

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

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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 wild-life [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

•Received 19 August 2014, revised 25 December 2014, accepted 21 January 2015.

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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-

Table 1. Seroprevalence of *Trichinella spiralis* in animals performed in Korea

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

^a1 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.

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