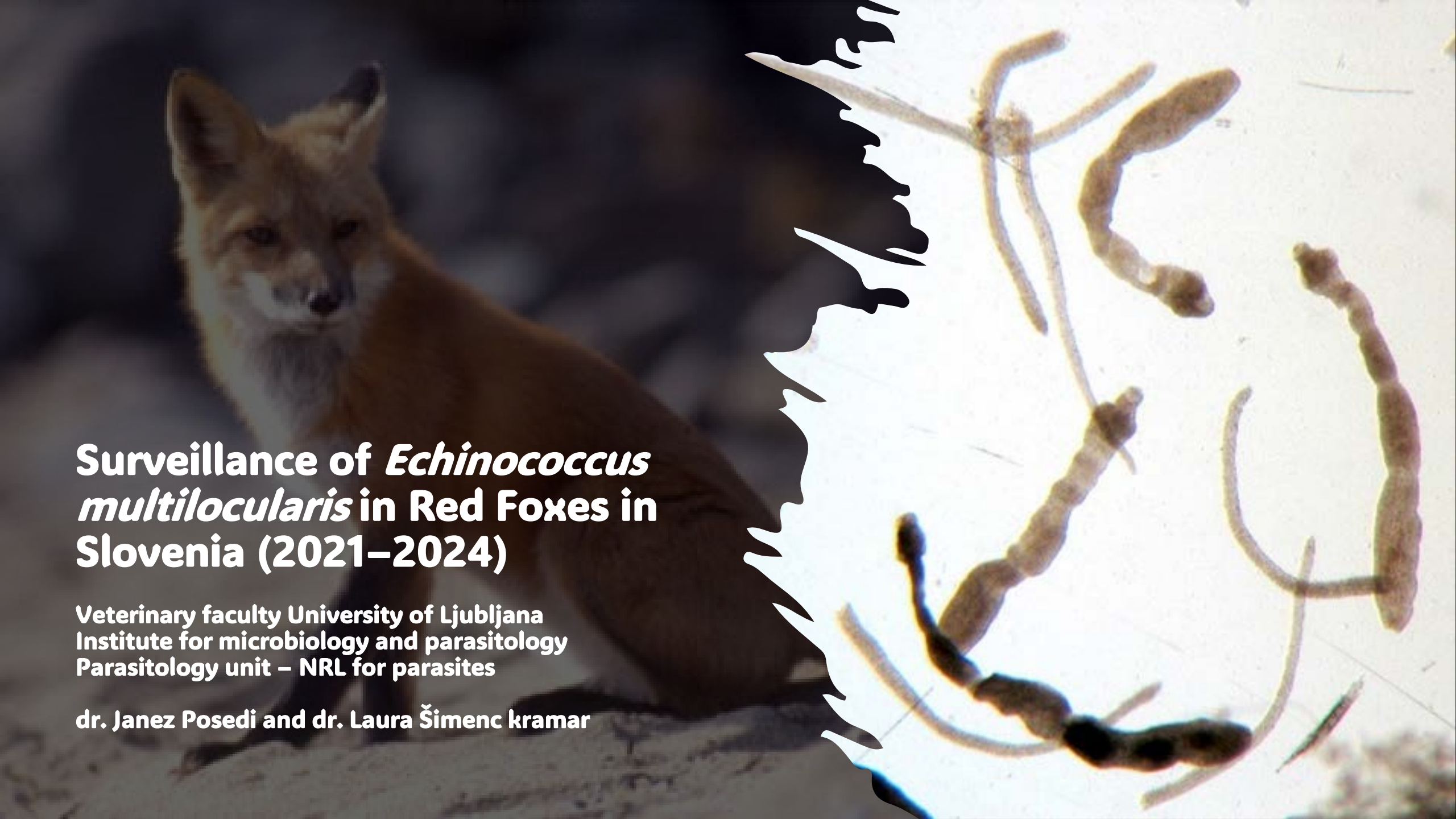




Surveillance of *Echinococcus multilocularis* in Red Foxes in Slovenia (2021–2024)

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Institute for microbiology and parasitology
Parasitology unit – NRL for parasites

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Slovenia



Echinococcus multilocularis is a small parasitic tapeworm belonging to the family *Taeniidae* and is the etiological agent of alveolar echinococcosis (AE), one of the most severe zoonotic helminthic infections worldwide.

Echinococcus multilocularis is morphologically defined as a small (approximately 1.2–4.5 mm in length), segmented tapeworm with a doubly armed scolex, radially striated eggs, and a larval stage forming a proliferative, alveolar hydatid structure characteristic of alveolar echinococcosis.



Echinococcus multilocularis – anterior end
(Vergles Rataj and Posedi, 2007)



Echinococcus multilocularis adult cestoda between
intestinal villi (Vergles Rataj and Posedi, 2007)

With a known distribution across the northern hemisphere throughout North America, Asia and Europe.

Its complex life cycle involves wild canids (mainly **foxes**) as definitive hosts, which harbour sexually reproducing adult worms, and small mammals (mainly **voles** as *Microtus spp.* and *Arvicola spp.*) as intermediate hosts in which the larval stage (metacestode) develops.



Humans act as accidental intermediate hosts after ingesting parasite eggs through contaminated food, water, or soil, leading to hepatic lesions that mimic malignant tumors.

