NOTE *Public Health*

Detection of pathogenic *Yersinia enterocolitica* in pet Djungarian hamsters in Japan

Mitsuhiro KAMEYAMA[1)]*, Junko YABATA[1,2)], Noriko OBANE[1)], Hitoshi OTSUKA[1)] and Yasuharu NOMURA[1)]

[1)]*Department of Health Science, Yamaguchi Prefectural Institute of Public Health and Environment, 2–5–67, Aoi, Yamaguchi 753–0821, Japan*

[2)]*Present address, Division of health and environment, Shunan health and welfare center, 2–38, Mori-cho, Shunan, Yamaguchi 745–0004, Japan*

(Received 16 November 2015/Accepted 27 June 2016/Published online in J-STAGE 11 July 2016)

ABSTRACT. The prevalence of *Yersinia enterocolitica* (*Y. enterocolitica*) and *Yersinia pseudotuberculosis* was examined in 151 pet animals including 108 rodents, 39 rabbits and four sugar gliders from 13 pet stores in the Yamaguchi Prefecture, Japan. *Y. enterocolitica* serogroup O:3 biotype 3 negative for the Voges-Proskauer reaction (O:3/3 variant VP-) was isolated from five Djungarian hamsters (*Phodopus sungorus*) raised at the same pet store. These pathogenic *Y. enterocolitica* isolates carried the virulence genes, *yadA*, *ail* and *virF*, and were shown to be clonal by pulsed-field gel electrophoresis with *Not*I digestion. This is a first report of pathogenic *Y. enterocolitica* O:3/3 variant VP- in pet Djungarian hamsters in Japan.

KEY WORDS: Djungarian hamster, O:3/3, *Yersinia enterocolitica*

doi: 10.1292/jvms.15-0654; *J. Vet. Med. Sci.* 78(10): 1639–1641, 2016

Yersinia enterocolitica (*Y. enterocolitica*) and *Yersinia pseudotuberculosis* (*Y. pseudotuberculosis*) are food- and water-borne bacteria that cause human yersiniosis, gastroenteritis, arthritis and septicemia [1, 18, 21]. Sporadic yersiniosis has been reported worldwide, and occasional major outbreaks have been reported in Japan [14, 15, 20]. Human yersiniosis is usually caused by the consumption of contaminated food, unpasteurized milk or untreated water [21]; however, infected animals have been increasingly recognized as infection sources [18, 23, 24]. Wild rodents are considered a common potential reservoir of *Y. enterocolitica* and *Y. pseudotuberculosis* [3, 12]. Although some reports have described the presence of these bacteria in pet animals, such as dogs and cats [2, 25], the prevalence of these pathogens in pet rodents and rabbits is still unknown. The present study was conducted to determine whether pet rodents and rabbits could harbor pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis*.

A total of 151 apparently healthy pet store animals including 108 rodents, 39 rabbits and four sugar gliders were examined for the prevalence of *Yersinia* spp. (Table 1). Animal stool samples were collected from 13 pet stores (store nos. A to M) in the Yamaguchi Prefecture, Japan, between September 2012 and November 2014. The samples were stored at 4°C for up to five days prior to being sent to our laboratory. Approximately 2 g of each stool sample was suspended in 20 m*l* of *Yersinia* enrichment broth according to Ossmer (Merck, Darmstadt, Germany) or 20 m*l* of phosphate buffered saline (PBS). These suspensions were incubated at 32°C for 24 hr and at 4°C for 3 weeks, respectively. Following incubation, each of the enrichment broths was streaked (with or without KOH treatment) [21] onto *Yersinia* selective agar supplemented with cefsulodin-irgasan-novobiocin (Oxoid Ltd., Hampshire, U.K.) and MacConkey agar (Oxoid Ltd.). The plates were incubated at 32°C for 20 hr and at 25°C for 48 hr, respectively. Following incubation, suspected colonies were selected and cultured on Columbia Blood Agar (Oxoid Ltd.) plates containing 5% sheep blood. Identification of the bacteria was performed as previously described [21]. The O serogroup of the isolates was determined by slide agglutination test using commercial antisera (Denka Seiken Co., Ltd., Tokyo, Japan). The biotype of the isolates was determined as previously described [21] using API 20E (BioMérieux, Tokyo, Japan), API 50CH (BioMérieux) and API ZYME (BioMérieux). The pathogenicity of the isolates was confirmed by PCR targeting the virulence genes, *yadA*, *ail* and *virF* [17, 22]. Pulsed-field gel electrophoresis (PFGE) analysis was performed as previously described [15] using the *Not*I restriction enzyme (Roche Diagnostics, Tokyo, Japan) and CHEF-MAPPER system (Bio-Rad Laboratories, Hercules, CA, U.S.A.) with a switching time of 5 to 8 sec for 19.5 hr at 14°C. *Salmonella enterica* serotype Braenderup strain H9812 digested with *Xba*I (Roche Diagnostics) was used as a standard size marker. Macrorestriction patterns were compared using BioNumerics 4.1 software (Applied Maths, Sint-Martens-Latem, Belgium). A Dice coefficient and the unweighted-pair group method with arithmetic mean (UPGMA) algorithm with a 1.0% band tolerance and 1.0% optimization were used to generate a dendrogram.

