International Journal for Parasitology: Parasites and Wildlife 22 (2023) 101–107

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Gray wolves as sentinels for the presence of *Echinococcus spp*. and other gastrointestinal parasites in France

Gérald Umhang [a,*], Christophe Duchamp [b], Jean-Marc Boucher [a], Christophe Caillot [a], Léo Legras [a], Jean-Michel Demerson [a], Jérémie Lucas [b], Dominique Gauthier [c], Franck Boué [a]

[a] *ANSES LRFSN, Wildlife Surveillance and Eco-epidemiology Unit, National Reference Laboratory for Echinococcus spp., Malzéville, France*
[b] *OFB (French Biodiversity Agency), Research Department, Predator and Depredator Unit, Gap, France*
[c] *Veterinary Diagnosis Laboratory, 05000, Gap, France*

ARTICLE INFO

Keywords:
Gray wolf
Echinococcus multilocularis
Echinococcus granulosus sensu stricto
Taenia spp.
Nematode
Copro-PCR

ABSTRACT

Over the past 30 years, the gray wolf population has recovered in France, initially to wolves from Italy passing through the Alps. The population is carefully monitored, but little information is available on their helminth fauna, which includes parasites of public health importance: *Echinococcus multilocularis* and *Echinococcus granulosus sensu lato*. Capitalizing on the availability of 911 fecal samples collected for the noninvasive genetic monitoring of French wolf populations, along with the intestines from 15 dead wolves, the presence of *Echinococcus* species among others helminth species was evaluated in French wolves. A copro-PCR approach amplifying a large spectrum of parasites was used for fecal samples while intestines were analyzed using SCT. The fecal occurrences of *E. granulosus sensu stricto* (2.4%) *and E. multilocularis* (0.3%), and indeedother parasitic species, are similar to those of other European wolf populations including *Taenia hydatigena* (7.2%), *Taenia krabbei* (2.4%), *Uncinaria stenocephala* (2.4%), *Mesocestoides litteratus* (1.9%), *Taenia ovis* (0.3%), *Taenia multiceps* (0.1%), and *Toxascaris leonina* (0.1%). The three most abundant species were also found in the intestines. Infections by *E. granulosus sensu stricto* are in accordance with the overlap of wolf pack areas and sheep breeding pastoral units. However, the wolf does not appear to play a significant role in the lifecycle of *E. granulosus sensu stricto*. The availability of this opportunistic fecal sampling of wolves in southeastern France means that they can be used as sentinels for the surveillance of *E. multilocularis* in the context of its southward expansion observed in recent years.

1. Introduction

The gray wolf is the largest of all canids in height and weight, and—after recolonizing several countries—enjoys a wide distribution in Europe (Ciucci et al., 2009). Its lifestyle is based on pack social structures with a dedicated home range. Additionally, several individuals regularly leave their original packs, reaching new territories sometimes several hundred kilometers away. In France, wolf recovery started from the early 1990s due to expansion of the Italian population in the Apennines (Fabbri et al., 2007). In the past 20 years, wolves have pushed northward and westward to colonize new territories in France and have now recovered about 12% of the country (OFB, personal communication). The current French wolf population is estimated at around 620 individuals mainly distributed over the Alpine mountain range (Drouet-Hoguet et al., 2020). The wolf is on the list of protected species in France and populations can only be regulated by official culling following a derogation in keeping with the Habitat Directive framework (https://ec.europa.eu/environment/nature/legislation/habitatsdirective/index_en.htm). The diet of French wolf packs has been studied by identifying macro-remains (mainly hairs) contained in fecal samples collected in the field. In general, the wolf's diet is composed of wild ungulates such as roe deer, deer, mouflon, and chamois (around 76%), domestic ungulates such as sheep, goats, and cattle (16%) and smaller mammals such as marmots, lagomorphs, and rodents (8%) (Flühr, 2011).

For the past forty years, much information has been obtained on the ecology of the French wolf population but few data are available on their helminthic fauna despite wolves worldwide having a very large diversity of more than 70 species from 40 different genera (Craig and Craig, 2005). Different infection routes can explain this large spectrum of

* Corresponding author.
E-mail address: gerald.umhang@anses.fr (G. Umhang).

https://doi.org/10.1016/j.ijppaw.2023.09.007
Received 13 June 2023; Received in revised form 12 September 2023; Accepted 18 September 2023
Available online 20 September 2023
2213-2244/© 2023 The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

