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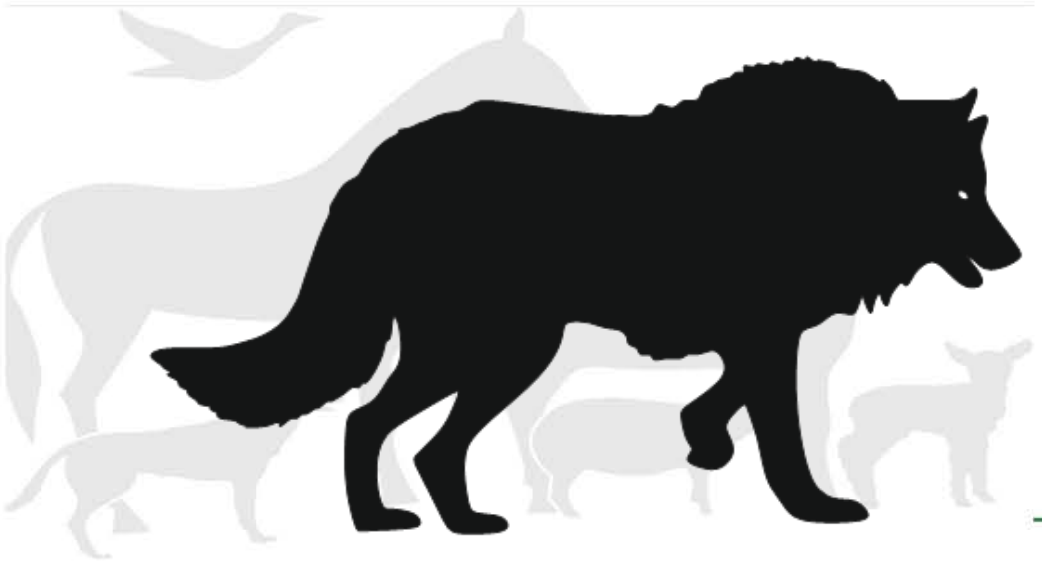
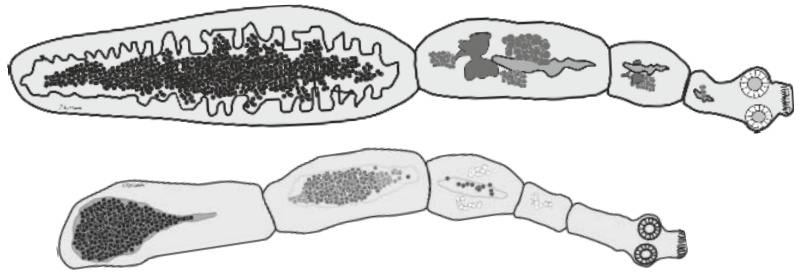
National Veterinary Research Institute

Grey wolves (*Canis lupus*)
as a host of *Echinococcus multilocularis, E.granulosus s.l.*
and other helminths
– new zoonotic threat in Poland

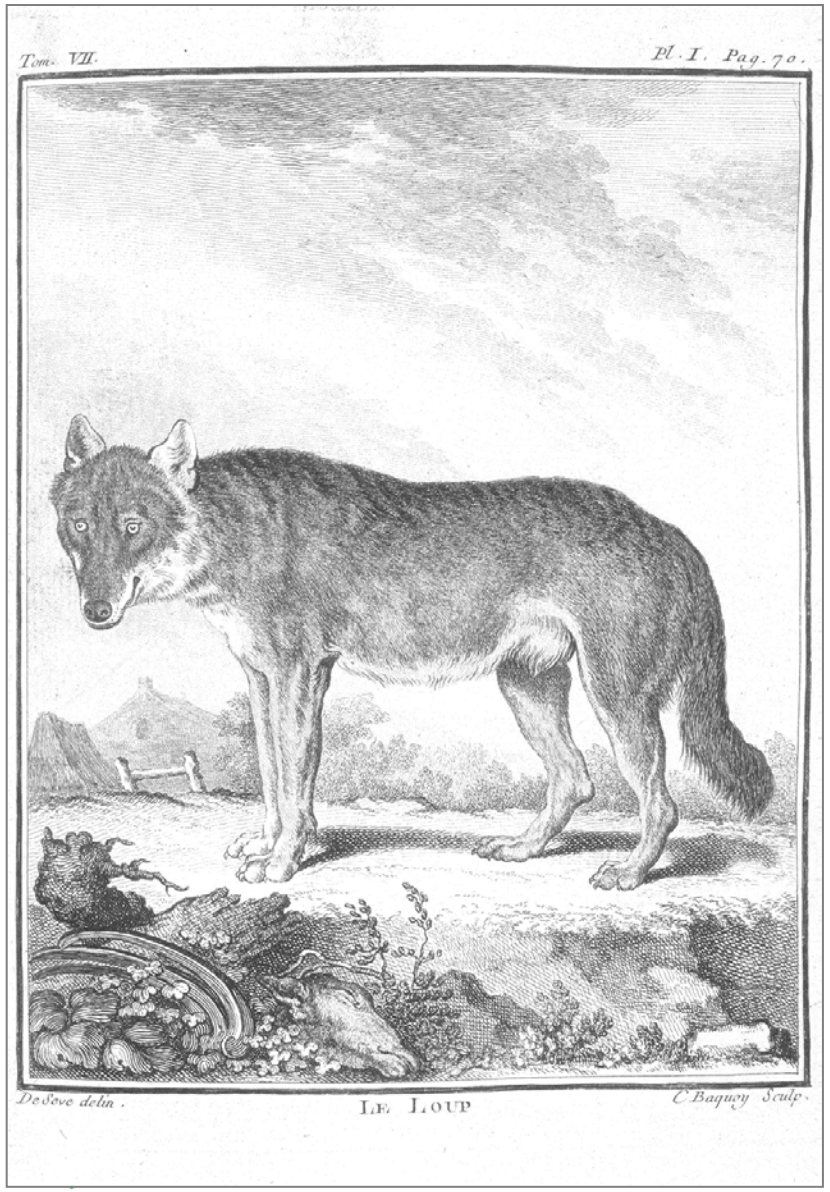


J.Karamon, M. Samorek-Pieróg, E. Bilska-Zając, J. Sroka, W. Korpysa-Dzirba, A. Bełcik, J. Zdybel, T. Cencek



19th Workshop of the National Reference Laboratories for Parasites, Rome, 6-7 November 2024

Grey wolf (*Canis lupus*)

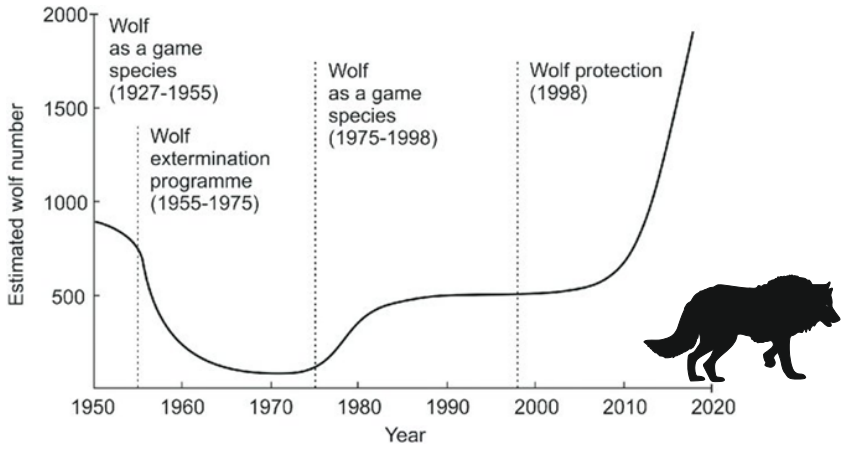


The grey wolf (*Canis lupus*) is a large canine native to Eurasia and North America.

