

Research Article

Cite this article: Zhang N-Z *et al.* (2024). Occurrence of *Trichinella spiralis* in farmed wild boars (*Sus scrofa*): an underrated risk in China. *Parasitology* **151**, 1351–1354. <https://doi.org/10.1017/S0031182024001288>

Received: 1 August 2024
Revised: 10 September 2024
Accepted: 25 September 2024
First published online: 3 December 2024



Keywords:

Artificial digestion; multiplex PCR; *Trichinella spiralis*; wild boars

Corresponding authors:

Nian-Zhang Zhang;
Email: nianzhang919@163.com;
Nguyen Thi Bich Thuy;
Email: bthuy.nivr@gmail.com;
Bao-Quan Fu;
Email: fubaoquan@caas.cn

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

Nian-Zhang Zhang¹ , Meng Wang¹, Wei-Gang Chen¹, Wen-Yan Gai², Thanh Thi Ha Dao³, Ting-Ting Li¹, Wen-Hui Li¹, Hai-Rui Zhang⁴, Hong Yin^{1,5}, Nguyen Thi Bich Thuy³ and Bao-Quan Fu^{1,5} 

¹State Key Laboratory for Animal Disease Control and Prevention, College of Veterinary Medicine, Lanzhou University, Key Laboratory of Veterinary Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China; ²Department of Pet Technology, Shandong Vocational Animal Science and Veterinary College, Weifang, China; ³National Institute of Veterinary Research, Hanoi, Vietnam; ⁴Animal Husbandry and Veterinary Management Service Center of Wudi County, Binzhou, China and ⁵Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Disease, Yangzhou, China

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).

© The Author(s), 2024. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives licence (<http://creativecommons.org/licenses/by-nc-nd/4.0>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided that no alterations are made and the original article is properly cited. The written permission of Cambridge University Press must be obtained prior to any commercial use and/or adaptation of the article.



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 north-eastern 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) \pm 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 GoldenViewTM.

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 $25\ \mu\text{L}$ contained $2\ \mu\text{L}$ of DNA, $12.5\ \mu\text{L}$ of $2 \times$ UniqueTM Taq MasterMix (Novogene, China) and each primer at $10\ \mu\text{M}$. 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

Areas	Collected samples	Age		Gender	
		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

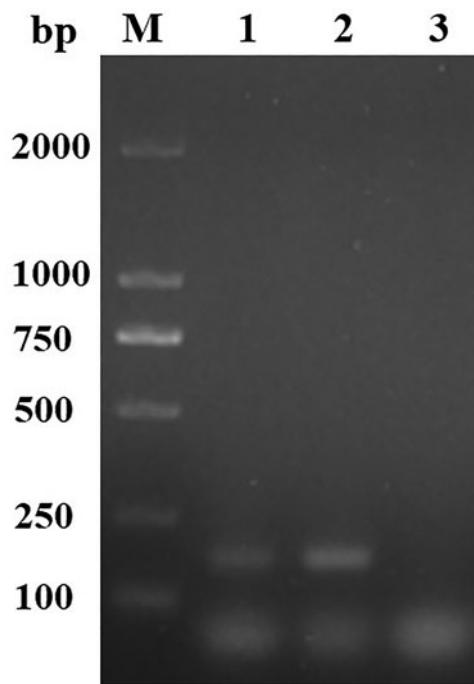


Figure 2. Identification of *Trichinella* isolates by PCR. M: DL2000 DNA marker; line 1: Multiplex PCR amplification of single larvae of *Trichinella* isolates in this study; line 2: Multiplex PCR amplification of single larvae of the *T. spiralis* Henan strain; line 3: Blank control.

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.

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.

Financial support. This study was funded by the National Key Research and Development Program of China (2023YFD1801000); Fundamental Research Funds for the Central Universities (Grant Number: lzujbky-2022-35); Science and Technology Major Project of Gansu Province (Grant Number: 22ZD6NA001); Natural Science Foundation of Gansu Province, China (Grant Number: 21JR7RA021) and Innovation Project for Scientific and Technological Youth Talents of Lanzhou City (2023-QN-21).

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).