This parasite is known to cause at least 18,000 new human cases of alveolar echinococcosis (AE) per year, and is considered one of the three most impactful food- and water-borne parasitic zoonoses worldwide.

Recent studies indicate expanding geographic ranges, driven by anthropogenic factors such as urbanization of red fox populations and environmental changes that increase human exposure to parasite eggs.

First finding of a metacestode of *E. multilocularis* in forest rodent (*Apodemus flavicollis*) in Slovenia (Brglez and Kryštufek, 1984).



Slika 1: Abdominalna votlina *Apodemus flavicollis* s konglomeratom mešičkov *Echinococcus multilocularis*

Fig.1: Abdominal cavity of *Apodemus flavicollis* with conglomerate of vesicles *Echinococcus multilocularis*



Zb. Biotehn. fak. Univ. E. Kardelja, Vet. 21 (1984) 1, 173—176
UDK 576.895.121.56:599.32=863

Cestoda / glodavci
Cestoda / rodentis

**METACESTOD *ECHINOCOCCUS MULTILOCULARIS* (LEUCKART, 1863)
VOGEL, 1955, PRI *APODEMUS FLAVICOLLIS* (MELCHIOR)
V SLOVENIJI**

J. BRGLEZ, B. KRYŠTUFEK

Pri glodavcu, *Apodemus flavicollis* (Melchior), *Muridae*, je bila v trebušni votlini in na jetrih ugotovljena larvalna oblika trakulje *Echinococcus multilocularis* (Leuckart, 1863) Vogel, 1955. To je prva ugotovitev omenjenega metacestoda pri *Apodemus flavicollis*. Razčlenjeni so nekateri epizootiološki dejavniki multilokularne ehinokokoze.

**METACESTODE *ECHINOCOCCUS MULTILOCULARIS* (LEUCKART, 1863)
VOGEL, 1955, IN *APODEMUS FLAVICOLLIS* (MELCHIOR)
IN SLOVENIA**

SLOVENSKA AKADEMIJA ZNANOSTI IN UMETNOSTI
SLOVENIAN ACADEMY OF SCIENCES AND ARTS
MEDAKADEMIJSKI ODBOR ZA PROUČEVANJE ZOONoz
INTER-ACADEMY COMMITTEE FOR THE STUDY OF ZOONOSES

JANEZ BRGLEZ
TEODOR WIKERHAUSER

EHINOKOKOZA



LJUBLJANA 1992

EHINOKOKOZA S POUDARKOM NA RAZISKAVAH V SLOVENIJI IN NA HRVAŠKEM

*IF THERE BE NOTHING NEW, BUT THAT WHICH IS,
HATH BEEN BEFORE...
THOU SHOULDST PRINT MORE, NOT LET THAT DIE.*

SHAKESPEARE, THE SONNETS

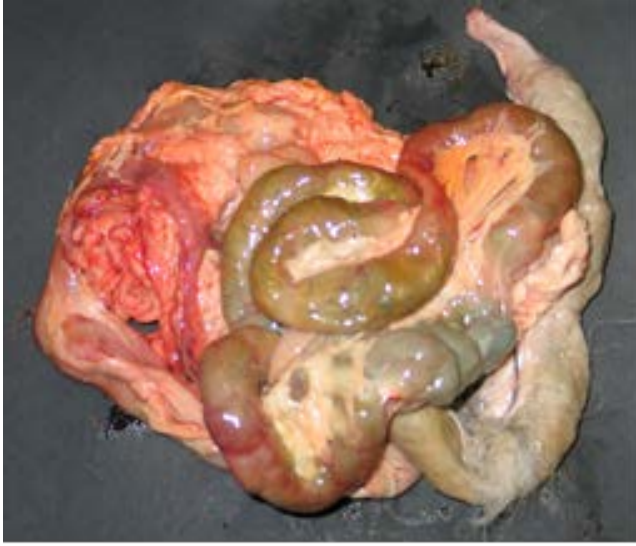
PREDGOVOR

Ehinokokoza je po svetu razširjena helmintska zoonoza, ki še vedno - ponekod bolj, drugod manj - ogroža zdravje ljudi in povzroča precejšnjo gospodarsko škodo rejcem in mesni industriji. Raziskovalci in strokovnjaki za različna področja so si doslej po svetu veliko prizadevali, da bi omenjeno bolezen izkoreninili ali v začetku vsaj zmanjšali njeno razširjenost na sprejemljivo raven.

Že dolgo vemo, da je ehinokokoza razširjena tudi pri nas. Manj pa je znano, kaj vse so naši strokovnjaki preučevali v zvezi s to boleznijo in do kakšnih rezultatov so prišli pri raziskavah. Ker pa so v Sloveniji in Hrvaški v zadnjih dvajsetih letih opravili številne med seboj povezane raziskave, nekateri rezultati le-teh pa presegajo okvire omenjenih dveh republik, smo se odločili, da v pričujoči publikaciji bolj celostno predstavimo strnjene in povezane rezultate in da zapolnimo vrzel na tem področju. Pri obravnavi pa povzemamo še nekatere pomembnejše dosežke iz drugih delov nekdanje Jugoslavije. Pričujoče delo sodi v raziskovalni program SAZU Parazitske zoonoze.

