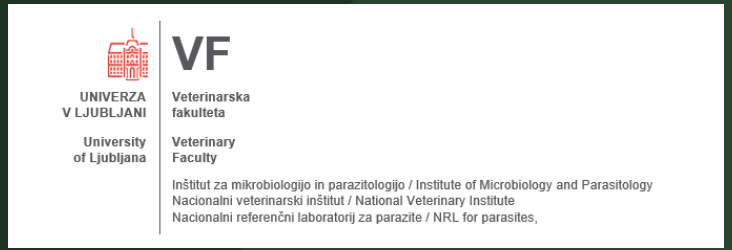
NATIONAL REFERENCE LABORATORY FOR PARASITES

Monitoring of *Trichinella spp*. in red foxes, golden jackals, badgers and lynx in Slovenia (2022-2025)

Laura Šimenc Kramar · Maja Gorišek · Janez Posedi

Institute of Microbiology and Parasitology · National Veterinary Institute · National Reference Laboratory for Parasites
Veterinary Faculty · University of Ljubljana, Slovenia



BACKGROUND

Slovenia – crossroad of four biogeographical regions

Slovenia lies at the junction of **four biogeographical regions**; about **60 %** of the area is covered with forests – an ideal habitat for the sylvatic cycle of parasites.

Alpine

8.541 km²

65% forest

Dinaric

5.706 km²

60% forest

Mediterranean

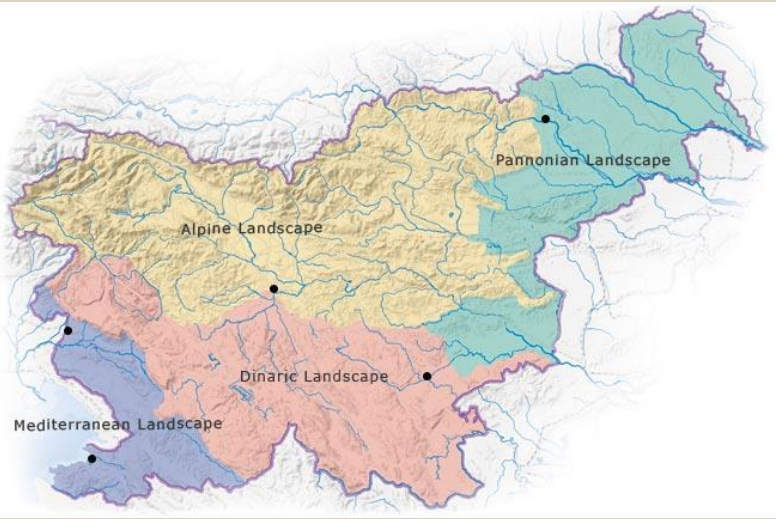
1.734 km²

35% forest · Karst

Pannonian

4.291 km²

34% forest



Macroregions of Slovenia (Perko 1998)

Red fox *(Vulpes vulpes)* = main sylvatic reservoir;
golden jackal (Canis aureus) and **badger (Meles meles)** as further hosts.

FUNDAMENTALS

Trichinella spp. – epidemiology

Zoonotic nematode; 13 genotypes, 8 recognised as species. Two transmission cycles govern the epidemiology.

Sylvatic cycle

Independent of human; transmission among wild carnivores via predator - prey.

Dominant species in Europe: ***T. britovi***.

Synanthropic cycle

Circulation within the pig population; main species ***T. spiralis***. Wildlife (fox, rat, mice) can be a source of introduction.

T. nativa – arctic/subarctic species, **freeze-resistant**: relevant for food safety.

OBJECTIVES

Objectives

1

Prevalence

To determine *Trichinella* prevalence in foxes, golden jackals and badgers. **WHY THESE SPECIES?**

2

Distribution & species

Geographic distribution and molecular species identification of the isolates.

3

Long-term trend

Placing the results within the 1965–2025 historical series, taking the methodological changes into objection.

MATERIALS & METHODS

Sampling – a change in study design

until 2021

Nationwide sampling
through the national rabies / wildlife surveillance programme.

→ large sample size (600–1,300 foxes/year), covering the whole country

from 2022

Only special-purpose hunting grounds (LPN)
of the Slovenia Forest Service – hunted by professional hunters (8 areas).

→ smaller sample, restricted spatially and by habitat; jackal and badger added (about 300 samples/year)

Important: The figures from 2022 onward are **not directly** comparable with the earlier nationwide estimates (changed coverage area).

