Prevalence of Helicobacter in Laboratory Mice in Thailand

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Abstract: Prevalence of Helicobacter is mostly unknown in laboratory animals in Thailand. The 221 mice feces/cecum from 8 universities, 2 pharmaceutical companies and 3 research institutions in Thailand were surveyed for the prevalence and distribution of Helicobacter species by using the Electrochemical DNA chip. Helicobacter were detected 23/46 samples in Specific Pathogen Free (SPF) and 168/175 in conventional condition. Prevalence of Helicobacter were 98%, 96%, 92% and 78% in South (n=40), Northeast (n=40), North (n=25) and Central area (n=116), respectively. Only Central area holds SPF facility resulting in Helicobacter prevalence that seems to be lower than other areas. Three species of Helicobacter were detected in feces/cecum samples by sequence analysis: H. rodentium (67.0%, 148 samples), Helicobacter sp. MIT 01-6451 (15.4%, 34 samples), and unidentified Helicobacter species (14.1%, 9 samples). The results suggested that H. rodentium is the most common species of Helicobacter in laboratory mice in Thailand.

Key words: DNA chip, Helicobacter, laboratory mice, prevalence

Introduction

Helicobacter is a genus of gram negative bacteria, family Helicobacteriaceae, spiral, microaerophilic isolated from the gastrointestinal system of mammals. Several Helicobacter species have been detected in laboratory animals. Among these, H. hepaticus is the most prominent pathogen of mice. Clinical signs are absent in immunocompetent mice in which only rectal prolapse may occur in immunodeficient mice [20]. Pathological changes include the chronic active hepatitis possibly of autoimmune etiology, occasional enterocolitis, and hepatocellular neoplasms [2, 3, 21]. H. hepaticus has also been associated with hepatic carcinomas and elevated levels of alanine aminotransferase in serum. Natural infection of laboratory mice with H. hepaticus, and possibly other Helicobacter spp., could confound carcinogenicity research and researches involving the gastrointestinal system [5, 21].

H. bilis has been isolated from intestine, bile and liver of aged inbred mice [4] and can cause enterohepatic disease and inflammatory bowel disease (IBD) in immunocompromised mice [7] and rats [8]. Co-infection with H. bilis and H. rodentium has been reported in severe combined immunodeficiency (SCID) mice with acute diarrhea [18].

H. rodentium is the first urease-negative Helicobacter species isolated from intestines of SCID mice with diar-
rhea that were co-infected with *H. bilis* [17]. In A/Jcr or C.B-17/ICr-scidBr mice, infection with *H. rodentium* alone does not cause hepatitis or enteritis; however, co-infection with *H. hepaticus* and *H. rodentium* has been associated with augmented cecal gene expression and clinical manifestation of disease in immunodeficient mice [11].

*H. ganmani* is another urease-negative *Helicobacter* species isolated from the intestines of laboratory mice from three of the four facilities in Sydney, Australia. This species is the most closely related with *H. rodentium* [15]. *H. ganmani* has been identified by PCR in the liver, small bowel, cecum, colon and feces of a breeding colony of IL-10 deficient B6.129P2-Il10tm1Cgn/J mice. Inflammation has also been found in cecum, colon and livers, most pronounced in the cecal areas of culture positive mice with a severe typhlitis [9].

*H. typhlonius* is the urease-negative *Helicobacter* isolated from colonies of laboratory mice independently by two laboratories in the United States [6]. *H. typhlonius* has also been detected in the sex organs of immunodeficient athymic nude-nu (nu/nu), Helicobacter-sensitive C3H/HeJ and Helicobacter-resistant C57BL/6J mouse strains but does not transmit vertically [16].

*H. muridarum* has been firstly isolated from the intestinal mucosa of rats and mice [10].

However, the prevalence of *Helicobacter* is mostly unknown in laboratory mice in Thailand. In this study, we surveyed the prevalence and distribution of *Helicobacter* species in laboratory mice collected from different facilities in Thailand.