parasitic species. Nematode infections are caused by direct ingestion of larvae (L3) in an environment contaminated by the excretion of eggs from the definitive host. On the other hand, cestode infections are due to predation or consumption of a mammalian intermediate host, which can range from small rodents to large wild or domestic herbivores. Trematode species can be picked up from intermediate hosts such as snails, fish or frogs. The two most prevalent helminths reported in wolves were the nematode *Uncinaria stenocephala* and the cestode *Taenia hydatigena* (Craig and Craig, 2005). Whiles some of these parasites are of veterinary importance, some are also of public health significance, such as the cestodes *Echinococcus multilocularis* and *Echinococcus granulosus sensu lato* (*s.l.*), which are both endemic in France. These parasites can infect humans after ingestion of microscopic eggs dispersed in the environment via the feces of definitive hosts. Their lifecycles are mainly maintained by red foxes after predation of small rodents in the case of *E. multilocularis* and through consumption of viscera from sheep, cattle, and pigs regarding *E. granulosus sensu stricto* (*s.s.*), *E. ortleppi* and *E. canadensis*, respectively. The presence of *E. multilocularis* is currently restricted to the northeastern half of the country (Combes et al., 2012). Wolves may be used as sentinels to detect the parasite at the border of today's known endemic areas where it has not yet been investigated. *E. granulosus s.s.* is present throughout France but more frequent in the southern Alps (Umhang et al., 2020b), where the French wolf population is mainly located. Both *E. ortleppi* and *E. canadensis* (G6/7) have already been reported in France but with more restricted distributions (Grenouillet al. 2014; Umhang et al., 2020b). The large collection of feces used by the French biodiversity agency (OFB) to monitor the wolf population throughout France (Duchamp et al., 2012) provided the opportunity to sample and collect initial data to evaluate wolves' infection by *E. granulosus s.l.* and *E. multilocularis*. Additionally, while *Echinococcus* species were mainly targeted, this study also provided an opportunity to obtain a larger overview of the gastrointestinal helminthic fauna of wolves in France.

2. Materials and methods

2.1. Collection of samples

Fecal samples were collected in the field from 2008 to 2016 through all four seasons by the wolf-lynx network managed by the OFB (Fig. 1). This network was set up as part of wolf molecular tracking to help detect the presence of new wolf packs and to contribute to non-invasive capture recapture studies focused on population dynamics (Cubaynes et al., 2010; Marescot, 2012). The study area involved 11 departments (i.e., the French administrative unit corresponding to the NUTS3 level in European Union standard territorial unit nomenclature) in southeastern France (65,647 km^2) corresponding to a temperate/montane biome (Fig. 2). During the sampling period, the French wolf population increased from 18 to 35 packs, mostly distributed over the Alpine range (Group, 2018). A total of 911 fecal samples were collected mainly during the winter (36.8%) and spring (35.4%), with fewer samples for summer (12.2%) and fall (15.6%). All the feces were genetically confirmed as coming from wolves according to the national wolf survey procedure (Duchamp et al., 2012) using mtDNA control region sequencing (Valière et al., 2003). All wolf samples were attributed to each of the corresponding packs following individual genotyping investigations (Duchamp and Queney, 2019) for 66.1% of the feces and/or sign surveys of spatial and temporal distributions (Duchamp et al., 2012). Fifteen wolf intestine samples were obtained from necropsied individuals that died either accidently or by official culls.

2.2. Laboratory analyses

All fecal and intestinal samples were frozen after collection for storage and decontaminated prior to analysis by deep-freezing at −80 °C for 7 days to prevent any zoonotic risk. The intestines were analyzed using the SCT (sedimentation and counting technique) (Hofer et al., 2000; Eckert, 2003) but after dividing the intestines into five equal parts as for segmental SCT (SSCT) (Umhang et al., 2011). All five intestinal segments were systematically analyzed. When present in a given segment, one parasite specimen morphologically identified as *Echinococcus* sp., *Taenia* sp., *Mesocestoides* sp. or from the Nematoda class was molecularly identified to species level.

For each parasitic worm identified, DNA was extracted with the DNeasy Blood and Tissue kit (Qiagen) from about 25 mg of parasite tissue. DNA was extracted from 500 mg of fecal sample using the QIAamp DNA Stool Mini Kit (Qiagen). A fragment of the cytochrome *c* oxidase subunit 1 (*cox1*) gene was amplified by PCR (Bowles et al., 1992) for DNA samples from feces and tissue. PCR products were sequenced by a private company (Eurofins) and the nucleotide sequences were aligned using the Vector NTI software (Invitrogen) prior to comparison with sequences available in GenBank using the BLASTn search tool (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the parasitic species.

2.3. Data analyses

Fecal sampling was initially carried out in a non-invasive capture

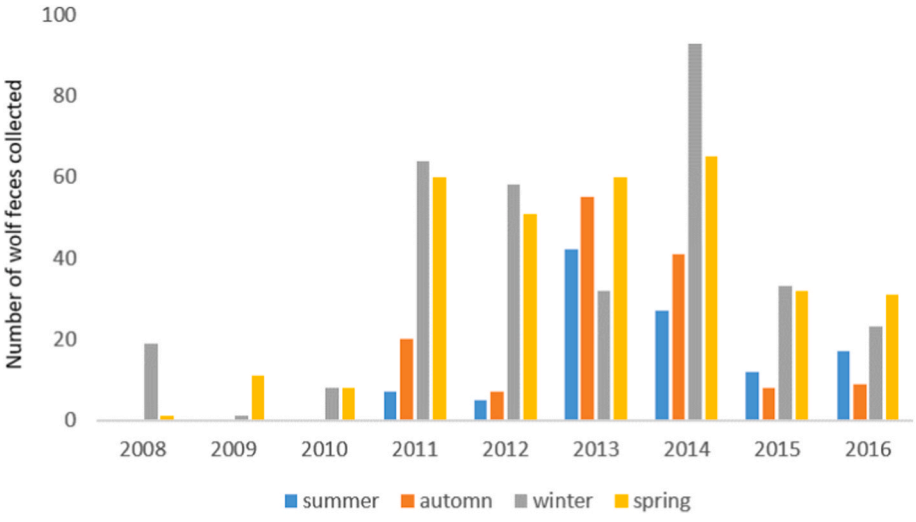

Fig. 1. Distribution of wolf fecal samples analyzed according to season and year.

