## Echinostome Infections in the Striped-Field Mouse, Apodemus agrarius, and the Ussuri White-Toothed Shrew, Crocidura lasiura, Caught Near the Demilitarized Zone, Gyeonggi-do (Province), Republic of Korea

Jong-Yil Chai<sup>1,\*</sup>, Jae-Hwan Park<sup>1,†</sup>, Bong-Kwang Jung<sup>1</sup>, Sang-Mee Guk<sup>1</sup>, Jae-Lip Kim<sup>1</sup>, Eun-Hee Shin<sup>1,2</sup>, Terry A. Klein<sup>3</sup>, Heung-Chul Kim<sup>4</sup>, Sung-Tae Chong<sup>4</sup>, Luck Ju Baek<sup>5</sup> and Jin-Won Song<sup>5</sup>

Department of Parasitology, Seoul National University College of Medicine, and Institute of Endemic Diseases, Seoul National University Medical Research Center, Seoul 110-799, Korea; Seoul National University Bundang Hospital, Gyeonggi-do 463-707, Korea; Forth Health Protection and Preventive Medicine, 65th Medical Brigade, Unit 15281, APO AP 96205-5281, Korea; Shedical Detachment, 168th Multifunctional Medical Battalion, 65th Medical Brigade, Unit 15247, APO AP 96205-5247, Korea; Department of Microbiology, College of Medicine, Korea University, Seoul 136-701, Korea

**Abstract:** A total of 1,498 small mammals (rodents and insectivores), including *Apodemus agrarius* (n = 1,366), *Crocidura lasiura* (54), *Mus musculus* (32), *Micronytus fortis* (28), *Eothenomys regulus* (9), *Micronys minutes* (6), and *Cricetulus triton* (3), were live-trapped in Gyeonggi-do (Province) (Paju-si, Pocheon-gun, and Yeoncheon-gun) near the demilitarized zone (DMZ) from December 2004 to September 2005. *A. agrarius* was found to be infected with 3 species of echinostomes (*Echinostoma hortense, Echinostoma cinetorchis*, and *Euparyphium murinum*), while *C. lasiura* was infected with 1 species (*Echinochasmus japonicas*) of echinostome. Other mammals were free from echinostome infections. Total 16 *E. hortense* were detected in 7 (0.5%) mice, 9 *E. cinetorchis* from 5 (0.4%), and 3 *E. murinum* from 2 (0.1%) out of 1.366 *A. agrarius* examined. *E. japonicus* was found only in 1 (1.9%; total 3 specimens) *C. lasiura*. These results demonstrate that *A. agrarius* and *C. lasiura*, inhabiting near the DMZ of Gyeonggi-do serve as the natural definitive hosts for several species of echinostomes, although their infection rates are low. This is the first record of natural infections of *A. agrarius* with *E. cinetorchis* and *C. lasiura* with *E. japonicus* in the Republic of Korea.

Key words: Echinostoma hortense, Echinostoma cinetorchis, Euparyphium murinum, Echinochasmus japonicus, striped field mouse, Apodemus agrarius, shrew. Crocidura lasiura, DMZ

Trematodes of the family Echinostomatidae, i.e., echinostomes, are intestinal parasites of animals, which can infect humans, are an important group of food-borne zoonotic parasites around the world [1]. A total of 20 species belonging to 9 genera are known to cause human infections, and among them, 7 species of *Echinostoma* and 5 species of *Echinochasmus* are the most common and important groups affecting humans and animals [1]. Natural definitive hosts for these echinostomes are known to be, in most cases, birds and mammals including rodents [1].

In the Republic of Korea (ROK), several studies have been performed on echinostome infections in rodents [2-6], cats [7, 8], and dogs [9]. Among rodent surveys, *Echinostoma hortense*, *Echinostoma cinetorchis*, and *Euparyphium murinum* infections were

The demilitarized zone (DMZ) of Gyeonggi-do and Gangwondo, ROK, is highly conserved and ecologically unique [10]. Many military field training sites are located near the DMZ, where civilian entry is prohibited. Echinostome infections had never been reported from this area. A comprehensive rodent surveillance program was conducted in 3 localities of northern Gyeonggi-do near the DMZ [11-13]. The present paper focused on the status of echinostome infections in field mice, *A. agrarius* and insectivores, *C. lasiura*, captured as a part of the rodent sur-

reported from *Rattus norvegicus* (Norway rat), *Rattus rattus alexandrines* (roof rat), *A. agrarius, Mus musculus yamashinai* (common field mouse), and *Microtus fortis pellceus* (reed vole), collected in Seoul and other localities in Korea [2-4]. In addition, *Echinostoma revolutum* and *Echinoparyphium recurvatum* were recovered from rats (probably *R. norvegicus*) captured in Yangyang-gun, Gangwondo (province) [5], and *E. japonicus* from *A. agrarius* from Goyang-gun, Gyeonggi-do and Iksan-gun, Jeollabuk-do [6].