Yersinia spp. were isolated from five Djungarian hamsters (*Phodopus sungorus*) raised at the same pet store (store B). All of these isolates were identified as *Y. enterocolitica* serogroup O:3 biotype 3 negative for the Voges-Proskauer reac-

*CORRESPONDENCE TO: KAMEYAMA, M., Department of Health Science, Yamaguchi Prefectural Institute of Public Health and Environment, 2–5–67, Aoi, Yamaguchi 753–0821, Japan.
e-mail: kameyama.mitsuhiro@pref.yamaguchi.lg.jp

Table 1. Number of samples examined in this study

Animal	No. of samples in pet stores													
	A	B	C	D	E	F	G	H	I	J	K	L	M	Total
Pet rodents														
Chinchilla (*Chinchilla lanigera*)				1	1		2	2	3	2	1	2	1	15
Chinese hamster (*Cricetulus griseus*)				1										1
Degu (*Octodon degus*)				1	1	1	1	3	3	4	2	1	2	19
Djungarian hamster (*Phodopus sungorus*)		10[a)]		4								1	1	16
Golden hamster (*Mesocricetus auratus*)	1	3		1		2			1	1			2	11
Guinea pig (*Cavia porcellus*)	1			1	1	2	2	3	3	4	1	3	2	23
Hamster (Subfamily Cricetinae)					1	1				2	4		1	9
House mouse (*Mus musculus*)												1		1
Japanese dwarf flying squirrel (*Pteromys momonga*)								1					1	2
Jerboa (Family Dipodidae)												1		1
Lemming (Genus *lemmus*)													3	3
Prairie dog (Genus *cynomys*)				1										1
Richardson's ground squirrel (*Urocitellus richardsonii*)				1										1
Roborovskii dwarf hamster (*Phodopus roborovskii*)		1												1
Siberian chipmunk (*Tamias sibiricus*)								1				1		2
Squirrel (Family Sciuridae)						1	1							2
Pet rabbits														
Domestic rabbit (*Oryctolagus cuniculus*)		1	2	4	1	6	8	3	5	1	2	4	2	39
Other														
Sugar glider (*Petaurus breviceps*)	1					1		1				1		4
Total	3	15	2	15	5	14	14	14	15	14	10	15	15	151

a) Of the ten Djungarian hamster samples, five were positive for *Yersinia enterocolitica* isolates.

tion (O:3/3 variant VP-) and possessed the three virulence genes (*yadA*, *ail* and *virF*). PFGE analysis was conducted to determine the genetic relationship of the pathogenic *Y. enterocolitica* isolates; although a few differences were found between the banding patterns of the isolates, their similarities were >93%, suggesting that the isolates could have originated from a common ancestor (data not shown).

Y. enterocolitica includes several phenotypic variants (O serogroup/biotype) of which O:3/4, O:3/3 variant VP-, O:5, 27/2, O:8/1b and O:9/2 are those most associated with the occurrence of human yersiniosis worldwide [1, 9, 19]. Serogroup O:3 is considered the predominant variant in Japan, and the number of outbreaks and sporadic cases due to serogroup O:8 have been increasing since this variant was first isolated from humans in 1990 [12, 13, 15, 21]. Although the O:3/3 variant VP- has been previously isolated from several animals (healthy pigs and dogs) and retail meat (pork imported from Taiwan and chicken from Thailand) in Japan [5–7, 15], to the best of our knowledge, this is the first report of the isolation of pathogenic *Y. enterocolitica* O:3/3 variant VP- from pet Djungarian hamsters in Japan.