Fig. 2. Spatial distribution of the 911 fecal samples collected from wolves (gray circle) in southeastern France submitted to copro-DNA analyses for identification of gastrointestinal parasites. The size of the circles is proportional to the number of samples collected per municipality. The departments (corresponding to NUTS3 level) are indicated by black lines.

recapture model of wolf population monitoring, focusing on repeated sampling among packs and between years. During this period, multiple samples were therefore collected and analyzed from the same individual, as confirmed by individual genotyping. However, these data were not available for 33.9% of the samples. The term 'occurrence' is therefore used for estimating the presence of parasites from feces rather than 'prevalence', which was only used for the intestine analysis from dead animals. Fecal occurrences of the parasitic species were described for the whole dataset, but also split into pack territories when available insofar as the sample pooled more than ten samples. Occurrence and prevalence with 95% confidence intervals were calculated using exact binomial tests.

Concerning a potential seasonal effect on parasite detection, data were processed using a Chi-square test for each category of parasites: all parasites, nematodes, and cestodes, going as far as the genus level for *Taenia* and species level for the most represented species (i.e.; *Uncinaria stenocephala*, *Taenia hydatigena* and *E. granulosus s.s*). If there were fewer than five seasonal occurrences, summer and fall were grouped together, as were spring and winter. If there were still fewer than five occurrences, a Fisher exact test was carried out. A p value below 0.05 was considered significant.

3. Results

The global occurrence of gastrointestinal parasitic species identified in wolf fecal samples was 17.4% (Table 1). Three different species of nematodes were identified from feces: *Uncinaria stenocephala* was the nematode with the highest occurrence, along with *Toxascaris leonina* and *Baylisascaris procyonis,* which was unexpected. Additionally, one out of the 15 available wolf intestines revealed the presence of *Toxocara canis* infection. No trematode species were identified. Among the cestodes, two *Echinococcus* species were identified: *E. multilocularis* and *E. granulosus s.s.*. *E. multilocularis* DNA was detected in three fecal samples (0.3%) and *E. granulosus s.s.* in 22 (2.4%) (Fig. 3 and Table 1). Two samples among the 15 intestines (i.e., 13% [2–40] 95% CI) also revealed the presence of 269 and 33 *E. granulosus s.s.* worms (Table 2). Four

Table 1
Occurrence and associated 95% confidence intervals of helminths detected in fecal samples from gray wolves identified using copro-PCR. The number of samples with a given species is indicated in parentheses.

	Occurrence	CI 95%
Cestodes		
E. granulosus ss	2.4% (22)	1.5-3.6
E. multilocularis	0.3% (3)	0.1-1.0
T. hydatigena	7.2% (66)	5.6-9.1
T. krabbei	2.4% (22)	1.5-3.6
T. ovis	0.3% (3)	0.1-1.0
T. multiceps	0.1% (1)	0.0-0.6
H. kamiyai	0.1% (1)	0.0-0.6
M.litteratus	1.9% (17)	1.0-3.0
Nematodes		
U. stenocephala	2.4% (22)	1.5-3.6
T. leonina	0.1% (1)	0.0-0.6
B. procyonis	0.1% (1)	0.0-0.6
Global occurrence of gastrointestinal parasite species	17.4%	15.0-20.1
Number of samples	911	

different *Taenia* species were detected in the wolf samples: *Taenia hydatigena*, *Taenia krabbei*, *Taenia ovis*, and *Taenia multiceps*. One other cestode species, identified as *Hydatygera kamiyai*, was observed in a single fecal sample. *Mesocestoides litteratus* was found in both wolf feces and intestines.

Co-infections were observed in three of the 15 wolf intestines analyzed. A maximum of three parasitic species was identified in the same intestine (*M. litteratus*, *T. krabbei* and *T. canis*). Co-infection was restricted to cestode species for the other two intestines: *T. hydatigena* and *T. krabbei* for one, *E. granulosus s.s.* and *T. hydatigena* for the other. No helminths were observed in four of the 15 intestinal samples.

The statistical analyses revealed that a seasonal effect was observed only for cestodes ($p = 0.04$), with a significantly higher detection in winter than fall ($p = 0.02$), while no significant difference was observed with summer ($p = 0.05$) and spring ($p = 0.53$). Fecal sampling covered 53 wolf pack territories, of which 29 accounted for fewer than ten fecal

Fig. 3. Location of the French wolf fecal samples positive for *Echinococcus granulosus sensu stricto* (green circles) and *Echinococcus multilocularis* (red circles). The *E. multilocularis*-positive fecal samples of dogs (red triangles) and wolves (small red diamonds) from Imperia (Italy) taken from Massolo et al., (2018) are also shown.

Table 2
Prevalence and associated 95% confidence intervals of helminths identified using the sedimentation and counting technique (SCT) on 15 Gy wolf intestines. The number of cases is indicated in parentheses.

