

Infections of Intestinal Helminth at Two Species of Field Mice, *Apodemus agrarius* and *A. Peninsulæ*, in Gangwon-do and Chungcheongnam-do, Korea

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Abstract: Rodents are important reservoirs of diseases affecting people and livestock, and are major sources of parasite contamination of agricultural products. We surveyed the infection status of intestinal helminths in 2 species of field mice, *Apodemus agrarius* and *A. peninsulæ*, captured in the agricultural fields of Gangwon-do and Chungcheongnam-do, Korea. Total 83 mice (57 *A. agrarius* and 26 *A. peninsulæ*) were collected in 2 surveyed areas, and the intestines of each mouse were opened with scissors, and then intestinal contents were examined with microscope. Total 6 species of intestinal helminth were detected in 61 (73.5%) out of 83 mice examined. Four species of nematode, i.e., *Nippostrongylus brasiliensis*, *Aspicularis tetraptera*, *Heterakis* spp. and ascarid, were found in 40 (48.2%), 14 (16.9%), 11 (13.3%) and 13 (15.7%) mice respectively. One species of cestode, *Hymenolepis diminuta* and 1 unidentified egg were also detected in the intestines of 14 (16.9%) and 1 (1.2%) mice, respectively. Conclusively, this study identified 5 helminth species in the gastrointestinal tracts of wild rodents captured in some areas in central and northern Korea, and *N. brasiliensis* was the most prevalent (dominant) species rather than zoonotic ones.

Key words: Intestinal helminth, wild rodent, Gangwon-do, Chungcheongnam-do

Rodents are vital components of various ecosystems, either as prey or as disease carriers and reservoirs. Parasite eggs disseminate via rodent droppings on agricultural fields, stored grains, and household foodstuff, and are responsible for the spread of various diseases [1,2]. There were some reports about rodent parasites in Korea [3-8]. Sohn et al. [3] identified more than 11 species of intestinal helminths (4 nematode spp., 5 trematode spp., 1 cestode spp. and unidentified egg) in *Apodemus agrarius* captured in Hapcheon-gun, Gyeongsangnam-do, Gurye-gun, and Jeollanam-do, Korea. Lee et al. [4] detected three nematode, 2 trematode, and 2 cestode species in the intestines of *A. agrarius* and *A. peninsulæ* captured in northern Gangwon-do, Korea. Chai et al. [5,6] reported *A. agrarius* infection with *Plagiorchis muris* and echinostomes near the demilitarized zone (DMZ) in northern Gyeonggi Province. Recently,

seven nematode species were identified from the gut contents and intestinal tracts of small mammals, including *A. agrarius*, captured near the DMZ [7]. However, most of the current surveys involve wild rodent parasites found near houses or the DMZ, while data on wild rodents captured in agricultural fields near the mountains are lacking. Rodents are responsible for the spread of various zoonotic diseases and also important reservoirs of various diseases affecting people and livestock. Several investigators have studied rodent parasites from southern regions and northern areas in Korea [3-8], however, data on wild rodents captured in central regions and agricultural fields near the mountains are lacking. Therefore, this study was attempted to provide preliminary data on the intestinal helminth fauna of wild rodents inhabiting agricultural fields near the mountains in Gangwon-do (Province) and Chungcheongnam-do, Korea.

To evaluate the role of wild rodents in agricultural product contamination, we selected survey sites in rural agricultural fields beneath Chiak-san (mountain) and Gyeryong-san. The agricultural fields were located in Haenggu-dong, Wonju, Gangwon-do, Banpo-myeon Bonggog-ri, and Gongju, Chungcheongnam-do, which are located at the foothills. Beans are

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widely cultivated in the agricultural fields. Six 20-m-wide traps were set at each agricultural field. Rodents were captured alive using Sherman traps (H.B. Sherman, Tallahassee, Florida, USA) baited with peanut butter-coated saltine crackers in July-October 2015 and May 2017. The rodents caught were identified using taxonomic keys [1]. The captured rodents were euthanized with chloroform following the approved animal use protocol while maintaining biosafety. Animal experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Chungnam National University (CNU-00706). Necropsies were performed soon after capturing the animals. The gastrointestinal tract from the small intestine to the end of the rectum of each mouse was dissected. Then, the gut region was cut open and the contents were

preserved in 10% buffered neutral formalin solution along with fecal pellets. The helminthic worms were collected with naked eyes or under a stereomicroscope from the sediment of the intestinal content. After clearing, the collected helminths were identified using appropriate systematic keys [9,10]. The chi-square test and Fisher's exact test were used for statistical analyses of infection rates according to mouse species and captured areas. Statistical significance was determined using ANOVA (StatView; Abacus Concepts Inc., Berkeley, California, USA). All experiments were performed at least twice; P -values < 0.05 were considered to indicate statistically significant differences.

Table 1 summarizes the intestinal helminth infection status of mice captured in agricultural fields near mountains in Gangwon-do (52 mice) and Chungcheongnam-do (31 mice), Korea.

