Optimization of Infectious Conditions with *Helicobacter pylori* in the Infection-highly Resistant Mongolian Gerbils Supplied in Japan

日本で供給されている *Helicobacter pylori* 難感染性の

スナネズミにおける最適な感染条件に関する研究

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【要旨】

1983 年に Warren と Marshall によって初めてヒトの胃粘膜から分離された *Helicobacter pylori*（*H. pylori* ）の動物実験には、BALB/c ヌードマウスあるいは BALB/c 無菌マウスなどが用いられたこともあるが、スナネズミがヒトの *H. pylori* 感染の病態を最もよく反映することから現在ではスナネズミが用いられている。

本邦では、*H. pylori* が容易に感染するスナネズミを最も多く供給していた繁殖会社（A 社）が、2012 年中頃からその供給を中止したため、現在は他の 1 社（B 社）から供給されているスナネズミしか入手できない。しかし、B 社から供給されているスナネズミには従来の *H. pylori* の接種条件ではほとんど感染しないため、現在わが国ではスナネズミを用いた *H. pylori* の研究を行うことが困難な状況下にある。

本研究では、B 社から供給されている *H. pylori* 難感染性のスナネズミに感染を容易にさせる条件を検討した。本研究の概要は以下の通りである。

本研究には、A 社（27 匹）及び B 社（162 匹）の雄性スナネズミ合計 189 匹を用いた。供試した *H. pylori* の菌株は、東海大学医学部感染症研究室の古賀康裕教授から分与された 1 株及び一般財団法人東京顕微鏡院から分与された臨床分離株 5 株である。本研究では *H. pylori* の接種量は 5×10^7 CFU/ml とし、その接種及び各種溶液のスナネズミへの経口投与には経口ゾンデを用いた。

本研究における *H. pylori* の感染の有無は、*H. pylori* に対する血清中の IgM 及び IgG 抗体の上昇に よって判定した。その血清抗体の上昇が *H. pylori* の感染成立の指標となることは、B 社のスナネズミを用いて次の方法で確認を行った。*H. pylori* 接種 2 週間後のスナネズミの胃の組織を培養して *H. pylori* の感染が確認された個体と確認されなかった個体の両方について血清抗体との関係を検討した。 *H. pylori* の感染が成立し易い群では、前処置として pH1.7 の胃液約 0.1ml を中性付近まで上昇させるために 0.1%炭酸水素ナトリウム溶液、pH9.5（重曹）0.3ml を投与したスナネズミ（10 匹）へ brain heart infusion（BHI）培地で調製した *H. pylori* を 1 日 1 回、2 日間連続接種した。他方、*H. pylori* が感染しにくい群では、スナネズミ（20 匹）へ famotidine を投与した後に滅菌生理食塩水（生理食塩水）で調製した *H. pylori* を 1 日 1 回接種した。これらのスナネズミは、*H. pylori* 接種の 2 週間後に麻酔下の採血によって安楽死させ、血清中の IgM 及び IgG 抗体を ELISA で測定した。それと同時に、摘出した胃の組織へ滅菌した PBS、pH7.4 を加えてホモジナイズし、それをウマ血清加 BHI 寒天培地 で培養した。その結果、前処置で 0.1%重曹 0.3ml を投与した 10 匹のスナネズミのうち血清中の IgM 及び IgG 抗体が有意に上昇した 9 匹のスナネズミ全ての胃から *H. pylori* が分離された。一方、famotidine を投与したスナネズミのうち *H. pylori* 接種後に血清中の IgM 及び IgG 抗体が有意に上昇 しなかった 18 匹から無作為に選んだ 10 匹のスナネズミの胃からは全く *H. pylori* が分離されなかった。 これによって *H. pylori* に対する血清中の IgM 及び

IgG 抗体の上昇が *H. pylori* の感染成立の判定を行う指標になることが確認された。それ故に、以下の実験では血清抗体の上昇を指標として *H. pylori* の感染を判定した。

