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Genetic identification of *Trichinella* species found in wild carnivores from the territory of Kazakhstan

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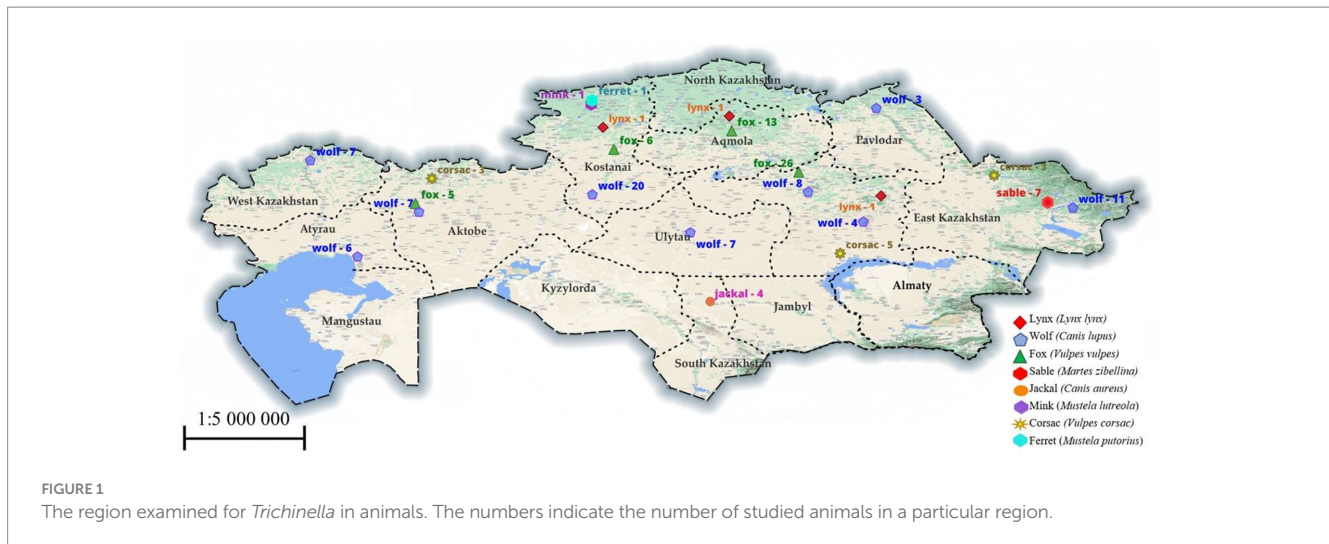
Trichinellosis, also called trichinosis, is a foodborne parasitic disease caused by eating raw or undercooked meat from animals infected with *Trichinella* spp. larvae and affects both animals and humans. Although on the territory of Kazakhstan, the species characteristics and prevalence of this helminth were studied back in the 90s, the data have not been updated since then. Given the above, our study was aimed at identifying *Trichinella* spp. using parasitological and molecular genetics methods. In our work, we studied 160 samples of muscle tissue of wild animals living in the natural zones of steppes and semi-deserts. Of the animals examined, 32 were positive for *Trichinella* spp., including 1 lynx (*Lynx lynx*), 17 wolves (*Canis lupus*), 11 foxes (*Vulpes vulpes*), 1 jackal (*Canis aureus*) and 2 corsac foxes (*Vulpes corsac*). Helminths were extracted using the digestion method. DNA was extracted using a Gene Jet commercial kit (Thermo Fisher Scientific, United Kingdom). For species identification a multiplex PCR, amplification of *ESV*, *ITS1*, and *ITS2* genes regions was performed. After that, uniplex PCR was performed on the 5S rDNA and *ITS1* genes region for sequencing analysis. The resulting sequences were subsequently used to construct a phylogenetic tree and the studied samples were identified as *Trichinella nativa* and *Trichinella britovi*. Thus, we can conclude that there is a circulation of two species of *Trichinella* in Kazakhstan, highlighting that constant control and monitoring of wild animals are necessary to prevent transmission and protect the health of people.

