The Journal of Veterinary Medical Science

FULL PAPER

Public Health

A long-term observation for ecology of pathogenic *Yersinia* in wild rodents living in Fukushima Prefecture, Japan

Thi Hien BUI[1)], Khanh Thuan NGUYEN[1,2)], Shunsuke IKEUCHI[1)], Haruka YANAGAWA[1)], Yomogi SATO[1)], Thi Hong To TRAN[1)], Minato OKUMURA[1)], Takeshi NIWA[1)], Takahide TANIGUCHI[1)] and Hideki HAYASHIDANI[1)]*

[1)]Institute of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu-shi, Tokyo 183-8509, Japan

[2)]Department of Veterinary Medicine, College of Agriculture, Can Tho University, Campus II, 3/2 Street, Ninh Kieu District, Can Tho City, Vietnam

J. Vet. Med. Sci.
83(11): 1790–1794, 2021
doi: 10.1292/jvms.21-0395

Received: 13 July 2021
Accepted: 19 September 2021
Advanced Epub:
13 October 2021

ABSTRACT. From 2012 to 2021, prevalence of pathogenic *Yersinia* in wild rodents captured in Fukushima Prefecture, Japan was investigated twice a year to clarify the ecology of this pathogen in wild rodent populations. Pathogenic *Yersinia enterocolitica* O8 was isolated from 13 (1.7%) of 755 wild rodents. The *Y. enterocolitica* O8 isolates harbored three virulent genes (*ail, fyuA*, and *virF*). This pathogen was isolated repeatedly from wild rodents in April 2015, 2016, and 2017, in June and November 2020, and in April 2021, which was 6 of 19 times of observations. All *Y. enterocolitica* O8 isolates showed the same PFGE patterns. These results indicated that the same clone of pathogenic *Y. enterocolitica* O8 has been maintained in wild rodent populations in Fukushima Prefecture. Therefore, wild rodent populations contribute substantially to the continuous transmission of *Y. enterocolitica* O8 and its persistence in the ecosystem. This is the first report on the isolation of pathogenic *Y. enterocolitica* O8 in wild rodents in Fukushima Prefecture, Japan.

KEY WORDS: ecology, epidemiology, prevalence, wild rodent, *Yersinia enterocolitica* O8

Pathogenic *Yersinia,* including *Yersinia enterocolitica* and *Y. pseudotuberculosis,* is recognized worldwide as an important foodborne and human zoonotic pathogen [2, 4]. Human *Yersinia* infection causes gastroenteritis, which clinical symptoms are abdominal pain, diarrhea, but highly pathogenic *Yersinia,* such as *Y. enterocolitica* O8 and *Y. pseudotuberculosis,* sometimes causes septicemia [3–5]. As a few reports, indicating that wild rodents harbor pathogenic *Yersinia,* has been published, wild rodent seems to be a natural reservoir for pathogenic *Yersinia* and the source of human infections [7, 11, 12, 18]. However, the prevalence of pathogenic *Yersinia* has not been reported in wild rodents living in Fukushima Prefecture, Japan. Moreover, ecology of pathogenic *Yersinia* in wild rodents is still unclear.

Therefore, we examined for the prevalence of pathogenic *Yersinia* in wild rodents living in Fukushima Prefecture to clarify the ecology of pathogenic *Yersinia* in nature.

MATERIALS AND METHODS

Sample collection

From July 2012 to April 2021 (total 19 times), a total of 755 wild rodents, including 464 large Japanese field mice (*Apodemus speciosus*), 232 small Japanese field mice (*Apodemus argenteus*), 37 Japanese grass voles (*Microtus montebelli*), and 22 Japanese shrew moles (*Urotrichus talpoides*), were captured in the mountainous areas of Nihonmatsu city in Fukushima Prefecture in Tohoku region of Japan. There are two mountains in this area, Mt. Kuchibuto (37°36'N 140°34'E) and Mt. Hayama (37°33'N 140°37'E). These mountains are 3 km apart. Wild rodents were captured twice a year. Wild rodents were captured mainly in spring (April) and autumn (November) except in 2012 (July) and 2020 (June). Wild rodents were captured at the same points during the survey period. Wild rodents were captured by live trap. After euthanasia by cervical dislocation method [9, 20], the rodents were preserved separately in sterile plastic bags and immediately transported to the Laboratory of Animal Health, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu city, Tokyo, Japan, under refrigeration condition (2–7°C) in

*Correspondence to: Hayashidani, H.: eisei@cc.tuat.ac.jp

the cold containers. Each wild rodent was measured, classified by species, sex, and age, and then dissected to collect rectal content samples. The age of *A. speciosus* was determined based on body weight by the method of Fukushima *et al.* [7]. Briefly, the rodents that weighed under 30 g or over 30 g were divided into juveniles or adults, respectively. Almost all other species of wild rodents that were captured were adults. Sampling procedures followed the Regulations for Animal Experiments at Tokyo University of Agriculture and Technology and were approved by the institution's Committee for Animal Research and Welfare.

