Seroprevalence of *Trichinella* sp. in Wild Boars (*Sus scrofa*) from Yanggu-gun, Gangwon-do, Korea

Hye-Jung Lee¹, Ok-Sik Chung², Jae-Lip Kim³, Seung-Ha Lee⁴, Young-Bok Yoo⁵, Min Seo¹*  
¹Department of Parasitology and Research Center for Mummy, College of Medicine, Dankook University, Cheonnan 330-714, Korea; ²Division of Ecology and Environment, Chungnam Development Institute, Gongju 314-140, Korea; ³Department of Parasitology and Tropical Medicine, and Institute of Infectious Diseases, Seoul National University College of Medicine, Seoul 110-799, Korea; ⁴Department of Biomedical Engineering, College of Medicine, Dankook University, Cheonnan 330-714, Korea; ⁵Department of Anatomy, College of Medicine, Dankook University, Cheonnan 330-714, Korea

**Abstract:** A total 7 outbreaks of trichinellosis have occurred in Korea, mostly as a result of consumption of raw wild boar (*Sus scrofa*) meat. Since only 1 serological survey on wild boars had yet been performed in Korea, the present study aimed to estimate the prevalence of trichinellosis in wild boars and some species of rodents by artificial digestion and serological examinations in Yanggu-gun, Gangwon-do, the endemic area of trichinellosis. Both the wild boar and rodent muscle samples revealed no *Trichinella* larvae by direct examination and artificial digestion method. However, serological examinations revealed that 4 wild boar sera samples out of 118 (3.4%) were positive to *Trichinella* antigen. Although the recovery of *Trichinella* larvae ended in a failure, it is proved for the first time that the sylvatic cycle of *Trichinella* has been maintained in wild boars of Gangwon-do, Korea.

**Key words:** *Trichinella*, wild boar, rodent, serology, Korea

Trichinellosis is a widespread zoonotic disease caused by *Trichinella* spp. infection. Humans are afflicted through consumption of undercooked meat of domestic animals or wildlife [1]. *Trichinella* has been reported in both domestic and wild animals in 66 countries, and human trichinellosis has been documented in 55 countries [2]. *Trichinella* spp. parasitize predominantly wild animals, and a switch from wild animals to domestic animals can occur due to improper management in segregating husbandry and wildlife. Hence, knowledge of *Trichinella* prevalence in wild animals is useful from an epidemiological perspective, and various pertinent studies have been performed in endemic countries. The annual prevalence of trichinellosis in wild boars during the 2002-2008 periods ranged from 0.0027% to 0.0032% in Germany, and was 0.0077% and 11.4% in Hungary and Argentina, respectively [3-5]. The ELISA positivity of wild boars was 8.7% in Switzerland and 4% in France, without discovery of *Trichinella* larvae [1,6].

In the Republic of Korea (= Korea), from the first outbreak in 1997 to 2013, total 52 cases in 7 outbreaks were recorded [7-13]. All of them were infected with *Trichinella* spp. through consumption of wild animals such as badger, wild boars, or soft-shelled turtle [7-13], suggesting the sylvatic cycle of *Trichinella* spp. in Korea. Although *Trichinella* larvae were once recovered from the leftover raw wild boar meat in an outbreak [12], the natural *Trichinella* infection status remained unknown for a long time in Korea, due to the absence of a proper survey. The serological surveillance on the pig breeding farms revealed that they were trichinellosis-free [14], but the survey on wild animals has been performed only once in Korea, by serology. The sera of wild boars hunted in 8 provinces of Korea were seropositive for *T. spiralis* by 1.7% [15]. In that study, *T. spiralis* was detected from 4 provinces of Jeonnam, Gyeongnam, Gyeonggi, and Gyeongbuk, showing the positivity in the range from 2.3% to 3.4% [15]. However, the absence of *T. spiralis*-positive wild boars in Gangwon-do is strange considering that most trichinellosis outbreaks have occurred in that province [9-12]. Furthermore, direct examination of wild boar muscles had not been performed in Korea, and no such survey of wild animals, prior to the present investigation, had been performed. Thus, the present study aimed to estimate the prevalence of trichinellosis in wild boars and a few species of rodents using the
methods of direct detection, artificial digestion, and serological examination, targeting Gangwon-do, the endemic area of trichinellosis.

