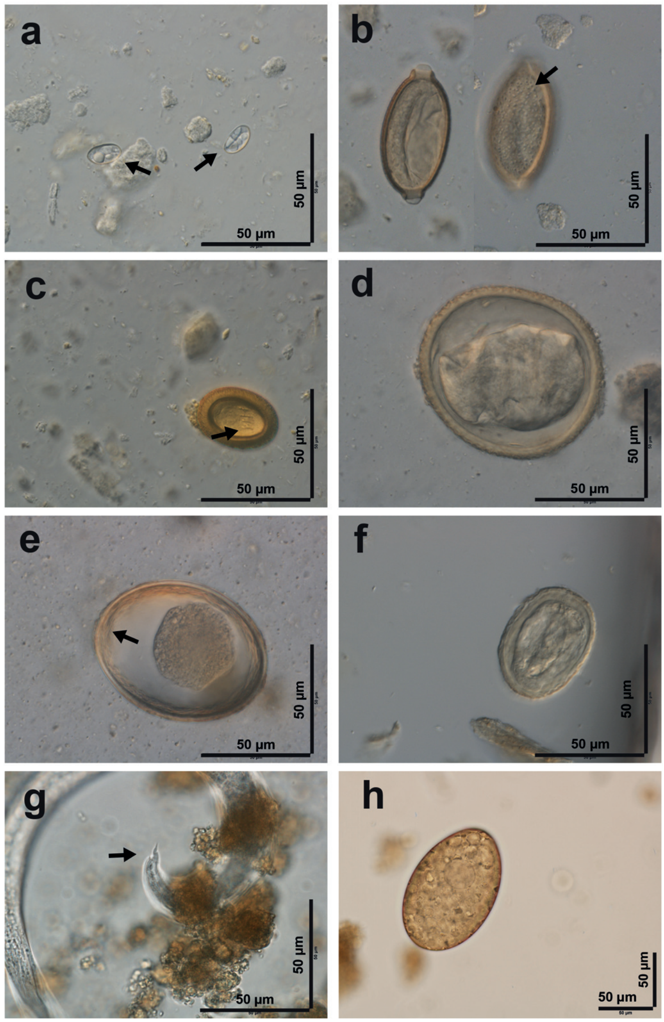

Fig. 3. Example of detected parasite stages in coproscopy.(a) *Sarcocystis* sp., sporocysts (arrows); (b) capillarid egg, identified as *Eucoleus aerophilus* based on typical shape and pattern of egg wall ornamentation (arrow); (c) taeniid tapeworm, egg with visible hooks of the oncosphere (arrow); (d) *Toxocara* sp., partially damaged egg - due to repeated freezing; (e) Toxascaris leonina, egg with typical ornamentation of inner egg wall; (f) *Metastrongylus* sp., partially damaged egg - due to repeated freezing, (g) *Elaphostrongylus* sp., detail of posterior end of L1 larvae (arrow); (h) *Alaria* sp., egg.

Discussion and conclusions

Studies on wolf feeding patterns in Central Europe consistently show a strong preference for wild ungulates (e.g. Nowak et al. 2005, 2011, Ansorge et al. 2006, Wagner et al. 2012, Guimarães et al. 2022, Kutal et al. 2024, Lippitsch et al. 2024). This trend is further supported by the findings of the present study, where the analysis of 361 scats revealed that wild ungulates comprised 92.8% of the total biomass consumed by wolves.

The structure of the wild ungulate community living in our study area is similar to other mountainous regions of Central Europe, such as Western and Eastern Carpathians (Nowak et al. 2005, Guimarães et al. 2022, Kutal et al. 2024). Although red deer was the dominant species in the wolf diet, it was generally avoided in proportion to its availability, while roe deer was clearly selected by wolves.