Zavod za gozdove Slovenije

ABOUT LPN | HUNTING GROUNDS | SPECIES | HUNTING METHODS | CONTACT | SI | EN | DE

Welcome to the website of the special purpose state hunting grounds

Dear visitors of the website of the special purpose state hunting grounds managed by Slovenia Forest Service, we have prepared background information on the special purpose state hunting grounds (LPN) managed by Slovenia Forest Service in eight locations in Slovenia. It is the extraordinary diversity of nature and species that provide each hunting guest and other visitors to our LPNs with the form and type of hunting and nature experience which personally suits them best. You are welcome to visit us and see for yourself!

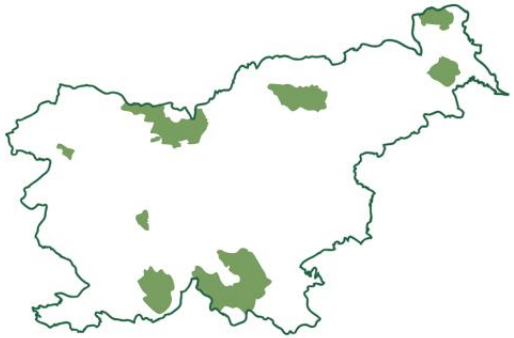
The Special Purpose State Hunting Grounds (managed by Slovenia Forest Service)

LPN Kozorog Kamnik

LPN Prodi Razor

LPN Ljubljanski vrh

LPN Jelen



LPN Kompas Peskovci

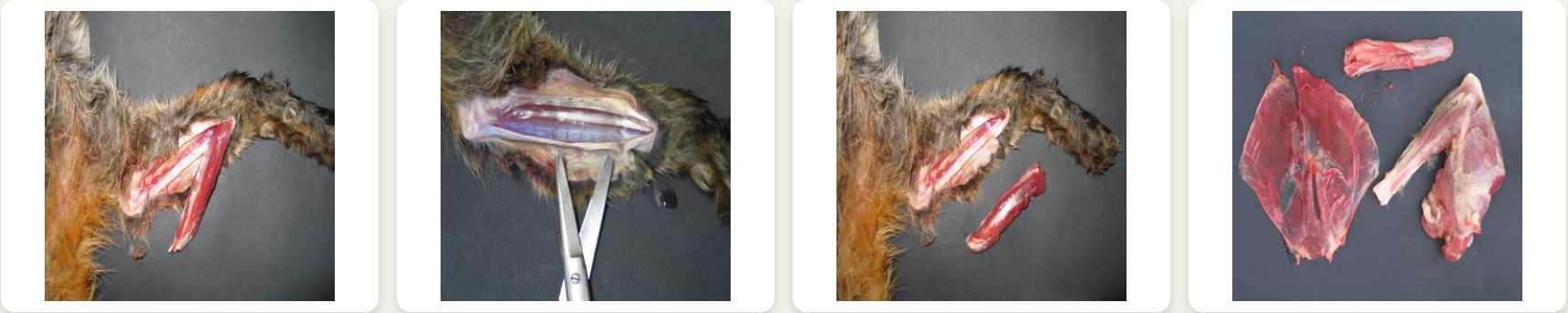
LPN Fazan Beltinci

LPN Pohorje

LPN Kočevsko

MATERIALS & METHODS

Sample material – foreleg muscle



Muscle sampling from the foreleg; right: diaphragm, tongue and foreleg (predilection sites).

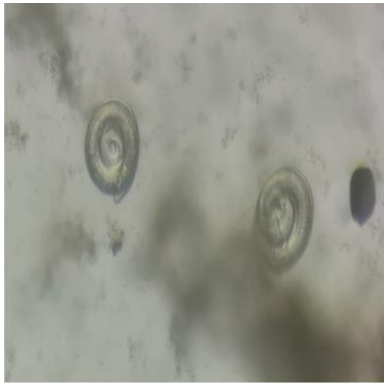
Sample material: Skeletal muscle of the foreleg; artificial digestion of the muscle sample. The extent of sampling changed over the years:

2006/2007	Foreleg + diaphragm; alternatively, tongue or masseter ≥ 10 g per animal.
2011/2012	≥ 10 g foreleg muscle (pooled).
2022–	20 g foreleg muscle per animal (ISO 18743:2015) – pooled - 5 samples/ pool)

MATERIALS & METHODS

Detection of muscle larvae

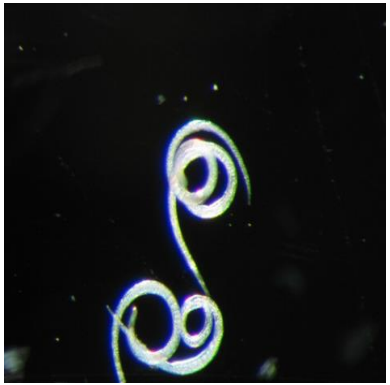
Artificial digestion method (**ISO 18743:2015/Amd 1:2023**) releases the muscle larvae, followed by microscopic detection. Unchanged across all periods.