Table 1. Helminth infection in the intestines of mice captured in agricultural fields near mountain in Gangwon-do and Chungcheongnam-do, Korea according to rodent host

Helminth species	No. of infected mice (%)								
	<i>A. agrarius</i>			<i>A. peninsulae</i>			Total		
	Gangwon-do (n=34)	Chungcheongnam-do (n=23)	Subtotal (n=57)	Gangwon-do (n=18)	Chungcheongnam-do (n=8)	Subtotal (n=26)	Gangwon-do (n=52)	Chungcheongnam-do (n=31)	Total (n=83)
Nematodes									
<i>Nippostrongylus braziliensis</i>	17 (53.1)	11 (47.8)	28 (50.9)	7 (38.9)	5 (62.5)	12 (46.2)	24 (46.2)	16 (51.6)	40 (48.2)
<i>Aspicularis tetraptera</i>	6 (18.8)	3 (13.0)	9 (16.4)	3 (16.7)	2 (25.0)	5 (19.2)	9 (17.3)	5 (16.1)	14 (16.9)
Ascarid	4 (12.5)	3 (13.0)	7 (12.7)	2 (11.1)	4 (50.0)	6 (23.1) ^a	6 (11.5)	7 (22.6) ^b	13 (15.7)
<i>Heterakis</i> spp.	3 (9.4)	3 (13.0)	6 (10.9)	3 (16.7)	2 (25.0)	5 (19.2)	6 (11.5)	5 (16.1)	11 (13.3)
Cestodes									
<i>Hymenolepis diminuta</i>	6 (18.8)	3 (13.0)	9 (16.4)	2 (11.1)	3 (37.5)	5 (19.2)	8 (15.4)	6 (19.4)	14 (16.9)
Unidentified	0 (0.0)	1 (4.3)	1 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	1 (1.2)
Total	26 (81.2)	17 (73.9)	43 (78.2)	13 (72.2)	5 (62.5)	18 (69.2)	39 (75.0)	22 (71.0)	61 (73.5)

^aSignificant difference of ascarid infection rates between *A. peninsulae* and *A. agrarius*.

^bSignificant difference of ascarid infection rates between Gangwon-do and Chungcheongnam-do.

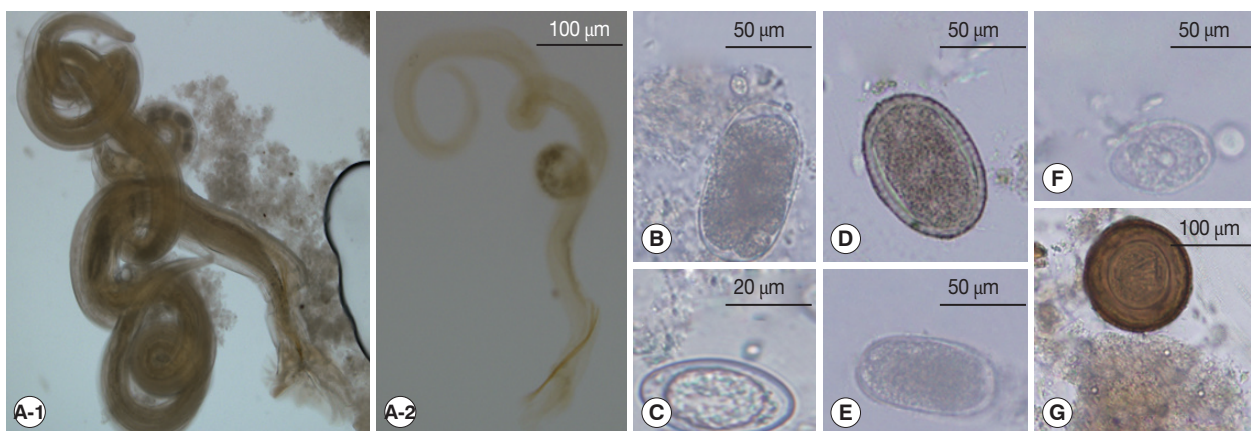


Fig. 1. Helminth and eggs from intestinal contents of wild rodents captured in the agricultural field near mountains in Gangwon-do (province) and Chungcheongnam-do, Korea. (A-1 and -2), *Nippostrongylus braziliensis* adult worms, (B) *A. braziliensis* egg, (C) *Aspicularis tetraptera* egg, (D) Ascarid eggs, (E) *Heterakis* spp. egg, (F) Unidentified egg, and (G) *Hymenolepis diminuta* egg.