<sup>•</sup> Received 21 May 2009, revised 30 July 2009, accepted 3 August 2009.

<sup>\*</sup> Corresponding author (cjy@snu.ac.kr)

<sup>&</sup>lt;sup>†</sup> Deceased.

veillance program.

A total of 1,498 small mammals (rodents and insectivores) were live-captured using Sherman traps (3 × 3.5 × 9 folding traps; H.B. Sherman, Tallahassee, Florida, USA) from military training sites at 3 areas in Gyeonggi-do, i.e., Paju-si (city), Pocheon-gun (township), and Yeoncheon-gun (township), located near the DMZ from December 2004 to September 2005. The captured mammals included the striped field mouse, *Apodemus agrarius* (n = 1,366), the Ussuri white-toothed shrew, *Crocidura lasiura* (54), *Mus musculus* (32), *Micronytus fortis* (28), *Myodes* (= *Eothenomys*) *regulus* (9), *Micronys minutes* (6), and *Tscherckia* (= *Cricetulus*) *triton* (3). Seasonal trapping was conducted in December 2004, March 2005, June 2005, and September 2005. Captured specimens were euthanized in accordance with the approved animal use protocol under biosafety level 3 (BSL-3) laboratory conditions.

The stomach, small intestine, and large intestine to the end of the rectum were removed and preserved in 70% alcohol until examination. The gastrointestinal content was examined for intestinal helminths under a stereomicroscope [11,12]. Trematodes were removed and placed on a microscope slide with a coverslip, fixed with 70% alcohol, stained with Semichon's acetocarmine, and identified using a research microscope. Data on *Plagiorchis muris* [11] and *Neodiplostomum seoulense* infections [12] were already published, and those on nematodes and cestodes will be published separately.

A total of 15/1,420 (1.1%) *A. agrarius* and *C. lasiura* were found to be infected with several species of echinostomes (Table 1; Fig. 1). The echinostome species included *E. hortense* (Fig. 1A),

**Table 1.** Infection status of echinostomes in *Apodemus agrarius* and *Crocidura lasiura* collected near the DMZ of Gyeonggi-do (province), Republic of Korea

	A. agrarius (n = 1,366) C. lasiura (n = 54)			
Species of echinostome	No. positive (%)	No. specimens	No. positive (%)	No. specimens
Echinostoma hortense	7 (0.5) <sup>a</sup>	16	0 (0.0)	0
Echinostoma cinetorchis	5 (0.4) <sup>b</sup>	9	0 (0.0)	0
Euparyphium murinum	2 (0.1)°	3	0 (0.0)	0
Echinochasmus japonicus	0 (0.0)	0	1 (1.9) <sup>d</sup>	3

<sup>&</sup>lt;sup>a</sup>Six of these 7 positive mice were caught in Yeoncheon-gun (positive rate; 1.8%, 6/325) and 1 mouse was captured in Pocheon-gun (0.3%, 1/288) of Gyeonggi-do.

E. cinetorchis (Fig. 1B), E. murinum (Fig. 1C), and E. japonicus (Fig. 1D). The infection rate of A. agrarius for E. hortense was 0.5%, (7/1,366 mice examined), and total 16 E. hortense specimens were recovered (Table 1). The infection rate was higher in mice (1.8%; 6/325) captured in Yeoncheon-gun than the mice captured in Pocheon-gun (0.3%; 1/288) and Paju-si (0%; 0/753). A total of 5 A. agrarius were infected with E. cinetorchis, and a total of 9 specimens were recovered (Table 1). E. cinetorchis infections were found exclusively in mice captured in Paju-si (0.7%; 5/753). Three specimens of E. murinum were recovered from 2 A. agrarius captured in Paju-si (0.3%; 2/753). E. japonicus (3 specimens) were found only in 1 C. lasiura captured in Paju-si (8.3%; 1/12).