### Materials and Methods

#### Samples

Two hundred and twenty one mice (*Mus musculus*) from facilities in each area of Thailand, including Central (6 facilities), South (2 facilities), North (2 facilities) and Northeast (3 facilities) were used in this study (Table 1). Mouse feces were obtained by random sampling from facilities of universities, pharmaceutical companies and research institutions which voluntarily participate in this study. There are over 25 facilities in Thailand. Mostly mouse facilities are maintained under conventional condition. For Specific pathogen free (SPF), all of the supplies and equipments are sterilized including feed whereas supplies and equipments in conventional are cleaned. In SPF facility, 10 bacteria, 18 viruses, 1 fungus and 5 parasites were monitored, excluding *Helicobacter* spp. Animal Care and Use Committee (IACUC) of National Laboratory Animal Center, Mahidol University (NLAC-MU) have approved the research protocols (RA2010-09).

#### Isolation of DNA

Genomic DNA was isolated from feces/cecum by using MagExtractor genome (TOYOBO CO., LTD, Osaka, Japan) according to the instruction. DNA was stored at −20°C until analysis.

#### Loop-mediated Isothermal Amplification (LAMP) and DNA chip detection

LAMP reaction is based on the principle of strand displacement under isothermal condition and used four
primers that recognized six distinct regions on the target DNA. This method uses a single tube and single temperature incubation for amplification of nucleic acid. The DNA chip is a new tool used to identify genetic difference based on principle of hybridization between two DNA strands. On the chip surface contains thousands of DNA probe, only strongly complementary DNA strands will remain hybridized after washing. LAMP product and DNA chip detection were carried out using Monigene™ Helico Multi for Helicobacter spp., Monigene™ Helico for 4 species of Helicobacters (H. typhlonius, H. bilis, H. hepaticus, and H. pylori), and Genelyzer GLG-2000 (Toshiba Hokuto Electronics Corporation, Asahikawa, Japan) according to the instruction manual. Positive samples in Monigene™ Helico Multi were subsequently tested with Monigene™ Helico. Helicobacter spp. positive samples in Monigene™ Helico were further studied to identify species of Helicobacter by sequence analysis.

**Sequence analysis**

Nested PCR was performed according to the previously described protocol [12]. PCR products were sequenced by using ABI prism 3730XL capillary Sequencer (Sequenced by Bio Basic Canada Inc., Makham Ontario, Canada). Homology analysis between DNA sequencing results and database were carried out using Basic Local Alignment Search Tool (BLAST) from the NCBI (National Center for Biotechnology Information) [http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web &PAGE_TYPE=BlastHome].

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**Results**

In Thailand, mostly facilities were maintained under conventional condition. We surveyed Helicobacter in 13 mouse facilities of 4 areas of Thailand; North, Northeast, Central and South. All facilities in this study were voluntarily participated. Helicobacter were tested using Electrochemical DNA chip and were found in 191 laboratory mice of all areas tested in Thailand. Sequence analysis reviewed the species of Helicobacter as H. rodentium, Helicobacter sp. MIT 01-6451 and unidentified Helicobacter species. The lowest prevalence of Helicobacter was found in Central and only H. rodentium was detected in this area (Fig. 1). Moreover, H. rodentium was found in all sampling areas while Helicobacter sp. MIT 01-6451 were detected in the samples from South, North and Northeast but not found in Central area. Unidentified Helicobacter species were presented only in Northeast of Thailand.

As shown in Table 1, we found Helicobacter in sample from all of mouse facilities participated in this study. H. rodentium was also detected in all facilities of Specific Pathogen Free (SPF) and conventional conditions except the facility 12. Helicobacter sp. MIT 01-6451 were detected in 2 facilities in North, 1 facility in Northeast and 1 facility in South.