Their main food source is medium and large ungulates (deer, roe deer, wild boars), additionally, smaller animals and dead animals. Rarely, they may also attack farm animals/domestic animals.

In Europe, its importance as a large predator has been increasing in last decades.

Wolves in Poland



Figures according to: ***Niedziałkowski et al. (2020)*** *What makes a major change of wildlife management policy possible? Institutional analysis of Polish wolf governance. PLoS ONE 15(4): e0231601. https://doi.org/10.1371/journal. pone.0231601*

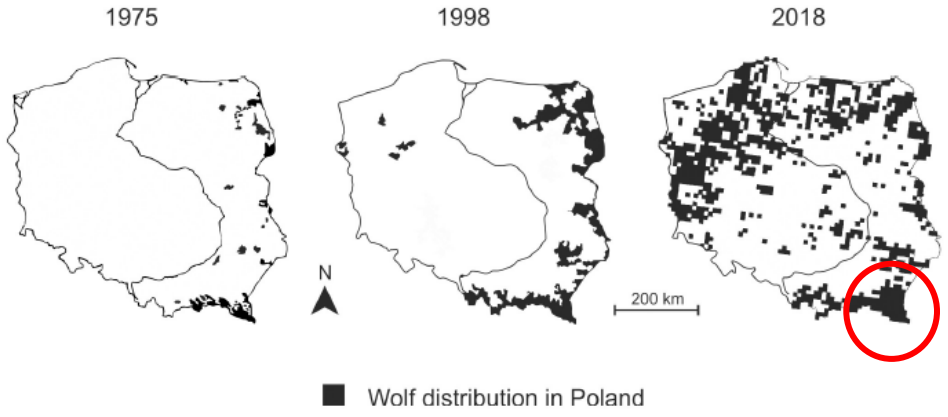


Fig 2. Wolf distribution in Poland in 1975, 1998, and 2018. Sources: Adapted from Wolsan, Bieniek [17]; Okarma, Jędrzejewski [19]; Śmietana

In Poland the grey wolf is one of the three large predators - along with the brown bear (Ursus arctos) and the lynx (Lynx lynx))

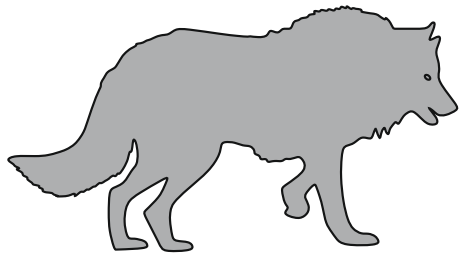
Before wolves were protected, their range was mainly limited to the regions of south-eastern Poland. After these animals were recognized as a protected species (1998), the population size is constantly increasing and their range is expanding

Since 2000, the population has increased app. by 300%. In 2022 the wolf population in Poland numbered 4,328 individuals of which 30% are located in the south-eastern part of the country.
It is one of the largest populations in Europe.

Wolf - host of many parasitic helminths species

Craig & Craig, Journal of Helminthology (2005) 79, 95–103

- **nematodes:** *Toxocara canis, Uncinaria/Ancylostoma, Trichuris vulpis, Toxascaris leonina, Spirocerca spp…*
- **flukes:** *Alaria spp. , Opistorchis spp., Clonorchis spp. …*
- **tapeworms** : *Taenia spp. , Mesocestoides spp., Dibothriocephalus, Echinococcus spp. …*



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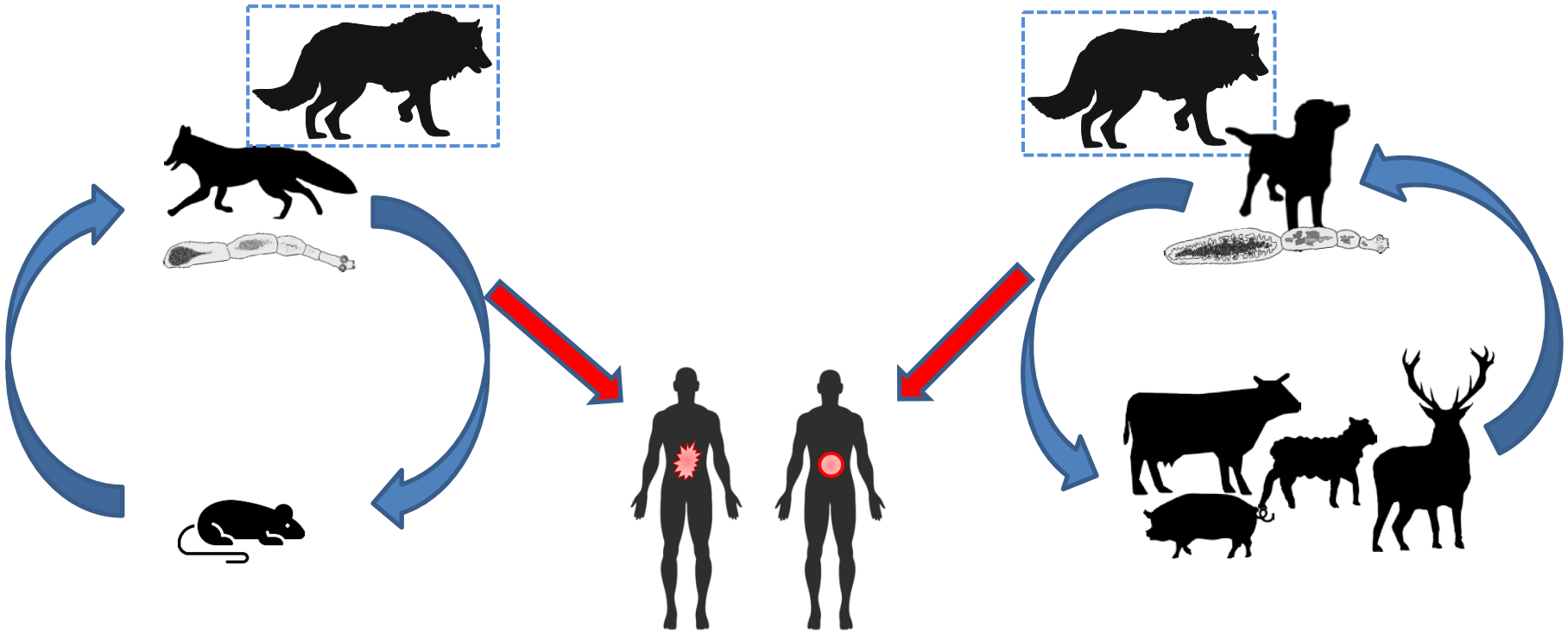
Echinococcus spp. life cycles