KEYWORDS

trichinellosis, parasitic transmission, wild animals, parasitology, Kazakhstan

1. Introduction

Trichinellosis is a foodborne parasitic zoonotic disease caused by the consumption of raw or semi-raw meat of animals infected by larvae of this nematode. The disease is both a public health hazard and a food safety problem in many parts of the world (1, 2). *Trichinella* species have a wide range of hosts, but predators and omnivores are most affected (3, 4). After eating infected meat, larvae are released and penetrate the intestinal mucosa. They develop into adult worms within days. Males and females copulate and then females releasing newborn larvae after 1 week. These



larvae travel *via* blood to the skeletal musculature where they either encapsulated or remain unencapsulated depending on the species (5).

To date, according to the literature, *Trichinella* species are divided into two clades: encapsulated (*T. spiralis*, *T. nativa*, *T. nelsoni*, *T. britovi*, *T. murrelli*, *T. patagoniensis*, and *T. chanchalensis*, *Trichinella* T6, T8, and T9) and non-encapsulated (*T. pseudospiralis*, *T. papuae*, and *T. zimbabwensis*) (6, 7).

In Kazakhstan, the problem of trichinellosis has not been studied well enough. *T. nativa*, *T. britovi*, and *T. pseudospiralis* are reported in wolves, jackal, foxes and cats living mainly in Southern Kazakhstan (5). One of the early studies on the spread of *Trichinella* is the work of Shaikenov. Their studies show pathogen infection of predators with *Trichinella* spp. in the deserts of Kazakhstan. As they note, the lowest infestation in predators was found in the sandy desert (3.7%), while the highest infestation of animals is noted in the mountainous (17.8–20.1%) and semi-desert (15.6–22.1%) zones (8).

Boev S.N. et al. described that the infestation of foxes (*Vulpes vulpes*) and corsac foxes (*Vulpes corsac*) in the steppe zone was 10.4 and 8.1%, respectively, and was inferior in terms of infestation of the same animals in the mountainous and semi-desert zones (9).

As was reported by Pozio (5), *T. nativa* and *T. britovi* circulate among foxes, wolves, jackals, martens, wild cats, lynxes, and wild boars in Kazakhstan. Also, *T. pseudospiralis* was recorded in corsac foxes, two rooks, and an eagle. Additionally, human trichinellosis has been documented following the consumption of meat from wild boars (10).

Wild carnivores such as wolves (*Canis lupus*), foxes (*Vulpes vulpes*), jackals (*Canis aureus*) and corsac foxes (*Vulpes corsac*) are native members of *Canidae* native to the Eurasia region (11). In Kazakhstan, the wild carnivore population is estimated to be around 14,000 animals (Unified Internet resource of the Ministry of Ecology, Geology and Natural Resources of the Republic of Kazakhstan, 2022) (12). These carnivores, at the top of the food chain, are excellent predators but also scavengers, and thus play an important role as a reservoir host of *Trichinella* species in the wild (13, 14).

The purpose of this article is to describe the occurrence of *Trichinella* spp. in wild animals of Kazakhstan and to study the species affiliation of the detected *Trichinella* larvae based on molecular analysis.

2. Materials and methods

2.1. *Trichinella* larvae sample collection

Work with samples of muscle tissues of animals caught from nature was carried out in the parasitological laboratory of the Faculty of Veterinary Medicine and was approved by the Animal Ethics Committee of the S. Seifullin Kazakh Agrotechnical University (extract from protocol No. 1 dated July 24, 2019). We obtained permission from the Department of Natural Resources and Environmental Management to excavation of animals. The method that was used to remove them is through shooting. All procedures were in accordance with the World Medical Association Code of Ethics (Declaration of Helsinki) for animal experiments.¹ The following animal species were studied: lynxes (*Lynx lynx*), wolves (*Canis lupus*), foxes (*Vulpes vulpes*), corsac foxes (*Vulpes corsac*), jackals (*Canis aureus*), mink (*Mustela lutreola*), ferret (*Mustela putorius*), sables (*Martes zibellina*), samples which were brought from 10 regions of Kazakhstan (Kostanay, Akmola, South Kazakhstan, Pavlodar, Karaganda, East Kazakhstan, West Kazakhstan, Aktobe, Atyrau and Ulytau regions), are shown in more detail in Figure 1. Animals were individually tested for the presence of *Trichinella* spp. larvae by the muscle compression method (15). Then, using artificial gastric juice, the muscle tissue (diaphragm and thigh muscle) was digested (16). The sediment was examined under a microscope, and *Trichinella* larvae were collected in test tubes with 70% ethanol. All morphological investigations were conducted using Micros Austria MCXI700.

