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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-

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Table 1. Distribution of *Helicobacter* in laboratory mice in Central, South, North and Northeast of Thailand

Source (Area)	Type of samples	Status	No. of <i>Helicobacter</i> positive / No. of samples tested	<i>Helicobacter</i> species
Facility 1 (Central)	Cecum	SPF	23/46	<i>H. rodentium</i>
Facility 2 (Central)	Feces	Conventional	19/20	<i>H. rodentium</i>
Facility 3 (Central)	Feces	Conventional	7/7	<i>H. rodentium</i>
Facility 4 (Central)	Feces	Conventional	19/20	<i>H. rodentium</i>
Facility 5 (Central)	Feces	Conventional	16/16	<i>H. rodentium</i>
Facility 6 (Central)	Feces	Conventional	7/7	<i>H. rodentium</i>
Facility 7 (South)	Feces	Conventional	20/20	<i>H. rodentium</i> and <i>Helicobacter</i> sp.MIT01-6451
Facility 8 (South)	Feces	Conventional	19/20	<i>H. rodentium</i>
Facility 9 (North)	Feces	Conventional	9/10	<i>H. rodentium</i> and <i>Helicobacter</i> sp.MIT01-6451
Facility 10 (North)	Feces	Conventional	14/15	<i>H. rodentium</i> and <i>Helicobacter</i> sp.MIT01-6451
Facility 11 (Northeast)	Feces	Conventional	9/10	<i>H. rodentium</i>
Facility 12 (Northeast)	Feces	Conventional	10/10	<i>Helicobacter</i> sp.MIT01-6451 and unidentified <i>Helicobacter</i>
Facility 13 (Northeast)	Feces	Conventional	19/20	<i>H. rodentium</i>

rhea that were co-infected with *H. bilis* [17]. In A/JCr or C.B-17/IcrCrl-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-*Il10^{tm1Cgn/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., Makhham 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].

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.

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*

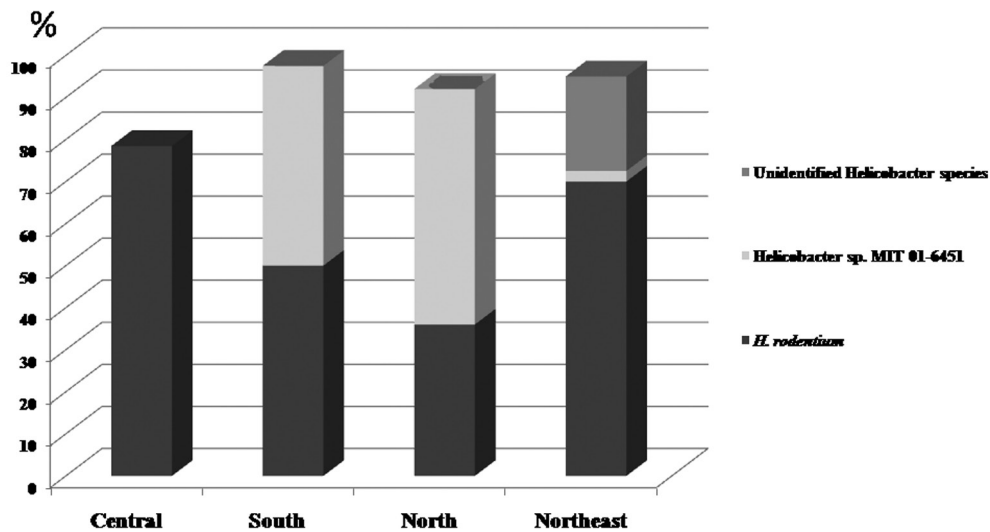


Fig. 1. Prevalence of *Helicobacter* in laboratory mice in Thailand

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.

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.

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