A 社及び B 社のスナネズミへ生理食塩水あるいは BHI 培地で調製した *H. pylori* をそれぞれ接種した場合の感染率を比較した結果、A 社のスナネズミ（27 匹）は全例が生理食塩水で調製した *H. pylori* に感染したが、B 社のそれ（40 匹）では全例で感染が成立しなかった。また、BHI 培地で調製した *H. pylori* を B 社のスナネズミへ接種した場合には、42%（5/12）に感染が確認された。感染が成立しなかった原因として、BHI 培地で調製した *H. pylori* の接種で 42%に感染が認められたことならびに *H. pylori* はウレアーゼを分泌して尿素をアンモニアと二酸化炭素に分解することで強酸性の胃内を自らが生存可能な環境に変えることから、胃内の pH が関係することが推察された。そこで、*H. pylori* を 接種する前に、H2-ブロッカーである famotidine あるいは重曹を用いて胃内の pH を上げる前処置を試みた。その条件下で行った感染実験の結果及びすでに得られていた成績を併せた結果は次の通りで あった。(1) BHI 培地（pH7.2）で調製した *H. pylori* を接種したところ、42%（5/12）で感染が認められた。(2) *H. pylori* 接種 3 時間前に 10 ㎎/㎏（体重）の famotidine を投与した後に、生理食塩水で 調製した *H. pylori* を接種した結果、10%（2/20）に感染が認められた。(3) 0.02%に尿素を含有した BHI 培地（pH7.3）で調製した *H. pylor*i を接種したところ、70%（7/10）で感染が確認された。(4) *H. pylori* 接種 10 分前に 0.1%の重曹 0.3ml を投与して胃内の pH を中性付近に調整した後、生理食塩水で調製した *H. pylori* を 1 日 1 回接種したところ、70%（7/10）で感染が認められ、これを 2 日間連続 接種したときには 80%（8/10）に感染が認められた。(5)(4)と同様に、0.1%重曹を投与した後に、BHI 培地で調製した *H. pylori* を 1 日 1 回接種した結果、80%（8/10）に感染が認められ、これを 2 日間連 続接種したときには 90%（9/10）で感染が確認された。

次に、継代の少ない臨床分離株の感染性を検討するために、生理食塩水で調製した *H. pylori* を B 社 の無処置のスナネズミ各 3 匹へそれぞれ接種したところ、5 株のうち 2 株で 3 匹中各 1 匹に感染が確認された（13.3%）が、他の 3 株では感染が成立しなかった。絶食させたスナネズミ 10 匹の胃液の pH は平均 1.7 で、その量は約 0.1ml であった。pH1.7 の 0.08M 塩酸 0.1ml に 0.1%重曹 0.3ml 及び BHI 培地 1.0ml を加えたときの pH は 6.9 に上昇した。そのことから、前処置によって胃内の pH が中性付近まで上昇したことで、継代を重ねた *H. pylori* の感染率が 高まったものと考えられた。その明確な機序は不明であるが、接種された *H. pylori* が胃粘膜に定着する際に、初めはその生存に必要な量のアンモニアを生成させるに充分なウレアーゼを分泌できないために、*H. pylori* が定着する部位の pH を生存可能な pH にまで上昇させることができないことが定着 を困難にしたと考えられた。また、継代の少ない *H. pylori* 臨床分離株が、継代を重ねた菌株よりもわずかに感染し易いと思われる成績が得られたが、その感染率（13.3%）は低く、継代を重ねた菌株との相違は明らかにできなかった。

以上のように、本研究では、菌体を生理食塩水に浮遊させた菌液を接種する一般的な接種方法では *H. pylori* の感染がきわめて困難な B 社のスナネズミにおいて、*H. pylori* の感染率を高める条件として菌接種前に胃内の pH を中性付近に調整する前処置が有効であることを確認した。

Optimization of Infectious Conditions with Helicobacter Pylori in the Infection-highly Resistant Mongolian Gerbils Supplied in Japan