2.2. DNA extraction

DNA was extracted from 310 larvae (as a pool of 10 larvae from each positive sample of an infected animal) using a GeneJet genomic DNA purification kit (Thermo Fisher, Cat.: K0701) with slight modifications.

¹ http://ec.europa.eu/environment/chemicals/lab_animals/legal_en.htm

Briefly, proteinase K was added to the larvae and incubated at 48°C for 60 min, followed by all steps according to the manufacturer’s protocol.

2.3. Multiplex and uniplex PCR

A multiplex PCR based on the use of five primer pairs amplifying the internal transcribed spacers *ITS1* and *ITS2*, and the expansion segment V region (*ESV*) of the large subunit ribosomal DNA (17) was used. Uniplex PCR primers set (5 s rDNA, *ITS1*) for differentiating species by sequencing were utilized (18). Details of the *ESV*, *ITS1*, *ITS2* and 5 s rDNA fragments produced by the PCR amplification are shown in Table 1.

Reactions were performed in 15 µL 2^x GoTaq Hot Start MasterMix, 9 µL nuclease-free water, 1 µL total primers, and 2.5 µL extracted DNA. The uniplex PCR was performed using the same mix as above but with the primer set II and VI for the *ITS1* and 5 s rDNA locus. The PCR cycles for multiplex PCR were performed as detailed in Table 2.

2.4. Electrophoresis and sequencing

Agarose gels (1.5%) were prepared in 1× TAE solution with 8 ng/µL ethidium bromide (Sigma, E1510). Electrophoresis was performed using 10 µL PCR products with a DirectLoad 100 bp Low ladder ready-to-use (Sigma, D3687-1VL) for 50 min at 120 V. The PCR-amplified target gene fragment was purified using a QIAquick PCR Purification Kit (QIAGEN, Germany, Cat.: 28106), following the manufacturer’s protocols. Sequencing was performed according to the manual for Seq Studio Genetic Analyzer (Thermo Fisher Scientific Applied

Biosystems). The resulting nucleotide sequences were visually checked by the Bio Capt program version 11.0. The nucleotide sequences of the studied species were compared with other sequences in the NCBI gene bank database by using the BLAST options. The nucleotide sequences of the studied species were deposited in NCBI GenBank database.

2.5. Bioinformatics analysis

Bioinformatic analysis of the obtained nucleotide sequences was carried out using computer software for statistical analysis of molecular evolution and construction of phylogenetic trees – MEGA 11. Multiple alignments were carried out using the CLUSTAL W program included in the MEGA software package. The evolutionary history and divergence between sequences was inferred by using the Maximum Likelihood method and Tamura-Nei model (19).

3. Results

Out of the 160 carnivores’ muscle tissue and diaphragm samples that were examined, 32 of them (20%, with a 95% confidence interval of 7.07–13.6) were found to be carriers of *Trichinella* larvae. Infection with *Trichinella* larvae was found in five out of the eight species that were examined. Specifically, 17 out of 83 wolves (20.5%), 11 out of 50 red foxes (22%), 2 out of 11 corsacs (18.2%), 1 out of 4 jackals (25%), and 1 out of 3 lynxes (33.3%) were infected, making a total of 32 samples. Examples of the detected helminths under the microscope can be seen in Figure 2. The specimens of ferret, sable, and mink that were examined were not infected with *Trichinella*. For more detailed information, please refer to Table 3.

During the multiplex PCR, positive samples were found for *Trichinella nativa* in lynx, wolves, foxes, and corsac foxes (identified by amplification of a 127 bp fragment), while a jackal tested positive for *Trichinella britovi* (identified by amplification of two fragments at 127 and 253 bp) (as shown in Figure 3). To identify the specific species, additional PCR was conducted using primer 5 s rDNA and *ITS1*. The resulting amplicons were purified and sequenced for their nucleotide sequences.