Small mammals, in particular, *A. agrarius* and *C. lasiura*, inhabiting near the DMZ of Gyeonggi-do have been found to serve as the natural definitive hosts for 4 species of echinostomes. Three

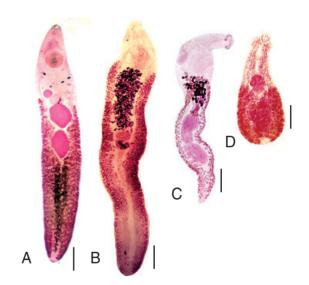


Fig. 1. (A) Echinostoma hortense adult recovered from a striped-field mouse, Apodemus agrarius, caught at Yeoncheon-gun, Gyeonggido. The characteristic features of this species include the presence of 27-28 collar spines on its head collar and a submedially located ovary. Scale bar = 1 mm. (B) Echinostoma cinetorchis adult recovered from A. agrarius caught at Paju-si, Gyeonggi-do. The characteristic features of this species include the presence of 37-38 collar spines on its head collar, abnormal location or disappearance of 1 or both testes, and a medially located ovary. Scale bar = 1 mm. (C) Euparyphium murinum adult recovered from A. agrarius caught at Paiu-si. Gveonggi-do. The characteristic features of this species include the presence of 45 collar spines on its head collar and a short uterine tubule. Scale bar = 0.5 mm. (D) Echinochasmus japonicus adult recovered from an insectivore, the Ussuri white-toothed shrew, Crocidura lasiura, caught at Paju-si, Gyeonggi-do. The characteristic features of this species include a short, plump body with the presence of 24 dorsally interrupted collar spines and 2 tandem arranged testes. Scale bar = 0.025 mm.

<sup>&</sup>lt;sup>b</sup>All 5 mice were caught in Paju-si (0.7%, 5/753)

<sup>&</sup>lt;sup>e</sup>Both mice were caught in Paju-si (0.3%, 2/753).

<sup>&</sup>lt;sup>d</sup>This infected insectivore was caught in Paju-si (8.3%, 1/12).

of the 4 species, E. hortense, E. cinetorchis, and E. japonicus, are zoonotic parasites capable of infecting humans, as human infections with these echinostome species have been reported in the ROK [14-17]. Infections of A. agrarius with E. cinetorchis and of C. lasiura with E. japonicus are reported for the first time in the ROK.

There was no difficulty in identifying the specimens of E. hortense, E. cinetorchis, and E. japonicus. However, there was difficulty in identifying E. murinum [18], because of the presence of several closely related species having 45 (43-47) collar spines. Such species included Echinostoma macrorchis [19], Echinostoma gotoi [19], Euparyphium paramurinum [20], and Euparyphium albuferensis [21]. The possibility of E. macrorchis for our specimens was excluded by the smaller body size, shorter and less coiled uterine tubule, and smaller egg size in our specimens. E. gotoi could be excluded because our specimens had globular testes, smaller sized eggs, and short uterus whereas E. gotoi has elongated elliptical or S-shaped testes, bigger sized eggs, and longer and more coiled uterus [19]. Two species of Euparyphium (E. paramurinum and E. albuferensis) were morphologically more similar to our specimens. However, the differential points between E. murinum and E. paramurinum given were minor [18,20], and these 2 species should be synonymous, so we excluded E. paramurinum for consideration. E. albuferensis was the most recently reared species of Euparyphium, which is similar to E. murinum [21]. However, the former differed from the latter in its larger body, larger suckers, more extensive distribution of vitelline follicles, and in the morphology of collar spines [21]. Our specimens were most similar to E. murinum in the body size and the distribution of vitelline follicles.

Infection rates of mice and insectivores with E. cinetorchis and E. japonicus, respectively, were higher in those captured at Paju-si compared with those captured at 2 other locations. Meanwhile, most of E. hortense infections were found in mice caught at Yeoncheon-gun. Whether this difference has any significant meaning is unclear at present. However, since all these 3 zoonotic echinostomes can infect humans through eating improperly cooked freshwater fish [1], attention should be paid to the possible presence of human infections with these echinostomes in these areas.

## **ACKNOWLEDGEMENTS**

Funding for portions of this work was provided by the Global Emerging Infections Surveillance and Response System, Armed Forces Health Surveillance Center, Center for Health Protection

and Preventive Medicine, Silver Spring, MD and the National Center for Medical Intelligence, Ft. Detrick, Mayland, USA.