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Optimization of infectious with H. pylori

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ABSTRACT

Optimization of infectious conditions with *Helicobacter pylori* (*H. pylori*) in the infection-highly resistant Mongolian gerbils was performed. Mongolian gerbils were inoculated with *H. pylori* without pre-treatment or administered with sodium bicarbonate prior to inoculation. Serum titers of IgG and IgM against *H. pylori* measured by ELISA. H. pylori were isolated from the stomachs of Mongolian gerbils with increased IgM and IgG titers. On the other hand, *H. pylori* were not isolated from the stomachs of Mongolian gerbils with unchanged titers. All of the Mongolian gerbils obtained from supplier A were infected with *H. pylori* suspended in sterilized saline without pre-treatment, while none of the Mongolian gerbils obtained from supplier B were infected with *H. pylori* suspended in sterilized saline, while 42%

of the Mongolian gerbils inoculated with *H. pylori* suspended in brain heart infusion medium were infected. Furthermore, infection with *H. pylori* in Mongolian gerbils previously administered with famotidine, an H2-blocker, was unsuccessful. Low infection rates were also observed in Mongolian gerbils administered 10% sodium bicarbonate solution prior to inoculation. However, infection with *H. pylori* in these Mongolian gerbils was successful after administration of 0.1% sodium bicarbonate solution prior to inoculation. The infection rate in Mongolian gerbils administered 0.1% sodium bicarbonate prior to inoculation with *H. pylori* suspended in brain heart infusion medium was 90%, and this rate was higher than with other pre-treatment methods. *H. pylori* infection of highly resistant Mongolian gerbils supplied in Japan is possible by adjusting gastric pH.

INTRODUCTION

Marshall was the first to report a link between *Helicobacter pylori* (*H. pylori*) infection and gastric cancer (Marshall 1983). *H. pylori* reportedly induces acute and chronic gastritis, gastric ulcer, duodenal ulcer and gastric cancer (Warren et al., 1983, Mcnulty et al., 1999, Allen 2001, Eslick 2006), and Karita et al.(Karita et al., 1991, Eslick 2006) established an animal model for human *H. pylori* infection using BALB/c nude mice, BALB/c euthymic mice or germ-free mice (Karita et al., 1991, 1994). A severe *H. pylori* infection and inflammation model was subsequently established in Mongolian gerbils (Karita et al., 1991, Yan et al., 2004, Tukamoto et al., 2013). Thus, Mongolian gerbil models are essential experimental animals for infection experiments using *H. pylori*. Until recently, Mongolian gerbils supplied in Japan were readily infected *H. pylori* and infectious experiments were conducted without problems. However, this supplier has discontinued marketing Mongolian gerbils, leaving only one supplier of Mongolian gerbils in Japan. Unfortunately, Mongolian gerbils from this supplier are not readily infected with *H. pylori* using standard inoculation methods (Hirayama et al., 1996), and infection experiments with *H. pylori* using Mongolian gerbils are therefore impossible in Japan at present. Thus, Mongolian gerbils purchased from this supplier require pre-treatment for infection with *H. pylori*. The aim of this study was to investigate suitable conditions for *H. pylori* infection in infection-highly resistant Mongolian gerbils supplied in Japan.

MATERIALS and METHODS

Bacterial strains

Six strains of *H. pylori* isolated from humans were used in this study. One strain was donated by Professor Yasuhiro Koga, Laboratory for Infectious Diseases, Tokai University School of Medicine and five strains were donated by Incorporated Foundation Tokyo Kenbikyo-in. *H. pylori* was cultured as described by Kabir et al.(Kabir et al., 1997). Colonies were confirmed using a commercial *H. pylori* urease kit (CLO Test; Sysmex Corporation, Hyogo, Japan). *H. pylori* was suspended in sterilized saline or brain heart infusion culture medium (Becton, Dickinson and Company, Franklin Lakes, NJ), at a pH of 7.4. Mongolian gerbils were inoculated with *H. pylori* within 1 hour of extraction from petri dishes.