	Prevalence (%)	CI 95%
E. granulosus ss	13 (2)	2–40
T. hydatigena	33 (5)	12–62
T. krabbei	27 (4)	8–55
M. litteratus	7 (1)	0–32
T. canis	7 (1)	0–32

samples and were consequently discarded from analysis of occurrence by pack. The number of samples used for pack-dependent occurrences ranged from 12 to 133 samples for the most represented "Haute-Tinée" pack (Fig. 4). No parasites were detected among the feces of 16 wolf packs, but these packs were generally poorly sampled. Up to five different species—including Nematoda and Cestoda—were identified from one pack in the core Alpine range (Galibier Thabor), with seven occurrences identified out of 68 fecal samples. The three cases of *E. multilocularis* correspond to wolves from three different packs, one from Savoie (northern Alps) and the other two from the Alpes-Maritimes department. Infection by *E. granulosus s.s.* occurred in seven different packs, revealed by the single detection of parasites for six of them. The "Haute-Tinée" wolf pack (subject to numerous sampling campaigns) located in the southern part of the Alps appears to be an outlier, there being 11 occurrences in samples collected from March 2011 to November 2012. In this pack, individual wolf genotyping data were available for eight of the 11 infected fecal samples, which corresponded to only three individuals: one adult (n = 4) and two of its offspring of the year (n = 3 and n = 1).

More globally, individual genotyping results were available for 602 fecal samples corresponding to 257 individuals, of which one parasitic infection was detected for 88 animals (34.2%). The number of fecal samples per individual ranges from one to 14, with four or more for 31 wolves in which at least one parasite was detected in 54.8% (n = 17) of these individuals. No parasites were detected after analysis of nine fecal samples each for two animals and 11 samples for another wolf. On the other hand, three different parasitic species were detected for two animals. The first one, with 14 fecal samples, was infected with *E. granulosus s.s.* (n = 4), *M. litteratus* (n = 2) and *T. Krabbei* (n = 1) whereas the other one, with 9 samples, was infected with *E. granulosus s.s.* (n = 3), *T. hydatigena* (n = 1) and *T. ovis* (n = 1). Regarding *E. granulosus s.s.* in particular, the 15 detections from fecal samples for which individual genotyping data were available correspond to ten different animals. These detections concerned only one fecal sample per animal except for two individuals, an adult and one of the young from the "Haute-Tinée" wolf pack previously mentioned. The 14 fecal samples from the adult were collected from October 2011 to February 2015. *E. granulosus s.s.* was detected in October 2011 (23rd and 28th) and February 2012 (19th and 20th) but not between these two periods despite an analysis of feces collected in December 2011 (18th), February 2012 (2 fecal samples collected on the 9th and 26th), one sample collected almost a month later (March 22nd) then another collected three months after that (2 fecal samples collected on June 18th). The last three fecal samples were collected between January and March 2015. Concerning the young wolf, the two detections of *E. granulosus s.s.* were from feces collected at a 4-day-interval (January 22nd and 26th, 2012) with no detection 2 months previously (November 16th and 18th, 2012), between the two positive results (January 22nd, 2012) or two weeks after (January 10th, 2013).

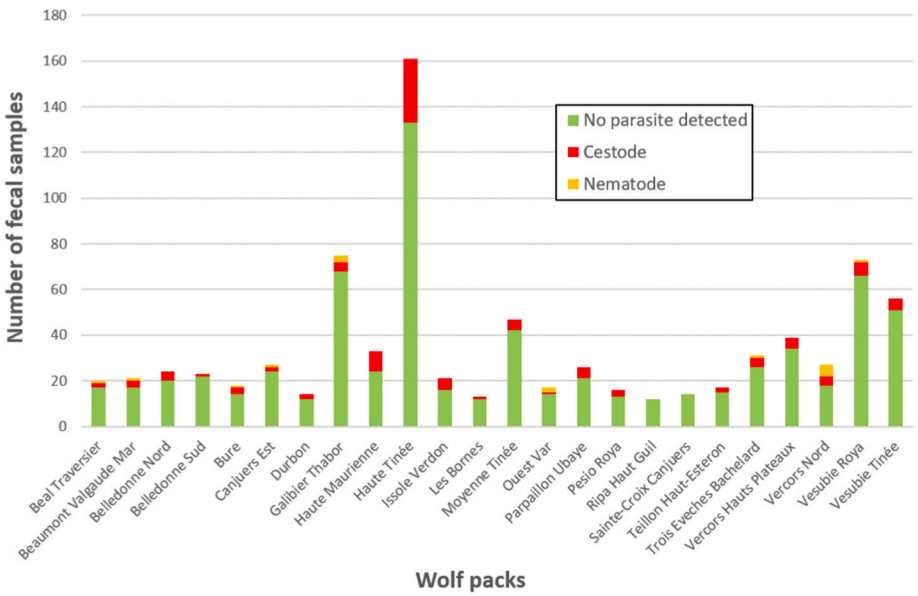

Fig. 4. Histogram of the total number of fecal samples analyzed for each French wolf pack with a minimum of 12 fecal samples available, with indication of the detection of cestodes (red), nematodes (orange) or absence of parasites (green).