E. multilocularis

AE CE

E. granulosus s.l.

E. granulosus s.s (G1-3)
E. equinus (G4)
E. ortleppi (G5)
E. canadensis (G6-8,10)
E. felidis

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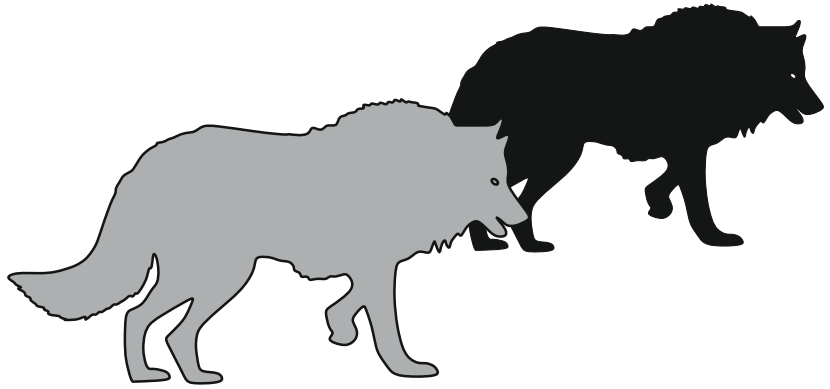
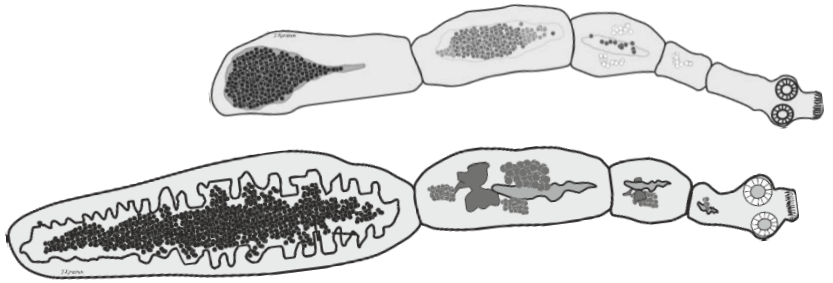
Echinococcus spp. in wolves

- ***E. multilocularis:*** France (0.3%) (Umhang et al. 2023), Latvia (5.9%) (Bagrade et al. 2009); Slovakia (35% (Jarosova et al. 2020); Iran (Beiromvand et al. 2011), Mongolia (28), Turkey (Akyuz et al. 2024), Canada (Shurer et al. 2016), Mongolia (3.4%) (Ito et al. 2006)
- ***E. granulosus* s.s. (G1)** Bulgaria (Breyer et al. 2004)
- ***E. canadensis* (G10)** Estonia 4% (Moks et al. 2006)
- ***E. canadensis* (G6/G7 , G10)** Mongolia 4.2% (Ito et al. 2013)
- ***E. canadensis* (G8/G10)** USA –Wyoming

***E. granulosus* s.l. (not identified to species/genotype)**

  - Italy (1987-1999) (Guberti et al. 2004)
  - Bieloarus (11%) (Shimalov 2000),
  - Finland (26%) (Hirvela-Koski eta al. 2003);
  - Lituania (70s XX.) .(Kauzlauskas et al. 1976)
  - USA (Idaho, Montana) 63% wilków (Foreyt et al. 2009)
  - Kazakhstan – 19.5% (Abdybekova et al. 2012)





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Echinococcus spp. in wolves in Poland

Tatra National Park - (PCR) – negative results for *Echinococcus* spp. (Gawor et al. 2020)

Microscopic examination for intestinal parasites – negative results for *Echinococcus* spp (e.g.: Kloch et al. 2005; Borecka et al. 2013; Szafrańska et al. 2010)

South-eastern Poland (Karamon et al. 2021) – wolf positive for ***Echinococcus ortleppi* (G5)**

pathogens MDPI

Article

The First Record of *Echinococcus ortleppi* (G5) Tapeworms in Grey Wolf (*Canis lupus*)

Jacek Karamon *, Małgorzata Samorek-Pieróg, Jacek Sroka, Ewa Bilska-Zając, Joanna Dąbrowska, Maciej Kochanowski, Mirosław Różycki, Jolanta Zdybel and Tomasz Cencek

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The aim of the study

The aim of the investigations was to estimate the occurrence of ***Echinococcus* spp.** and other helminths in wolves in south-eastern Poland

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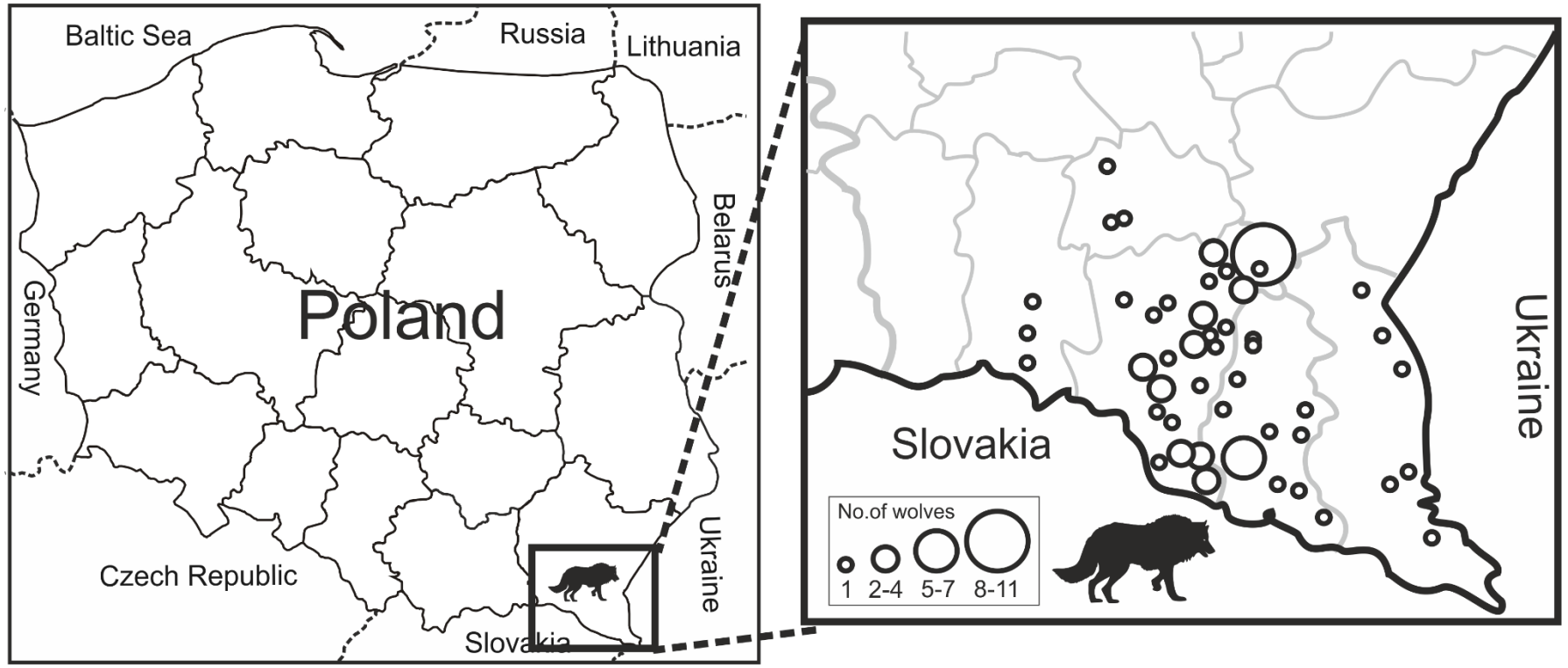
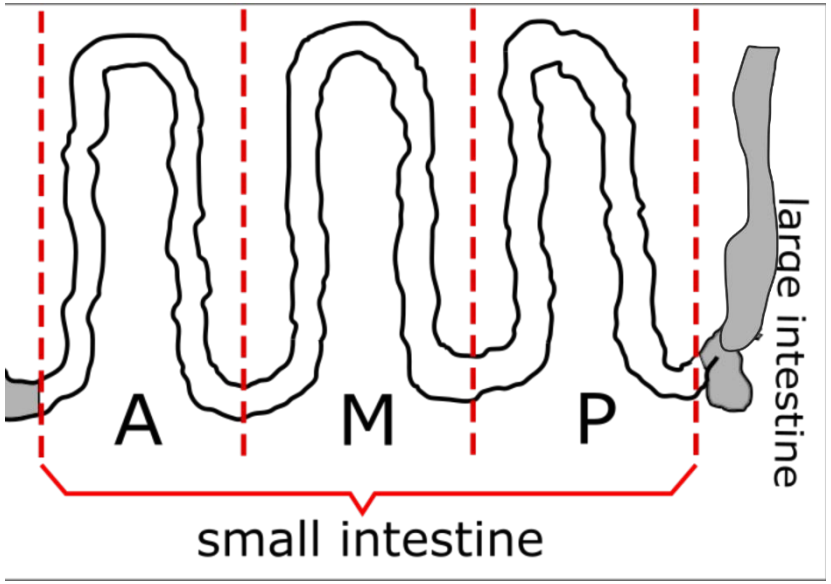
Material & Methods