Yersinia isolation and identification

The rectal contents (ca 0.5–1.0 g) of animals were homogenized in 9 times amount of phosphate-buffered saline (PBS; pH 7.2) and incubated at 4°C for 3–4 weeks. After alkali (KOH) treatment [1], the sample suspension was then cultured on irgasan-novobiocin (IN) [7] and CHROMagar™ Yersinia (Currently CHROMagar™ Y.enterocolitica) (CHROMagar Microbiology, Paris, France) plates and incubated at 25°C for 48 hr. After that, 4 morphologically suspicious colonies from each selective agar were picked up and subcultured onto trypticase soy agar (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) for further tests. *Yersinia* identification was accomplished by the methods described previously [13]. Serotyping of *Yersinia* strains isolated from wild rodents was performed by slide agglutination with commercial rabbit anti-*Y. enterocolitica* sera and rabbit anti-*Y. pseudotuberculosis* sera (Denka-Seiken, Tokyo, Japan). All *Yersinia* isolates were examined for their temperature-dependent autoagglutination by the method of Laird and Cavanaugh [16] and the presence of the virulence plasmid by the modified method of Kado and Liu [14] to evaluate their potential pathogenicity. The PCR was carried out to detect pathogenic *Yersinia* virulence genes, including *fuy*A, *ail, inv,* and *virF* (unpublished data) of *Yersinia* isolate.

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed to compare the genetic characteristics of *Y. enterocolitica* O8 isolates. The PFGE was carried out as described by Iwata *et al.* [13]. Briefly, chromosomal DNAs were digested by the restriction enzyme *Not*I (TaKaRa, Kusatsu, Japan) for 3 hr at 37°C. The DNA fragments were separated in 1.2% agarose NA (GE Healthcare, Bioscience AB, Uppsala, Sweden) on a CHEF-DRII Pulsed Field Electrophoresis System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Electrophoresis was carried out for 24 hr at 14°C and 200 V with pulse times of 2 to 25 sec. A CHEF DNA Size Standard Lambda Ladder (Bio-Rad) was used as molecular size marker. The gels were stained with AtlasSight DNA Stain (Bioatlas, Tartu, Estonia) and photographed using a Gel Doc camera system (Bio-Rad).

Statistical analysis

Differences in the prevalence were analyzed by Fisher's exact test using R statistical software, R version 4.0.5, 2021 [19].

RESULTS

Thirteen *Y. enterocolitica* O8 and 1 *Y. pseudotuberculosis* 5b strains were isolated from 14 (1.9%) of 755 wild rodents captured in Fukushima Prefecture from 2012–2021 (Table 1). All *Y. enterocolitica* O8 isolates harbored virulence genes such as *fyuA, ail,* and *virF.* However, the *Y. pseudotuberculosis* 5b isolate did not harbor *virF* gene. Therefore, this isolate was identified as non-pathogenic *Y. pseudotuberculosis*. For 10-year observations, the *Y. enterocolitica* O8 was isolated from *A. speciosus* and *A. argenteus* in 6 of 19 times surveys. This pathogen was isolated from both *A. speciosus* and *A. argenteus* at the same opportunity of capture in April 2016 and June 2020. No *Yersinia* isolate was recovered from *M. montebelli* and *U. talpoides*. No significant difference in the prevalence of *Y. enterocolitica* O8 among seasons was observed.

Of 13 pathogenic *Y. enterocolitica* O8 positive rodents, 6 (1.2%) were from 464 *A. speciosus*, and 7 (3.0%) were from 232 *A. argenteus,* respectively (Table 2). No significant difference in the isolation rate of *Y. enterocolitica* O8 between *A. speciosus* and *A. argenteus* was observed. Of 6 *Y. enterocolitica* O8 positive *A. speciosus*, 3 were females and 3 were males. Moreover, of 6 those *A. speciosus*, 1 female and 2 males were juveniles. Of 3 *Y. enterocolitica* O8 positive juvenile, 1 was captured in Mt. Hayama and 2 were in Mt. Kuchibuto. No significant differences in *Y. enterocolitica* O8 isolation rate were observed among sex in both rodent species.

