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

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**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...
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 alkal (KOH) treatment [1], the sample suspension was then cultured on agar-novobioin (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 fyuA, 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 NotI (TaKaRa, Kusatsu, Japan) for 3 hr at 37°C. The DNA fragments were separated on 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 (Bioatlasant, 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 NotI 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...
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

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

| Survey time | 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)        | 0/8 (0.0)         | 0/7 (0.0)         | 0                 | 1/50 (2.0) |
| 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)        | 0/3 (0.0)         | 0/1 (0.0)         | 3/100 (3.0) |
| Nov.        | 0/36 (0.0)        | 0/4 (0.0)         | 0                 | 0                 | 0/40 (0.0) |
| 2016 Apr.   | 2/38 (5.3)        | 1/18 (5.6)        | 0/4 (0.0)         | 0/2 (0.0)         | 3/62 (4.8) |
| 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)        | 0                 | 0/4 (0.0)         | 1/17 (5.9) |
| 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)         | 0/34 (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)        | 2/23 (8.7)        | 0/1 (0.0)         | 0/1 (0.0)         | 3/45 (6.7) |
| Nov.        | 1/25 (4.0)        | 0/23 (0.0)        | 0                 | 0/3 (0.0)         | 1/51 (2.0) |
| 2021 Apr.   | 2/37 (28.6)       | 0/10 (0.0)        | 0                 | 0                 | 2/47 (11.8) |
| 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 (%) |
|----------------|---------|-----------------------------------------------------|
| Apodemus speciosus | Male Juvenile | 2/59 (3.4) |
|                | Adult     | 0/51 (0.0) |
|                | Subtotal  | 2/110 (1.8) |
|                | Female Juvenile | 0/45 (0.0) |
|                | Adult     | 2/41 (4.9) |
|                | Subtotal  | 2/86 (2.3) |
|                | Subtotal  | 4/196 (2.0) |
|               |           | 2/268 (0.7) |
|               |           | 6/464 (1.2) |
| Apodemus argenteus | Male   | 3/74 (4.1) |
|                | Female   | 3/75 (4.0) |
|                | Subtotal | 6/149 (4.0) |
|                |           | 1/83 (1.2) |
|                |           | 7/232 (3.0) |
|                |           | 10/345 (2.9) |
|                |           | 3/351 (0.9) |
|                |           | 13/696 (1.9) |
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 \textit{Y. pseudotuberculosis} from juvenile wild rodents was significantly higher than that in adults. In addition, Fukushima [6] challenged pathogenic \textit{Y. pseudotuberculosis} to \textit{A. speciosus} intragastrically and found that the juvenile rodents showed significantly higher susceptibility to this bacterium rather than that of adults and \textit{A. speciosus} excreted \textit{Y. pseudotuberculosis} in the feces for 1–2 weeks after oral challenge. Furthermore, Hayashidani et al. [10] also challenged \textit{Y. enterocolitica} \textit{O8} to \textit{A. speciosus} intragastrically and reported that those \textit{A. speciosus} shed this bacterium in their feces for more than 2 weeks. The wild rodent’s feces including pathogenic \textit{Yersinia} might contaminate the environment such as feeds, soil, and water. Those results indicated that horizontal and vertical transmission of pathogenic \textit{Y. enterocolitica} \textit{O8} seem to occur among wild rodent populations. Further studies are needed to clarify clearly the ecology of pathogenic \textit{Y. enterocolitica} \textit{O8} in wild rodent populations.

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

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

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