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Occurrence of *Trichinella spiralis* in farmed wild boars (*Sus scrofa*): an underrated risk in China

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Research Article

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Abstract

Natural infection by *Trichinella* sp. has been reported in humans and more than 150 species of animals, especially carnivorous and omnivorous mammals. Although the presence of *Trichinella* sp. infection in wild boars (*Sus scrofa*) has been documented worldwide, limited information is known about *Trichinella* circulation in farmed wild boars in China. This study intends to investigate the prevalence of *Trichinella* sp. in farmed wild boars in China. Seven hundred and sixty-one (761) muscle samples from farmed wild boars were collected in Jilin Province of China from 2017 to 2020. The diaphragm muscles were examined by artificial digestion method. The overall prevalence of *Trichinella* in farmed wild boars was 0.53% [95% confidence interval (CI): 0.51–0.55]. The average parasite loading was 0.076 ± 0.025 larvae per gram (lpg), and the highest burden was 0.21 lpg in a wild boar from Fusong city. *Trichinella spiralis* was the only species identified by multiplex polymerase chain reaction. The 5S rDNA inter-genic spacer region of *Trichinella* was amplified and sequenced. The results showed that the obtained sequence (GenBank accession number: OQ725583) shared 100% identity with the *T. spiralis* HLJ isolate (GenBank accession number: MH289505). Since the consumption of farmed wild boars is expected to increase in the future, these findings highlight the significance of developing exclusive guidelines for the processing of slaughtered farmed wild boar meat in China.

Introduction

Trichinellosis is a food-borne zoonotic parasitic disease caused by ingestion of *Trichinella* sp. infective larvae *via* consuming raw or undercooked meat (Pozio, 2015). The genus *Trichinella* consists of 13 taxa (10 species and 3 genotypes) distributed into 2 clades: encapsulated and nonencapsulated (Pozio, 2021). To date, 4 species have reportedly been isolated in China: *T. spiralis*, *T. nativa*, *T. pseudospiralis* and *T. papuae* (Bai *et al.*, 2017).

Pork meat and its products are China's largest source of human *Trichinella* infection (Cui *et al.*, 2006; Pozio, 2014). During 2009–2020, up to 87.5% (7/8) of outbreaks of human trichinellosis were caused by the consumption of raw or undercooked pork (Zhang *et al.*, 2022). Other cases of human trichinellosis were shown to be sporadic infections resulting from the ingestion of meat from dogs, wild boar and other game (Wang *et al.*, 2006; Bai *et al.*, 2017).

Wild boar meat and meat-derived products are considered a further important source of *Trichinella* sp. infection in humans (Pozio, 2015; Rostami *et al.*, 2017). Approximately two million commercial wild boars are reared on farms for trade in China every year. Most of them are kept under non-controlled conditions. Improper manage of farmed wild boars increases the risk of occurrence of trichinellosis in humans. *Trichinella* sp. infection in hunted wild boars has been widely reported in Europe (Vieira-Pinto *et al.*, 2021), South America (Ribicich *et al.*, 2020), the Middle East (Haim *et al.*, 1997) and Southeast Asia (Yera *et al.*, 2022). However, the prevalence of *Trichinella* sp. infection in farmed wild boars is unknown in China.

The current study aimed to determine the presence of *Trichinella* infections in farmed wild boars (*Sus scrofa*) in northeastern China, which would increase awareness of the prevalence of *Trichinella* sp. in farmed wild boars and encourage surveillance measures.

Materials and methods

Study area and animals

The present study was conducted between August 2017 and December 2020 to investigate the presence of *Trichinella* infections in wild boars that could freely forage on small farms from Gongzhuling, Fusong and Jilin cities in Jilin Province, northeastern China (Fig. 1).

Figure 1. Map of China indicating the locality investigated in this study. The area marked in red represents the location of the investigated region.

The seroprevalence of *Trichinella* infections in pigs in northeastern China was reported to vary from 0.02% to below 4% (Cui *et al.*, 2006; Liu and Boireau, 2002). With a confidence level of 95% ($z = 1.96$) and an allowed deviation of the true prevalence of 5% (d), the projected seroprevalence is 4% (P). Consequently, 59 was used to calculate the sample size [according to $n = P\,(1 - P)z^2/d^2$] (Zhang *et al.*, 2015). Information regarding geographical position, age and sex is provided in Table 1.

Muscle samples

Diaphragm muscles were collected from 761 farmed wild boar carcasses for post-mortem inspection. The diaphragms (60 g–120 g from each boar) were trimmed of fat and fascia and stored at −15°C for pending testing by artificial digestion.