The strong selectivity for roe deer observed in this study, despite its relatively low availability (~8% of the ungulate community), can be explained by several likely overlapping, ecological and behavioural factors. First, roe deer offer a favourable trade-off between energy reward and predation risk. Compared to larger ungulates, such as red deer or wild boar, roe deer are smaller-sized prey, which reduce both hunting time and risk of injury, especially for smaller wolf packs or dispersing individuals (Peterson & Ciucci 2003). Furthermore, roe deer tends to be solitary and lack of coordinated defense behaviour, making them easier targets, especially in dense forest cover where visibility is limited. Second, seasonal snow conditions in the Šumava region may further enhance the vulnerability of roe deer during winter. Their small body size and limited mobility in deep snow increase their susceptibility to predation under such conditions, as was observed in other regions (e.g. in Bieszczady Mts., Gula 2004). Third, roe deer preference may reflect learned hunting strategies within recently recolonizing wolf populations. In areas where wolf packs are establishing territories after several decades of absence, pairs and newly formed packs often initially rely on smaller, more manageable prey until their hunting proficiency and social cohesion are sufficient to target larger ungulates (Capitani et al. 2004, Zlatanova et al. 2014). This pattern may help explain the observed selectivity for roe deer in the Šumava region, where wolves have recolonized and established stable territories over the past decade (Bufka & Červený 2021, Vorel et al. 2024, Vorel et al. 2025). Comparable patterns have been reported in other European regions (e.g. in Eastern Germany, Western Poland or Northern Italy), where recolonizing wolf populations have positively selected roe deer (Capitani et al. 2004, Ansorge et al. 2006, Nowak et. al 2011, Wagner et al. 2012). This contrasts with patterns reported in other regions with long term stable wolf population such as Białowieża Forest or parts of the Western and Eastern Carpathians, where red deer is often the preferred prey within diverse and abundant ungulate communities (Okarma 1995, Jędrzejewski et al. 2000, Nowak et al. 2005, Guimarães et al. 2022).

In species-rich ecosystems, wolves tend to refine their foraging strategies to maximize energetic efficiency (Zlatanova et al. 2014). This suggests that in the future, as Šumava's wolf population matures and stabilizes, a dietary shift toward larger prey such as red deer could emerge, particularly as roe deer are also the main prey of the local Eurasian lynx population (Belotti et al. 2015). The availability of roe deer may thus fluctuate under combined predation pressure from both apex predators, as well as hunting management practices within and around the national parks.

Additionally, wild boar was totally avoided by wolves in our study area, following feeding patterns observed in other Central European regions (e.g. WAGNER et al. 2012, KUTAL et al. 2024). However, this exclusion may not be permanent. Should the availability of preferred prey decline, wolves may adaptively shift their diet to include wild boar, highlighting their ecological plasticity (e.g. DAVIS et al. 2012). Nevertheless, a rise in wild boar predation in the Šumava region appears unlikely in the near future, due to the ample availability of suitable prey, primarily cervid species, combined with the relatively low population density of wild boar comparing to other areas in Czech Republic and the effects of intensive hunting management.

Among other prey types, medium-sized mammals such as beavers were the most frequently detected food item after ungulates. In certain areas of north-western or north-eastern Poland (NOWAK et al. 2011, JĘDRZEJEWSKI et al. 2012, MYSŁAJEK et al. 2019, 2021), the European beaver serves an alternative prey after wild ungulates, comprising between 5.1% and 24.6% of the total biomass consumed. A similar trend was observed in our study area, where beavers accounted for nearly 4% of the total biomass consumed. With their successful recovery in the last two decades (VOREL et al. 2014), beavers may become an increasingly important food source in the Šumava region.

Livestock constituted a very small portion of wolf diet in our study area (1.8% of Bio), similar to findings in other countries, such as Germany, Poland or Slovakia, where livestock made up up to 5% of the total biomass consumed (NOWAK et al. 2005, WAGNER et al. 2012, NOWAK et al. 2011, GUIMARÃES et al. 2022, KUTAL et al. 2024, LIPPITSCH et al. 2024). These results align with the general pattern of wolf feeding habits across Europe, where wolves in the northern part of their range primarily feed on wild ungulates (ZLATANOVA et al. 2014). Consistent with other studies that analysed of large proportion of scat samples (e.g. WAGNER et al. 2012 or LIPPITSCH et al. 2024), other food sources such as small mammals or birds comprised only negligible parts of wolf diet. Given that annual game census data obtained from hunting inventories may contain inaccuracies that can affect both the calculation and interpretation of the selectivity index, the resulting values should be treated with caution. We therefore recommend that future studies on wolf feeding preferences rely on more robust data on prey availability in the study area. Suitable approaches include, for example, pellet transects, which can provide a more reliable picture of the abundance and population density of the ungulate community and allow comparison with census data from hunting statistics. Combining these methods is likely to yield more comprehensive results that can be directly applied to the effective conservation and management of both the apex predator and its ungulate prey in the area of interest.