From July 2011 to December 2012, volunteer hunters residing in Yanggu-gun, Gangwon-do caught wild boars (Sus scrofa) on the mountains near their residences. The total number of wild boars was 118, all of which were taken in Yanggu-gun. Muscle specimens removed from the foreleg and hind leg of each animal, were transported along with blood samples to a laboratory set-up for the purposes of the present study. The average weight of removed muscle was 333 g (126-710 g). A total of 54 rodents were caught at the foot of a mountain in Yanggu-gun using traps; 41 striped field mice (Apodemus agrarius coreae), 12 Korean wood mice (Apodemus peninsulae peninsulae), 1 red-backed vole (Clethrionomys rufocanus), and 1 squirrel (Tamias sibiricus). All of their muscles were collected for Trichinella examination.

Muscle larvae detection was undertaken by direct examination, artificial digestion, and serological analysis. The muscle samples removed from each wild boar and rodent, numbering 20 and 10, respectively, were observed by the pressure method using 2 slide glasses. Subsequently, all of the remaining muscle tissue of each animal was finely trimmed with a pair of scissors, ground in a mortar with pestle, and digested at 37°C for 2 hr in the digestive solution. The digestive solution was 6 g of pepsin and 8 ml of HCl in 1,000 ml of distilled water, and the ratio of muscle weight to the digestive solution was 100 g:1,000 ml. To eliminate impurities, the meat juice was passed through 500 µm and 250 µm sieves, and the content thus purified was investigated under a stereomicroscope (Olympus). Finally, wild boar sera samples were obtained and stored at -70°C until detection of specific antibodies against Trichinella antigens by ELISA at the Department of Parasitology, Seoul National University College of Medicine, Seoul, Korea. The sera were 1:200 diluted in PBS-Tween; IgG whole molecule polyclonal antibody (MP Biomedicals, Irvin, California, USA) was used as the conjugate for wild boar antibodies. The positivity criterion was over 0.250 in optical density.

Both the wild boar and rodent muscle samples revealed no Trichinella larvae by direct examination and artificial digestion method. Unidentified nematode larvae were recovered from 9 wild boar muscle samples (figure not shown). They were 804 (690-980) µm in length and 29 (26-32) µm in width, and were found to be slowly moving in saline. Their cuticle was thick, and had transverse striations. The pharynx was visible in the anterior part, but stichocytes were not found. Subsequently, multiplex PCR was performed on those larvae by using primers targeted for 7 Trichinella species, i.e., T. spiralis, T. native, T. britovi, T. pseudospiralis, T5, T6, and T. nelsoni [16], revealing that they were not belonged to any of them. However, serological examinations revealed that 4 wild boar sera samples out of 118 (3.4%) were positive, and their ODs were distributed in the range of 0.245 to 0.335.

This study intended to provide the first such report on the prevalence of Trichinella in wild boars specifically from Yanggu-gun, Gangwon-do, where 5 outbreaks have occurred. However, the recovery of Trichinella larvae failed. Instead, ELISA showed that 4 wild boar sera were positive for T. spiralis, although the actual presence of larvae was not proved in this survey. Among the investigative methods suitable for Trichinella larvae, artificial digestion is the most sensitive, efficient, and reliable method [17]. The average amount of digested muscle tissues examined in the present study was over 300 g on average, higher than in previous studies [1,5,18]. Although ELISA might be positive at a low level such as 1 larva per 100 g of muscle of a wild boar [19], it was reasonable that larvae of T. spiralis did not exist in present samples, even in the seropositive samples. This low infection rate might explain the rare occurrence of Trichinella outbreaks in Korea although the consumption of raw wild boar meat is quite widely distributed in Korea (Table 1).