References

- Bai X, Hu X, Liu X, Tang B and Liu M (2017) Current research of trichinellosis in China. *Frontiers in Microbiology* **8**, 1472. doi: 10.3389/fmicb.2017.01472.
- Balić D, Marucci G, Agičić M, Benić M, Krovina Z, Miškić T, Aladić K and Škrivanko M (2020) *Trichinella* spp. in wild boar (*Sus scrofa*) populations in Croatia during an eight-year study (2010–2017). *One Health (Amsterdam, Netherlands)* **11**, 100172. doi: 10.1016/j.onehlt.2020.100172.
- Cohen M, Costantino SN, Calcagno MA, Blanco GA, Pozio E and Venturiello SM (2010) *Trichinella* infection in wild boars (*Sus scrofa*) from a protected area of Argentina and its relationship with the presence of humans. *Veterinary Parasitology* **169**, 362–366. doi: 10.1016/j.vetpar.2010.01.005.
- Conlan JV, Vongxay K, Khamlome B, Gomez-Morales MA, Pozio E, Blacksell SD, Fenwick S and Thompson RC (2014) Patterns and risks of *Trichinella* infection in humans and pigs in northern Laos. *PLoS Neglected Tropical Diseases* **8**, e3034. doi: 10.1371/journal.pntd.0003034.
- Cui J, Wang ZQ and Kennedy MW (2006) The re-emergence of trichinellosis in China? *Trends in Parasitology* **22**, 54–55. doi: 10.1016/j.pt.2005.12.006.
- Dimzas D, Chassalevris T, Ozolina Z, Dovas CI and Diakou A (2021) Investigation of the food-transmitted parasites *Trichinella* spp. and *Alaria* spp. in wild boars in Greece by classical and molecular methods and development of a novel real-time PCR for *Alaria* spp. detection. *Animals (Basel)* **11**, 2803. doi: 10.3390/ani11102803.
- Fu BQ, Liu MY, Yao CY, Li WH, Li YG, Wang YH, Wu XP, Zhang DL, Cai XP, Blaga R and Boireau P (2009) Species identification of *Trichinella* isolates from China. *Veterinary Parasitology* **159**, 214–217. doi: 10.1016/j.vetpar.2008.10.033.
- Gajadhar AA, Noeckler K, Boireau P, Rossi P, Scandrett B and Gamble HR (2019) International commission on trichinellosis: recommendations for quality assurance in digestion testing programs for *Trichinella*. *Food and Waterborne Parasitology* **16**, e00059. doi: 10.1016/j.fawpar.2019.e00059.
- Gamble HR, Bessonov AS, Cuperlovic K, Gajadhar AA, Van Knapen F, Noeckler K, Schenone H and Zhu X (2000) International commission on trichinellosis: recommendations on methods for the control of *Trichinella* in domestic and wild animals intended for human consumption. *Veterinary Parasitology* **93**, 393–408. doi: 10.1016/s0304-4017(00)00354-x.
- Haim M, Efrat M, Wilson M, Schantz PM, Cohen D and Shemer J (1997) An outbreak of *Trichinella spiralis* infection in southern Lebanon. *Epidemiology and Infection* **119**, 357–362. doi: 10.1017/s0950268897007875.
- Hatta T, Imamura K, Yamamoto T, Matsubayashi M, Tsuji N and Tsutsui T (2017) A large-scale survey of *Trichinella* spp. infection in Japanese wild boars. *Japanese Journal of Infectious Diseases* **70**, 219–220. doi: 10.7883/yoken.JJID.2016.258.
- Hidalgo A, Villanueva J, Becerra V, Soriano C, Melo A and Fonseca-Salamanca F (2019) *Trichinella spiralis* infecting wild boars in southern Chile: evidence of an underrated risk. *Vector Borne and Zoonotic Diseases* **19**, 625–629. doi: 10.1089/vbz.2018.2384.
- Kärssin A, Häkkinen L, Vilem A, Jokelainen P and Lassen B (2021) *Trichinella* spp. in wild boars (*Sus scrofa*), brown bears (*Ursus arctos*), Eurasian Lynxes (*Lynx lynx*) and badgers (*Meles meles*) in Estonia, 2007–2014. *Animals (Basel)* **11**, 183. doi: 10.3390/ani11010183.
- Kirjušina M, Deksnė G, Marucci G, Bakasejevs E, Jahundoviča I, Daukste A, Zdanovska A, Bērziņa Z, Esīte Z, Bella A, Galati F, Krūmiņa A and Pozio E (2015) A 38-year study on *Trichinella* spp. in wild boar (*Sus scrofa*) of Latvia shows a stable incidence with an increased parasite biomass in the last decade. *Parasites and Vectors* **8**, 137. doi: 10.1186/s13071-015-0753-1.