Bright field



Bright field



Dark field



Single larva

Trichinella muscle larvae after artificial digestion.

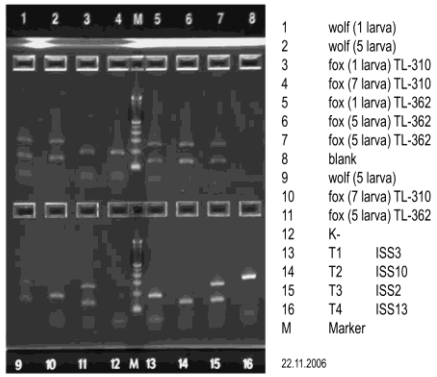
MATERIALS & METHODS

Genotyping – multiplex PCR

Species identification of isolated larvae following the **EURL-P protocol**. Method change in 2020:

≤ 2019 directly from larvae (proteinase K)

≥ 2020 with DNA extraction

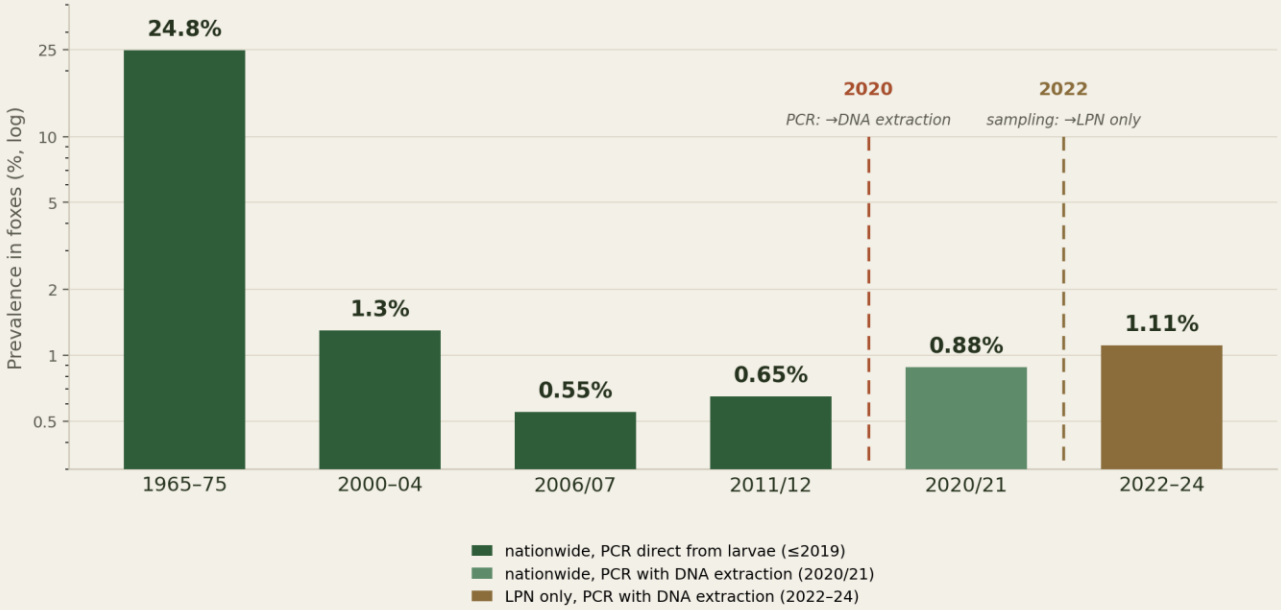




Banding pattern (ISS markers) for species determination; ***T1–T4*** *= reference strains (T. spiralis, T. nativa, T. britovi, T. pseudospiralis), M = marker, K– = negative control.*