The resulting nucleotide sequences confirmed that all *Trichinella* samples were *T. nativa* and *T. britovi*, and were deposited in the international GenBank database (OP829907, OP829905, OP829906, OP829903, OP829904, OQ716806, OR159834). The tree with the highest log likelihood (–4977.99) is presented (Figure 4), and the initial tree was obtained using the Maximum Parsimony method. The tree is to scale, with branch lengths measured in the number of substitutions per site. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendant clade is shown next to each internal node in the tree.

TABLE 1 Multiplex and uniplex PCR fragment size of the studied taxa of the genus *Trichinella*.

| Primer | Locus set | Sequence (5’ → 3’) |
|--------|-----------|--|
| I | ESV | F: GTTCCATGTGAACAGCAGT R: CGAAAAACATACGACAACTGC |
| II | ITS1 | F: GCTACATCCTTTTGATCTGTT R: AGACACAATATCAACCACAGTACA |
| III | ITS1 | F: GCGGAAGGATCATTATCGTGTA R: TGGATTACAAAAGAAAACCATCACT |
| IV | ITS2 | F: GTGAGCGTAATAAAGGTGCAG R: TTCATCACACATCTCCACTA |
| V | ITS2 | F: CAATTGAAAACCGCTTAGCGTGTTT R: TGATCTGAGGTCGACATTTCC |
| VI | 5S rDNA | F: GCGAATTCTTGGATCGGAGACGGCCTG R: GCTCTAGACGAGATGTCGTGCTTTCAACG |

TABLE 2 Thermocycler parameters for 5S rDNA and multiplex primers gene amplification.

| | Pre-denaturation | 40 cycles | | | Final elongation |
|-----------|------------------|--------------|--------------|--------------|------------------|
| | | Denaturation | Annealing | Elongation | |
| | Temp./Time | | | | |
| Multiplex | 95°C / 2 min | 95°C / 10 s | 55°C / 30 s | 72°C / 30 s | 72°C / 5 min |
| 5 s rDNA | 94°C / 1,5 min | 94°C / 30 s | 48°C / 1 min | 72°C / 1 min | 72°C / 10 min |



FIGURE 2 *Trichinella* larvae detected after digestion: (A) Specimen found in a lynx. (B) Specimen found in a fox. Magnification 10x.

Our analysis involved 15 nucleotide sequences. The final dataset had a total of 1800 positions. The tree was rooted with an outgroup of *Trichosomoides crassicauda*. We also included sequences from *Trichinella* spp. species to show how closely related they are. We made sure to include *T. nativa* species from countries bordering Kazakhstan, such as China and Russia, where these species have been identified.

Obtained sequence data were used to calculate a pairwise fixation index (Fst), which helped us determine the level of genetic differentiation between *T. nativa* and *T. britovi* samples collected from carnivorous animals in Kazakhstan. Our analysis showed that the Fst values ranged from 0 to 1.084045 (as shown in Table 4), with 0 indicating complete identity among the samples.

4. Discussion

From 2020 to 2023, we conducted studies on the occurrence and species identification of *Trichinella*, common in wild animals of Kazakhstan. During the study, the muscle tissue and diaphragm of 160 animals were studied. Infection of animals with *Trichinella* larvae was

TABLE 3 *Trichinella* infection, larval burden and species identification in wild animals in Kazakhstan.

| Animal species | Examined (n) | Infected (n) | Infected (%) | grams of tissue examined for digestion (g) | 95% CI (%) | LPG ± SD | <i>Trichinella</i> species |
|-------------------------------------|--------------|--------------|--------------|--|--------------------|--------------|----------------------------|
| Lynx (<i>Lynx lynx</i>) | 3 | 1 | 33.3 | 5 | 20 (-12-52) | 8 ± 28.28 | <i>T. nativa</i> |
| Wolf (<i>Canis lupus</i>) | 83 | 17 | 20.5 | 5 | 12.59 (7.13-18.1) | 12.5 ± 25.38 | <i>T. nativa</i> |
| Fox (<i>Vulpes vulpes</i>) | 50 | 11 | 22 | 5 | 9.2 (4.24-14.2) | 9.5 ± 10.06 | <i>T. nativa</i> |
| Corsac fox (<i>Vulpes corsac</i>) | 11 | 2 | 18.2 | 5 | 3.8 (-1.1-8.7) | 3.5 ± 8.3 | <i>T. nativa</i> |
| Jackal (<i>Canis aureus</i>) | 4 | 1 | 25 | 5 | 11.25 (-7.85-30.4) | 9 ± 19.48 | <i>T. britovi</i> |
| Mink (<i>Mustela lutreola</i>) | 1 | 0 | 0 | 5 | - | - | - |
| Ferret (<i>Mustela putorius</i>) | 1 | 0 | 0 | 5 | - | - | - |
| Sable (<i>Martes zibellina</i>) | 7 | 0 | 0 | 5 | - | - | - |
| Total | 160 | 32 | 20 | | 7.07-13.6 | 8.5 ± 21 | |