Magnetic stirrer artificial digestion

Diaphragm muscles were digested by the magnetic stirrer method utilizing HCl-pepsin to examine the presence of muscle larvae (Gamble *et al.*, 2000). Each muscle sample was initially cut into 5 g pieces to create a pool of 50 g mixture for screen. Subsequently, all samples of positive pools were digested individually weighing 97 ± 6 g to identify *Trichinella* larvae. Briefly, the minced muscle sample was mixed with a solution containing 1% pepsin (1: 10 000 NF, Sigma, USA), 1% HCl and 0.9% NaCl. This mixture was stirred using a magnetic stirrer at 45 °C for 1 h. The homogenized digestive fluid was settled in a 2-litre separatory funnel for 1 h. The *Trichinella* larvae were obtained from the bottom of the settling funnel and kept in 90% ethyl alcohol after being washed 5 times using 0.9% NaCl. The parasite load was calculated as larvae per gram (lpg) ± S.D..

Multiplex polymerase chain reaction (PCR) assays of Trichinella larvae

The species of collected muscle larvae were identified by multiplex PCR as previously described (Zarlenga *et al.*, 1999). Briefly, extracted DNA from single *Trichinella* larvae (Pozio and La Rosa, 2003) was subjected to amplification of specific regions (ESV, ITS1 and ITS2) of the ribosomal DNA repeats using 5 primer sets (Zarlenga *et al.*, 1999). The species-specific DNA banding pattern was visualized on 1% (w/v) agarose gels stained with GoldenView™.

Sequencing analysis

The 5S rDNA inter-genic spacer region of *Trichinella* was amplified as previously described for phylogenetic analysis (Fu *et al.*, 2009). Briefly, the PCR system in a volume of 25 μL contained 2 μL of DNA, 12.5 μL of 2 × Unique™ Taq MasterMix (Novogene, China) and each primer at 10 μM. The cycling conditions were 35 cycles of 94°C for 1 min, 55.8°C for 1 min and 72°C for 1 min. The PCR products were directly sequenced by Sangon Biotech (Shanghai) Co., Ltd., China.

Statistical analysis

Exact binomial 95% confidence intervals (CIs) were established for the rate of infection in this study. The *t*-test was used to assess statistical differences of infection rates according to the animals' sex, with values of $P < 0.05$ considered as statistically

Table 1. Information about farmed wild boars used in this study from 3 cities of Northeast China

		Age		Gender	
Areas	Collected samples	22–66days	>66 days	Male	Female
Gongzhuling	198	35	163	23	175
Fusong	302	110	192	46	256
Jilin	261	75	186	40	221
Total	761	220	541	109	652

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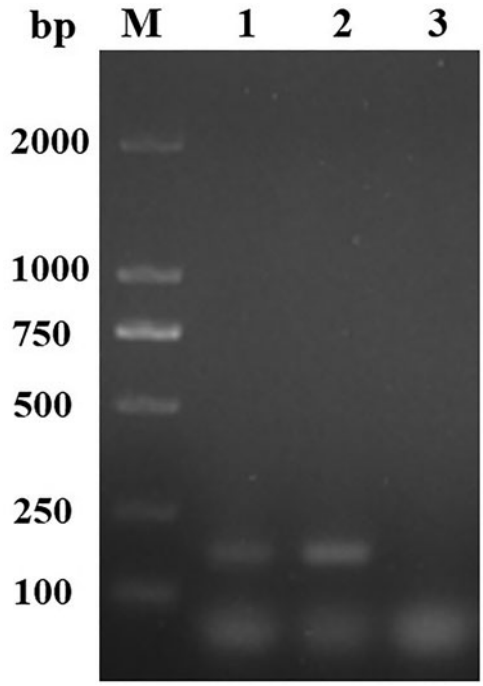

Figure 2. Identification of *Trichinella* isolates by PCR. M: DL2000 DNA marker; line 1: Multiplex PCR amplification of single larvae of *Trichinella* isolates in this study; line 2: Multiplex PCR amplification of single larvae of the *T. spiralis* Henan strain; line 3: Blank control.

significant. Statistical analyses were performed by using the online software (Epitools-Epidemiological Calculators, http://epitools.ausvet.com.au).

Results

The overall prevalence of *Trichinella* in farmed wild boars was 0.53% [95% confidence interval (CI): 0.51–0.55]. Of the 4 positive results, 1 sample came from a male (1/109, 0.92%), and the positive rate in males was higher than that in females (3/652, 0.46%), but the difference was not statistically significant ($P > 0.05$, $t = 0.047$). Three out of the 4 *Trichinella*-positive wild boars were from Fusong city, and the other positive boar was from Jilin city. A higher prevalence of *Trichinella* infection in wild boars at the age > 66 days, compared to those at 22–66 days ($P > 0.05$, $t = 0.013$). The average parasite burden was 0.076 ± 0.025 larvae per gram (lpg), and the highest burden was 0.21 lpg detected in a female boar (age >66 days) from Fusong city.