LONG-TERM TREND

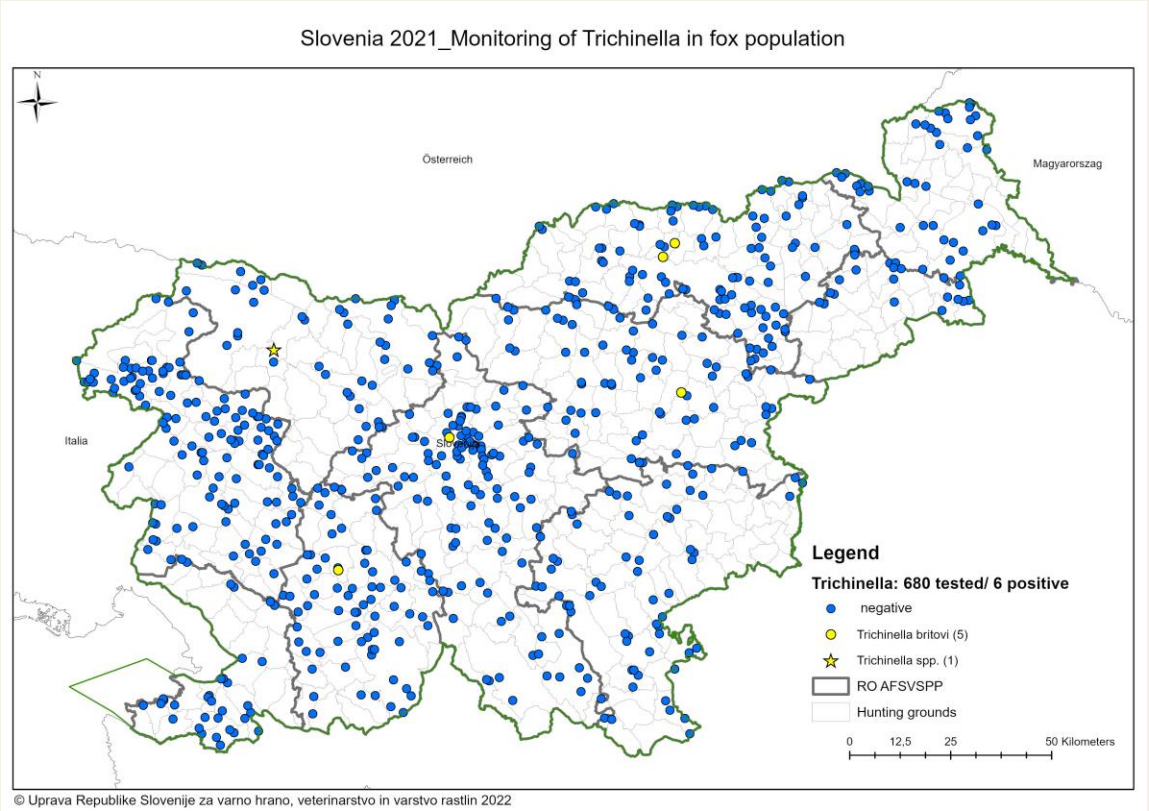
Historical overview 1965–2024



Two non-coincident breaks: method change (2020) and sampling change (2022) → not a directly comparable time series.

RESULTS

Geographic distribution 2021–2025



2021 nationwide

680 foxes tested

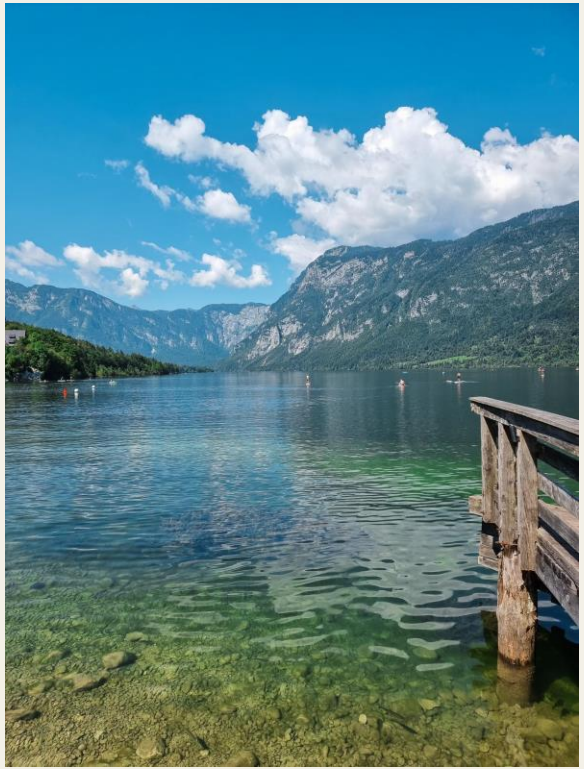
5 positive – *T. britovi*

1 positive – *T. nativa*

Blue dot = examined
Yellow dot = positive
Yellow star = Trichinella nativa

RESULTS

Geographic distribution 2021–2025



Bohinj



2021

nationwide



Bled

Veterinary Parasitology 335 (2025) 110424



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





First detection of *Trichinella nativa* in red foxes (*Vulpes vulpes*) in Slovenia

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[b] *Unit for Parasitology, Institute of Microbiology and Parasitology, Veterinary Faculty, University of Ljubljana, Ljubljana 1000, Slovenia*
[c] *National Reference Laboratory for Parasites, National Veterinary Institute, Veterinary Faculty, University of Ljubljana, Ljubljana 1000, Slovenia*