CI, confidence interval; LPG ± SD, larvae per gram of muscle tissue ± standard deviation.

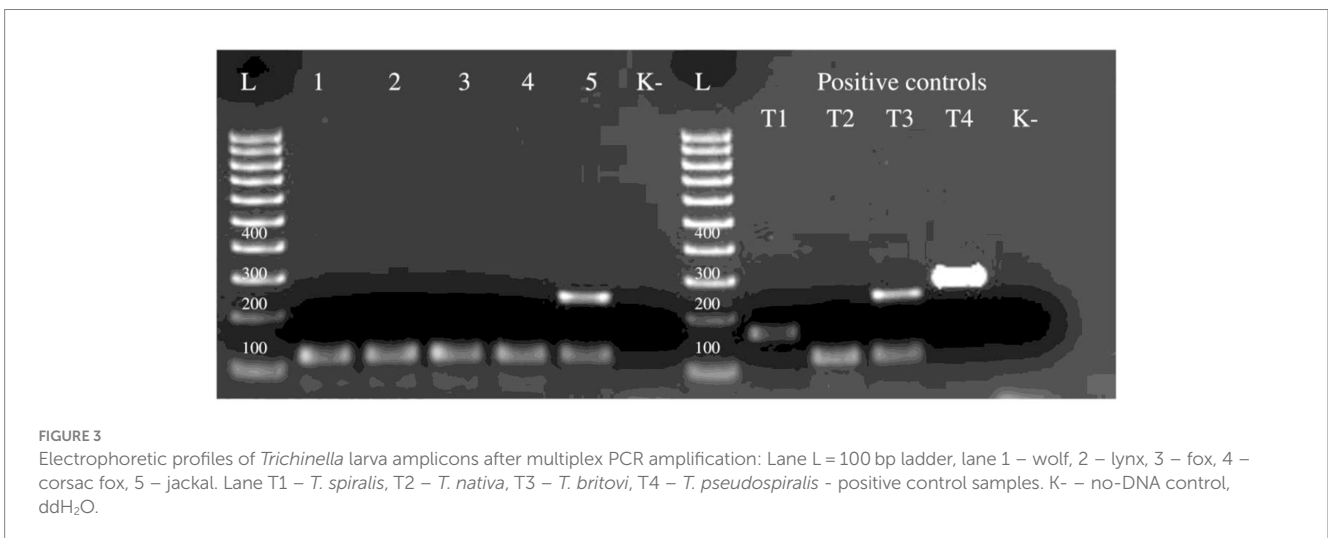


FIGURE 3 Electrophoretic profiles of *Trichinella* larva amplicons after multiplex PCR amplification: Lane L = 100 bp ladder, lane 1 – wolf, 2 – lynx, 3 – fox, 4 – corsac fox, 5 – jackal. Lane T1 – *T. spiralis*, T2 – *T. nativa*, T3 – *T. britovi*, T4 – *T. pseudospiralis* - positive control samples. K- - no-DNA control, ddH₂O.