- Samples from wolves were collected in south-eastern Poland (NUTS PL821)
- Overall, 74 samples of wolf feaces were collected (55 from environment and 19 from the rectum of necropsed wolves)
- The study on intestinal helminthofauna were conducted on intestines of 20 wolves (10 males, 9 female, 1 sex not-reported; age: 0.5-15 years)



Material & Methods (microscopy)

- Intestines of 20 wolves were examined with SCT (intestines were divided on 4 parts: A, M, P of small intestine and large intestine - each of 4 parts were examined separately)
- Echinococcus tapeworms isolated during the SCT were preserved in 70% ethanol for further molecular identification
- Additionally, faecal samples (environmental and from rectum) were examined by flotation



Material & Methods (molecular part)

- DNA extracted from faeces (n=74)(QIAamp Fast stool Mini kit (Qiagen) were examined with:
  - multiplex PCR for the detection of *E. multilocularis*, *E. granulosus s.l.*, and *Taenia* spp.) (Trachesel et al. 2007);
  - qPCR for detection of *E. multilocularis* (Knapp et al. 2016);
  - Set of qPCRs for detection *E. granulosus* s.l.: *E. granulosus s.s.* (G1-3), *E. equinus* (G4), *E. ortleppi* (G5), E. *canadensis* (G6-10) (Maksimov et al. 2020)
- Additionally we extracted DNA from Echinococcus worms isolated form intestines (3 worms /positive wolf) and made PCRs for mitochondrial genes. Following fragments were amplified: *nad1* (wg. Bowles and McManus 1993) i *cox1* (wg. Casulli et al. 2008) for *E. granulosus* s.l. worms; *cox1* and *nad2* gene (Nakao et al (1998) for *E. multilocularis* worms.
- The selected PCR products were sequenced by standard Sanger sequencing.

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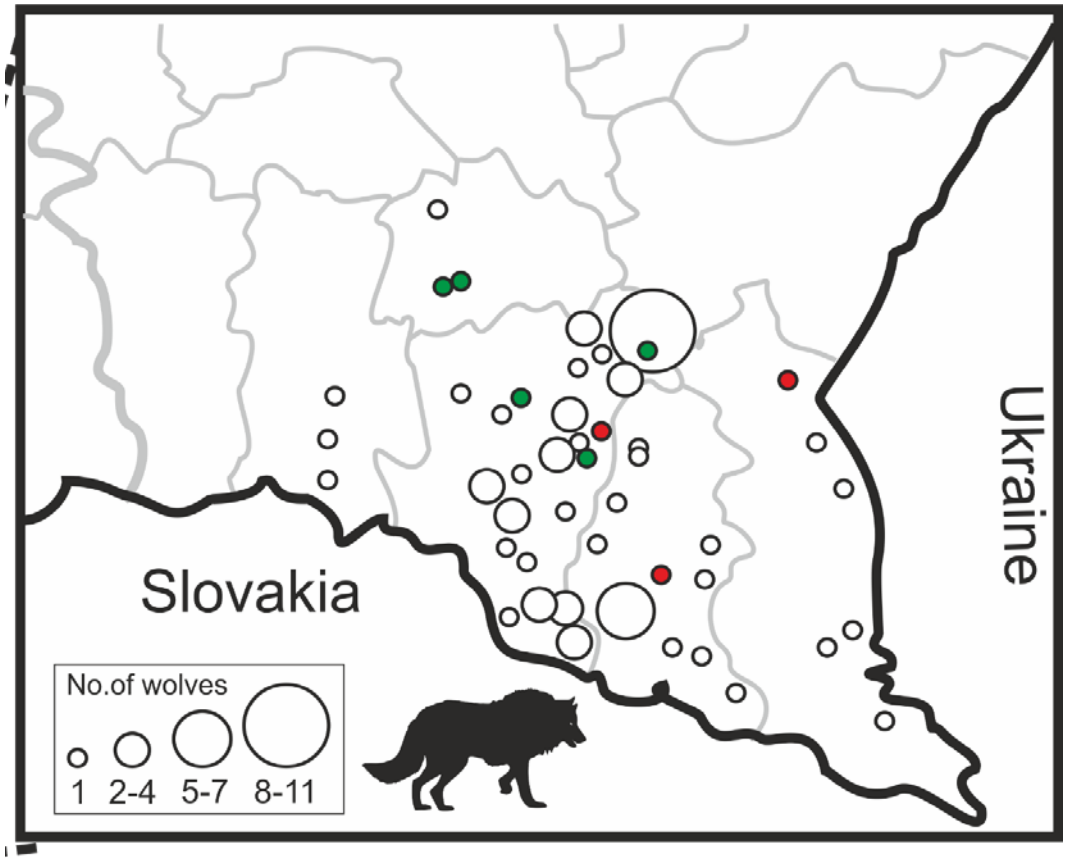
Results (molecular screeining)

- ***E. multilocularis:*** **5 positive (6.8%)**
- ***E. granulosus s.l.*** **: 3 positive (4.1%)**
  - **All identified finally as *E. ortleppi* (G5)**
- *Taenia spp.*: 32 positive (43.2%) [*T. serialis* (33.8%), *T. hydatigena* (8.1%), *Hydatigera taeniaeformis* (1.4%)]
- *Mesocestoides litteratus*: 3 positive (4.1%)