4. Discussion

Many European surveys targeting the identification of gastrointestinal parasites in wolves have been conducted in Italy (Guberti et al., 1993; Gori et al., 2015; Poglayen et al., 2017; Macchioni et al., 2021), as well as in southern (Guerra et al., 2013; Munoz et al., 2018), eastern (Martínek et al., 2001; Bagrade et al., 2009; Borecka et al., 2013; Gawor et al., 2021) and northern European countries (Lavikainen et al., 2011; Al-Sabi et al., 2018). The diversity of helminthic species detected in France and their fecal occurrences are very similar to those previously reported in other European wolf populations from fecal analyses based on flotation and PCR (Guerra et al., 2013; Gori et al., 2015; Poglayen et al., 2017; Massolo et al., 2018; Munoz et al., 2018; Macchioni et al., 2021). Nevertheless, a big difference was observed in the detection of helminths between feces and intestines, with 17% and 73% (11 out of 15), respectively. This gap can be explained by a difference in sensitivity for the two matrices with a polyspecific method able to detect all parasites for intestines and the limited presence of parasitic DNA in feces. Furthermore, the choice of PCR target (i.e., short *cox1*) meant that a broad panel of parasitic species could be detected, but this detection was limited to a single species in each fecal sample even when there was co-infection. Thus, the sensitivity of the *cox1* copro-PCR approach does not appear suited to temporal monitoring of infection in individuals due to the numerous non-detections despite a parasitic species being identified a few weeks or even a few days before or after. This was illustrated with the wolves infected by *E. granulosus s.s.* from the "Haute-Tinée" pack, which prevented correct extrapolation of the length of the infestation. In these cases, a period of 6 weeks and 3 months were observed between the two periods of detection but the absence of detection during this period, and especially afterward, prevents confidence in these estimations, as it is difficult to rule out the possibility of a second infection as the cause of detection at the end of the period. In natural conditions, it is possible for wolves to have a second, concomitant, infection. The use of a very sensitive and specific tool like real-time qPCR should enable uninterrupted detection and open up the possibility of evaluating the number of infections for each individual.

The significant difference between cestode infections in winter compared with fall is difficult to interpret, as it groups several parasitic species with various intermediate hosts (such as rodents, sheep, and cervids). It may be linked more to the predation behavior of wolves during these periods than to the cestode infection rate in intermediate hosts. It may also be hypothesized that the conservation of parasitic DNA in fecal samples from the field is better in winter due to cold temperatures than in fall, when heavy rainfall can rapidly leach feces.

Overall, 11 parasite species were detected in wolf fecal samples, including eight cestode and three nematode species, with *T. hydatigena* and the nematode *U. stenocephala* among the most frequent, as generally observed in wolf populations (Craig and Craig, 2005). *T. canis* is usually common in wolves, but no cases were observed here in any of the fecal samples and its occurrence was revealed only by one intestine sample. The presence of *B. procyonis* was totally unexpected because it had never previously been reported in France and is not known to occur in wolves (Umhang et al., 2020a). The location of this case remains unexplained as it did not match any of the three main raccoon populations monitored in France, although raccoons have been reported in the French Alps (Léger and Ruette, 2014). Although infection was probably caused by predation on an infected host (such as a rodent or even raccoon), this detection of DNA in the absence of eggs in the fecal samples prevent any conclusions to be drawn regarding the wolf as a potential definitive host, but raises questions about the presence of this parasite in France and neighboring countries (i.e., Switzerland and Italy).

. Wolves frequently hunt red deer and roe deer as revealed by diet analysis (Flühr, 2011) and further corroborated by the detection of *T. krabbei*, for which wild cervids are intermediate hosts. The highest cestode occurrence is for *T. hydatigena*. Although cervids can be infected with *T. hydatigena*, the identification of *T. ovis* and *T. multiceps* in wolf feces clearly confirms the presence of sheep in the wolves' diet. The lifecycle of *E. granulosus s.s.* is also based on consumption of sheep viscera, with a global occurrence in feces of 2.4% (IC95%: 1.5–3.6) and an intestinal prevalence of 13% (IC95%: 2–40). The 22 cases identified from fecal samples originated mainly (n = 17) from the Alpes-Maritimes department, but also from Isère and Alpes-de-Haute-Provence, all parts of the French Alps that have been the main historical focus of *E. granulosus s.s.* in sheep (Umhang et al., 2020b). Attacks on sheep flocks are the main cause of conflict between pastoral activities and the presence of wolves. Based on the low frequency of occurrence of infection among 911 wolf samples and knowledge on the epidemiology of *E. granulosus s.s.* present throughout France, the wolf appears to play a negligible role in maintaining the lifecycle of *E. granulosus s.s.* in the French Alps. The same conclusion has been reached in Italy (Poglayen et al., 2017), although the wolf population there is higher than the French one (Galaverni et al., 2016) and with very high prevalence of

cystic echinococcosis in sheep estimated to lie between 20% and 75% in the country (Cardona and Carmena, 2013).