Zaradi boljšega razumevanja problematike opisujemo v uvodnem delu tiste probleme in raziskave, ki so povezani z ehinokokoza drugod po svetu. Številni bibliografski podatki ob koncu publikacije pa omogočajo, da lahko bralec poišče dodatne podatke v izvirnih objavah. Zavedamo se, da zaradi izbirnega pristopa nismo mogli zajeti vsega, kar je bilo doslej objavljeno pri nas in tudi po svetu. Zato prosimo bralce za razumevanje in se jim vnaprej zahvaljujemo.

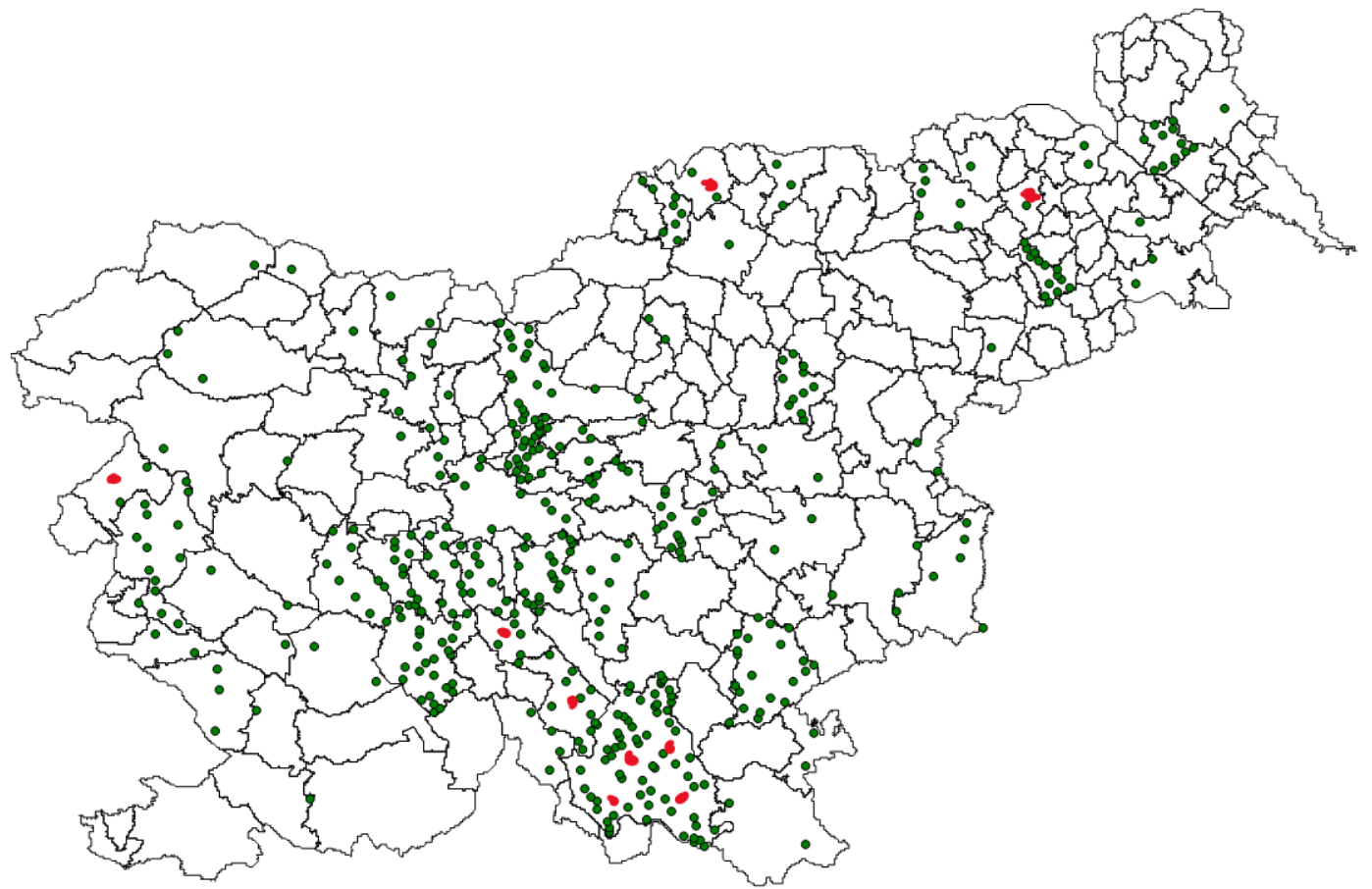
Avtorja



Fox intestine (Posedi, 2005)



Fox carcass, abdominal cavity (Posedi, 2005)