Digestion of genomic DNA from 13 *Y. enterocolitica* O8 isolates with *Not*I gave 21 to 22 fragments (Fig. 1). Of 13 *Y. enterocolitica* O8 isolates, strain YE15-29 isolated in 2015 showed the PFGE pattern P1, and the other 12 *Y. enterocolitica* O8 isolates showed the same PFGE pattern P2. However, patterns P1 and P2 differ in only one band. P1 and P2 were identified as the same clone, following the guideline of Tenover *et al.* [21].

DISCUSSION

In the present study, *Y. enterocolitica* O8 had been frequently isolated from wild rodents living in Fukushima Prefecture, although non-pathogenic *Y. pseudotuberculosis* 5b was isolated only 1 time. The pathogenic *Y. enterocolitica* O8 was isolated from wild rodents living in Niigata and Aomori Prefecture, located eastern part of Honshu Island in Japan [11, 12, 18]. On the other hand, Fukushima *et al.* [7] reported that pathogenic *Y. pseudotuberculosis* 1b and 4b were isolated from wild rodents in Shimane Prefecture, located western part of Honshu Island, Japan. Moreover, pathogenic *Y. pseudotuberculosis* was detected from a wild rodent in Hokkaido Island of Japan. Fukushima *et al.* [8] also reported that the wild rodent which harbored *Y. enterocolitica* O9 in China is protected against *Y. pestis* infection. Until now, no report on the prevalence of pathogenic *Yersinia* in wild rodents

Table 1. Prevalence of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* in wild rodents in Fukushima prefecture, Japan by year

Survey time		No. of *Yersinia* positive animals/No. of animals examined (%)				
		Apodemus speciosus	*Apodemus argenteus*	*Microtus montebelli*	*Urotrichus talpoides*	Total
2012	Jul.	0/9 (0.0)	0/9 (0.0)	0	0	0/18 (0.0)
	Nov.	0/9 (0.0)	0/10 (0.0)	0	0	0/19 (0.0)
2013	Apr.	0/27 (0.0)	0/16 (0.0)	0	0	0/43 (0.0)
	Nov.	1/35 (2.9)[a]	0/8 (0.0)	0/7 (0.0)	0	1/50 (2.0)[a]
2014	Apr.	0/28 (0.0)	0/19 (0.0)	0/3 (0.0)	0/7 (0.0)	0/57 (0.0)
	Nov.	0/21 (0.0)	0/11 (0.0)	0	0/2 (0.0)	0/34 (0.0)
2015	Apr.	0/61 (0.0)	3/35 (8.6)[b]	0/3 (0.0)	0/1 (0.0)	3/100 (3.0)[b]
	Nov.	0/36 (0.0)	0/4 (0.0)	0	0	0/40 (0.0)
2016	Apr.	2/38 (5.3)[b]	1/18 (5.6)[b]	0/4 (0.0)	0/2 (0.0)	3/62 (4.8)[b]
	Nov.	0/17 (0.0)	0/13 (0.0)	0	0	0/30 (0.0)
2017	Apr.	0/9 (0.0)	1/4 (25.0)[b]	0	0/4 (0.0)	1 /17 (5.9)[b]
	Nov.	0/12 (0.0)	0/2 (0.0)	0	0	0/14 (0.0)
2018	Apr.	0/72 (0.0)	0/16 (0.0)	0/17 (0.0)	0/1 (0.0)	0/106 (0.0)
	Nov.	0/11 (0.0)	0/2 (0.0)	0/2 (0.0)	0/1 (0.0)	0/16 (0.0)
2019	Apr.	0/12 (0.0)	0/3 (0.0)	0	0	0/15 (0.0)
	Nov.	0/15 (0.0)	0/6 (0.0)	0	0	0/21 (0.0)
2020	Jun.	1/20 (5.0)[b]	2/23 (8.7)[b]	0/1 (0.0)	0/1 (0.0)	3/45 (6.7)[b]
	Nov.	1/25 (4.0)[b]	0/23 (0.0)	0	0/3 (0.0)	1/51 (2.0)[b]
2021	Apr.	2/7 (28.6)[b]	0/10 (0.0)	0	0	2/17 (11.8)[b]
Total		7/464 (1.5)	7/232 (3.0)	0/37 (0.0)	0/22 (0.0)	14/755 (1.9)

[a] *Y. pseudotuberculosis* O5b. [b] *Y. enterocolitica* O8.