ARTICLE INFO

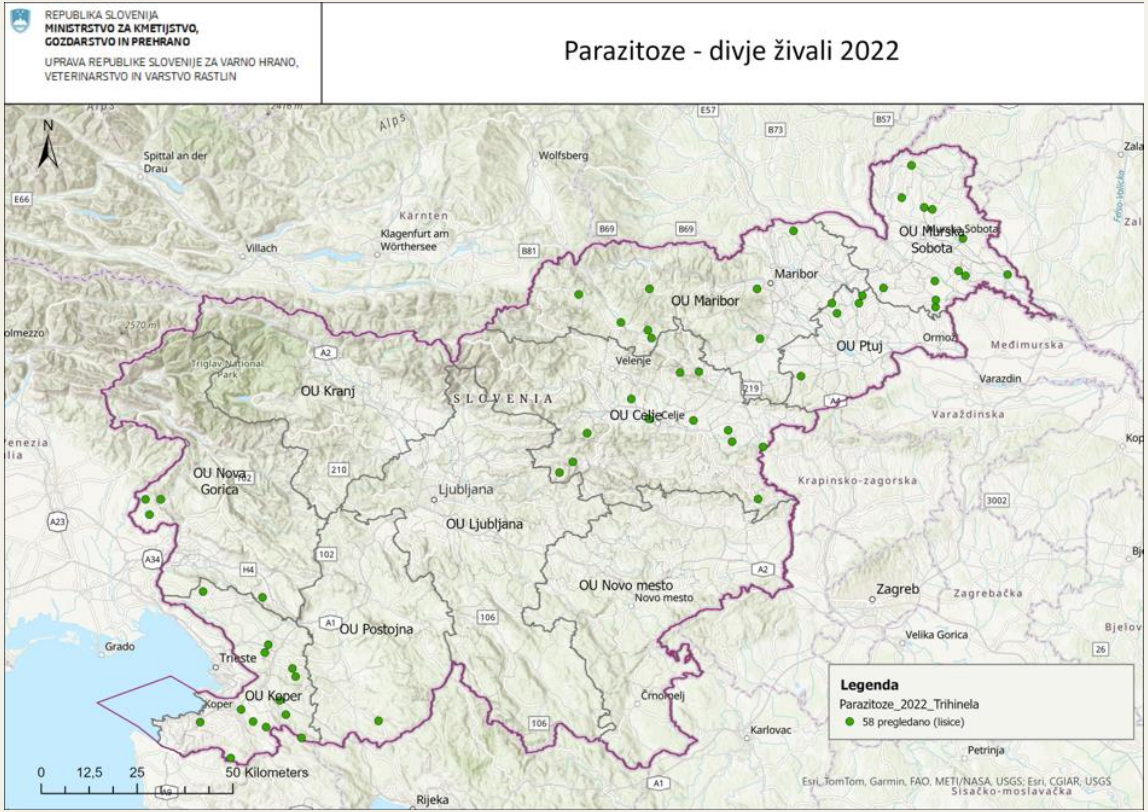
Keywords:
Red fox
Trichinella nativa
Trichinella britovi
Slovenia

ABSTRACT

Trichinella spp. is the zoonotic agent that causes trichinellosis in humans. It is traditionally associated mainly with undercooked meat from susceptible species such as domestic and wild pigs and other wild animals containing *Trichinella* larvae. In the present study, we investigated the prevalence and species distribution of *Trichinella spp.* in red foxes (*Vulpes vulpes*) in Slovenia during the 2020–2021 hunting season. Six hundred and eighty red fox samples were examined and the presence of *Trichinella spp*. larvae was confirmed in six cases (0.8 %). Using multiplex PCR, *T. britovi* was detected in five cases and *T. nativa* in one case. The occurrence of *T. nativa* on the southern side of the Alps is of great epidemiological importance and raises very important public health issues.