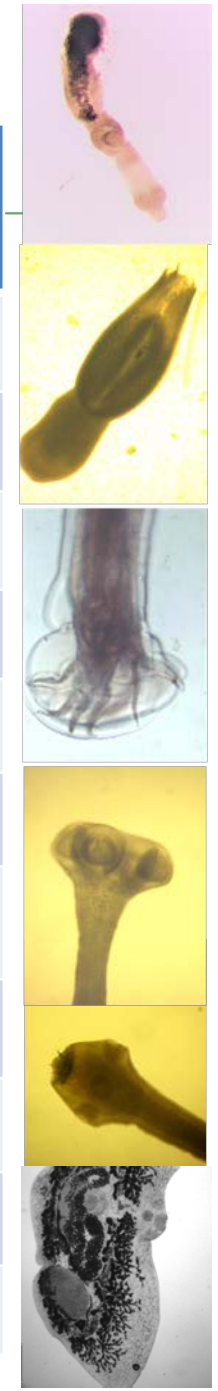
Co-infections:

- *E. ortleppi + T. serialis (n=2)*
- *E. multilocularis + T. serialis (n=2)*
- *E. multilocularis + Mesocestoides (n=1)*



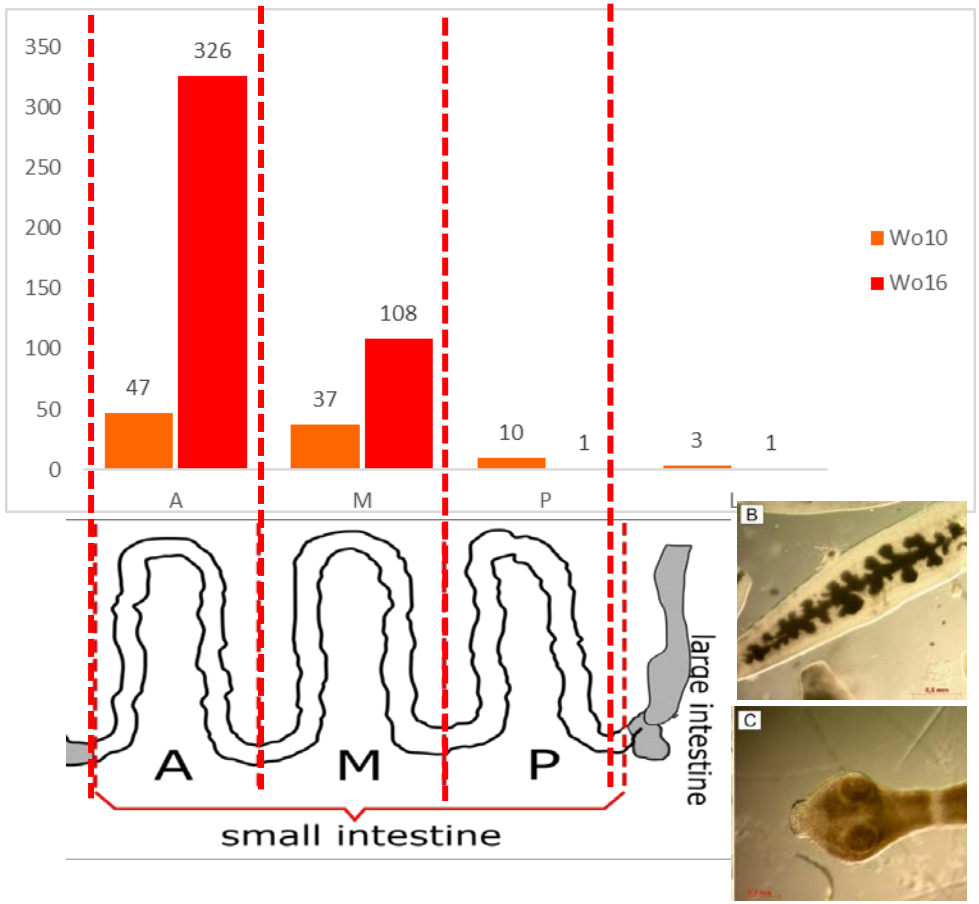
Results (SCT)

	Entire intestines (small + large)		Small intestine						Large intestine	
			Anterior part (A)		Middle part (M)		Posterior part (P)			
	% pos. (95%CI)	Int. (range)	% pos. (95%CI)	Int. (range)	% pos. (95%CI)	Int. (range)	% pos. (95%CI)	Int. (range)	% pos. (95%CI)	Int. (range)
Echinococcus multilocularis	**10** (3-30)	27837 (6-55660)	**10 (3-30)**	1832 (3-3660)	**10 (3-30)**	26002 (3-52000)	**0**	-	**5 (1-24)**	8
E. granulosus s.l.[a]	**10** (3-30)	250 (64-436)	**10 (3-30)**	184 (42-326)	**10 (3-30)**	61 (14-108)	**10 (3-30)**	3 (1-5)	**10 (3-30)**	2 (1-3)
Taenia spp.	**100**	33 (1-208)	**90 (70-97)**	5 (1-30)	**90 (70-97)**	24 (1-150)	**80 (58-92)**	8 (1-30)	**35**	1.3 (1-3)
Mesocestoides spp.	**10** (3-30)	15 (1-28)	**5 (1-24)**	1	**5 (1-24)**	28	**0**	-	**0**	-
Euryhelmis sp.	**5** (1-24)	477	**0**	-	**0**	-	**5 (1-24)**	477	**0**	-
Alaria alata	**20** (8-40)	35 (1-135) [190%]	**20 (8-40)**	35 (1-135) [190%]	**0**	-	**0**	-	**0**	-
Uncinaria / Ancylostoma	**30** (15-50)	45 (1-219)	**10 (3-30)**	1	**30 (15-50)**	32 (1-148)	**15 (5-36)**	26 (1-70)	**5 (1-24)**	2
Molineus spp.	**5** (1-24)	47	**5 (1-24)**	1	**5 (1-24)**	1	**5 (1-24)**	45	**0**	-
Trichuris vulpis	**15** (5-36)	1	**0**	-	**0**	-	**0**	-	**15 (5-36)**	1
Total (all parasites)	**100**	-	**90 (70-97)**	-	**90 (70-97)**	-	**85 (64-95)**	-	**55 (34-74)**	-