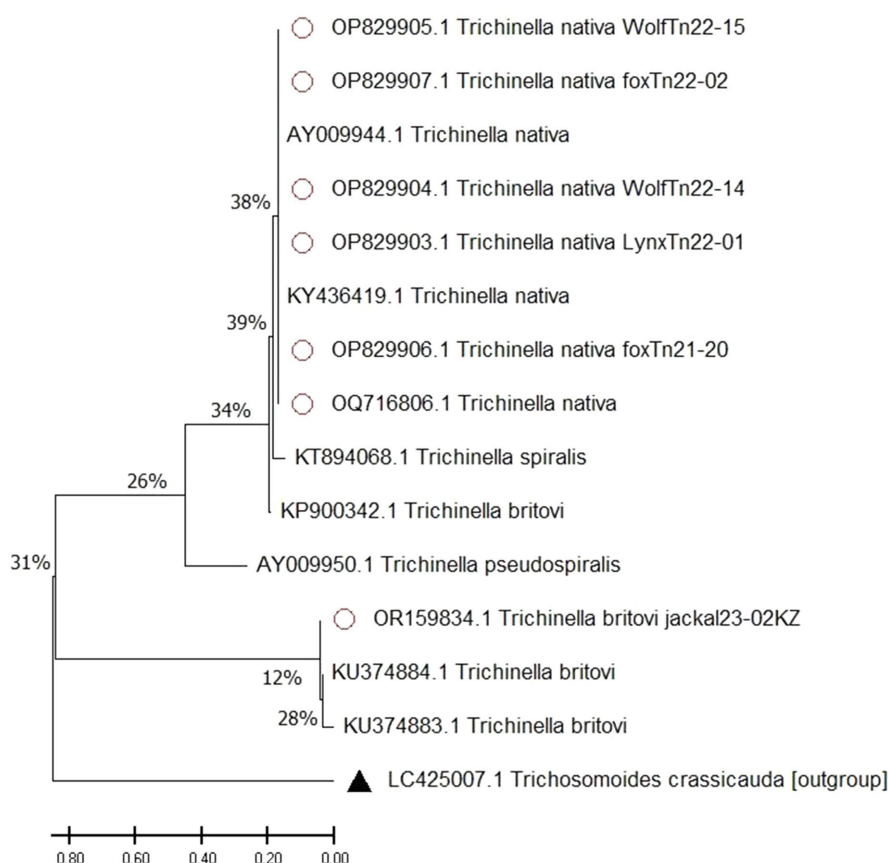


FIGURE 4

Maximum likelihood tree of *Trichinella* spp. A scale bar (divergence of 0.80) is shown. Sequences with red dots obtained in this study, outgroup marked with triangle, without any marks reference samples from GenBank.

20%, i.e., 32 tests were positive. The greatest detection of helminths was in areas located in the zone of steppes and semi-deserts.

To genetically identify the detected *Trichinella* larvae using sequencing, analysis was performed on the 5 s rDNA large ribosomal subunit and ITS1 Internal transcribed spacer (18, 20–22). After conducting research on wild mammals in Kazakhstan, we found that the species *T. nativa* is predominantly distributed throughout the territory. However, in the southern region of the country, we discovered the presence of the species *T. britovi*.

The process of identifying differences between species involves building a phylogenetic tree using the maximum likelihood method. *Trichosomoides crassicauda* (LC425007) was used as an outgroup to create a rooted tree. It is important to note that the studied sequence of *T. britovi* (OR159834) with reference sequences was divided into a separate clade, as the sequences were obtained by the ITS1 gene. The studied sequences of *T. nativa* wolves, foxes, lynxes and corsac foxes (OP829907, OP829905, OP829906, OP829903, OP829904, OQ716806) show deviations from the most recent common ancestor. The most recent common ancestor is the reference sample of *T. spiralis* (KT894068). The studied *T. nativa* sequences (Fst values of 0.00652%) are grouped together with reference samples (AY009944, KY436419), forming one subclade, with a difference between the last common ancestor of 38%.

In general, the use of the maximum likelihood method to create a phylogenetic tree with *Trichosomoides crassicauda* as an outgroup

and reference specimens of *T. spiralis*, *T. pseudospiralis*, *T. britovi*, and *T. nativa* provided insight into the unique evolutionary history of these species. The distinction of *T. nativa* from wolves, foxes, and lynx from the most recent common ancestor highlights the importance of considering evolutionary relationships when studying the genetic makeup of a species.