Animals

Mongolian gerbils (body weight; 20 to 30 g) were purchased from two different suppliers in Japan. One supplier (supplier A) has since stopped supplying Mongolian gerbils. The other supplier (supplier B) is currently the only supplier of Mongolian gerbils in Japan. Mongolian gerbils were kept in cages at a temperature of 23 ± 2°C, and a relative humidity of 55% ± 10%, on a 12/12 dark (18:00-6:00)/light (6:00-18:00) cycle with the air exchanged 12 times or more per hour. Mongolian gerbils were fed MF (Oriental Yeast Co., Ltd., Tokyo, Japan), and were allowed free access to water. For 18 hours before inoculation, Mongolian gerbils were deprived of food, but had free access to water.

All experiments were approved by the

Institutional Review Board of Azabu University and were conducted in accordance with the institute's Animal Experimentation guidelines (Japanese Association for Laboratory Animal Science, JALAS, 1987).

Experimental infection with *H. pylori*

In the first experiment, Mongolian gerbils from supplier A were inoculated with a 1-ml suspension of 5.0 × 107 CFU/ml *H. pylori* suspended in sterilized saline without pre-treatment. Mongolian gerbils from supplier B were inoculated with a 1-ml suspension of 5.0 × 107 CFU/ml *H. pylori* suspended in sterilized saline or brain heart infusion medium (Becton, Dickson and Company) without pre-treatment.

In the second experiment, all Mongolian gerbils from supplier B were inoculated with *H. pylori* after pre-treatment. First, *H. pylori* suspended in sterilized saline was given to Mongolian gerbils administered famotidine at 10 mg/kg body weight (Astellas Pharma Inc., Tokyo, Japan) 3 hours before inoculation. Furthermore, *H. pylori* suspended in brain heart infusion medium (Becton, Dickson and Company) including 0.2 mg/ml urea (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was given to Mongolian gerbils.

Next, Mongolian gerbils were inoculated with H.pylori after administration of sodium bicarbonate solution (Wako Pure Chemical Industries, Ltd.) as pre-treatment. *H. pylori* suspended in sterilized saline was inoculated into Mongolian gerbils administered 0.5 ml of 10% sodium bicarbonate solution (Wako Pure Chemical Industries, Ltd.) 10 or 30 minutes before inoculation. Subsequently, *H. pylori* suspended in sterilized saline or brain heart infusion medium (Becton, Dickinson and Company) was inoculated into Mongolian gerbils administered 0.3 ml of 0.1% of sodium bicarbonate solution 10 minutes before inoculation. *H. pylori* was inoculated either once or once a day for two days.

Measurement of antibodies against *H. pylori*

Infection was confirmed by elevation of serum titers of IgG and IgM against *H. pylori*. Blood was collected by ventricular puncture before inoculation and at 1 week after inoculation, under anesthesia with pentobarbital (Kyoritsu Seiyaku Corporation, Tokyo, Japan).

Serum titers of IgG and IgM against *H. pylori* were measured by enzyme-linked immunosorbent assay (ELISA). *H. pylori* were disrupted with ultrasonic treatment, and were dissolved in phosphate buffered saline for use as somatic antigen. Somatic antigen was diluted in 0.05 M sodium bicarbonate buffer (pH 9.6) and was incubated at 100 µl/ml at room temperature for 1 hour. After blocking with 1% bovine serum albumin in sodium bicarbonate buffer (pH 9.6), sera from Mongolian gerbils inoculated with *H. pylori* were added at 100 µl/well. Plates were incubated at room temperature for 1 hour. HRPO conjugated goat anti mouse IgG (Bethyl Laboratories Inc., Montogomery,

Table 1 *Infection rates of Helicobacter pylori in Mongolian gerbils*

Breeding supplier	Suspension with *H. pylori*	Inoculation frequency	n	Case of infection	Infection rate (%)
A	Sterilized saline		27	27	100
B	Sterilized saline	once a day	40	0	0
B	Brain heart infusion medium		12	5	42