Table 2. Prevalence of pathogenic *Yersinia enterocolitica* O8 by sampling area and rodents species

Animal species	Sex/age		No. of positive animals/No. of animals examined (%)		
			Mt. Kuchibuto	Mt. Hayama	Total
Apodemus speciosus	Male	Juvenile	2/59 (3.4)	0/57 (0.0)	2/116 (1.7)
		Adult	0/51 (0.0)	1/78 (1.3)	1/129 (0.8)
	Subtotal		2/110 (1.8)	1/135 (0.7)	3/245 (1.2)
	Female	Juvenile	0/45 (0.0)	1/71 (1.4)	1/116 (0.9)
		Adult	2/41 (4.9)	0/62 (0.0)	2/103 (1.9)
	Subtotal		2/86 (2.3)	1/133 (0.8)	3/219 (1.4)
Subtotal			4/196 (2.0)	2/268 (0.7)	6/464 (1.2)
Apodemus argenteus	Male		3/74 (4.1)	1/40 (2.5)	4/114 (3.5)
	Female		3/75 (4.0)	0/43 (0.0)	3/118 (2.5)
Subtotal			6/149 (4.0)	1/83 (1.2)	7/232 (3.0)
Total			10/345 (2.9)	3/351 (0.9)	13/696 (1.9)

has been reported in Fukushima Prefecture although pathogenic *Y. enterocolitica* O8 is known to be distributed in eastern part of Honshu Island of Japan. This is the first report of the isolation of pathogenic *Y. enterocolitica* O8 in wild rodents living in this Prefecture.

Almost all researchers have tried to isolate pathogenic *Yersinia* in wild rodents to know the prevalence of this pathogen 1- or 2-times surveys [11, 12, 18]. We observed the prevalence of pathogenic *Yersinia* in wild rodent populations in Fukushima Prefecture for 10 years. For 10-year observations, *Y. enterocolitica* O8 was isolated from wild rodents in 6 of 19 times surveys. A total of 13 pathogenic *Y. enterocolitica* O8 isolates were detected from wild rodents during those periods. All 13 *Y. enterocolitica* isolates showed the same PFGE patterns. Those results indicated that the same clone of *Y. enterocolitica* O8 has been maintained in wild rodent populations in this area for at least 6 years. Generally, pathogenic *Yersinia* seems to show "habitat isolation" in wild rodents in the world [17]. This phenomenon, "habitat isolation", means that one wild rodent population usually maintain one pathogenic *Yersinia* species although the mechanism of this phenomenon is still unknown. Therefore, the wild rodent populations in this survey area of Fukushima Prefecture seem to maintain only *Y. enterocolitica* O8. Notably, the *Y. enterocolitica* O8 was isolated from wild rodents captured in Mt. Hayama and Mt. Kuchibuto at the same time in 2016, 2020 and 2021, and showed the same genetic type. Two mountains, Mt. Hayama and Mt. Kuchibuto, are 3 km apart from each other, and wild rodents may migrate