As for wild boars, the results of low seroprevalence and no Trichinella larvae have frequently been observed in various Eu-

| Province          | Samples                        | No. examined | No. positive (%) | Reference |
|-------------------|--------------------------------|--------------|------------------|-----------|
| Unknown           | Pigs from 7 breeding farms     | 803          | 0 (0)            | [14]      |
| Gyeonggi, Chungbuk| Wild boars                     | 521          | 9 (1.7)          | [15]      |
| Chungnam, Gyeongbuk, Gyeongnam, Gangwon, Jeonnam, Jeonbuk | Wild boars | 118          | 4 (3.4)          | Present study |

*1 sample was positive by ELISA, but later proved to be negative by western blot analysis.
ropean countries such as France, Germany, and Switzerland [1,6,20]. In these cases, the serological results can be interpreted as an indicator of population exposure to T. spiralis, highlighted in wildlife monitoring programme by EU [21]. While previous Korean studies on wild boars showed no seropositivity in Gangwon-do [15], the present study showed 3.4% of seroprevalence in wild boars caught in Yanggu-gun, Gangwon-do (Table 1). This supported the presumption that sylvatic cycle for Trichinella has also been maintained in wild boars of Gangwon-do, although the infection rate was very low. Besides the wild boars, other mammalian hosts for T. spiralis should be necessary to maintain the sylvatic cycle, but inspection of the rodent muscle samples revealed no Trichinella larvae in the present study. Since Trichinella had been found in 14 animal species such as rats, raccoons, yellow weasels in China, and in other mammals such as minks and Siberian weasels in Japan [22,23], examination of samples from other animals is urgently required for determination of the sylvatic cycle of Trichinella spp. in Korea.

Until the present time, the genus Trichinella consists of 9 different species, i.e., T. spiralis, T. nativa, T. britovi, T. pseudospiralis, T. murrelli, T. nelsoni, T. papuae, T. zimbabwensis, and T. patagoniensis, and 4 genotypes, i.e., T5, T6, T8, and T9 [24]. Among them, T. spiralis has been the only species confirmed to be distributed in Korea. The muscle larvae from 2 Korean outbreaks were analyzed by PCR-RFLP, and showed a band for T. spiralis larvae [25]. Considering the existence of 2 Trichinella spp. in China (i.e., T. spiralis and T. nativa) [26], it would not be unreasonable to posit that another species of Trichinella might be distributed in Korea. The recovery of Trichinella larvae from wild animals and molecular-based species identification studies are required before the question can be settled.

From this study, it could be concluded that the sylvatic cycle of T. spiralis has been maintained in wild boars of Gangwon-do, and that further research on wild animals is urgently required.

ACKNOWLEDGMENT

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (MEST) (2011-0009312).

CONFLICT OF INTEREST

We have no conflict of interest related to this work.

REFERENCES

1. Richomme C, Lacour SA, Ducrot C, Gilot-Fromont E, Casabianca E Maestrini O, Vallée I, Grasset A, van der Giessen J, Boireau P. Epidemiological survey of trichinellosis in wild boar (Sus scrofa) and fox (Vulpes vulpes) in a French insular region, Corsica. Vet Parasitol 2010; 172: 150-154.

2. Murrell KD, Pozio E. Worldwide occurrence and impact of human trichinellosis, 1986-2009. Emerg Infect Dis 2011; 17: 2194-2202.

3. Szell Z, Marucci G, Ludovisi A, Gómez-Morales MA, Sréter T, Pozio E. Spatial distribution of Trichinella britovi, T. spiralis and T. pseudospiralis of domestic pigs and wild boars (Sus scrofa) in Hungary. Vet Parasitol 2012; 183: 393-396.

4. Pannwitz G, Myer-Scholl A, Balicka-Ramisz A, Nöckler K. Increased prevalence of Trichinella spp., northeastern Germany, 2008. Emerg Infect Dis 2010; 16: 936-942.

5. Cohen M, Costantino SN, Calcagno MA, Blanco GA, Pozio E, Venturiello SM. Trichinella infection in wild boars (Sus scrofa) from a protected area of Argentina and its relationship with the presence of humans. Vet Parasitol 2010; 169: 362-366.

6. Gottstein B, Pozio E, Connolly B, Gamble HR, Eckert J, Jakob HP. Epidemiological investigation of trichinellosis in Switzerland. Vet Parasitol 1997; 72: 201-207.

7. Sohn WM, Kim HM, Chung DI, Yee ST. The first human case of Trichinella spiralis infection in Korea. Korean J Parasitol 2000; 38: 111-115.

8. Lee HC, Kim JS, Oh HY, Kim JH, Kim HG, Lee MS, Kim WJ, Kim HT. A case of trichinosis caused by eating a wild badger. Korean J Med 1999; 56: 134-138.