Store B investigated in this study is located in an urban area in the western part of Yamaguchi Prefecture. Several mammals (dogs, cats, rodents and rabbits), birds and aquarium fish are sold at this store, all of which are bought from a number of breeders. In this study, we collected 15 animal stool samples from 10 Djungarian hamsters, three Golden hamsters (*Mesocricetus auratus*), one Roborovski dwarf hamster (*Phodopus roborovskii*) and one domestic rabbit (*Oryctolagus cuniculus*) at store B (Table 1). Pathogenic *Y. enterocolitica* was isolated from five Djungarian hamsters raised in individual cages (three in 2012 and two in 2013). PFGE analysis suggested that these pathogens were clonal. All Djungarian hamsters at store B (both positive and negative for *Y. enterocolitica)* had been bought from the same breeder. The cages used for breeding the rodents were cleaned daily and are washed and disinfected after the animals were purchased. The Djungarian hamsters were provided with the same type of feed as the other rodents, and all animals were given tap water as drinking water. Thus, it is unlikely that the common source of the clonal pathogen contamination existed in the store between 2012 and 2013; although we did not examine the prevalence of pathogens in environmental samples, such as the feed, drinking water and cages. Therefore, it is unclear why the clonal pathogens were isolated solely from Djungarian hamsters.

A number of studies using experimental animals have revealed that pathogenic *Y. enterocolitica* serogroups O:3 and O:8 penetrate the intestinal mucosa *in vivo* [10, 16]. Although serogroup O:8 isolates have been shown to colonize the intestinal tract of wild rodents, O:3 isolates do not colonize the intestinal tract of the large Japanese field mouse (*Apodemus speciosus*) [4, 11]. This suggested that the Djungarian hamsters from which *Y. enterocolitica* O:3/3 variant VP- was isolated in this study constitute transient carriers**;** further studies will be required to determine whether this pathogen can infect hamsters.

Pet animals have long been suspected as a source of human yersiniosis, because of their close contact with humans, especially young children; however, pet to human

transmission has yet to be proven [3]. Fukushima *et al.* [8] have demonstrated the transmission of *Y. pseudotuberculosis* through environmental substances contaminated by feces from pathogen-infected cats. Since hamsters are popular pets worldwide, it is vital to alert pet owners and pet shops that pet hamsters could potentially carry pathogenic bacteria that might infect humans indirectly. This would enable the implementation of proper precautionary measures to prevent unwanted transmission.