RESULTS

Geographic distribution 2021–2025



2022 LPN only

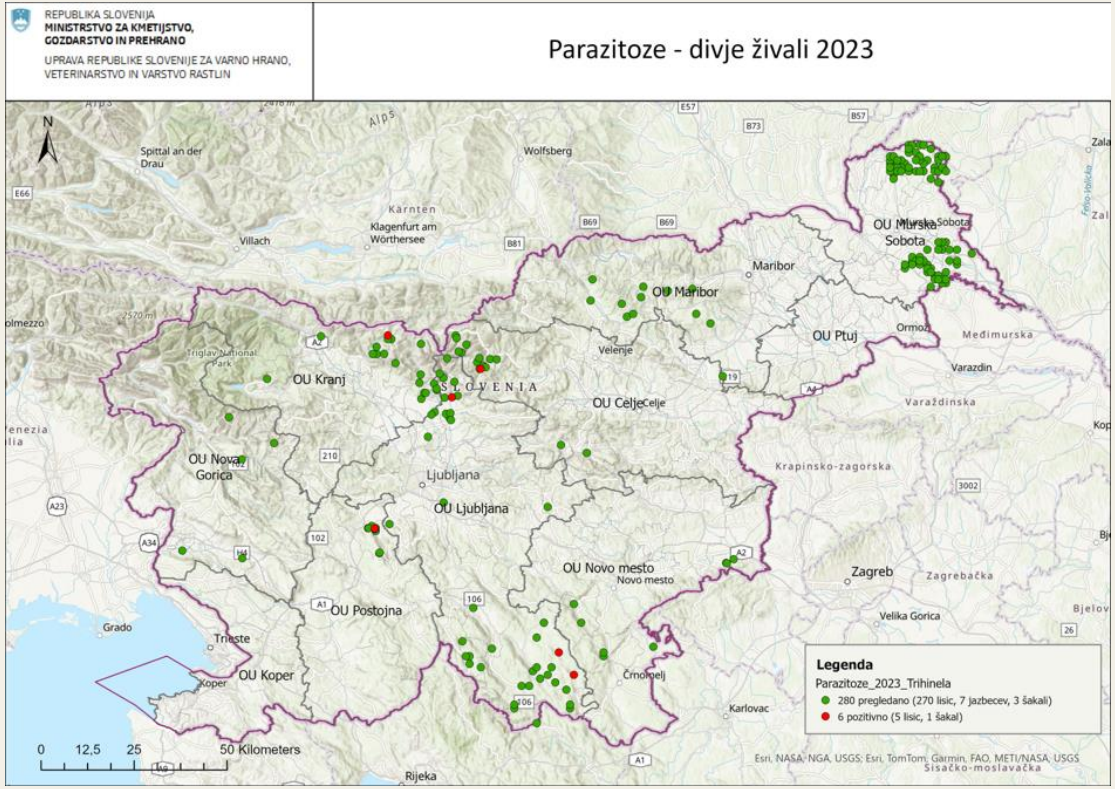
green dot = examined
Red dot = positive
NO POSITIVE
SMALL SAMPLE

2021 nationwide,
from 2022 only within the LPN

– the reduction in spatial coverage is clearly visible.

RESULTS

Geographic distribution 2021–2025



2023 LPN only

green dot = examined
red dot = positive

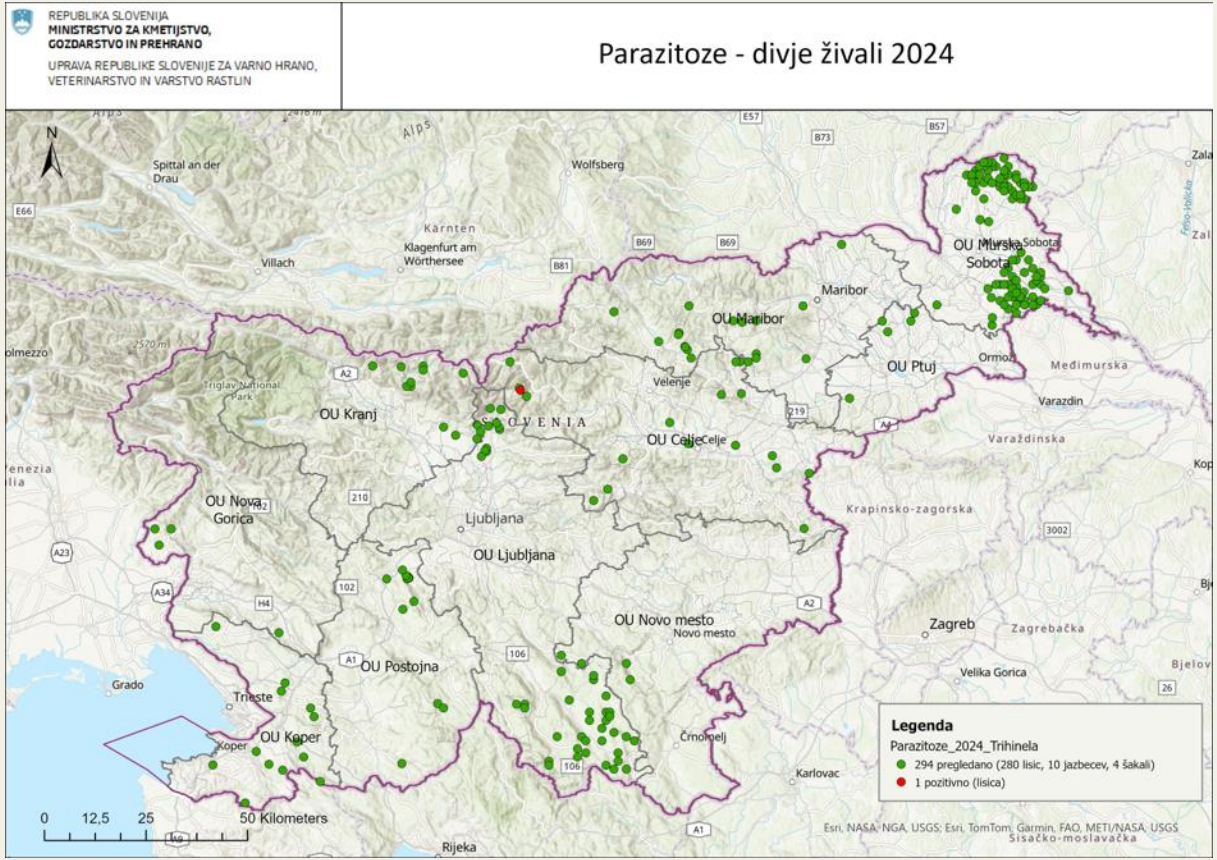
6 POSITIVE FOXES – *Trichinella britovi*
1 POSITIVE GOLDEN JACKAL – *Trichinella britovi*