It is not surprising that *E. ortleppi* and *E. canadensis* were not detected because of the epidemiological situation of these two species in France. Surveys have confirmed the exclusive presence of *E. canadensis* (G6/7) on the French island of Corsica in a classically domestic lifecycle involving dogs, pigs, and even wild boars (Umhang et al., 2014, 2020b; Grech-Angelini et al., 2019). *E. ortleppi* has been reported at a very low prevalence in areas not concerned by the sampling of wolf feces; two such foci were in cattle but other areas were involved in human cases (Grenouillet et al., 2014). As the exact knowledge of its distribution in the country is currently unknown, it may be possible to detect this species in wolves in the future, especially as two cases in wolves have been identified in Italy close to the French border (Massolo et al., 2018). Small rodents are considered only a minimal part of the wolf's diet, the 8% concerning small mammals being mainly composed of lagomorphs and marmots. In addition to the other parasitic species caused by predation on rodents, the detection of *E. multilocularis*—which generally has a very low prevalence in rodents—and *M. litteratus* provides further support for a potentially greater predation on rodents than previously estimated, at least during certain periods and specific areas. The presence of *E. multilocularis* DNA in two fecal samples collected in 2011 and 2013 from the Alpes-Maritimes department extends the southern range of the known endemic area in France, as this parasite had only previously been detected in the Hautes-Alpes department in *Arvicola terrestris* (Umhang et al., 2021) and red fox fecal samples (Umhang et al., 2016). No cases have yet been detected further south in the Var and Bouches-du-Rhône departments (Umhang et al., 2022). The report of *E. multilocularis* being detected in Imperia Province, which is in the southwestern Italian Alps (Massolo et al., 2018) located only 5–15 km from the closest French wolf case, confirms the southward expansion of the parasite. Previous genetic investigations using the EmsB microsatellite marker on *A. terrestris* samples also suggested a southerly expansion into the French Alps from the historically endemic area of eastern France (Umhang et al., 2021). Despite the absence of relevant molecular data from fecal samples from either side of the French and Italian Alps, it may be hypothesized that this parasite's presence in the southwestern Italian Alps may be the result of a southern expansion in France overflowing into Italy. Additional molecular investigations are needed on both sides of the border to better understand the recent expansion of the parasite into these areas. However, these results argue that wolves can be used as a sentinel species for the detection of *E. multilocularis* in southeastern France, notably as surveillance is greatly facilitated by the large number of wolf fecal samples available due to the active monitoring of this species. Once detected, specific investigations on the main definitive host—the red fox—will then be required to estimate prevalence and parasite flows in time and space. Information on both the zoonotic risk and preventive measures to be taken should be disseminated in the southern Alpine areas where the parasite is generally unknown to the human population. Taking advantage of the global overview of helminth diversity obtained here, future studies targeting *E. multilocularis* and *E. granulosus s.l.* with specific and sensitive molecular tools such as real-time PCR can use the wolf as a sentinel from a public health perspective.

Declaration of competing interests

The authors declare no competing interests in association with this study.

Acknowledgments

We are grateful to the "Réseau Loup-Lynx" for collecting feces, the team of the veterinary diagnosis laboratory of the Hautes-Alpes department for providing wolf intestines, and Emmaunelle Robardet for help in the statistical analyses.

References

Al-Sabi, M.N.S., Rääf, L., Osterman-Lind, E., Uhlhorn, H., Kapel, C.M.O., 2018. Gastrointestinal helminths of gray wolves (Canis lupus lupus) from Sweden. Parasitol. Res. 117, 1891–1898. https://doi.org/10.1007/s00436-018-5881-z.

Bagrade, G., Kirjuina, M., Vismanis, K., Ozoli, J., 2009. Helminth parasites of the wolf Canis lupus from Latvia. J. Helminthol. 83, 63–68.

Borecka, A., Gawor, J., Zieba, F., 2013. A survey of intestinal helminths in wild carnivores from the Tatra National Park, southern Poland. Ann. Parasitol. 59, 169–172.

Bowles, J., Blair, D., McManus, D.P., 1992. Genetic variants within the genus Echinococcus identified by mitochondrial DNA sequencing. Mol. Biochem. Parasitol. 54, 165–173. https://doi.org/10.1016/0166-6851(92)90109-W.

Cardona, G.A., Carmena, D., 2013. A review of the global prevalence, molecular epidemiology and economics of cystic echinococcosis in production animals. Vet. Parasitol. 192, 10–32. https://doi.org/10.1016/j.vetpar.2012.09.027.

Ciucci, P., Reggioni, W., Maiorano, L., Boitani, L., 2009. Long-Distance dispersal of a rescued wolf from the northern Apennines to the western alps. J. Wildl. Manag. 73, 1300–1306. https://doi.org/10.2193/2008-510.

Combes, B., Comte, S., Raton, V., Raoul, F., Boué, F., Umhang, G., Favier, S., Dunoyer, C., Woronoff, N., Giraudoux, P., 2012. Westward spread of Echinococcus multilocularis in foxes, France, 2005–2010 Emerg. Inf. Disp. 18, 2059–2062. https://doi.org/10.3201/eid1812.120219.

Craig, H.L., Craig, P.S., 2005. Helminth parasites of wolves (Canis lupus): a species list and an analysis of published prevalence studies in Nearctic and Palaearctic populations. J. Helminthol. 79, 95–103. https://doi.org/10.1079/joh2005282.