9. Kim E, Pyun RH, Park JH, Kim KH, Choi I, Park HH, Lee YH, Yong TS, Hong SK. Family outbreak of trichinosis after eating a raw meat of wild swine. Infect Chemother 2003; 35: 180-184.

10. Hur GI, Hwang BI, Lee JG, Lee MG, Cheong HJ, Cho SW, Joo KH. An outbreak of trichinellosis caused by ingestion of raw wild boar. Korean J Med 2004; 67 (Suppl 3): S97-S922.

11. Rhee JY, Hong ST, Lee HJ, Seo M, Kim SB. The fifth outbreak of trichinosis in Korea. Korean J Parasitol 2011; 49: 405-408.

12. Kim G, Choi MH, Kim JH, Kang YM, Jeon HJ, Jung Y, Lee MJ, Oh MD. An outbreak of trichinellosis with detection of Trichinella larvae in leftover wild boar meat. J Korean Med Sci 2011; 26: 1630-1633.

13. Lee SR, Yoo SH, Kim HS, Lee SH, Seo M. Trichinosis caused by ingestion of raw soft-shelled turtle meat in Korea. Korean J Parasitol 2013; 51: 219-221.

14. Wee SH, Lee CG, Joo HD, Kang VB. Enzyme-linked immunosorbent assay for detection of Trichinella spiralis antibodies and the surveillance of selected pig breeding farms in the Republic of Korea.
15. Kang SW, Doan HT, Noh JH, Choe SE, Yoo MS, Kim YH, Reddy KE, Nguyen TT, Van Quyen D, Nguyen LT, Kweon CH, Jung SC. Seroprevalence of *Toxoplasma gondii* and *Trichinella spiralis* infections in wild boars (*Sus scrofa*) in Korea. Parasitol Int 2013; 62: 583-585.

16. Zarlenga DS, Chute MB, Martin A, Kapel CM. A multiplex PCR for unequivocal differentiation of all encapsulated and non-encapsulated genotypes of *Trichinella*. Int J Parasitol 1999; 29: 1859-1867.

17. Gottstein B, Pozio E, Nöckler K. Epidemiology, diagnosis, treatment and control of trichinellosis. Clin Microbiol Rev 2009; 22: 127-145.

18. Li F, Cui J, Wang ZQ, Jiang P. Sensitivity and optimization of artificial digestion in the inspection of meat for *Trichinella spiralis*. Foodborne Pathog Dis 2010; 7: 879-885.

19. Kapel CM. Sylvatic and domestic *Trichinella* spp. in wild boars: infectivity, muscle larvae distribution, and antibody response. J Parasitol 2001; 87: 309-314.

20. Nöckler K, Reckinger S, Pozio E. *Trichinella spiralis* and *Trichinella pseudospiralis* mixed infection in a wild boar (*Sus scrofa*) of Germany. Vet Parasitol 2006; 137: 364-368.

21. Gajadhar AA, Pozio E, Gamble HR, Nöckler K, Maddox-Hyttel C, Forbes LB, Vallée I, Rossi P, Marinculis A, Boireau P. *Trichinella* diagnostics and control: mandatory and best practices for ensuring food safety. Vet Parasitol 2009; 159: 197-205.

22. Liu M, Boireau P. Trichinellosis in China: epidemiology and control. Trends Parasitol 2002; 18: 553-556.

23. Kobayashi T, Kanai Y, Ono Y, Matoba Y, Suzuki K, Okamoto M, Taniyama H, Yagi K, Oku Y, Katakura K, Asakawa M. Epidemiology, histopathology, and muscle distribution of *Trichinella* T9 in feral raccoons (*Procyon lotor*) and wildlife of Japan. Parasitol Res 2007; 100: 1287-1291.

24. Pozio E, Zarlenga DS. New pieces of the *Trichinella* puzzle. Int J Parasitol 2013; 43: 983-997.

25. Sohn WM, Huh S, Chung DI, Pozio E. Molecular identification of Korean *Trichinella* isolates. Korean J Parasitol 2003; 41: 125-127.

26. Fu BQ, Liu MY, Yao CY, Li WH, Li YG, Wang YH, Wu XP, Zhang DL, Cai XP, Blaga R, Boireau P. Species identification of *Trichinella* isolates from China. Vet Parasitol 2009; 159: 214-217.