Of the 83 captured mice, 61 (73.5%) had one or more types of helminths in their intestines, which included 39 of 52 (75.0%) and 22 of 31 (71.0%) mice captured in Gangwon-do and Chungcheongnam-do, respectively. Among mouse species, 78.2% of *A. agrarius* (n=57) and 69.2% of *A. peninsulae* (n=26) were infected with various species of intestinal helminths. Six helminth species (4 nematodes, 1 trematode, and 1 cestode species) were identified (Fig. 1). Among nematodes, *Nippostrongylus brasiliensis* (adult worms and eggs), *Aspiculuris tetraptera* (eggs), ascarid (eggs), and *Heterakis* spp. (eggs) were detected in 40 (48.2%), 14 (16.9%), 13 (15.7%), and 11 (13.3%) mice, respectively. The cestode *Hymenolepis diminuta* (eggs) was detected in 14 (16.9%) mice, and unidentified egg was also isolated. *N. brasiliensis* infection was more prevalent in *A. agrarius* than in *A. peninsulae* (50.9% vs 46.2%, $P > 0.05$), whereas *H. diminuta* and ascarid infections were more prevalent in *A. peninsulae* (19.2% vs 16.4% for *H. diminuta*, $P > 0.05$; 23.1% vs 12.7% for ascarids, $P < 0.05$). Comparison of capture regions showed that the prevalence of ascarid infection was much higher in Chungcheongnam-do (22.6%) than in Gangwon-do (11.5%) ($P < 0.05$). *N. brasiliensis* (48.2%) was the most prevalent nematode, followed by *H. diminuta* (16.9%), *A. tetraptera* (16.9%), ascarids (15.7%), *Heterakis* spp. (13.3%) and unidentified eggs (1.2%).

Rodents play important roles in seed and spore dispersal, pollination, seed predation, energy and nutrient cycling, plant succession, and species composition, and are food for many predators [1]. Few studies report the status of intestinal nematode infection in wild rodents in Korea [3-8]. Sohn et al. [3] identified four nematode species in *A. agrarius* in southern Korea: a heligmosomid, *Syphacia obvelata*, an ascarid, and a hookworm. Lee et al. [4] detected three nematode species in *A. agrarius* in Gangwon-do, Korea, namely, a hookworm, *Syphacia* sp., and *Plagiorchis muris*. Kim et al. [7] identified at least 1 nematode species per individual from *A. agrarius* captured near the DMZ in Gyeonggi-do, Korea, including *Capillaria* sp., *H. spumosa*, *Heligmosomoides polygyrus*, *N. brasiliensis*, *Protospirura muris*, *Rictularia affinis*, *S. obvelata*, and *Trichuris muris*.

In our study, 4 nematode species, namely, *N. brasiliensis*, *A. tetraptera*, ascarid, and *Heterakis* spp. were detected in *A. agrarius* and *A. peninsulae*, and *N. brasiliensis* was the dominant species. There were no significant capture site- or mouse species-specific differences in the prevalence of *N. brasiliensis* infection. The prevalence of *N. brasiliensis* infection in *A. agrarius* in our study (48.2%) was lower than that reported by Kim et al. (70.9%) [7]. The differences in nematode infection rate may

be closely related to the environment inhabited by the rodent hosts. We surveyed agricultural fields near mountains in central and northern Korea, whereas Kim et al. [7] studied sites in the demilitarized zone of Gyeonggi Province, Korea. Ascarids are intestinal nematodes commonly found in rodents. In this study, the ascarid infection rate was 15.7%, which was higher than that (2.5%) reported by Sohn et al. [3] and Lee et al. (11.6%) [4]. The higher prevalence in our study may be because the wild rodents were captured from agricultural fields, where contamination with stools of small mammals may be higher than in other areas. *Heterakis* is a genus of minute parasitic roundworms (pinworms) that is ubiquitous in rats and mice. In our study, 13.3% of the captured mice harbored intestinal *Heterakis* spp. This is similar to the observations of Kim et al. [7], who reported that 22.1% of *A. agrarius* captured in the DMZ in Gyeonggi Province were infected with *Heterakis* spp.

Unlike nematodes, trematodes and cestodes can be zoonotic pathogens. In Korea, *I. hortense*, *P. muris*, *H. diminuta*, and *H. nana* are common in wild rodents, which act as reservoir hosts for human infections [3-5,8,11]. In our survey, the infection rate of *H. diminuta* was 16.4% among *A. agrarius* and 19.2% among *A. peninsulae* captured in Chungcheongnam-do and Gangwon-do, while the dwarf tapeworm *H. nana* was not identified. This might be due to the relatively small number of mice examined.

In this study, the overall intestinal helminth infection rates of mice captured in agricultural fields near mountains were 71.0% and 75.0% in Chungcheongnam-do and Gangwon-do, respectively. These infection rates are lower than those of mice captured in southern Korea (97.0% in Hapcheon and 98.1% in Gurye) [3] and the DMZ in Gyeonggi Province (100%) [7], but higher than those of northern Korean mice (72.1% for *A. agrarius* and 63.6% for *A. peninsulae* in Gyeonggi and Gangwon Provinces, respectively) [4]. Although *A. peninsulae* had higher ascarid and *H. diminuta* infection rates than *A. agrarius*, the overall helminth infection rate of *A. peninsulae* (78.2%) was not significantly different from that of *A. agrarius* (69.2%).

From this study, it was confirmed that there were not significant differences of intestinal helminth infection rates between wild *A. agrarius* and *A. peninsulae*, and some species of rodent intestinal helminth, *N. brasiliensis*, *H. diminuta*, *A. tetraptera* and *Heterakis* spp. are prevalent rather than zoonotic ones. However, this study has some limitations such as the surveyed number of mice were not enough, and the parasite numbers were not presented quantitatively.

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CONFLICT OF INTEREST

We have no conflict of interest related to this study.

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