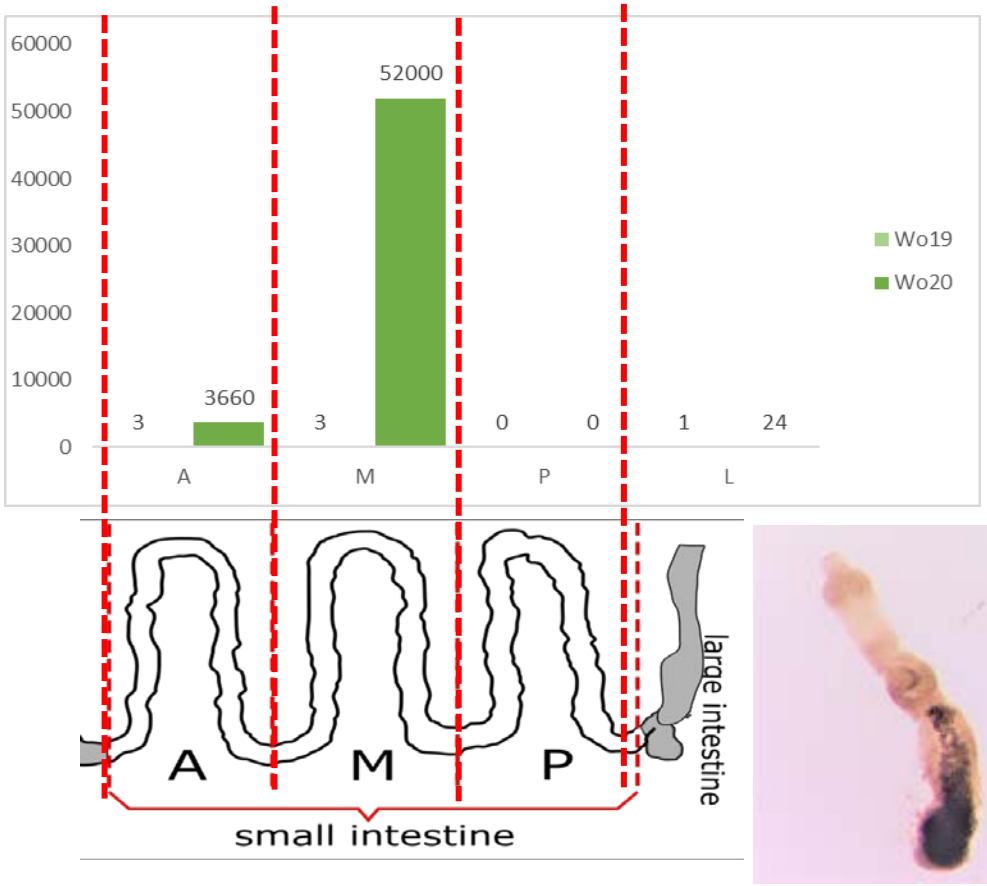


Results (SCT) – distribution in the intestinal parts

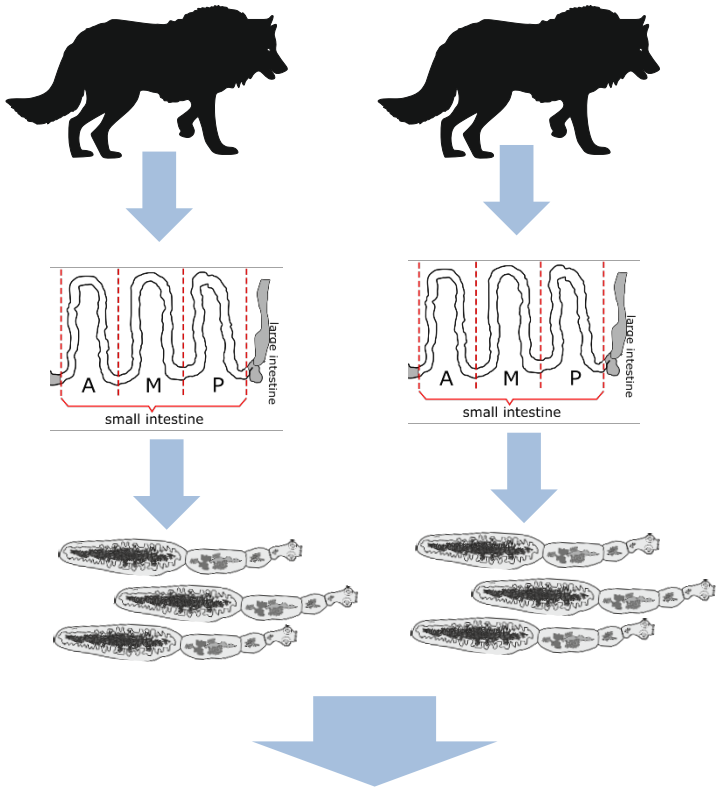
E. granulusus s.l. (E. ortleppi) (intsensity)



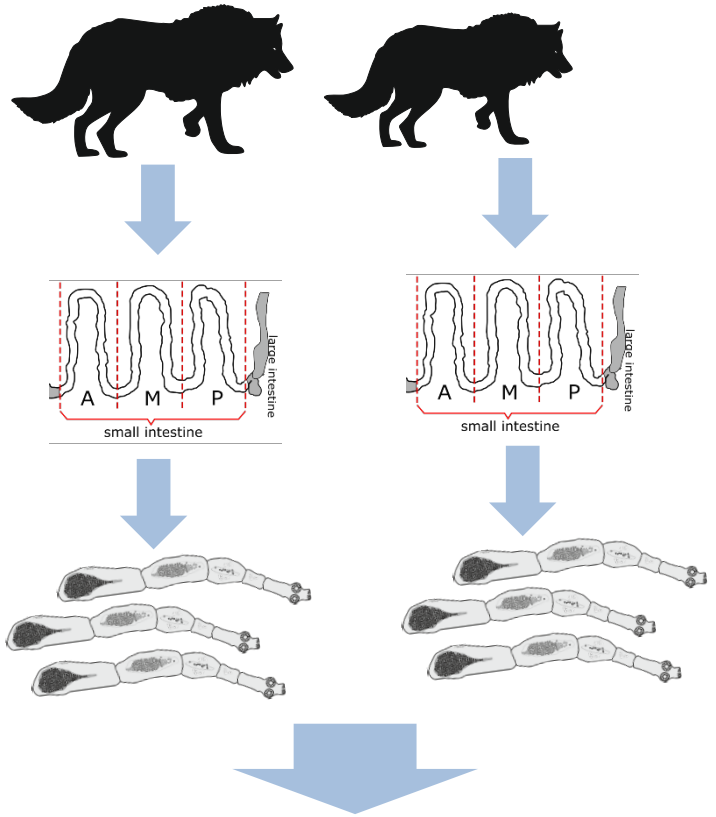
E. multilocularis (intensity)



Molecular analysis (*Echinococcus* spp. worms)