H. pylori was suspended suspension of 5.0 × 107 CFU/ml.

Figure 1. *Typical cultivation observations of stomachs from Mongolian gerbils inoculated with Helicobacter pylori. (A) Stomach from Mongolian gerbil showing increased titers of IgG and IgM against H. pylori. (B) Stomach from Mongolian gerbil showing no increase in titers of IgG and IgM against H. pylori.*

TX) or HRPO-conjugated goat anti mouse IgM antibodies (American Qualex, San Clemente, CA) were added at 100 µl/well. After incubation for 1 hour, peroxidase conjugated rabbit anti-mouse IgG antibodies were added at 100 µl/well. Substrate was 2, 2-azino-di-(3-ethyl-benzthiazoline sulphonic acid-6) (ABTS), and after incubation for 1 hour (Zymed Laboratories, South San Francisco, CA), absorbance at 415 nm was measured.

Cultivation of *H. pylori* from Mongolian gerbils

Two groups of Mongolian gerbils were sacrificed after measurement of antibody titers against *H. pylori* in order to assess the differences in antibodies against *H. pylori* and isolated *H. pylori* from the stomach. One group was administered 0.1% sodium bicarbonate prior to inoculation once a day for two days and the other group was administered famotidine prior to inoculation. Stomachs of these groups were removed and homogenized with sterilized phosphate buffered saline (PBS, pH 7.4). *H. pylori* was then cultured according to the method described by Kabir et al. (Kabir et al., 1991).

Measurement of gastric pH

When Mongolian gerbils were sacrificed, gastric pH was measured using a pH Spear for food testing (Eutech Instruments Pte, Ltd., Singapore). Furthermore, the pH of various suspended solutions containing hydrochloric acid (Wako Pure Chemical Industries, Ltd.) to simulate gastric pH (1.52) was measured.

Statistical analysis

Serum titers of IgG and IgM against *H. pylori* between pre-inoculation and post-inoculation were analyzed using the paired Student's t-test. Differences in p values <0.01 were considered significant.

RESULTS

Infection rates in Mongolian gerbils purchased from supplier A or B without pre-treatment after inoculation with *H. pylori* are shown Table 1. All Mongolian gerbils purchased from supplier A were infected with *H. pylori*. On the other hand, Mongolian gerbils purchased from supplier B were not infected with *H. pylori* suspended in sterilized saline, while 42% of Mongolian gerbils inoculated with *H. pylori* suspended in brain heart infusion medium (Becton, Dickson and Company)were infected without pre-treatment.

The infection rate in Mongolian gerbils administered famotidine was only 10%, while it was 70% in Mongolian gerbils inoculated with *H. pylori* suspended in brain

Table 2 *Infection rate of Helicobacter pylori in the infection highly resistant mongolian gerbils with pre-treatment*

Pre-treatment method	Suspension with *H. pylori*	Inoculation frequency	n	Case of infection	Infection rate (%)
H_2-blocker (famotidine)	Sterilized saline	once a day	20	2	10
Urea			10	7	70

All mongolian gerbils were purchased from supplier B. H. pylori was suspended suspension of 5.0× 10^7 CFU/ml.

Table 3 *Infection rates of Helicobacter pylori in the infection-highly resistant mongolian gerbils administered before sodium bicarbonate solution*

Pre-treatment method			Inoculation method				
Concentration of sodium bicarbonate (%)	Administration volume of sodium bicarbonate (ml)	Suspension with *H. pylori*	Time after pre-treatment (minutes)	Inoculation frequency	n	Case of infection	Infection rate (%)
10	0.5	Sterilized saline	30	once a day	20	5	25
10	0.5	Sterilized saline	10	twice a day	10	4	40
10	0.5	Sterilized saline	10	once a day for 2 days	10	5	50
0.1	0.3	Sterilized saline	10	once a day	10	7	70
0.1	0.3	Sterilized saline	10	once a day for 2 days	10	8	80
0.1	0.3	Brain heart infusion medium	10	once a day	10	8	80
0.1	0.3	Brain heart infusion medium	10	once a day for 2 days	10	9	90

All mongolian gerbils were purchased from supplier B. H. pylori was suspended suspension of 5.0×10^{7} *CFU/ml.*

heart infusion medium (Becton, Dickson and Company)including urea (Table 2).