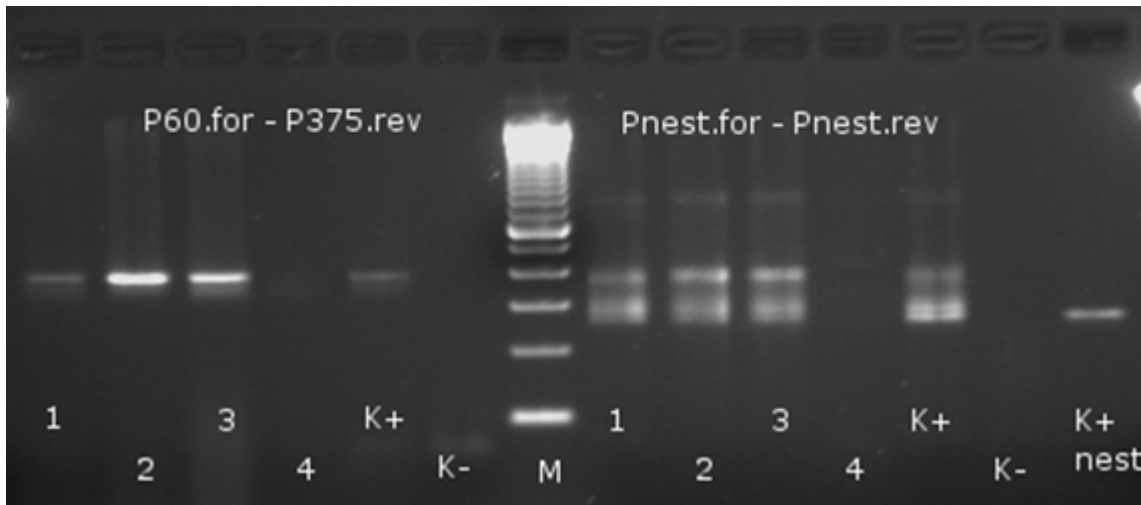
Between 2002 and 2005, 428 small intestines of red foxes (*Vulpes vulpes*) were examined for adult *Echinococcus multilocularis* worms using the parasitological washing out method.

2.6% (11 foxes) prevalence was confirmed (Vergles Rataj et al., 2010).

This is the first extended research reporting on the presence of *E. multilocularis* in the Slovenian fox population.

NESTED PCR

73 samples from previous research were examined also by **nested PCR** method. Some of them were stool samples and other adult *E.multilocularis* samples. **9** of them were positive and were also **sequenced by Sanger method**. All sequences were the same.



PCR I – PCR II. M marker, 1 – feces fox No. 269, 2 - feces fox 330, 3 - feces fox No. 397, 4 - feces fox No. 386, K+ - DNA from cestoda *E. multilocularis*

In 2022 molecular methods were used to assess the prevalence of the tapeworms *Echinococcus multilocularis*, *E. canadensis*, and *E. granulosus sensu stricto* in the stool of red foxes (210), wolves (42), golden jackals (39), martens (18), lynxes (2), badgers (2), and otter (1). **Red foxes (29.1%) and golden jackals (18%) were positive for *E. multilocularis* (EM)**, while all other animals were negative for all *Echinococcus* species tested in this study. Statistical analysis showed that the prevalence of EM **is associated only with the region** where the sample originated. **Central and south regions of Slovenia have a higher EM prevalence and risk of infection.** Due to habitat expansion and an increasing population, golden jackal may soon become an important source for human infection with EM (Bandelj et al., 2022).

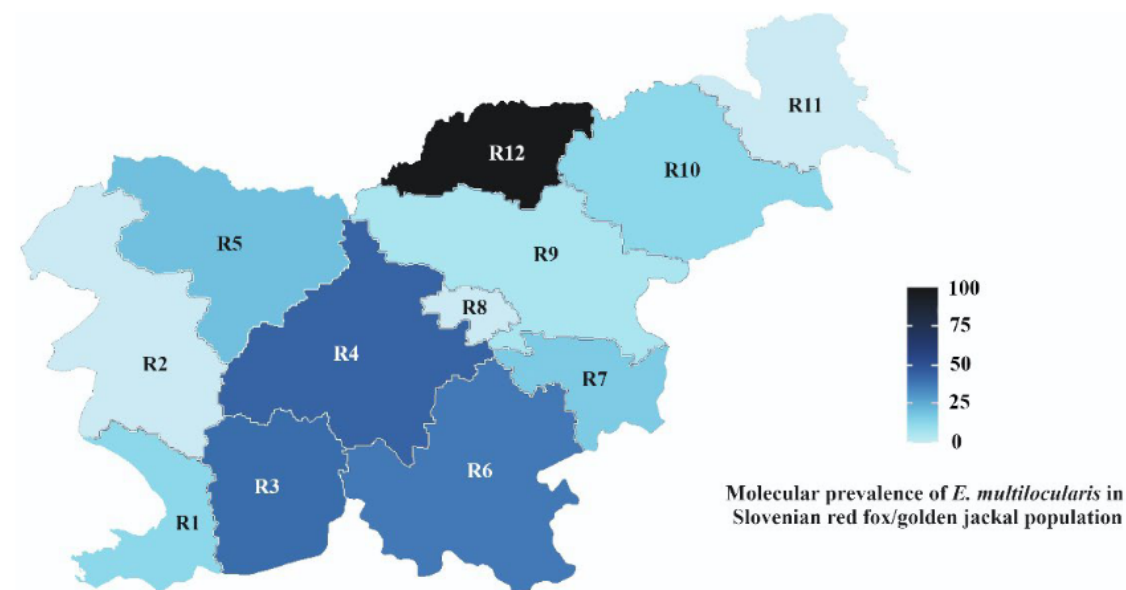


Figure 1. Molecular prevalence of *E. multilocularis* in the Slovenian red fox/golden jackal population (%). Regions (R): R1—obalno kraska, R2—goriska, R3—primorsko notranjska, R4—osrednjeslovenska, R5—gorenjska, R6—jugovzhodna Slovenija, R7—posavska, R8—zasavska, R9—savinjska, R10—podravska, R11—pomurska, R12—koroška.