DNA
PCR cox1, nad1
sequencing



DNA
PCR cox1, nad2
sequencing

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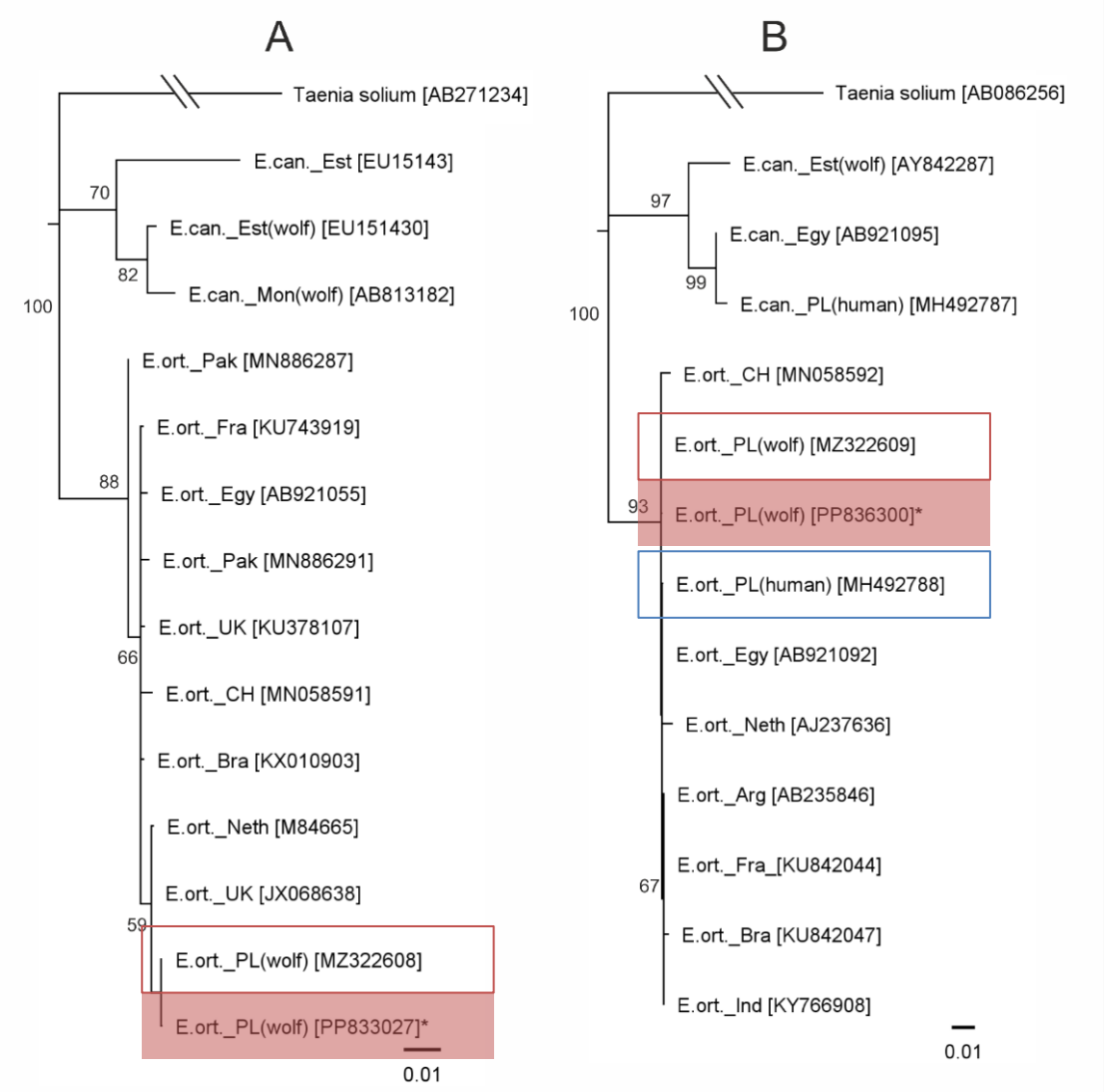
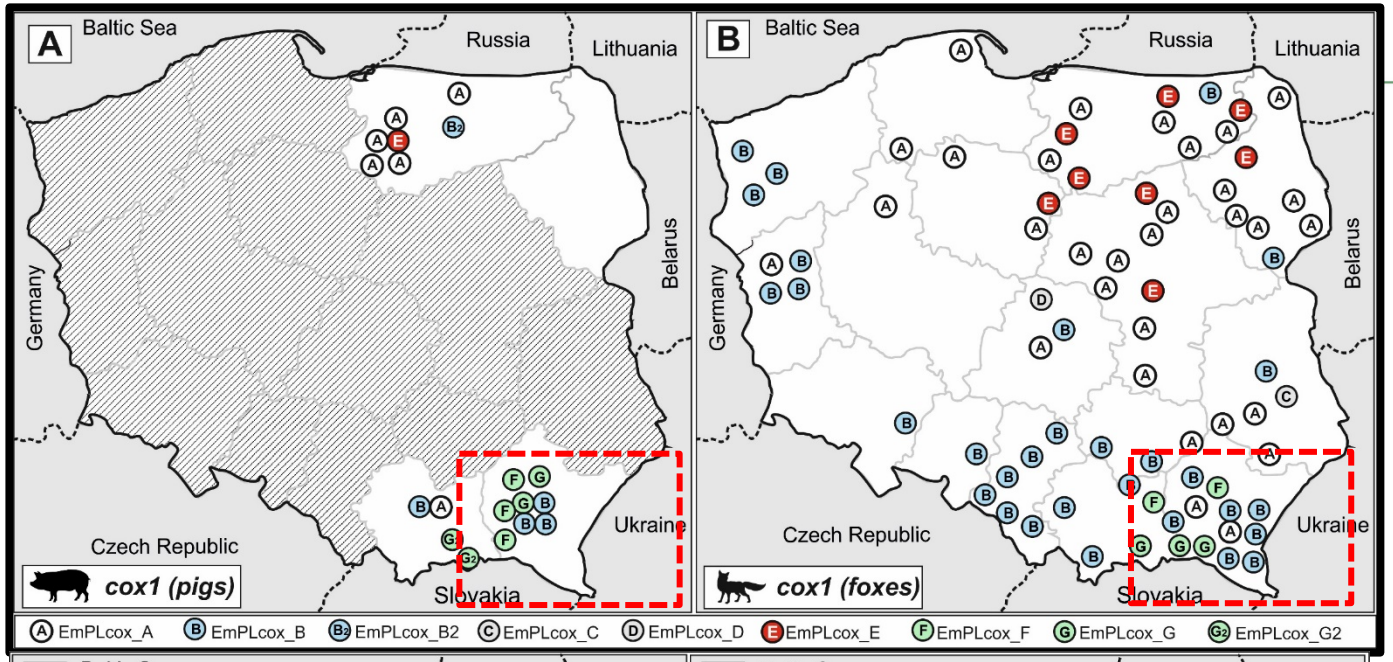
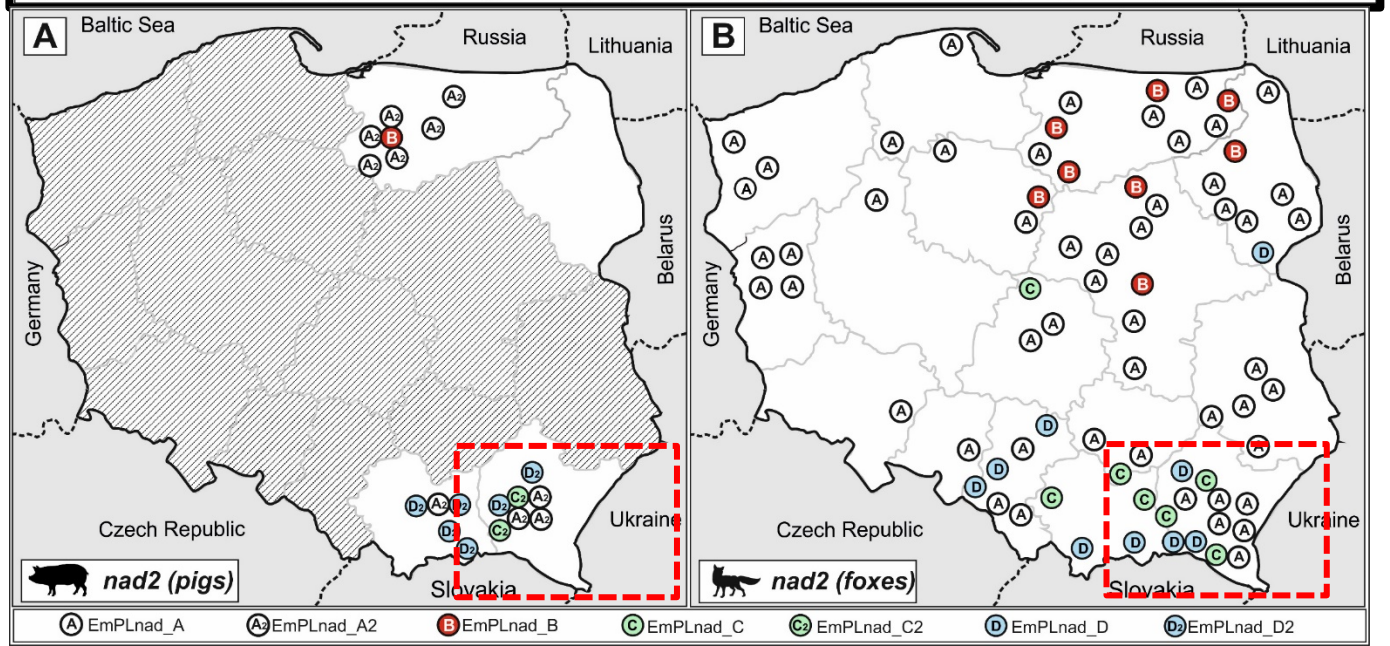
E. ortleppi haplotypes