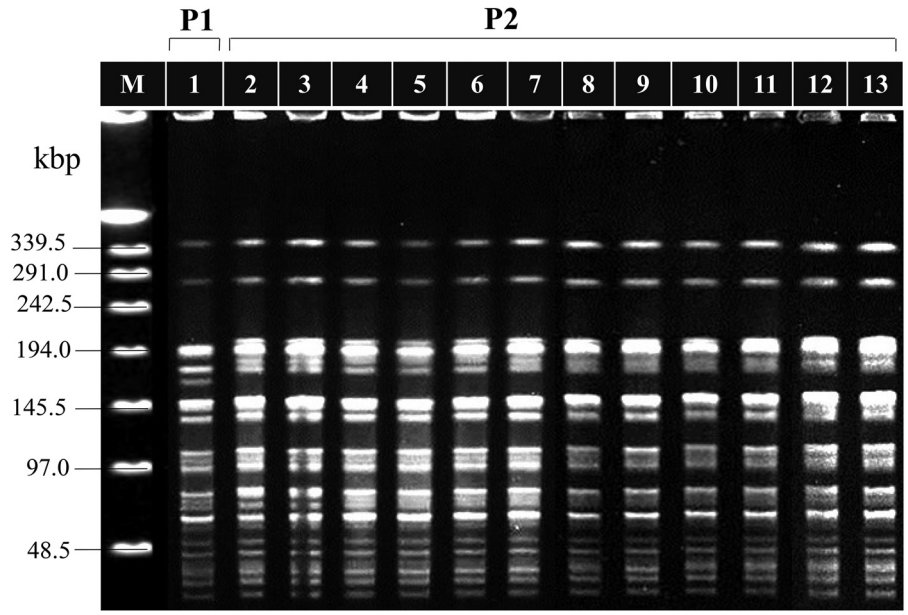

Fig. 1. Pulsed-field gel electrophoresis (PFGE) patterns obtained from 13 *Yersinia enterocolitica* O8 isolates originated from wild rodents in Fukushima Prefecture, Japan by *Not*I enzyme. Lane M, CHEF DNA Size Standard Lambda Ladder. Lane 1 shows PFGE pattern P1 produced from YE15-29 strain isolated in 2015. Lanes 2 to 13 show PFGE pattern P2 produced from 12 strains isolated in 2015, 2016, 2017, 2020, and 2021: lanes 2–3 are isolates in 2015, coded YE15-61, and YE15-65, respectively; lanes 4–6 are isolates in 2016, coded YE16-07, YE16-14, and YE16-58, respectively; lane 7 is isolate in 2017, coded YE17-08; lanes 8–11 are isolates in 2020, coded YE20-06, YE20-07, YE20-23, and YE20-44, respectively; and lanes 12–13 are isolates in 2021, coded YE21-4 and YE21-17, respectively.

among both mountains although wild rodents' sphere of activity is not usually wide except breeding season [15]. Moreover, Fukushima *et al.* [7] reported that the isolation rate of *Y. pseudotuberculosis* from juvenile wild rodents was significantly higher than that in adults. In addition, Fukushima [6] challenged pathogenic *Y. pseudotuberculosis* to *A. speciosus* intragastrically and found that the juvenile rodents showed significantly higher susceptibility to this bacterium rather than that of adults and *A. speciosus* excreted *Y. pseudotuberculosis* in the feces for 1–2 weeks after oral challenge. Furthermore, Hayashidani *et al.* [10] also challenged *Y. enterocolitica* O8 to *A. speciosus* intragastrically and reported that those *A. speciosus* shed this bacterium in their feces for more than 2 weeks. The wild rodent's feces including pathogenic *Yersinia* might contaminate the environment such as feeds, soil, and water. Those results indicated that horizontal and vertical transmission of pathogenic *Y. enterocolitica* O8 seem to occur among wild rodent populations. Further studies are needed to clarify clearly the ecology of pathogenic *Y. enterocolitica* O8 in wild rodent populations.

In this study, the pathogenic *Y. enterocolitica* O8 strains were isolated from *Apodemus* species but not from *M. montebelli* and *U. talpoides,* although they were captured in the same areas. Some researchers reported that pathogenic *Yersinia* such as *Y. enterocolitica* and *Y. pseudotuberculosis* were isolated from *Apodemus* species but not from *M. montebelli* and *U. talpoides* [7, 11, 18]. Fukushima [6] and Hayashidani *et al.* [10] intragastrically challenged *A. speciosus* with pathogenic *Y. pseudotuberculosis* or *Y. enterocolitica* O8 respectively and found that *A. speciosus* showed susceptibility to *Y. pseudotuberculosis* and *Y. enterocolitica* O8. However, there is no data related to the susceptibility of *M. montebelli* and *U. talpoides* to pathogenic *Yersinia*. Further research should be done to clarify the susceptibility of *M. montebelli* and *U. talpoides* to pathogenic *Yersinia*.

CONFLICT OF INTEREST STATEMENT. The authors declare no conflict of interest.

REFERENCES

1. Aulisio, C. C., Mehlman, I. J. and Sanders, A. C. 1980. Alkali method for rapid recovery of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from foods. *Appl. Environ. Microbiol.* **39**: 135–140. [Medline] [CrossRef]
2. Bari, M. L., Hossain, M. A., Isshiki, K. and Ukuku, D. 2011. Behavior of *Yersinia enterocolitica* in foods. *J. Pathogens* **2011**: 420732. [Medline] [CrossRef]
3. Bottone, E. J. 1997. *Yersinia enterocolitica*: the charisma continues. *Clin. Microbiol. Rev.* **10**: 257–276. [Medline] [CrossRef]
4. Carniel, E., Autenrieth, I., Cornelis, G., Fukushima, H., Guinet, F., Isberg, R., Pham, J., Prentice, M., Simonet, M., Skurnik, M. and Wauters, G. 2006. *Y. enterocolitica* and *Y. pseudotuberculosis*. pp. 270–398. In: The Prokaryotes, A Handbook on the Biology of Bacteria (Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. and Stackebrandt, E. eds.), Springer, New York.
5. Fredriksson-Ahomaa, M. 2007. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis.* pp. 79–113. In: Foodborne Diseases, Infectious Disease (Simjee, S. ed.), Humana Press, Totowa.

