別記様式第３号

学　位　論　文　要　旨

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題　　目　Epidemiological studies on pathogenic *Yersinia* in wild rodents and development of rapid detection methods for those pathogens

（ノネズミにおける病原性エルシニアに関する疫学的研究ならびにこれら病原体の迅速検出法の開発）

Yersinia enterocolitica and *Yersinia pseudotuberculosis* are recognized worldwide as important foodborne and human zoonotic pathogens. Human *Yersinia* infection causes gastroenteritis with clinical symptoms including abdominal pain, diarrhea, and fever; however, highly pathogenic *Yersinia,* such as *Y. enterocolitica* O8 and *Y. pseudotuberculosis,* sometimes causes septicemia. Wild rodents were suggested to be natural reservoirs for pathogenic *Yersinia.* A few reports indicating that wild rodents harbor pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* have been published. However, the ecology and epidemiology of pathogenic *Yersinia* in wild rodents is still unclear. In addition, no reports are available on the prevalence of pathogenic *Yersinia* in wild rodents living in Fukushima Prefecture, Japan.

Three groups of pathogenic *Yersinia,* including highly pathogenic *Y. enterocolitica,* low pathogenic *Y. enterocolitica,* and *Y. pseudotuberculosis*, have been caused yersiniosis in Japan. Moreover, recently, the pathogenic *Y. enterocolitica* O8, belonging to the highly pathogenic *Y. enterocolitica,* has been isolated from human patients in Japan and some European countries such as Germany, Poland, and France. Therefore, the rapid and sensitive methods for the detection of these pathogens are required. However, the method for rapid detection and differentiation of these 3 groups of pathogens has not been developed.

In the present study, we aimed to clarify the ecology and epidemiology of pathogenic *Yersinia* originated from wild rodents in Fukushima prefecture, Japan, and to develop rapid, sensitive, and specific detection methods for three groups of pathogenic *Yersinia.*

[Chapter I] **Prevalence of pathogenic *Yersinia* in wild rodents in Fukushima Prefecture, Japan**

From 2012 to 2021, 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

twice a year in Fukushima Prefecture of Japan to examine for the prevalence of pathogenic *Yersinia* and clarify the ecology and epidemiology of this pathogen in nature. Pathogenic *Yersinia enterocolitica* O8 was isolated from 13 (1.7%) of 755 wild rodents. All *Y. enterocolitica* O8 isolates were pathogenic strains, harboring 3 virulent genes, including *ail, fyuA*, and *virF*. 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*. No *Y. enterocolitica* O8 isolate was recovered from *M. montebelli* and *U. talpoides*. For 10-year observations, the *Y. enterocolitica* O8 was isolated from wild rodents in 6 of 19 times surveys, which were in April 2015, 2016, and 2017, in June and November 2020, and in April 2021. All *Y. enterocolitica* O8 isolates showed the same PFGE (Pulse-filed Gel Electrophoresis) patterns. These results indicate that the same clone of pathogenic *Y. enterocolitica* O8 has been maintained in wild rodent populations in Fukushima Prefecture. Wild rodent populations seem to contribute substantially to the persistence of pathogenic *Y. enterocolitica* O8 in the ecosystem. This is the first report on the prevalence of pathogenic *Y. enterocolitica* O8 in wild rodents in Fukushima Prefecture, Japan.

[Chapter II] **Development of multiplex PCR method for differentiating highly pathogenic *Yersinia enterocolitica* and low pathogenic *Yersinia enterocolitica,* and *Yersinia pseudotuberculosis***

A multiplex PCR method for rapid and sensitive diagnosis, differentiating 3 pathogenic *Yersinia* groups such as highly pathogenic *Y. enterocolitica,* low pathogenic *Y. enterocolitica,* and *Y. pseudotuberculosis* was developed. The multiplex PCR targets 4 genes, *fyuA*, *ail*, *inv*, and *virF,* responsible for the virulence in pathogenic *Yersinia* species. Under the multiplex PCR conditions, the unique band patterns for the highly pathogenic *Y. enterocolitica,* low pathogenic *Y. enterocolitica,* and *Y. pseudotuberculosis* were generated from *Yersinia* strains, respectively. The limits of detection were 10^1–10^3 CFU per reaction tube from pure culture and 10^2–10^3 CFU per reaction tube from spiked rabbit blood samples. This multiplex PCR method could detect pathogenic *Y. enterocolitica* O8 from the wild rodent fecal samples that were culture-positive. Therefore, the new multiplex PCR method developed in this study is a useful tool for rapid and sensitive diagnosis, distinguishing 3 pathogenic *Yersinia* groups.

[Chapter III] **Development of multiplex real-time PCR methods for differentiating highly pathogenic *Yersinia enterocolitica* and low pathogenic *Yersinia enterocolitica,* and *Yersinia pseudotuberculosis***

The novel SYBR Green and TaqMan multiplex real-time PCR methods for rapid and sensitive diagnosis and differentiating 3 pathogenic *Yersinia* groups were developed in this Chapter. Three virulence genes, including *fyuA*, *ail*, and *inv,* were selected for the development of these multiplex real-time PCR methods. The SYBR Green and TaqMan multiplex real-time PCR methods showed highly specificity for the detection and differentiation of 3 pathogenic *Yersinia* groups. Both SYBR Green and TaqMan multiplex real-time PCR methods showed the similar detection limits (10^1 CFU per reaction tube from pure cultures and 10^1–10^2 CFU per

reaction tube from spiked rabbit blood samples). This sensitivity is 10 times higher than those of the multiplex PCR method developed in Chapter 2. In addition, the SYBR Green and TaqMan multiplex real-time PCR methods could detect pathogenic *Y. enterocolitica* O8 from wild rodent fecal samples that were culture-positive and conventional multiplex PCR-positive. Both multiplex real-time PCR run take approximately only 2–3 h although the conventional multiplex PCR method required 1 day to detect and distinguish the pathogenic *Yersinia* strains. Therefore, the novel SYBR Green and TaqMan multiplex real-time PCR methods developed in this study are more rapid and sensitive to diagnose and distinguish 3 pathogenic *Yersinia* groups compared to the conventional multiplex PCR method developed in Chapter 2.

In the present study, the prevalence of pathogenic *Yersinia* in wild rodents was observed for 10 years and the pathogenic *Y. enterocolitica* O8 has been found to be maintained at least 6 years within the wild rodent populations in Fukushima Prefecture, Japan. These findings can be useful for understanding the epidemiology and ecology of pathogenic *Yersinia*, especially the pathogenic *Y. enterocolitica* O8 in Japan. Moreover, 3 molecular methods, including multiplex PCR, SYBR Green and TaqMan multiplex real-time PCR, were developed for rapid and sensitive diagnosis, differentiating 3 pathogenic *Yersinia* groups such as the highly pathogenic *Y. enterocolitica*, including serotype O8, low pathogenic *Y. enterocolitica*, and *Y. pseudotuberculosis*. These methods can be valuable tools for clarifying the ecology and epidemiological of pathogenic *Yersinia* in nature and for the diagnosis of *Yersinia* infection in humans.

（備考）１　学位論文題目が日本語の場合は英訳を、英語の場合は和訳を（　　）内に記入すること。（英語の表記は，論文目録と同じ。）

２　日本語の場合は、２，０００字程度とする。

３　英語の場合は、１，２００語程度とする。

４　図表は、この要旨には記載しないこと。

５　枚数は１枚を超えても差し支えありません。