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**Discussion**

In this study, H. rodentium, Helicobacter sp. MIT 01-6451, and unidentified Helicobacter species were detected in mice tested. Helicobacter were found in 98%, 96%, 92% and 78% of samples from South, Northeast, North and Central area, respectively. These results indicated that Helicobacter spread to all areas tested in Thailand. However, only in Central area where laboratory mice have been maintained under SPF condition, represented the lower prevalence of Helicobacter than other areas. If comparing the Helicobacter prevalence in conventional facilities, there seems to be no differences in all areas tested in Thailand. This evidence might potentially support that SPF condition could limit the spread of Helicobacter in laboratory animals.

Overall, Helicobacter was found in 86% of sample from SPF and conventional condition of mouse colonies obtained from the universities, pharmaceutical companies and research institutions around Thailand. Most facilities in Thailand have still maintained mice in conventional condition. The prevalence of Helicobacter in conventional condition (96%) was higher than in SPF (50%) condition (Table 1). This information could support that the health quality of laboratory animals maintained under SPF condition still is the most reliable option.

In agreement with our finding, a study in mice from commercial and academic institutions in Asia, Europe and North America has showed that 30 of 34 institutions (88%) were infected with Helicobacter spp. [19]. Nilsson et al. [13] have surveyed 42 mice in 4 conventional laboratory animal houses. They found that 36 of 42 (86%) samples were positive with Helicobacter genus-specific PCR in fecal samples. Bohr et al. have investigated the prevalence and spread of enterohepatic Helicobacter species in SPF mice in which Helicobacter...
species were identified in 87.5% of the mouse lines tested [1]. These reports indicated that a broad spread of Helicobacter spp. was found in laboratory mouse colonies.

Regarding our results, H. rodentium is the most common Helicobacter species infected in laboratory mice in Thailand. Although, the infection of H. rodentium alone has not been reported the association with disease, however, co-infection of H. rodentium with H. hepaticus or H. bilis has been shown the association with clinical manifestation of disease in immunodeficiency mice [11, 18]. The pathogenicity of H. rodentium is still unclear and tends to be the normal flora of the gastrointestinal tract.

No visible lesions have been observed in the liver and intestines of mice from SPF condition that were infected with Helicobacter. Most of Helicobacter species have not been reported the association with disease in immunocompetent mice. H. hepaticus and H. bilis have been report the most prevalence species in laboratory animals [14] and associated with inflammatory bowel disease (IBD) in SCID mice [7, 20]. Interestingly, mice infected with H. bilis and H. hepaticus were not found in our study and Helicobacter were not detected in 2 SCID mice (data not shown).

Our finding showed that Helicobacter sp. MIT01-6451 was found in South, North and Northeast but not found in Central. Taylor et al. have surveyed Helicobacter in mice and also found Helicobacter sp. MIT01-6451 presented in both colonies of two institutions from Japan [19]. These results may be supported that Helicobacter sp. MIT01-6451 is one of the common species of Helicobacter in Asia.

Apart from H. rodentium and Helicobacter sp. MIT01-6451, we also found unidentified Helicobacter species by homology analysis in 9 samples from facility 12 of Northeast which may be new species of Helicobacter and necessary for further investigation.

In conclusion, laboratory mice maintained under SPF condition has lower prevalence of Helicobacter (50%) than those mice maintained under conventional condition (96%) supporting that SPF is the most reliable options for laboratory animal. H. rodentium, Helicobacter sp. MIT 01-6451, and unidentified Helicobacter species have been detected in laboratory mice and H. rodentium is the most common species of Helicobacter in laboratory mice in Thailand.