REFERENCES

1. Bottone, E. J. 1997. *Yersinia enterocolitica*: the charisma continues. *Clin. Microbiol. Rev.* **10**: 257–276. [Medline]
2. Fredriksson-Ahomaa, M., Korte, T. and Korkeala, H. 2001. Transmission of *Yersinia enterocolitica* 4/O:3 to pets via contaminated pork. *Lett. Appl. Microbiol.* **32**: 375–378. [Medline] [CrossRef]
3. Fredriksson-Ahomaa, M., Stolle, A. and Korkeala, H. 2006. Molecular epidemiology of *Yersinia enterocolitica* infections. *FEMS Immunol. Med. Microbiol.* **47**: 315–329. [Medline] [CrossRef]
4. Fukushima, H. 1991. Susceptibility of wild mice to *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*. *Zentralbl. Bakteriol.* **275**: 530–540. [Medline] [CrossRef]
5. Fukushima, H. and Gomyoda, M. 1991. Intestinal carriage of *Yersinia pseudotuberculosis* by wild birds and mammals in Japan. *Appl. Environ. Microbiol.* **57**: 1152–1155. [Medline]
6. Fukushima, H., Hoshina, K., Itogawa, H. and Gomyoda, M. 1997. Introduction into Japan of pathogenic *Yersinia* through imported pork, beef and fowl. *Int. J. Food Microbiol.* **35**: 205–212. [Medline] [CrossRef]
7. Fukushima, H., Nakamura, R., Iitsuka, S., Tsubokura, M., Otsuki, K. and Kawaoka, Y. 1984. Prospective systematic study of *Yersinia* spp. in dogs. *J. Clin. Microbiol.* **19**: 616–622. [Medline]
8. Fukushima, H., Gomyoda, M., Ishikura, S., Nishio, T., Moriki, S., Endo, J., Kaneko, S. and Tsubokura, M. 1989. Cat-contaminated environmental substances lead to *Yersinia pseudotuberculosis* infection in children. *J. Clin. Microbiol.* **27**: 2706–2709. [Medline]
9. Galindo, C. L., Rosenzweig, J. A., Kirtley, M. L. and Chopra, A. K. 2011. Pathogenesis of *Y. enterocolitica* and *Y. pseudotuberculosis* in human yersiniosis. *J. Pathogens* **2011**: 182051 [CrossRef]. [Medline]
10. Hanski, C., Kutschka, U., Schmoranzer, H. P., Naumann, M., Stallmach, A., Hahn, H., Menge, H. and Riecken, E. O. 1989. Immunohistochemical and electron microscopic study of interaction of *Yersinia enterocolitica* serotype O8 with intestinal mucosa during experimental enteritis. *Infect. Immun.* **57**: 673–678. [Medline]
11. Hayashidani, H., Kitahara, E. and Ogawa, M. 1994. Infectivity and pathogenicity of *Yersinia enterocolitica* serovar 0:8 to wild rodents in Japan. *Zentralbl. Veterinarmed. B.* **41**: 504–511. [Medline]
12. 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]
13. Ichinohe, H., Yoshioka, M., Fukushima, H., Kaneko, S. and Maruyama, T. 1991. First isolation of *Yersinia enterocolitica* serotype O:8 in Japan. *J. Clin. Microbiol.* **29**: 846–847. [Medline]
14. Inoue, M., Nakashima, H., Ueba, O., Ishida, T., Date, H., Kobashi, S., Takagi, K., Nishu, T. and Tsubokura, M. 1984. Community outbreak of *Yersinia pseudotuberculosis*. *Microbiol. Immunol.* **28**: 883–891. [Medline] [CrossRef]
15. Isobe, J., Kimata, K., Shimizu, M., Kanatani, J., Sata, T. and Watahiki, M. 2014. [Water-borne outbreak of *Yersinia enterocolitica* O8 due to a small scale water system]. *Kansenshogaku Zasshi* **88**: 827–832 (in Japanese). [Medline]
16. Lian, C. J., Hwang, W. S., Kelly, J. K. and Pai, C. H. 1987. Invasiveness of *Yersinia enterocolitica* lacking the virulence plasmid: an in-vivo study. *J. Med. Microbiol.* **24**: 219–226. [Medline] [CrossRef]
17. Nakajima, H., Inoue, M., Mori, T., Itoh, K., Arakawa, E. and Watanabe, H. 1992. Detection and identification of *Yersinia pseudotuberculosis* and pathogenic *Yersinia enterocolitica* by an improved polymerase chain reaction method. *J. Clin. Microbiol.* **30**: 2484–2486. [Medline]
18. Rahman, A., Bonny, T. S., Stonsaovapak, S. and Ananchaipattana, C. 2011. *Yersinia enterocolitica*: epidemiological studies and outbreaks. *J. Pathogens* **2011**: 239391. [Medline] [CrossRef]
19. Sabina, Y., Rahman, A., Ray, R. C. and Montet, D. 2011. *Yersinia enterocolitica*: mode of transmission, molecular insights of virulence, and pathogenesis of infection. *J. Pathogens* **2011**: 429069. [Medline] [CrossRef]
20. Sakai, T., Nakayama, A., Hashida, M., Yamamoto, Y., Takebe, H. and Imai, S. 2005. Outbreak of food poisoning by *Yersinia enterocolitica* serotype O8 in Nara prefecture: the first case report in Japan. *Jpn. J. Infect. Dis.* **58**: 257–258. [Medline]
21. Sugiyama, K. 2000. *Yersinia enterocolitica*. pp. 298–320. *In*: Food- and Water-borne Infection and Food Poisoning. new ed. (Sakazaki, R. ed.), Chuohoki, Tokyo (in Japanese).
22. Thoerner, P., Bin Kingombe, C. I., Bögli-Stuber, K., Bissig-Choisat, B., Wassenaar, T. M., Frey, J. and Jemmi, T. 2003. PCR detection of virulence genes in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* and investigation of virulence gene distribution. *Appl. Environ. Microbiol.* **69**: 1810–1816. [Medline] [CrossRef]
23. Wang, X., Cui, Z., Wang, H., Tang, L., Yang, J., Gu, L., Jin, D., Luo, L., Qiu, H., Xiao, Y., Xiong, H., Kan, B., Xu, J. and Jing, H. 2010. Pathogenic strains of *Yersinia enterocolitica* isolated from domestic dogs (*Canis familiaris*) belonging to farmers are of the same subtype as pathogenic *Y. enterocolitica* strains isolated from humans and may be a source of human infection in Jiangsu Province, China. *J. Clin. Microbiol.* **48**: 1604–1610. [Medline] [CrossRef]
24. Wilson, H. D., McCormick, J. B. and Feeley, J. C. 1976. *Yersinia enterocolitica* infection in a 4-month-old infant associated with infection in household dogs. *J. Pediatr.* **89**: 767–769. [Medline] [CrossRef]
25. Yanagawa, Y., Maruyama, T. and Sakai, S. 1978. Isolation of *Yersinia enterocolitica* and *Yersina pseudotuberculosis* from apparently healthy dogs and cats. *Microbiol. Immunol.* **22**: 643–646. [Medline] [CrossRef]