Alveolar echinococcosis in nutria (*Myocastor coypus*) (Križman et al., 2022)

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Alveolar echinococcosis in nutria (*Myocastor coypus*), invasive species in Slovenia

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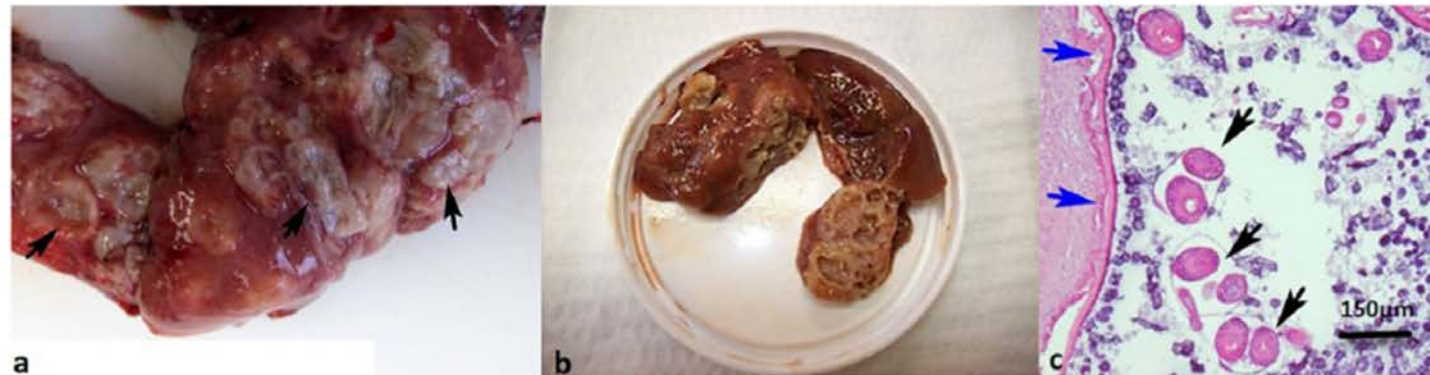


Fig. 1. Pathomorphological finding in the liver of a nutria (*Myocastor coypus*) with echinococcosis. Numerous cysts of varying sizes on the surface of the liver (arrows). b Numerous fluid-filled cysts in the cut section of the liver. c Haematoxylin and eosin (HE) stain of single cyst with several protoscolices (black arrows). The cyst is lined by an eosinophilic, hyaline outer membrane (blue arrows) and an inner germinal epithelial layer. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

METHODS

Sample collection

Fresh fox carcasses from hunting families from all over Slovenian territory/ Hunting grounds with a special purpose (from 2022-) were collected at National veterinary Institute unites in different regions of Slovenia.

Veterinary pathologists there prepared the samples of last part of the intestines (anal part) in special sealed plastic bags and brought them to our laboratory in cooling boxes.

Sample preparation

The samples were frozen under -70 °C for at least 10 days before the preparation. Later the samples were thawed and 2g of stool including intestinal mucosa from each sample were transferred into 2ml safe lock tubes.

1ml of InhibitEX Buffer (Qiagen) was added to each stool sample and thoroughly homogenized. The suspensions were heated to 70-95 °C for 10 minutes and then they were put on dry ice until frozen (few minutes). This step was repeated for 3 times to be sure to crack the potential parasitic eggs.

IMPORTANT STEP !!

METHODS

DNA extraction

DNA extraction was performed by **QIAamp Fast DNA Stool Mini Kit** following manufacturers instructions. About 200 µl of DNA was eluted in ATE Buffer and stored at -20 °C.

Real-Time PCR

The Real Time PCR was performed from each sample using commercial **ParoReal Kit *Echinococcus multilocularis* (Ingenetix)** adding internal positive control, DNA from a positive a positive stool and a negative control (purified water).

Sensitivity:

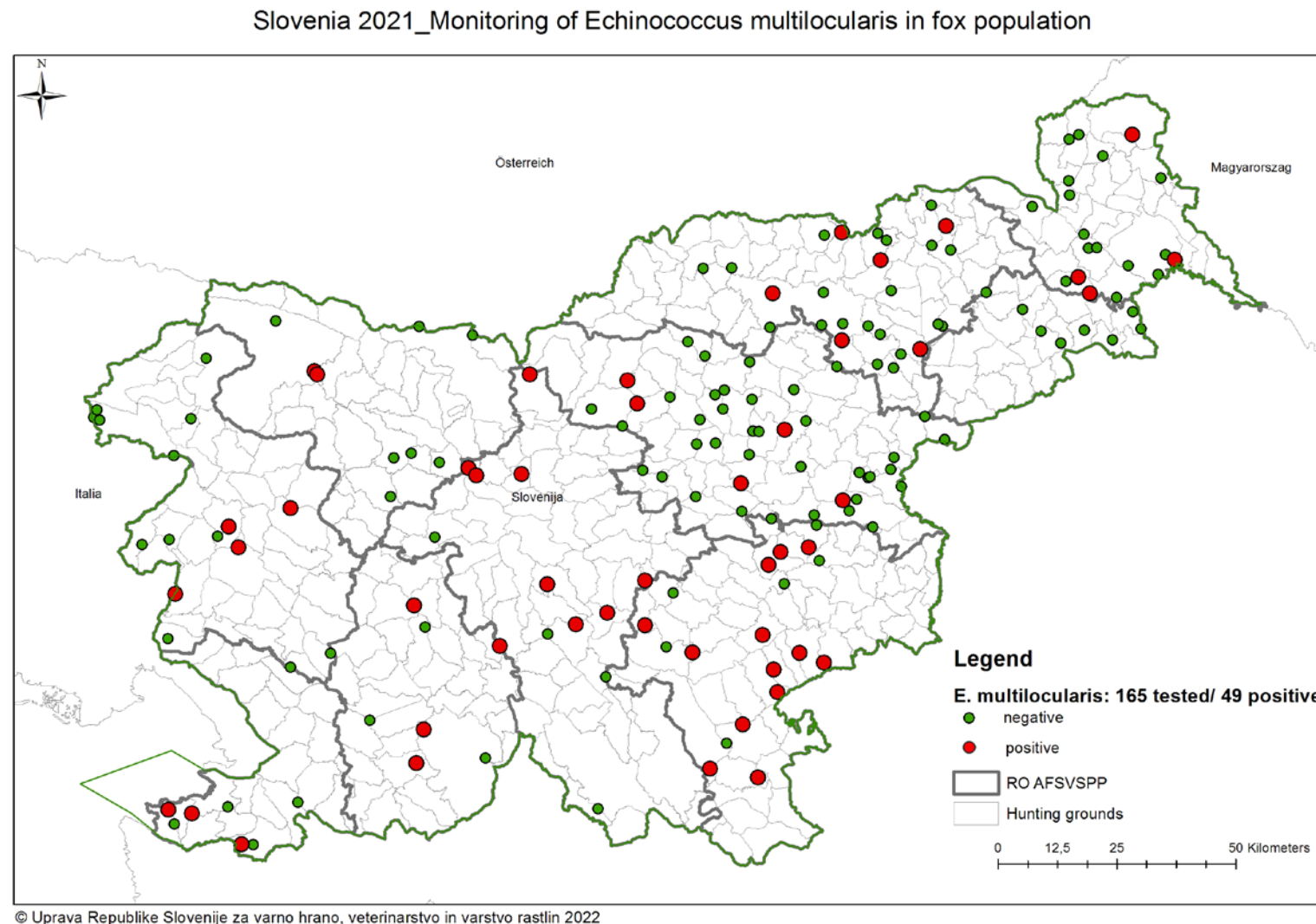
Limit of detection: LOD95%=5 copies/reaction (smallest number of copies of target DNA which can be detected in 95% of cases).ParoReal® Kit Echinococcus multilocularis has a slope of -3.411 ± 0.02662 and a R2 of 0.9971.

Specificity:

To investigate potential cross-reactivity, primers and probes were analyzed for potential homologies to currently published sequences. Specificity of primers and probes were validated in silico by carrying out the basic local alignment tool (BLAST) against the NCBI database. This also validated the detection of so far known *Echinococcus multilocularis* strains. No relevant sequence variabilities were observed in the primer and probe region. No cross-reactions have been observed. The kit is specific for *Echinococcus multilocularis*.

The PCR was performed on QuantStudio5 machine (Thermo Fisher Scientific).

RESULTS



2021

165 samples of red fox stool sampled throughout Slovenia were examined by real-time PCR method for presence of *E. multilocularis* DNA. 49 samples were confirmed positive (29.51%).