- Sequences (cox1, nad1) obtained from E.g. sl worms (PP836300, PP833027) were in 100% silimalar to E. orletppi sequences isolated from first positve wolf described earlier in Poland by us (MZ322608, MZ322609)
- 100% identity (nad1) was observed also in relations to larvae from Polish human case of *E. ortleppi*

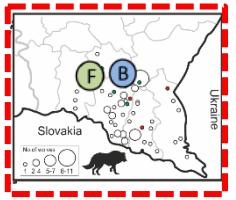


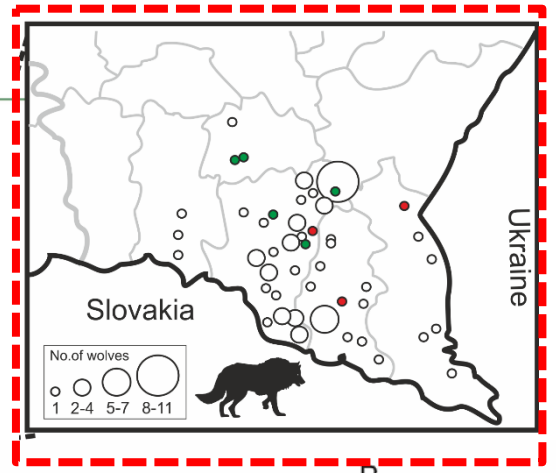
E. multilocularis haplotypes in Poland



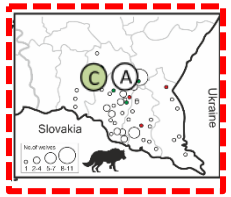


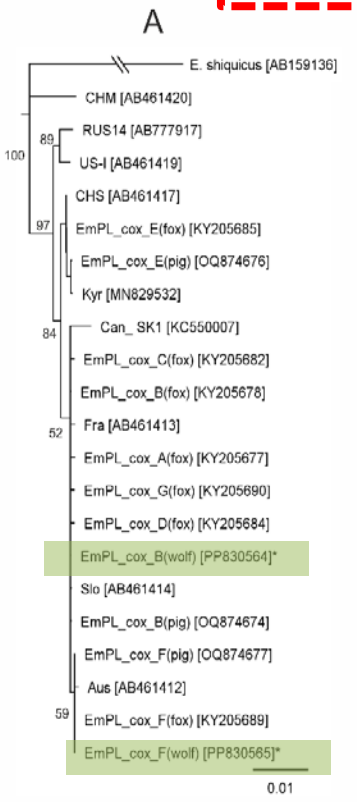
E. multilocualris
- EmPL_cox_B (PP830564)
- EmPL_cox_F (PP830565)

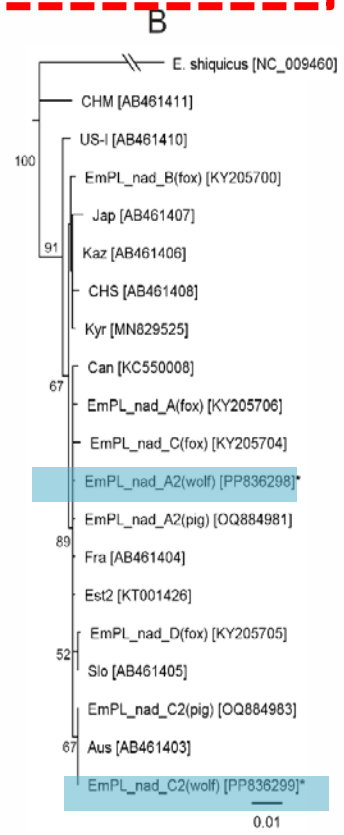




E. multilocualris
- EmPL_nad_C (PP836298)
- EmPL_nad_A (PP836298)

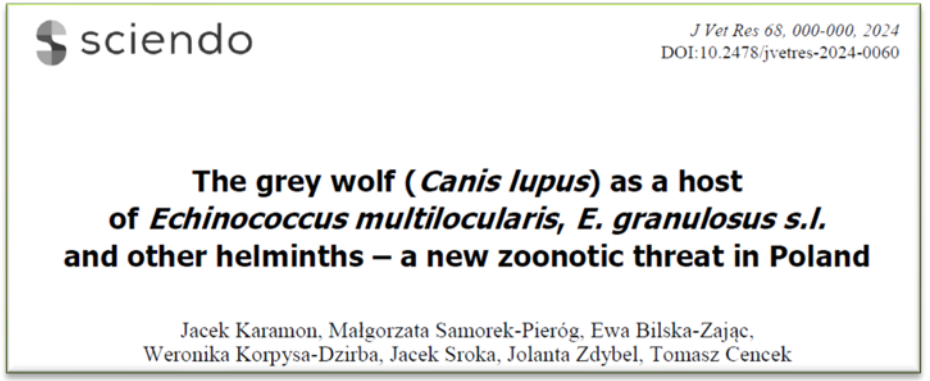






Conclusions

- Our study showed the presence of *E. multilocularis* in wolves for the first time in Poland
- And confirmed our earlier observations regarding the occurrence of *E. ortleppi*.
- This is important information from an epidemiological point of view – this new zoonotic threat from two *Echinococcus* species in the south-eastern wolf population (the largest in Poland) should be taken into account assessing the risk of infection to humans.
- In addition, the research enriched the knowledge about other helminths found in wolves, including zoonotic ones, but also those that were recorded for the first time in these animals.



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Thank you for attention

	% of positive samples (95% CI)	Mean EPG/OPG (range) [CV]
Taeniidae	27.0 (17.6–39.0)	399 (15-3000) [186%]
Tramatoda	3.2 (0.9–10.9)	26 (15-50) [76%]
Capillariidae	58.7 (46.4–70.0)	316 (7-2250) [173%]
Uncinaria/Ancylostoma	1.6 (0.2–8.5)	15
Trichuris vulpis	1.6 (0.2–8.5)	15
Toxocara sp.	1.6 (0.2–8.5))	15
Coccidia	1.6 (0.2–8.5)	300

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