

## 学 位 論 文 要 旨

博士課程 甲	第	号	氏 名	SUBANGKIT MAWAR
<p>[論文題名]</p> <p>Genotyping of swine <i>Mycobacterium avium</i> subsp. <i>hominissuis</i> isolates from Kyushu, Japan 九州産の豚 <i>Mycobacterium avium</i> subsp. <i>hominissuis</i> の遺伝子型判定</p> <p>Journal of Veterinary Medical Science, in press, 20, Vol. 81 no. 7 DOI: <a href="https://doi.org/10.1292/jvms.19-0048">https://doi.org/10.1292/jvms.19-0048</a></p> <p>[要 旨]</p> <p>The incidence of diseases caused by nontuberculous mycobacteria (NTM) is increasing annually worldwide, including Japan. <i>Mycobacterium avium</i> subsp. <i>hominissuis</i> (MAH) is one of the most common NTM species responsible for chronic lung diseases in animals and humans. MAH strains are maintained or persist in humans, other animals, and the environment. In humans, NTM cause pulmonary nontuberculous mycobacterial disease (PNTMD), the incidence of which is increasing worldwide, including Japan. Pigs are the animals most susceptible to <i>Mycobacterium avium</i> complex (MAC) infection, especially MAH. Mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) is a useful technique for characterizing the genetic diversity of isolates of MAC, <i>M. avium</i> subsp. <i>paratuberculosis</i>, and MAH isolated from humans, animals, and the environment. Eight MIRU-VNTR loci (292, X3, 25, 47, 3, 7, 10, and 32) can potentially be used for high-throughput, reproducible, effective discriminatory analysis of MAH. In the current study, MIRU-VNTR typing was employed to characterize the genetic diversity of swine MAH isolates from Kyushu, Japan.</p> <p>A total of 107 mesenteric lymph nodes were not displaying any clinical signs of disease, with granuloma lesions from pigs aged ~6 months old and suspected of carrying <i>Mycobacterium</i> infections were used in this study. The samples from pigs raised on 15 farms in Kyushu were obtained during 2012–2016. Bacteria were cultured on Middlebrook 7H10 agar (BD Difco, Sparks, MD, U.S.A) and the acid-fastness of colonies was confirmed by Ziehl-Neelsen staining. Multiplex PCR with MYCOGEN-F, MYCOGEN-R, MYCAV-R and MYCINT-F primer set were used for <i>Mycobacterium</i> genetic identification and IS901 flanking region (FR) 300 PCR was performed for subspecies determination. Eight MIRU-VNTR loci typing was performed for genotyping analysis, allelic diversity measurements and HGDI calculations. Clustering analysis was performed by dendrogram analysis and minimum spanning tree were analyzed in R software ver. 3.5.1.</p>				

In total, 309 isolates were obtained from the lymph nodes of 107 pigs, of which 307 (99.35%) were identified as MAH and comprising 173 strains. Based on eight established MIRU-VNTR loci, the MAH strains represented 50 genotypes (ky01-ky50) constituting three lineages, and 29 had not been previously described in the *Mac French National Institute for Agricultural Research Nouzilly MIRU-VNTR* (Mac-INVM) database, one of MAC genotype profiles database in the world. Three genotype profiles with significantly higher strain populations than others ( $p < 0.05$ ) were represented in genotyping analysis of Kyushu strains. These three predominant genotype profiles were ky07 (22331118; 18.49%), ky08 (22431118; 17.34%), and ky33 (24231128; 9.24%). In three predominant genotype profiles, only ky08 were reported isolated from pig in Japan. Ky07 never reported previously in Japan and ky33 strains, showed as a popular genotype profile in Europe and reported was isolated in human and pig in Finland and France. Even three predominant strains were isolated from healthy pig in Kyushu, but two of three strains were reported caused clinical symptom in pig in other countries. Ky07 profiles have been isolated from swine lymph nodes, genital swabs, aborted fetuses, uterus, sperm, and umbilical cord from a breeding sow in Germany. Ky33 has been isolated from pig, cattle, human, and other samples in France, and pig, human, hen, and chicken samples in Finland. Currently, no additional information is available for ky08, since the sources of this strain have not yet been published. These inconsistent results may be due to the age of pigs. We isolated MAH with these genotype profiles from growing-finishing pigs (around 6-months-old), while other groups isolated these MAH strains from sows. The infection period of MAH in pigs may correlate with the development of clinical signs. Further work to confirm the pathogenicity of the predominant MAH strains in Kyushu is in progress in our laboratory.

The allelic diversity data revealed that locus 25 displayed the highest allelic diversity, with high diversity for locus 25 ( $h = 0.668$ ), followed by locus X3 ( $h = 0.539$ ) and locus 10 ( $h = 0.520$ ). Loci 3 and 7 did not exhibit allelic diversity ( $h = 0.000$ ), and locus 292 displayed low allelic diversity ( $h = 0.300$ ). Single locus analysis by MIRU-VNTR resulted in poor discrimination of the genetic diversity of MAH in Kyushu. Loci 3 and 7 were monomorphic. The best single locus discriminatory values were locus 25 (HGDI = 0.695) followed by locus 10 (HGDI = 0.530). Other loci resulted in low and moderate discrimination. Eight combinations of loci successfully discriminated MAH among Kyushu isolates (HGDI = 0.921). Allelic diversity data showed that MAH in Kyushu shared similar characteristics in allelic diversity with MAH isolated in other parts of Japan.

In minimum spanning tree (MST) analysis, some Kyushu strains genotype profiles were reported in human isolates. Five Kyushu strains (ky12, ky13, ky25, ky26, and ky33) showed identical with human isolate from Finland, four Kyushu strains (ky10, ky13, ky20, and ky26) identical with human isolates from France and two Kyushu strains (ky26 and ky34) identical with human isolates in

Japan. Although, these two strains were identical with human in Japan were not belong in predominant genotype profiles.

This study revealed that high genetic diversity of MAH between farms in Kyushu, indicated that there was no cross contamination between farm. Reinfection with same genotype profiles were observed in two farm in Kyushu during experiments. Pig barn environments probably contain most of the factors that result in repeated autoinfection of pigs with MAH. Sawdust, peat, or other kinds of bedding are reportedly one of the main sources of infections that result in repeated autoinfection of pigs with MAH. Reusing sawdust for bedding in pig farms is common practice in Kyushu, and this may be one of the factors responsible for horizontal transmission. The failure of cleaning systems to eliminate MAH from pig farms leads to continuous infection over several farming periods. Some imported materials such as sawdust were imported to Japan from other countries, including European nations. These imported materials may be responsible for the high relatedness between MAH in isolates from Kyushu and European countries.

In conclusion, MAH was the dominant *M. avium* complex (MAC) in pigs from Kyushu, and there was high genetic diversity among genotype profiles of MAH from Kyushu. We identified three predominant genotype profiles in the tested area sharing high relatedness with genotype profiles of strains isolated in European countries. Several genotype profiles, including one of the predominant profiles, matched those obtained from human isolates. MAH was the most common NTM in pigs from Kyushu and exhibited high diversity, with new strain-derived genotypes.

備考 論文要旨は、和文にあつては2,000字程度、英文にあつては1,200語程度