Through the analysis of the pairwise fixation index, it was discovered that there were minor variations between *T. nativa* species extracted from a variety of animals such as wolves, foxes, corsac foxes, and lynxes. Additionally, there were variations in nucleotide sequences between *T. nativa* collected from foxes (OP829906) and lynxes (OP829903). These findings offer significant insights into the genetic diversity of these species and could assist researchers in comprehending the ecological and evolutionary aspects that determine their distribution in different animal populations.

According to the map data in Figure 1, the samples we studied were obtained from four natural zones in Kazakhstan: forest-steppe and steppe in the northern areas, and semi-desert and desert in the western, central, and southern regions. We believe that weather and natural conditions do not affect the distribution of *Trichinella* species among wild carnivores in Kazakhstan. Although fewer animal specimens were studied in the southern part of the country, we found the *T. britovi* species in only 1 out of 4 jackals from the South Kazakhstan region. However, in all other samples of muscle tissue from wild carnivores in regions such as Karaganda, Ulytau, Kostanay,

TABLE 4 Pairwise fixation index (Fst values) between *T. nativa* and *T. britovi*.

| | OP829905 | OP829904 | OP829907 | AY009944 | OP829906 | OQ716806 | OP829903 | KY436419 | KP900342 | KT894068 | AY009950 | LC425007 | OR159834 | KU374884 | KU374883 |
|--|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| OP829905 <i>T. nativa</i> wolf | | | | | | | | | | | | | | | |
| OP829904 <i>T. nativa</i> wolf | 0.00000 | | | | | | | | | | | | | | |
| OP829907 <i>T. nativa</i> fox | 0.00000 | 0.00000 | | | | | | | | | | | | | |
| AY009944 <i>T. nativa</i> | 0.00000 | 0.00000 | 0.00000 | | | | | | | | | | | | |
| OP829906 <i>T. nativa</i> fox | 0.00000 | 0.00000 | 0.00000 | 0.00000 | | | | | | | | | | | |
| OQ716806 <i>T.nativa</i> corsac-fox | 0.00000 | 0.00000 | 0.00000 | 0.00000 | 0.00000 | | | | | | | | | | |
| OP829903 <i>T.nativa</i> lynx | 0.00628 | 0.00628 | 0.00628 | 0.00628 | 0.00628 | 0.00628 | | | | | | | | | |
| KY436419 <i>T. nativa</i> | 0.00628 | 0.00628 | 0.00628 | 0.00628 | 0.00628 | 0.00628 | 0.00000 | | | | | | | | |
| KP900342 <i>T. britovi</i> | 0.01905 | 0.01905 | 0.01905 | 0.01905 | 0.01905 | 0.01905 | 0.02547 | 0.02547 | | | | | | | |
| KT894068 <i>T. spiralis</i> | 0.05872 | 0.05872 | 0.05872 | 0.05872 | 0.05872 | 0.05872 | 0.05871 | 0.05871 | 0.06592 | | | | | | |
| AY009950 <i>T. pseudospiralis</i> | 0.37745 | 0.37745 | 0.37745 | 0.37745 | 0.37745 | 0.37745 | 0.37537 | 0.37537 | 0.36630 | 0.36492 | | | | | |
| LC425007 <i>Trichosomoides crassicauda</i> | 1.00884 | 1.00884 | 1.00884 | 1.00884 | 1.00884 | 1.00884 | 1.03736 | 1.03736 | 1.02709 | 1.039606 | 1.31151 | | | | |
| OR159834 <i>Tbritovi</i> jackal | 1.12977 | 1.12977 | 1.12977 | 1.12977 | 1.12977 | 1.12977 | 1.10229 | 1.10229 | 1.04405 | 1.123990 | 0.92394 | 1.07820 | | | |
| KU374884 <i>T. britovi</i> | 1.12977 | 1.12977 | 1.12977 | 1.12977 | 1.12977 | 1.12977 | 1.10229 | 1.10229 | 1.04405 | 1.123990 | 0.92394 | 1.07820 | 0.00000 | | |
| KU374883 <i>Tbritovi</i> | 1.12977 | 1.12977 | 1.12977 | 1.12977 | 1.12977 | 1.12977 | 1.10229 | 1.10229 | 1.04405 | 1.123990 | 0.92394 | 1.07820 | 0.00000 | 0.00000 | |

Sequences with red dots obtained in this study.