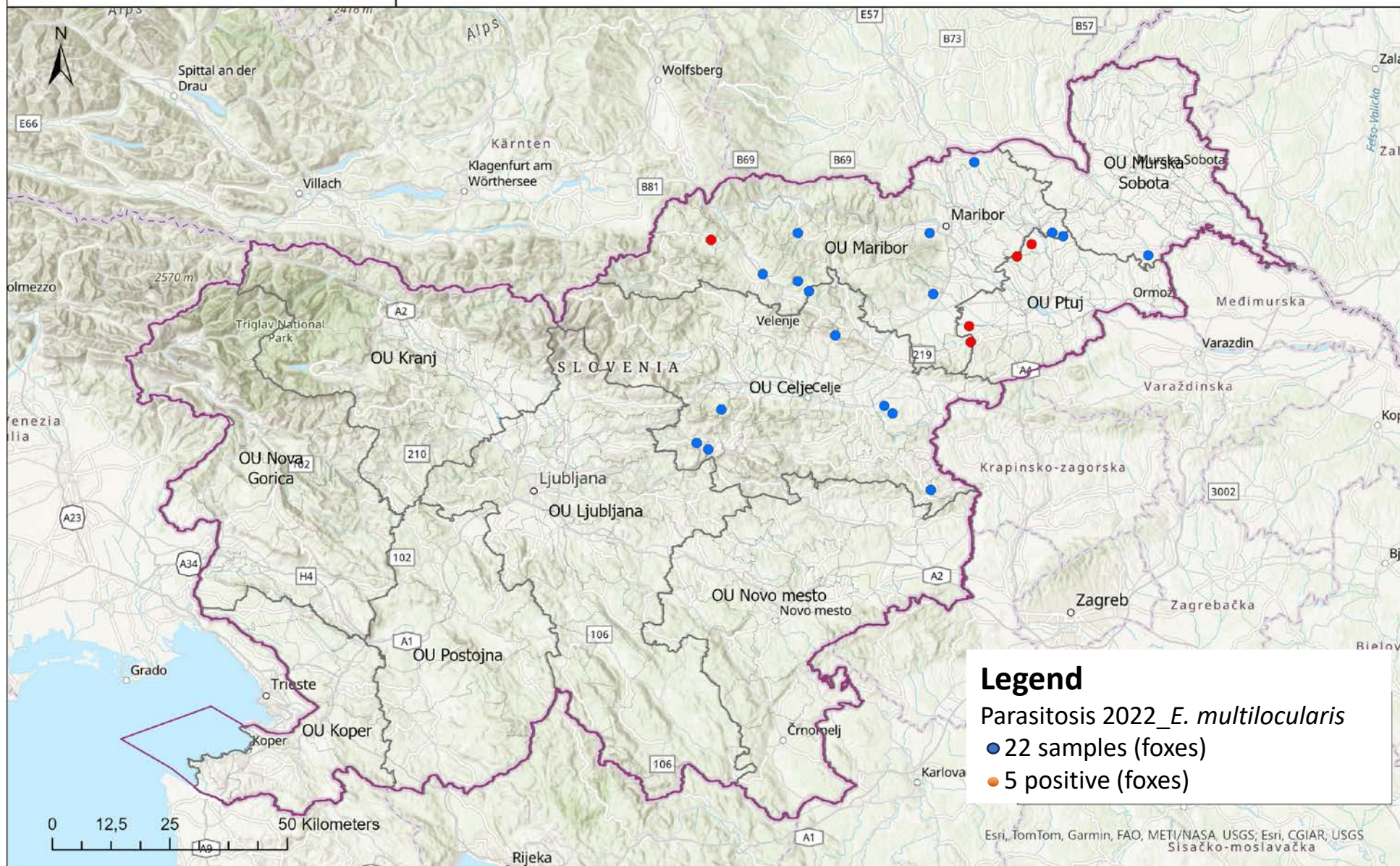
Hunting grounds with a special purpose

In Slovenia, "Hunting grounds with a special purpose" are designated areas managed separately from regular hunting grounds and are operated by specialized entities for focused game management, research, education, and breeding purposes. There are currently **8** such special purpose hunting grounds in the country which include almost **10%** of all Slovenian area.

These grounds aim to implement sustainable hunting practices, contribute to wildlife conservation, and support scientific, educational, or breeding activities beyond ordinary game management.