- Liu M and Boireau P (2002) Trichinellosis in China: epidemiology and control. *Trends in Parasitology* **18**, 553–556. doi: 10.1016/s1471-4922(02)02401-7.
- Nicorescu IM, Ionita M, Ciupescu L, Buzatu CV, Tanasuica R and Mitrea IL (2015) New insights into the molecular epidemiology of *Trichinella* infection in domestic pigs, wild boars, and bears in Romania. *Veterinary Parasitology* **212**, 257–261. doi: 10.1016/j.vetpar.2015.07.021.
- Pozio E (2014) Searching for *Trichinella*: not all pigs are created equal. *Trends in Parasitology* **30**, 4–11. doi: 10.1016/j.pt.2013.11.001.
- Pozio E (2015) *Trichinella* spp. imported with live animals and meat. *Veterinary Parasitology* **213**, 46–55. doi: 10.1016/j.vetpar.2015.02.017.
- Pozio E (2021) Scientific achievements of the last 60 years: from a single to a multispecies concept of the genus *Trichinella*. *Veterinary Parasitology* **297**, 109042. doi: 10.1016/j.vetpar.2020.109042.
- Pozio E and La Rosa G (2003) PCR-derived methods for the identification of *Trichinella* parasites from animal and human samples. *Methods in Molecular Biology* **216**, 299–309. doi: 10.1385/1-59259-344-5-299.
- Ribicich MM, Fariña FA, Aronowicz T, Ercole ME, Bessi C, Winter M and Pasqualetti MI (2020) A review on *Trichinella* infection in South America. *Veterinary Parasitology* **285**, 109234. doi: 10.1016/j.vetpar.2020.109234.
- Rostami A, Gamble HR, Dupouy-Camet J, Khazan H and Bruschi F (2017) Meat sources of infection for outbreaks of human trichinellosis. *Food Microbiology* **64**, 65–71. doi: 10.1016/j.fm.2016.12.012.
- Sgroi G, D'Alessio N, Marucci G, Pacifico L, Buono F, Deak G, Anastasio A, Interisano M, Fraulo P, Pesce A, Toscano V, Romano AC, Toce M, Palazzo L, De Carlo E, Fioretti A and Veneziano V (2022) *Trichinella britovi* in wild boar meat from Italy, 2015–2021: a citizen science approach to surveillance. *One Health (Amsterdam, Netherlands)* **16**, 100480. doi: 10.1016/j.onehlt.2022.100480.
- Szél Z, Marucci G, Ludovisi A, Gómez-Morales MA, Sréter T and Pozio E (2012) Spatial distribution of *Trichinella britovi*, *T. spiralis* and *T. pseudospiralis* of domestic pigs and wild boars (*Sus scrofa*) in Hungary. *Veterinary Parasitology* **183**, 393–396. doi: 10.1016/j.vetpar.2011.07.035.
- Thi NV, Nguyen VD, Praet N, Claes L, Gabriël S, Huyen NT and Dorny P (2014) *Trichinella* infection in wild boars and synanthropic rats in north-west Vietnam. *Veterinary Parasitology* **200**, 207–211. doi: 10.1016/j.vetpar.2013.11.011.
- Vieira-Pinto M, Fernandes ARG, Santos MH and Marucci G (2021) *Trichinella britovi* infection in wild boar in Portugal. *Zoonoses and Public Health* **68**, 103–109. doi: 10.1111/zph.12800.
- Wang ZQ, Cui J and Xu BL (2006) The epidemiology of human trichinellosis in China during 2000–2003. *Acta Tropica* **97**, 247–251. doi: 10.1016/j.actatropica.2005.03.012.
- Yera H, Bory S, Khieu V and Caron Y (2022) Human trichinellosis in Southeast Asia, 2001–2021. *Food and Waterborne Parasitology* **28**, e00171. doi: 10.1016/j.fawpar.2022.e00171.
- Zarlenga DS, Chute MB, Martin A and Kapel CM (1999) A multiplex PCR for unequivocal differentiation of all encapsulated and non-encapsulated genotypes of *Trichinella*. *International Journal for Parasitology* **29**, 1859–1867. doi: 10.1016/s0020-7519(99)00107-1.
- Zhang NZ, Zhang XX, Zhou DH, Huang SY, Tian WP, Yang YC, Zhao Q and Zhu XQ (2015) Seroprevalence and genotype of *Chlamydia* in pet parrots in China. *Epidemiology and Infection* **143**, 55–61. doi: 10.1017/S0950268814000363.
- Zhang XZ, Wang ZQ and Cui J (2022) Epidemiology of trichinellosis in the People's Republic of China during 2009–2020. *Acta Tropica* **229**, 106388. doi: 10.1016/j.actatropica.2022.106388.