East Kazakhstan, and West Kazakhstan, we identified the *T. nativa* species.

As reported by Pozio (10), *T. nativa* and *T. britovi* are present among various wild animals such as red and corsac foxes, wolves, jackals, martens, wild cats, lynxes and wild boars (8, 9, 23) in Kazakhstan. In 1975, *T. pseudospiralis* was found in corsac foxes, two ravens and an eagle from the Chimkent region (25, 26). In 2016, 20 cases of human trichinosis were detected in the Kyzylorda region reported by Kenzhebaev. Out of the 20 patients, two were children under 14 years old, 14 lived in urban areas and 6 in rural areas. This group infection outbreak was of an epidemiological nature (24). The source of the infection was wild boar meat killed by an amateur hunter. There are likely both “wild” and “synanthropic” foci of trichinosis in south-west Kazakhstan. These foci initially formed in local reservoirs where the infection accumulated and grew in wild animals due to the “predator–prey” principle, ultimately leading to a group human infection with *Trichinella*. The sources of infection for animals and humans with *Trichinella* in Kazakhstan are diverse, and the pathogen continues to circulate due to the population of various field rodents, predators, and wild omnivores (boars) in the region (27).

In reference to the current situation regarding trichinosis in border countries with Kazakhstan, recent research conducted in mainland China has revealed that out of the 16 isolates obtained, 13 were identified as *T. spiralis*. These samples were collected from pigs all across the country. The remaining two isolates were obtained from dogs and one from a cat, and these were identified as *T. nativa*. These were collected in northeast China. It is worth noting that *Trichinella* has been found in 15 different animal species, including but not limited to pigs, dogs, cats, rats, cows, foxes, and bears, and these are distributed throughout China (28). Furthermore, a study conducted in Russia by Glazunov YV et al. found that *T. spiralis* was present in 58.8% of badgers (*Meles meles*), 35.3% of brown bears (*Ursus arctos*), and 5.9% of wild boars (*Sus scrofa*) (29).

In Kyrgyzstan, there have been reports of the discovery of *T. nativa* in red foxes (23). However, there is currently no information available regarding the infection of humans or domestic animals with *Trichinella*. In Uzbekistan, *T. britovi* was found in jackals (23), and there have been reports of a significant outbreak of trichinosis caused by consuming pork from a wild boar (Kairov, 1965; Nadzhimiddinov et al., 1965). However, there is no information available regarding cases of infection in domestic animals (10).

Our research findings revealed that *Trichinella*, a causative agent of a parasitic disease, is widely distributed among wild mammals in all provinces of our country. This indicates that the natural biocenoses of Kazakhstan are at risk of invasion by *Trichinella*. Even with a relatively small sample size of animals under study, the results demonstrate the wide distribution of this parasite. As such, there is a real risk of human infection with *Trichinella* in the territory where the animals were caught. The main cause of human infections is often related to the illegal hunting of wild animals such as wild boar and badger, where the meat of poached mammals is not subjected to veterinary and sanitary examination. This study is of utmost importance, as data on *Trichinella* prevalence in wildlife is scarce in our region. By gaining more information, we can develop strategies to prevent and control the spread of *Trichinella* in our natural biocenoses.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: <https://www.ncbi.nlm.nih.gov/genbank/>, OP829907, <https://www.ncbi.nlm.nih.gov/genbank/>, OP829905, <https://www.ncbi.nlm.nih.gov/genbank/>, OP829906, <https://www.ncbi.nlm.nih.gov/genbank/>, OP829903, <https://www.ncbi.nlm.nih.gov/genbank/>, OP829904.

Ethics statement

The animal study was approved by the Animal Ethics Committee S.Seifullin Kazakh Agrotechnical University (extract from the protocol No. 1 from 24.07.2019). All of the procedures complied with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiments (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

RU: Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft. AM-S: Conceptualization, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. CS: Writing – original draft, Formal analysis, Software, Validation, Visualization. AS: Writing – original draft, Formal analysis, Methodology, Software, Validation, Visualization. LL: Conceptualization, Methodology, Supervision, Validation, Writing – original draft. SL: Writing – original draft, Formal analysis, Methodology, Resources, Validation, Visualization. VK: Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Funding acquisition, Investigation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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