WHICH DOT IS THE JACKAL?

2021 nationwide,
from 2022 only within the LPN
– the reduction in spatial coverage is clearly visible.

RESULTS

Geographic distribution 2021–2025



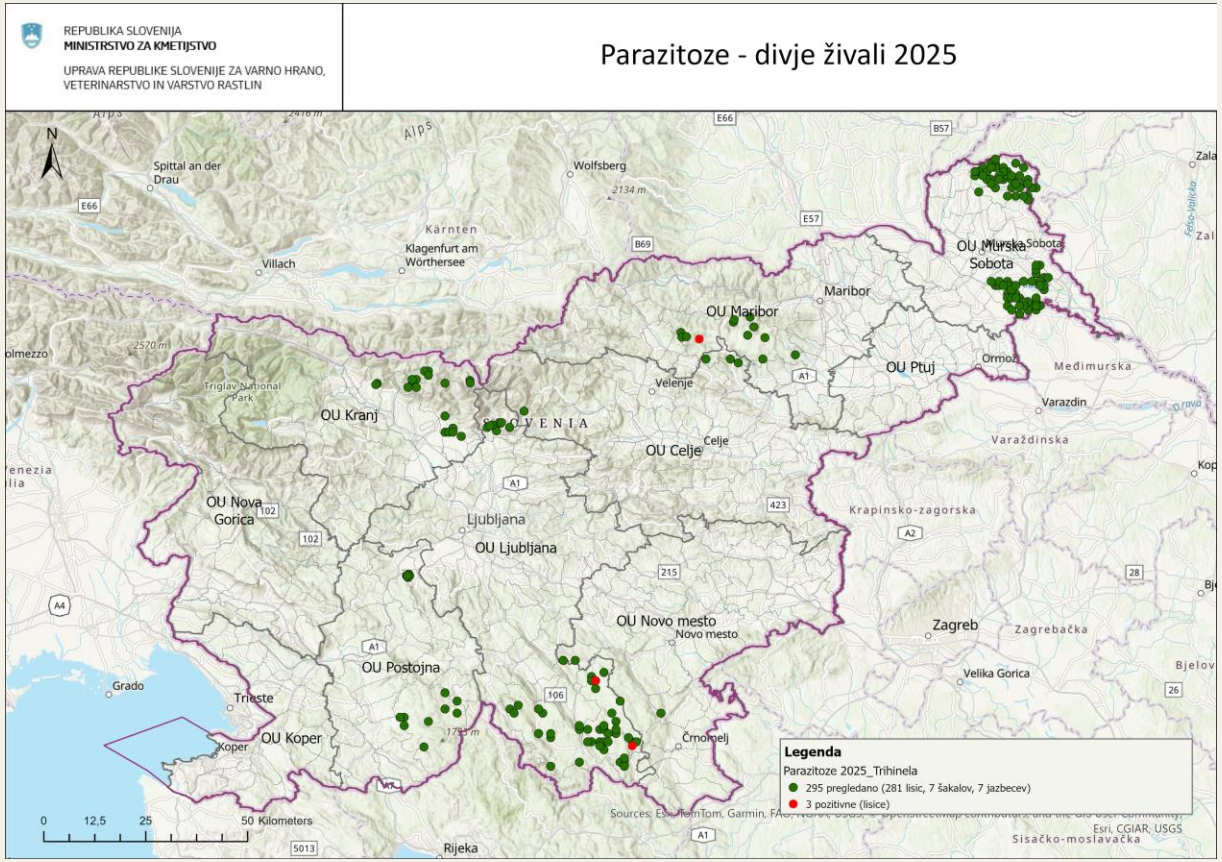
2024 LPN only

green dot = examined (280 foxes, 4 jackals, 10 badgers)
red dot = positive

1 POSITIVE FOX – *Trichinella britovi*

RESULTS

Geographic distribution 2021–2025



2025

LPN only

green dot = examined (281 foxes, 7 jackals, 7 badgers)

red dot = positive

3 POSITIVE FOXES – *Trichinella britovi*

RESULTS

Results 2022–2025

All ten isolates ***T. britovi***; golden jackal confirmed as a host for the first time.

927
animals examined

10
positive

1.07%
overall prevalence

Year	Species	Examined	Positive	Prevalence	Species (PCR)
2022	Foxes	58	0	0%	–
2023	Foxes	270	5	1.85%	*T. britovi*
	Golden jackals	3	**1**	**33.3%**	*T. britovi*
	Badgers	7	0	0%	–
2024	Foxes	280	1	0.36%	*T. britovi*
	Jackals / badgers	14 (10/4)	0	0%	–
2025	Foxes	281	3	1.07%	*T.britovi*
	Jackals / badgers	14 (7/7)	0	0%	-
Total	**all species**	**927**	**10**	**1.08%**	***T. britovi***

CONTEXT

First record of T. nativa (2020/2021)

680

foxes examined

6 positive (0.88%)

5 × T. britovi

1 × T. nativa

First record in Slovenia

- Confirmed by multiplex PCR and sequencing (BLAST: 100% identity).
- T. nativa is freeze-resistant – hence particularly relevant to meat hygiene.
- Earlier, methodologically homogeneous surveys (2006/07, 2011/12) found no *T. nativa* → pointing to a probably recent introduction or range expansion.