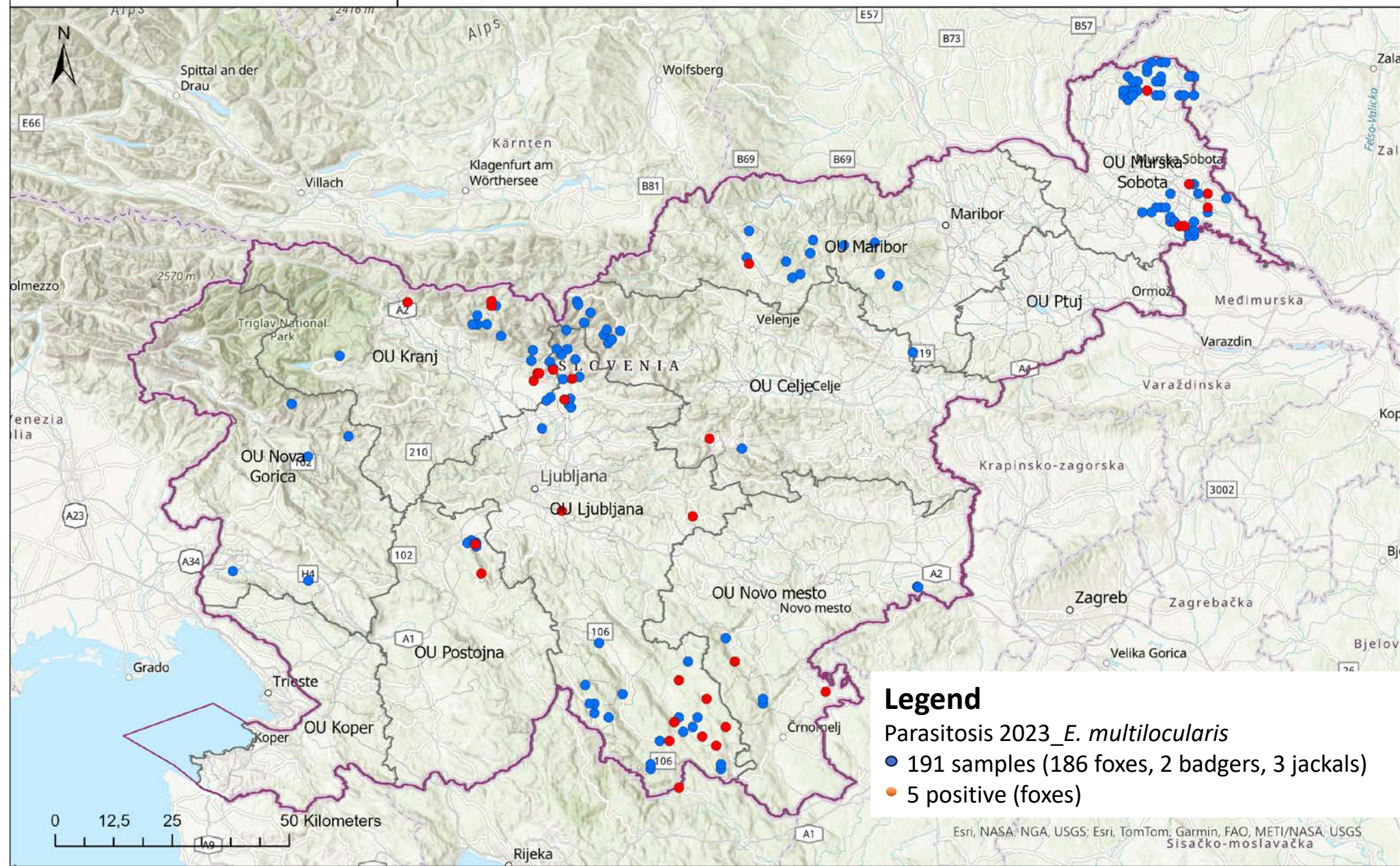


Parasitosis – Wild life 2022



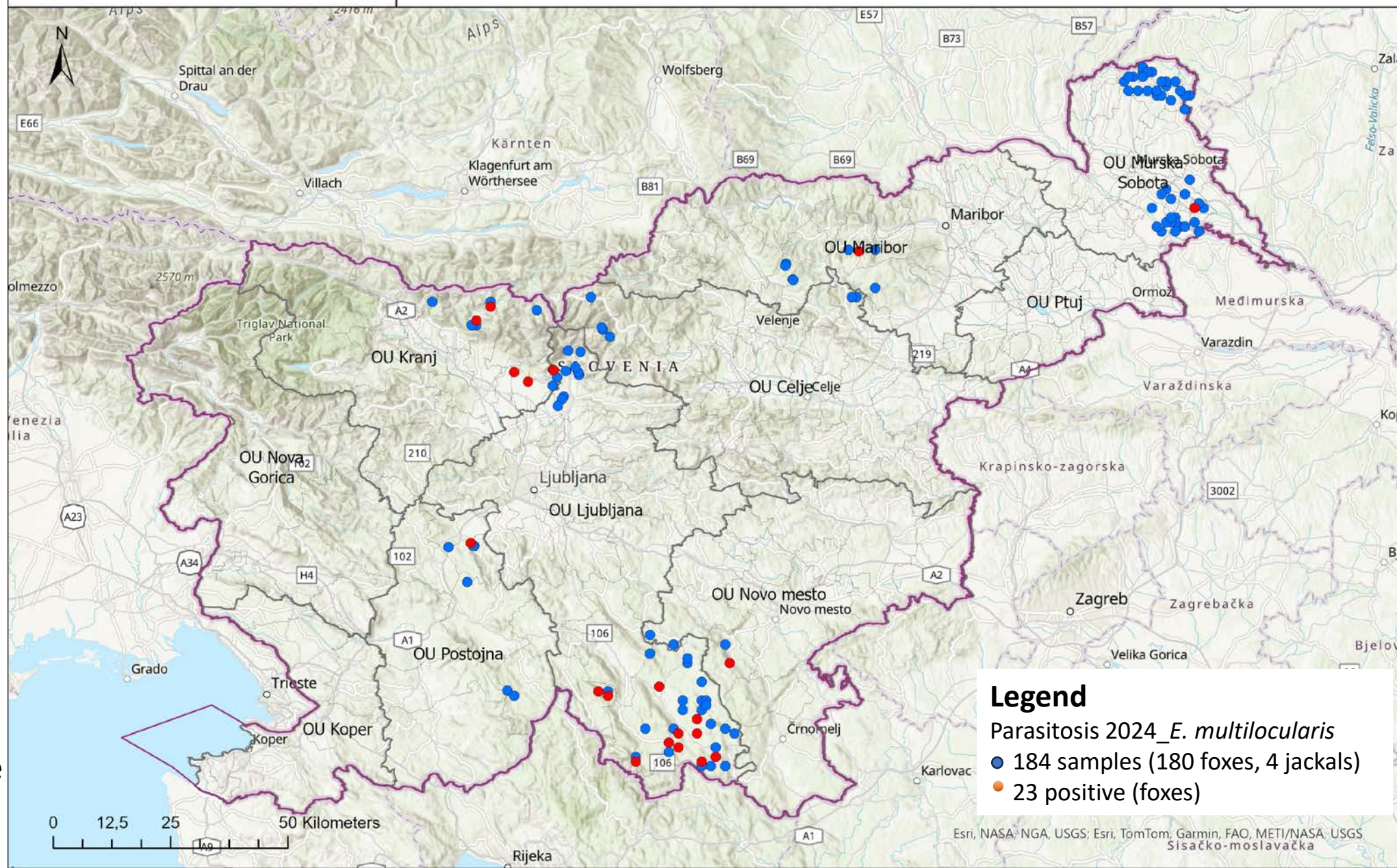
22.7 %
prevalence
in foxes

Parasitosis – Wild life 2023



17.7 %
prevalence
in foxes

Parasitosis – Wild life 2024



12.8 %
prevalence
in foxes

Summary

- ❖ *Echinococcus multilocularis* is a very important food born parasite in Slovenia.
- ❖ Monitoring in wildlife is of high importance.
- ❖ To date the prevalence in the last 4 years is staying constant. Some differences can be observed due to seasonal changes of fox and voles populations.
- ❖ The population of foxes and other potential hosts in Slovenia is expanding and getting closer to urban areas.

**Thank you to our
Parasitology Team <3**

Dr. Janez Posedi
Tatjana Pačnik
Martina Germek
Maja Gorišek





Thank you
for your
attention!