Cubaynes, S., Pradel, R., Choquet, R., Duchamp, C., Gaillard, J.M., Lebreton, J.D., Marboutin, E., Miquel, C., Reboulet, A.M., Poillot, C., Taberlet, P., Gimenez, O., 2010. Importance of accounting for detection heterogeneity when estimating abundance: the case of French wolves. Conserv. Biol. 24, 621–626. https://doi.org/10.1111/j.1523-1739.2009.01431.x.

Drouet-Hoguet, N., Gimenez, O., Duchamp, C., 2020. Mise à jour des effectifs et paramètres démographiques de la population de loups en France : conséquences sur la viabilité de la population à long terme.

Duchamp, C., Boyer, J., Briaudet, P.-E., Leonard, Y., Moris, P., Bataille, A., Dahier, T., Delacour, G., Millisher, G., Miquel, C., Poillot, C., Marboutin, E., 2012. Wolf monitoring in France : a dual frame process to survey time- and space-related changes in the population. Hystrix 23, 14–28. https://doi.org/10.4404/hystrix-23.1-4559.

Duchamp, C., Queney, G., 2019. Le suivi génétique des loups en 2018, bilan de la 1ère année de mise en oeuvre du nouveau marché public.

Eckert, J., 2003. Predictive values and quality control of techniques for the diagnosis of Echinococcus multilocularis in definitive hosts. Acta Trop. 85, 157–163.

Fabbri, E., Miquel, C., Lucchini, V., Santini, A., Caniglia, R., Duchamp, C., Weber, J.-M., Lequette, B., Marucco, F., Boitani, L., Fumagalli, L., Taberlet, P., Randi, E., 2007. From the Apennines to the Alps: colonization genetics of the naturally expanding Italian wolf (Canis lupus) population. Mol. Ecol. 16, 1661–1671. https://doi.org/10.1111/j.1365-294X.2007.03262.x.

Flühr, J., 2011. Analyse spatio-temporelle du régime alimentaire du loup (Canis lupus) dans les Alpes françaises.

Galaverni, M., Caniglia, R., Fabbri, E., Milanesi, P., Randi, E., 2016. One, no one, or one hundred thousand: how many wolves are there currently in Italy? Mammal. Res. 61, 13–24. https://doi.org/10.1007/s13364-015-0247-8.

Gawor, J., Laskowski, Z., Myczka, A.W., Zwijacz-Kozica, T., Sałamatin, R., 2021. Occurrence of Echinococcus spp. in red foxes and wolves in the protected area of the Tatra National Park in southern Poland - a threat to human health. Ann. Agric. Environ. Med. 28, 579–584. https://doi.org/10.26444/aaem/131649.

Gori, F., Armua-Fernandez, M.T., Milanesi, P., Serafini, M., Magi, M., Deplazes, P., Macchioni, F., 2015. The occurrence of taeniids of wolves in Liguria (northern Italy). Int. J. Parasitol. Parasites Wildl. 4, 252–255. https://doi.org/10.1016/j.ijppaw.2015.04.005.

Grech-Angelini, S., Richomme, C., Peytavin de Garam, C., Boucher, J.M., Maestrini, O., Grenouillet, F., Casabianca, F., Boué, F., Umhang, G., 2019. Identification and molecular characterization of Echinococcus canadensis G6/7 in dogs from Corsica, France. Parasitol. Res. 118, 1313–1319. https://doi.org/10.1007/s00436-019-06261-6.

Grenouillet, F., Umhang, G., Arbez-Gindre, F., Mantion, G., Delabrousse, E., Millon, L., Boué, F., 2014. Echinococcus ortleppi infections in humans and cattle, France. Emerg. Infect. Dis. 20, 2100–2102. https://doi.org/10.3201/eid2012.140641.

Group, W.A., 2018. Wolf poulations status in the alps: pack distribution and trend up to 2016 - with focus on year 2015-2016 - report. Published in LCIE Web site. http://www.lcie.org.

Guberti, V., Stancampiano, L., Francisci, F., 1993. Intestinal helminth parasite community in wolves (Canis lupus) in Italy. Parassitologia 35, 59–65.

Guerra, D., Armua-Fernandez, M.T., Silva, M., Bravo, I., Santos, N., Deplazes, P., Carvalho, L.M., 2013. Taeniid species of the Iberian wolf (Canis lupus signatus) in Portugal with special focus on Echinococcus spp. Int. J. Parasitol. Parasites Wildl. 2, 50–53. https://doi.org/10.1016/j.ijppaw.2012.11.007.

Hofer, S., Gloor, S., Müller, U., Mathis, A., Hegglin, D., Deplazes, P., 2000. High prevalence of Echinococcus multilocularis in urban red foxes (Vulpes vulpes) and voles (Arvicola terrestris) in the city of Zurich, Switzerland. Parasitology 120, 135–142.

Lavikainen, A., Laaksonen, S., Beckmen, K., Oksanen, A., Isomursu, M., Meri, S., 2011. Molecular identification of Taenia spp. in wolves (Canis lupus), brown bears (Ursus arctos) and cervids from North Europe and Alaska. Parasitol. Int. 60, 289–295. https://doi.org/10.1016/j.parint.2011.04.004.