Infection rates in Mongolian gerbils purchased from supplier B administered sodium bicarbonate before inoculation of *H. pylori* are shown in Table 3. The highest infection rate was 90% in Mongolian gerbils administered 0.1% sodium bicarbonate before inoculation with *H. pylori* suspended in brain heart infusion medium.

Titers of IgG and IgM against *H. pylori* did not increase in 18 Mongolian gerbils prior to inoculation with famotidine. *H. pylori* were not isolated from the stomach of ten randomly selected Mongolian gerbils from the 18 showing no changes in titer (Fig. 1). However, titers of IgG and IgM against *H. pylori* in nine Mongolian gerbils increased after administration of 0.1% sodium bicarbonate solution once a day for two days prior to inoculation of *H. pylori* suspended in brain heart infusion medium. Furthermore, these titers showed significant differences between pre-inoculation and post-inoculation. Moreover, *H. pylori* was isolated from the stomachs of these Mongolian gerbils (Fig. 1). On the other hand, *H. pylori* was not isolated from the stomach of one Mongolian gerbil that did not show increased titers against *H. pylori* (Fig. 1).

The infection rates for other *H. pylori* strains isolated from humans, donated by Incorporated Foundation Tokyo Kenbikyo-in, are shown in Table 4. One of the three Mongolian gerbils inoculated with two strains showed increased titers of IgM and IgG. The other strains did not show increased titers.

The pH of hydrochloric acid-containing simulated gastric solutions with various suspended solutions is

Table 4 *Infection rates of Helicobacter pylori isolated from human in the infection-highly resistant mongolian gerbils without pre-treatment*

Strain number of *H. pylori*	n	Suspension with *H.pylori*	Inoculation frequency	Case of infection	Infection rate (%)
1	3			1	33
2	3			0	0
3	3	Sterilized saline	once a day	1	33
4	3			0	0
5	3			0	0

All mongolian gerbils were purchased from supplier B. These five strains were donated by Incorporated Foundation Tokyo Kenbikyo-in. H. pylori was suspended suspension of 5.0× 10^7 CFU/ml.

shown in Table 5. The pH of the solution with saline was 2.13. However, the pH of the *H. pylori* suspension with bicarbonate sodium or brain heart infusion was almost neutral.

DISCUSSION

H. pylori are considered to induce various gastric disorders in humans (Mrshall 1994, Konturek et al., 2009). Mongolian gerbils are used as pathologic models for *H. pylori* infection (Hirayama et al., 1996). However, it is now difficult to obtain *H. pylori*-sensitive Mongolian gerbils in Japan. Thus, suitable conditions for infection with *H. pylori* in the infection-highly resistant Mongolian gerbils need to be elucidated.

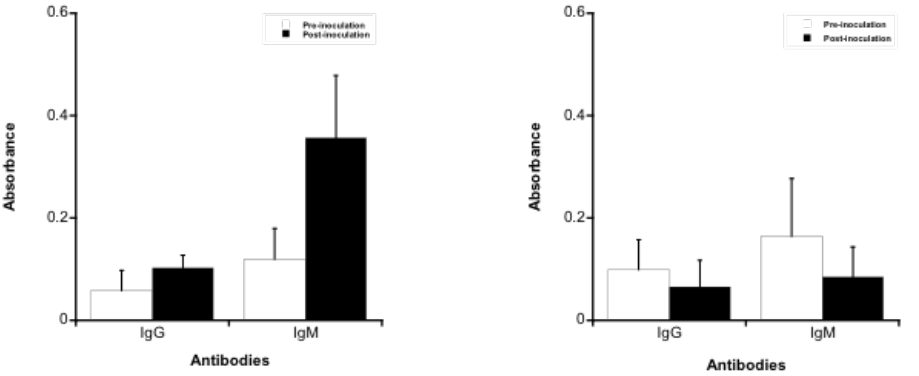
Table 5 *The ph of hydrochloric solution adjusted gastric pH added with various suspended solution*