6. Fukushima, H. 1991. Susceptibility of wild mice to Yersinia pseudotuberculosis and Yersinia enterocolitica. *Zentralbl. Bakteriol.* **275**: 530–540. [Medline] [CrossRef]
7. Fukushima, H., Gomyoda, M. and Kaneko, S. 1990. Mice and moles inhabiting mountainous areas of Shimane Peninsula as sources of infection with *Yersinia pseudotuberculosis*. *J. Clin. Microbiol.* **28**: 2448–2455. [Medline] [CrossRef]
8. Fukushima, H., Hao, Q., Wu, K., Hu, X., Chen, J., Guo, Z., Dai, H., Qin, C., Lu, S. and Gomyoda, M. 2001. *Yersinia enterocolitica* O9 as a possible barrier against *Y. pestis* in natural plague foci in Ningxia, China. *Curr. Microbiol.* **42**: 1–7. [Medline] [CrossRef]
9. Gannon, W. L. and Sikes, R. S. and Animal Care and Use Committee of the American Society of Mammalogists. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *J. Mammal.* **88**: 809–823. [CrossRef]
10. Hayashidani, H., Kitahara, E. and Ogawa, M. 1994. Infectivity and pathogenicity of Yersinia enterocolitica serovar 0:8 to wild rodents in Japan. *Zentralbl. Veterinärmed. B.* **41**: 504–511. [Medline]
11. Hayashidani, H., Ohtomo, Y., Toyokawa, Y., Saito, M., Kaneko, K., Kosuge, J., Kato, M., Ogawa, M. and Kapperud, G. 1995. Potential sources of sporadic human infection with *Yersinia enterocolitica* serovar O:8 in Aomori Prefecture, Japan. *J. Clin. Microbiol.* **33**: 1253–1257. [Medline] [CrossRef]
12. Iinuma, Y., Hayashidani, H., Kaneko, K., Ogawa, M. and Hamasaki, S. 1992. Isolation of *Yersinia enterocolitica* serovar O8 from free-living small rodents in Japan. *J. Clin. Microbiol.* **30**: 240–242. [Medline] [CrossRef]
13. Iwata, T., Une, Y., Okatani, A. T., Kaneko, S., Namai, S., Yoshida, S., Horisaka, T., Horikita, T., Nakadai, A. and Hayashidani, H. 2005. *Yersinia enterocolitica* serovar O:8 infection in breeding monkeys in Japan. *Microbiol. Immunol.* **49**: 1–7. [Medline] [CrossRef]
14. Kado, C. I. and Liu, S. T. 1981. Rapid procedure for detection and isolation of large and small plasmids. *J. Bacteriol.* **145**: 1365–1373. [Medline] [CrossRef]
15. Kuroda, T. and Katsuno, T. 2007. Relationship between differential land use type and habitation of Japanese field mouse in suburban area. *J. Jpn. Inst. Landsc. Archit.* **70**: 479–482 (in Japanese). [CrossRef]
16. Laird, W. J. and Cavanaugh, D. C. 1980. Correlation of autoagglutination and virulence of yersiniae. *J. Clin. Microbiol.* **11**: 430–432. [Medline] [CrossRef]
17. Mollaret, H. H. 1995. Fifteen centuries of Yersiniosis. *Contrib. Microbiol. Immunol.* **13**: 1–4. [Medline]
18. Oda, S., Kabeya, H., Sato, S., Shimonagane, A., Inoue, K., Hayashidani, H., Takada, N., Fujita, H., Kawabata, H. and Maruyama, S. 2015. Isolation of pathogenic *Yersinia enterocolitica* 1B/O:8 from *Apodemus* mice in Japan. *J. Wildl. Dis.* **51**: 260–264. [Medline] [CrossRef]
19. R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna.
20. Science council of Japan. 2006. Guidelines for proper conduct of animal experiments. http://www.scj.go.jp/ja/info/kohyo/pdf/kohyo-20-k16-2e.pdf [accessed on September 14, 2021].
21. Tenover, F. C., Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H. and Swaminathan, B. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* **33**: 2233–2239. [Medline] [CrossRef]