## **REFERENCES**

- 1. Chai JY. Echinostomes in humans (Chapter 7). In Fried B, Toledo R eds, The Biology of Echinostomes. New York, USA. Springer. 2009. p 147-183.
- 2. Park JT. A rat trematode, Echinostoma hortense Asada, from Korea. Keijo J Med 1938; 9: 283-286.
- 3. Seo BS, Rim HJ, Lee CW. Studies on the parasitic helminths of Korea, I. Trematodes of rodents. Korean J Parasitol 1964: 2: 20-26
- 4. Seo BS, Cho SY, Hong ST. Studies on parasitic helminths of Korea. V. Survey on trematodes of house rats. Korean J Parasitol 1981; 19: 131-136.
- 5. Lee SH, Sohn WM, Chai JY. Echinostoma revolutum and Echinoparyphium recurvatum recovered from house rats in Yangyang-gun, Kangwon-do. Korean J Parasitol 1990; 28: 235-240.
- 6. Yong TS, Chung KH, Ree HI. Infection status of intestinal parasites of field rodents in Korea. Yonsei Reports Trop Med 1991; 22: 55-
- 7. Lee HS. A survey on helminth parasites of cats in Gyeongbug area. II. Trematodes. Korean J Vet Res 1979; 19: 57-61 (in Korean).
- 8. Sohn WM, Chai IY. Infection status with helminthes in feral cats purchased from a market in Busan, Republic of Korea. Korean J Parasitol 2005; 43: 93-100.
- 9. Cho SY, Kang SY, Ryang YS. Helminthes infections in the small intestine of stray dogs in Ejungbu City, Kyunggi Do, Korea. Korean J Parasitol 1981; 19: 55-59 (in Korean).
- 10. Kim KC. Preserving biodiversity in Korea's demilitarized zone. Science 1997; 278: 242-243.
- 11. Chai JY, Park JH, Guk SM, Kim JL, Kim HJ, Kim WH, Shin EH, Klein TA, Kim HC, Chong ST, Song JW, Baek LJ. Plagiorchis muris infection in Apodemus agrarius from northern Gyeonggi-do (Province) near the demilitarized zone. Korean J Parasitol 2007; 45: 153-156.
- 12. Chai JY, Park JH, Guk SM, Kim JL, Kim HJ, Kim WH, Shin EH, Klein TA, Kim HC, Chong ST, Song JW, Baek LJ. Apodemus agrarius as a new definitive host for Neodiplostomum seoulense. Korean J Parasitol 2007; 45: 157-161.
- 13. O'Guinn ML, Klein TA, Lee JS, Kim HC, Baek LJ, Chong ST, Turell MJ, Burkett DA, Schuster A, Lee IY, Yi SH, Sames WJ, Song KJ, Song JW. Ecological surveillance of small mammals at firing points 10 and 60, Gyeonggi Province, Republic of Korea, 2001-2005. J Vect Ecol 2008; 33: 370-384.
- 14. Seo BS, Cho SY, Chai JY. Studies on intestinal trematodes in Korea. I. A human case of Echinostoma cinetorchis infection with an epidemiological investigation. Seoul J Med 1980; 21: 21-29.
- 15. Seo BS, Hong ST, Chai JY, Lee SH. Studies on intestinal trematodes in Korea. VIII. A human case of Echinostoma hortense infection. Korean J Parasitol 1983; 21: 219-223.

- 16. Seo BS, Lee SH, Chai JY, Hong SJ. Studies on intestinal trematodes in Korea. XX. Four cases of natural human infection by *Echinochasmus japonicus*. Korean J Parasitol 1985; 23: 214-220.
- 17. Chai JY, Lee SH. Food-borne intestinal trematode infections in the Republic of Korea. Parasitol Int 2002; 51: 129-154.
- 18. Lie KJ. Studies on echinostomatidae (Trematoda) in Malaya. XV. The life history of *Echinostoma murinum* (Tubangui, 1931). Proceed Helminthol Soc Wash 1967; 34: 139-143.
- 19. Ando R, Ozaki Y. On four new species of trematodes of the family Echinostomatidae. Dobutsugaku Zasshi 1923; 35: 109-119

- (in Japanese).
- 20. Velasquez CC. Observations on the life cycle of *Euparyphium paramurinum* sp. n. (Trematoda: Echinostomatidae). J Parasitol 1964; 50: 106-111.
- 21. Esteban JG, Toledo R, Sánchez L, Muñoz-Antoli. Life-cycle of *Euparyphium albuferensis* n. sp. (Trematoda: Echinostomatidae) from rats in Spain. Syst Parasitol 1997; 38: 211-219.
- 22. Yamaguti S. Studies on the helminth fauna of Japan. Part 25. Trematodes of birds, IV. Jpn J Zool 1939; 8: 131-210.