Léger, F., Ruette, S., 2014. Raton laveur et chien viverrin : le point sur leur répartition en France. Faune Sauv 302, 9–16.

Macchioni, F., Coppola, F., Furzi, F., Gabrielli, S., Baldanti, S., Boni, C.B., Felicioli, A., 2021. Taeniid cestodes in a wolf pack living in a highly anthropic hilly agro-ecosystem. Parasite 28, 10. https://doi.org/10.1051/parasite/2021008.

Marescot, L., 2012. Dynamique et conservation des populations difficilement observables : cas d'étude de la recolonisation du loup dans les Alpes françaises.

Martínek, K., Kolářová, L., Hapl, E., Literák, I., Uhrin, M., 2001. Echinococcus multilocularis in European wolves (Canis lupus). Parasitol. Res. 87, 838–839. https://doi.org/10.1007/s004360100452.

Massolo, A., Valli, D., Wassermann, M., Cavallero, S., D'Amelio, S., Meriggi, A., Torretta, E., Serafini, M., Casulli, A., Zambon, L., Boni, C.B., Ori, M., Romig, T., Macchioni, F., 2018. Unexpected Echinococcus multilocularis infections in shepherd dogs and wolves in south-western Italian Alps: a new endemic area? Int. J. Parasitol. Parasites Wildl. 7, 309–316. https://doi.org/10.1016/j.ijppaw.2018.08.001.

Munoz, S., Ramos, P.L., Carreton, E., Diosdado, A., Gonzalez-Miguel, J., Simon, F., Morchon, R., 2018. Intestinal helminths in Iberian wolves (Canis lupus signatus) from Northwest Spain. Parasitol. Open 6. https://doi.org/10.2174/1874421401806010106.

Poglayen, G., Gori, F., Morandi, B., Galuppi, R., Fabbri, E., Caniglia, R., Milanesi, P., Galaverni, M., Randi, E., Marchesi, B., Deplazes, P., 2017. Italian wolves (Canis lupus italicus Altobello, 1921) and molecular detection of taeniids in the Foreste Casentinesi national Park, northern Italian Apennines. Int. J. Parasitol. Parasites Wildl. 6, 1–7. https://doi.org/10.1016/j.ijppaw.2017.01.001.

Umhang, G., Comte, S., Hormaz, V., Boucher, J.M., Raton, V., Favier, S., Raoul, F., Giraudoux, P., Combes, B., Boue, F., 2016. Retrospective analyses of fox feces by real-time PCR to identify new endemic areas of Echinococcus multilocularis in France. Parasitol. Res. 115, 4437–4441. https://doi.org/10.1007/s00436-016-5220-1.

Umhang, G., Demerson, J.M., Legras, L., Boucher, J.M., Peytavin de Garam, C., Bastid, V., Vannard, E., Pinot, A., Giraudoux, P., Boué, F., 2021. Rodent control programmes can integrate Echinococcus multilocularis surveillance by facilitating parasite genotyping: the case of Arvicola terrestris voles screening in France. Parasitol. Res. 120, 1903–1908. https://doi.org/10.1007/s00436-021-07126-7.

Umhang, G., Duchamp, C., Boucher, J.M., Ruette, S., Boué, F., Richomme, C., 2020a. Detection of DNA from the zoonotic raccoon roundworm Baylisascaris procyonis in a French wolf. Parasitol. Int. 78, 102155 https://doi.org/10.1016/j.parint.2020.102155.

Umhang, G., Richomme, C., Bastid, V., Boucher, J.-M., de Garam, C.P., Itié-Hafez, S., Danan, C., Boué, F., 2020b. National survey and molecular diagnosis of Echinococcus granulosus sensu lato in livestock in France, 2012. Parasitology. https://doi.org/10.1017/S0031182020000190 (in press).

Umhang, G., Richomme, C., Caillot, C., Bastid, V., Boucher, J.-M., Moyen, J.-L., Novella, C., Richoux, B., Davoust, B., Boué, F., 2022. Towards delimitation of the Echinococcus multilocularis parasite's southernmost range in France. Vet. Parasitol.: Reg. Stud. Rep. 30, 100724 https://doi.org/10.1016/j.vprsr.2022.100724.

Umhang, G., Richomme, C., Hormaz, V., Boucher, J.M., 2014. Pigs and wild boar in Corsica harbor Echinococcus canadensis G6/7 at levels of concern for public health and local economy. Acta Trop. 133, 64–68. https://doi.org/10.1016/j.actatropica.2014.02.005.

Umhang, G., Woronoff-Rhen, N., Combes, B., Boue, F., 2011. Segmental sedimentation and counting technique (SSCT): an adaptable method for qualitative diagnosis of Echinococcus multilocularis in fox intestines. Exp. Parasitol. 128, 57–60. https://doi.org/10.1016/j.exppara.2011.01.004.

Valière, N., Fumagalli, L., Gielly, L., Miquel, C., Lequette, B., Poulle, M.-L., Weber, J.-M., Arlettaz, R., Taberlet, P., 2003. Long-distance wolf recolonization of France and Switzerland inferred from non-invasive genetic sampling over a period of 10 years. Animal Conservation forum 6, 83–92. https://doi.org/10.1017/S1367943003003111.