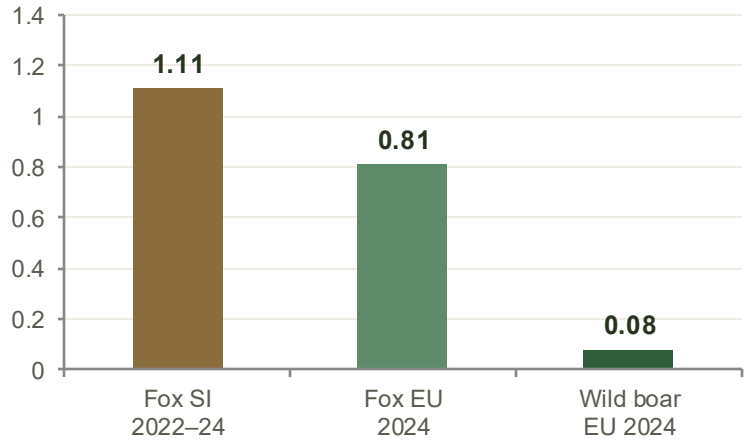
EUROPEAN CONTEXT

Trichinella in the EU, 2024 (EFSA One Health)

Key figures 2024

- 102 confirmed human cases (0.02/100,000).
- Domestic pigs under controlled housing: 0 positive.
- Non-controlled housing: 30 of >41 million positive.
- Croatia: T. spiralis in 2 raw-sausage samples.
- Reference method: ISO 18743:2015/Amd 1:2023.

Indicator animals – prevalence (%)



EFSA: *Wildlife data are not harmonised and not comparable across countries – robust trend analyses are not possible. This supports a cautious interpretation of our time series.*

DISCUSSION

Discussion

T. britovi dominates

Consistently the predominant sylvatic species; low but persistent circulation.

Golden jackal as host

Confirmed for the first time – consistent with the jackal's range expansion in Central Europe.

T. nativa as a new element

Freeze-resistant; warrants attention to meat hygiene and continued surveillance.

Cautious interpretation

The 2022 sampling change and 2020 method change limits direct comparability.

CONCLUSIONS

Continuous monitoring remains essential

- Low but persistent sylvatic circulation; *T. britovi* consistently dominant.
- New elements – *T. nativa* (2020/21) and the golden jackal as a host – underlines the importance of ongoing surveillance.
- One Health relevance: wildlife → pig/human; education on safe meat handling and preparation.

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