In total, 312 out of 369 samples (84.57%) were found to be positive for faecal stages of parasites across all three coproscopic methods, representing a higher frequency of positive samples compared to studies conducted in neighbouring countries such as Slovakia and Germany (ČABANOVÁ et al. 2017, LESNIAK et al. 2018). While this may indicate a relatively high prevalence of parasitic infections in wolves in the Šumava region, the showed frequency of occurrence includes passage of parasites of prey. The overall intensity of parasite infection in the examined samples was low, however it could be at least partially attributed to the sample processing, freezing in particularly.

Regarding typical heteroxenous parasites transmitted through carnivores, our study detected eggs of *Taenia* spp., including *T. krabbei* and *T. lynciscapreoli*, both of which primarily use roe deer as intermediate hosts. *Taenia hydatigena*, which has a broader range of intermediate hosts (including wild and domestic ruminants, as well as wild boar and domestic pigs), was also detected. *Taenia hydatigena* and *T. krabbei* were previously recorded in free-ranging wolves in the Czech Republic (JURÁNKOVÁ et al. 2021), this is the first confirmation of *T. lynciscapreoli* in wolves in the country. *Taenia hydatigena* and *T. krabbei* have also been documented in wolves in Germany, Finland and France (LAVIKAINEN et al. 2011, UMHANG et al. 2023). Eggs of ascarids, common in young animals, were also detected in our study, with results consistent with a similar study in Poland (POPIOLEK et al. 2007).

Our larvoscopic examination including sequencing indicated the presence of the pulmonary nematode *C. vulpis*. Since *C. vulpis* is typically considered as parasite of red fox and and foxes were detected as part of the diet in some cases, not all positive samples necessarily proved infection of wolves. However, the prey content analysis for several of *C. vulpis* positive samples were indicative of wolf origin.

The frequency of *Alaria* sp. (15.44%) in our study aligns with data from Germany, where a similar prevalence of 15.94% was reported (BINDKE et al. 2019).

Stages of *Sarcocystis* species, which infest multiple hosts and depend on predation for their life cycle, were among the most commonly detected parasite stages in our flotation analysis (55.28% of samples). Taeniid tapeworm eggs were found in 18.42% of samples. These findings indirectly reflect the level of infection in intermediate hosts, which are prey for wolves. In Germany, a study focusing on *Sarcocystis* identified 11 species by metabarcoding, with *Sarcocystis taeniata* and *S. grueneri* being more common in wolves than expected based on infection patterns in ungulates which are part of the wolf diet. These species appear to be highly adapted to wolves, making them examples of "parasitic specialists". Notably, the species richness of *Sarcocystis* spp. in wolves was significantly higher in pups than in adults, with wolf-specific species persisting throughout wolf adolescence. However, one study suggests that age-dependent maturation of the immune system may contribute to the control of parasitic protozoa in wolves (LESNIAK et al. 2018). Thus, our study provides a basis for the further molecular investigation into *Sarcocystis* infection in wolves.

A particularly intriguing aspect of our findings is the detection of stages of parasites from prey animals, such as wild ungulates and wild boar. This highlights the role of parasite stages passages from prey in shaping the overall understanding of wolf prey preferences, as these parasites indirectly reflect the diet and ecological interactions of wolves. The present study demonstrates that wolves in the Šumava National Park maintain a strongly cervid-based diet, constituting the vast majority of consumed biomass. Livestock contributed only marginally, indicating a low level of human-wolf conflict. The combination of a rich and diverse ungulate assemblage, the presence of alternative prey such as the Eurasian beaver, and extensive areas of contiguous forest habitat provides an ecologically favorable conditions for stable wolf population.

The parasite community detected in wolf scats closely mirrors this specialized trophic niche. The dominance of heteroxenous endoparasites, particularly *Taenia* spp. or *Sarcocystis* spp., and pulmonary helminths, reflects a transmission cycle tightly linked to cervid intermediate hosts. These findings are consistent with parasite assemblages reported in other Central and Northern European wolf populations and underscore the trophic dependency of parasite diversity on prey selection. Moreover, this study highlights the broader ecological role of wolves not only as apex predators but also as keystone hosts in complex multi-host parasite systems. By sustaining and connecting parasite life cycles across trophic levels, wolves contribute to the structuring of parasitic communities within ecosystems.

Finally, the integration of scat-based dietary and parasitological analyses proves to be a valuable approach in carnivore ecology. When interpreted in tandem, these complementary datasets provide a more nuanced understanding of predator-prey dynamics, host-parasite interactions, and the ecological consequences of diet selection. Such integrative methods are essential for informing evidence-based conservation strategies and for understanding the multifaceted ecological functions of large carnivores in recolonizing and human-modified landscapes.

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