Added solution	pH
Bicarbonate sodium	6.90
Urea	1.94
Brain heart infusion medium	6.83
Bicarbonate sodium and Brain heart infusion medium	6.92
Saline	2.13
Bicarbonate medium and saline	6.56
Urea and Brain heart infusion medium	6.86

Titers of IgG and IgM against in Mongolian gerbils isolated *H. pylori* from stomach increased significantly. A correlation between increases in IgG and IgM titers against *H. pylori* and isolation of *H. pylori* from stomach was confirmed. These results suggest that establishment of infection with *H. pylori* could be confirmed by detecting increases in these titers.

All Mongolian gerbils purchased from supplier A, which has since left the market, were infected with *H. pylori* without pre-treatment. On the other hand, Mongolian gerbils purchased from supplier B, the only supplier in Japan at present, were not infected with *H. pylori* without pre-treatment. The other five *H. pylori* strains isolated from humans, donated from Incorporated Foundation Tokyo Kenbikyo-in, were given to Mongolian gerbils from supplier B. The purpose of this experiment was to evaluate differences in strain sensitivity among the Mongolian gerbils purchased from supplier B. One-third of Mongolian gerbils inoculated with two strains were infected. Differences in infection rates between strains were assumed to depend on the ease of establishing infection; however, it was not possible to stably infect Mongolian gerbils purchased from supplier B. Differences in strain were

Figure 2. *Titers of IgG and IgM against H. pylori pre- and post-inoculation. (A) H. pylori was isolated from the stomach (B) H. pylori was not isolated from stomach : significant difference between pre- and post-inoculation (p<0.01).*



not considered to be a principal factor. *H. pylori* survives in the gastric environment by secreting urease to decompose ammonia and carbon dioxide from urea (Ha et al., 2001, Amin et al.,2010, Fahy et al., 2013). Mongolian gerbils administered antibodies against fragments of *H. pylori* urease could be infected with *H. pylori* (Mahdieh et al., 2012). Moreover, the survival of *H. pylori* incubated for 1 hour at pH 7.4 was higher than at pH 3.0 (Marcus et al., 2013). The pH of gastric juice was 1.52 in Mongolian gerbils in this study. Gastric pH was thus considered to be an important factor in *H. pylori* infection of highly resistance Mongolian gerbils.

Administration of 0.1% sodium bicarbonate solution to Mongolian gerbils inoculated with *H. pylori* suspended in brain heart infusion medium once daily for two days showed the highest infection rate. Infection rates increased with administration of sodium bicarbonate solution or urea prior to inoculation. In vitro, the pH of hydrochloric acid solution-containing simulated gastric solution increased by adding sodium bicarbonate, brain heart infusion medium or urea. Thus, it is possible to infect highly resistance Mongolian gerbils by elevating gastric pH prior to inoculation. The famotidine is an efficacious H2-blocker that elevates gastric pH by inhibiting gastric acid (Özer et al., 2012, Okabe et al., 2001). However, infection rates were lower with famotidine than with other pre-treatment methods. This may be because famotidine did not sufficiently adjust gastric pH for infection with *H. pylori*.

Mongolian gerbils are essential experimental animals for infection studies with *H. pylori*. However, it is now difficult to obtain Mongolian gerbils that can be stably infected with *H. pylori* in Japan. In the present study, infection-highly resistant Mongolian gerbils could be infected with *H. pylori* by elevating gastric pH with pre-treatment. Thus, gastric pH is an important factor in *H. pylori* infection of highly-resistant Mongolian gerbils.

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