Fig. 1. Prevalence of Helicobacter in laboratory mice in Thailand
221 mice feces-cecum were tested for Helicobacter species by using the Electrochemical DNA chip followed by sequencing analysis. Helicobacter contamination rates are discriminately shown in percent bar according to the geographical location of facilities tested (Central, South, North and Northeast areas of Thailand). Constitution rates of H. rodentium, Helicobacter sp. MIT 01-6451 and unidentified Helicobacter species were indicated in the bar. Note that only Central area of Thailand holds a SPF facility but other three areas possess conventional ones.
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References

1. Bohr, U.R., Selgrad, M., Ochmann, C., Backert, S., Konig, W., Fenske, A., Wex, T., and Malferttheiner, P. 2006. Prevalence and spread of enterohpatic Helicobacter species in mice reared in a specific-pathogen-free animal facility. J. Clin. Microbiol. 44: 738–742. [Medline] [CrossRef]

2. Canella, K.A., Diwan, B.A., Gorlick, P.L., Donovan, P.J., Sipowicz, M.A., Kasprzak, K.S., Wegerhorst, C.M., Snyderwine, E.G., Davis, C.D., Keefer, L.K., Kyrtopoulos, S.A., Hecht, S.S., Wang, M., Anderson, L.M., and Rice, J.M. 1996. Liver tumorigenesis by Helicobacter hepaticus: considerations of mechanism. In Vivo 10: 285–292. [Medline]

3. Fox, J.G., Li, X., Yan, L., Cahill, R.J., Hurley, R., Lewis, R., and Murphy, J.C. 1995. Chronic proliferative hepatitis in A/JCr mice associated with persistent Helicobacter hepaticus infection: a model of helicobacter-induced carcinogenesis. Infect. Immun. 64: 1548–1558. [Medline]

4. Fox, J.G., Yan, L.L., Dewhirst, F.E., and Fox, J.G. 2007. Enterohepatic helicobacter species are prevalent in laboratory mouse strains by multiplex PCR-denaturing gradient gel electrophoresis and pyrosequencing. J. Clin. Microbiol. 42: 3781–3788. [Medline] [CrossRef]

5. Robertson, B.R., O'Rourke, I.S., Stenram, U., Moran, A.P., Wadstrom, T., and Al-Soud, W.A. 2004. High prevalence of Helicobacter Species detected in laboratory mouse strains by multiplex PCR-denaturing gradient gel electrophoresis and pyrosequencing. J. Clin. Microbiol. 42: 3781–3788. [Medline] [CrossRef]

6. Franklin, C.L., Gorelick, P.L., Donovan, J.C., Anderson, K.A., Da Motta, M.A., Gilden, R.V., Tully, J.G., Russell, R.J., and Besch-Walter, T.J. 1992. Helicobacter typhlonius sp. nov., a novel Murine urease-negative anaerobe isolated from the intestines of laboratory mice. Int. J. Syst. Bacteriol. 42: 942–946. [Medline] [CrossRef]

7. Franklin, C.L., Ryley, L.K., Hook, R.R. Jr., and Besch-Walter, T.J. 1992. Helicobacter typhlonius sp. nov., a microaerophilic helical bacterium with a novel ultrastructure isolated from the intestinal mucosa of rodents. Int. J. Syst. Bacteriol. 42: 27–36. [Medline] [CrossRef]

8. Myles, M.H., Livingston, R.S., and Franklin, C.L. 2004. Pathogenicity of Helicobacter rodentium in A/JCr and SCID mice. Comp. Med. 54: 549–557. [Medline]

9. Nakamura, N., Ito, K., Hashimoto, M., Nakamura, A., Hayashimoto, N., Takakura, A., Hashimoto, K., Naido, M., and Gemma, N. 2011. Development of multiaspect detection system using a tag insertion primer and an electrochemical DNA chip. Anal. Biochem. 419: 190–195. [Medline] [CrossRef]

10. Lee, A., Phillips, M.W., O'Rourke, J.L., Paster, B.J., Dewhirst, F.E., Fraser, G.J., Fox, J.G., Sly, L.I., Romaniuk, P.J., Trust, T.J., and Kouprach, S. 1992. Helicobacter muridarum sp. nov., a microaerophilic helical bacterium with a novel ultrastructure isolated from the intestinal mucosa of rodents. Int. J. Syst. Bacteriol. 42: 27–36. [Medline] [CrossRef]