The multiplex PCR allowed to identify all the larvae collected from the farmed wild boars as belonging to *T. spiralis* species (Fig. 2). The 5S rDNA obtained sequence (GenBank accession no. OQ725583) showed a 100% similarity with the corresponding sequence of the *T. spiralis* HLJ isolate (accession no. MH289505).

Discussion

Wild boar meat is the second largest source of human infections of *Trichinella* worldwide (Pozio, 2015; Rostami *et al.*, 2017). An increasing number of wild boars are being kept in captivity because of the rising demands for consumable wild boar meat in China. The present survey aimed to investigate the risk of human exposure to these products intended for human consumption. The overall prevalence of *Trichinella* in the present study was lower than that revealed by similar studies in Argentina (11.4%) (Cohen *et al.*, 2010), northwest Vietnam (3.2%) (Thi *et al.*, 2014), Latvia (2.2%) (Kirjušina *et al.*, 2015), Laos (2.1%) (Conlan *et al.*, 2014), Chile (1.8%) (Hidalgo *et al.*, 2019), Romania (1.66%) (Nicorescu *et al.*, 2015), and Estonia (0.9%) (Kärssin *et al.*, 2021) but was higher than that in Hungary (0.0077%) (Széll *et al.*, 2012), Italy (0.01%) (Sgroi *et al.*, 2022), Croatia (0.17%) (Balić *et al.*, 2020), and some other countries, where *Trichinella* parasites were not detected (Hatta *et al.*, 2017; Dimzas *et al.*, 2021). The different prevalence levels of *Trichinella* infection in wild boars could be ascribed to differences in management conditions, food source, geographic region, climatic conditions, living environments, parasite species and animal welfare. Furthermore, the possibility that low infection could have passed unnoticed because the tested frozen meat drastically reduce the larvae recovery and easily lead to false negatives, although we attempted to increase the weight of frozen samples along with the sedimentation time to compensate reduction in test sensitivity (Gajadhar *et al.*, 2019).

Although no *Trichinella* species were detected in Gongzhuling city in this study, adequate supervisions are also necessary to control circulation of the worm from farmed wild boars to humans. The tested farmed wild boars were all reared on non-controlled, small farms. Under this type of system, the animals would be exposed to vehicles that transfer *Trichinella* from the sylvatic environment to the domestic cycle, such as synanthropic rodents, mustelids, or other small carnivorous and omnivorous mammals (Pozio, 2015).

In the present study, only *T. spiralis* was identified using the multiplex PCR method in the farmed wild boars. *T. spiralis* is the most common epidemic strain in China and is the exclusive species in pigs (Bai *et al.*, 2017). In this study, *T. spiralis* was reported for the first time in Jilin Province, where in the past *T. nativa* was identified in dogs (Liu and Boireau, 2002), demonstrating the co-presence of the 2 species in this province.

T. spiralis isolated from farmed wild boars in the present data indicates a small but uncontrollable risk associated with transfer to local residents. The low burdens of *Trichinella* from the diaphragm, the predilection site, of wild boars would indicate a negligible load in the rest of the carcass, contributing to the low risk for transmission. The risks come from failure to valid monitor *Trichinella* infection in wild boars consumed in China. No human clinical cases of trichinellosis have been reported in Jilin Province due to ingestion of wild boar meat infected by *Trichinella* larvae. The possible reasons are (1) the number of slaughtered farmed wild boars has not been very large in China in the last several years compared to 50 million tons of pork per year; (2) a relatively small proportion of carcasses is eaten by consumers as fresh meat because well-cooked food is more popular for Chinese individuals; and (3) asymptomatic infections due to consumption of positive meat are ignored in clinical practice. Cases of human trichinellosis in Jilin Province were reported 20 years ago (Liu *et al.*, 2002). As the consumption of meat from farmed wild boars is predicted to increase in the future in China, an improvement in breeding conditions as well as an increase in veterinary controls is needed to reduce the risk of human infection. Also, it is suggested to implementing education of farmers and the public on good farming practices and responsible consumption.

Conclusions

In Jilin Province, China, *T. spiralis* was firstly detected in farmed wild boars. The prevalence rate was found to be 0.53%, indicating a low yet underestimated danger given the existing breeding conditions. This incident highlights the significance of creating unique regulations for China's processing of wild boar meat from farms. The frequency of *Trichinella* infection in farmed wild boars needs to be further investigated nationwide.

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Author's contributions

NZZ and BQF conceived and designed the study. MW, WGC, HRZ and WYG collected and prepared samples. WHL gained the GenBank accession number. TTL performed statistical analyses. NZZ, MW and WGC prepared figures, prepared tables, wrote original drafts. TTHD, HY, NTBT and BQF edited and reviewed manuscript drafts.

Data availability statement. No additional data available.

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Competing interests. None.

Ethical standards. All procedures involving animals in the present study were approved and this study was approved by the Ethics Committee of the Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (Approval No. LVRIAEC2017-028).

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