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A better method for confirming *Helicobacter pylori* infection in Mongolian gerbils

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Background. Our aim was to evaluate the accuracy of the stool antigen test and the optimal time point for detecting *Helicobacter pylori* infection in a Mongolian gerbil model. ***Methods.*** We inoculated 8-week-old Mongolian gerbils with *H. pylori* (Vac A (+)/CagA(+)). The gerbil-infected model was developed as follows: *H. pylori* was put into broth (about 10^9 CFU/ml), and 50 gerbils were then fed with 1 ml intragastrically twice within a 3-day interval. Another ten gerbils were fed broth only. Twenty-six weeks after the inoculation, the gerbils were killed. The gastric mucosa was sampled for a series of examinations including culture, histology, rapid urease test, and polymerase chain reaction. Stool samples for a stool antigen test, *H. pylori*-specific stool antigen assay (HpSA), were collected during weeks 4, 6, 8, 12, and 26 after inoculation. Of the 50 gerbils inoculated with *H. pylori,* the inoculation was successful in 88%. Severe active gastritis, ulceration, and intestinal metaplasia were obvious. ***Results.*** The HpSA test results were sensitivity, 88.6%; specificity, 100%; positive predictive value (PPV), 100%; negative predictive value (NPV), 54.5%, and accuracy, 90%. The HpSA test began to be more sensitive and accurate ($P < 0.05$) beginning during week 6 after inoculation. We also found that *H. pylori* could be detected earlier and more easily in the group with high *H. pylori* density. ***Conclusions.*** HpSA seems to be suitable for confirming colonization of gerbils with *H. pylori*. The optimal testing time point is around 6 weeks after inoculation. This test is a good choice for long-term observation of *H. pylori* infection in Mongolian gerbils.

Key words: *Helicobacter pylori*, Mongolian gerbil, stool antigen test

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Introduction

Since Warren and Marshall[1] succeeded in detecting a Gram-negative bacillus in human gastric mucosa in 1983, the role of *Helicobacter pylori* infection in gastric disorders has been well demonstrated. It is also well known that a small population of *H. pylori*-infected people develop gastric cancers. Studies[2–4] published in 1991 showed higher anti-*H. pylori* titers in patients with gastric cancer than in controls. A Working Group of the World Health Organization International Agency for Research on Cancer concluded in 1994 that *H. pylori* is a group I carcinogen in humans.

Gastric cancer is known to be a multifactorial disease;[5] therefore, genetic factors and environmental factors other than *H. pylori* can complicate the elucidation of the association between *H. pylori* infection and gastric cancer. The pathogenic mechanisms leading from chronic active inflammation of the gastric mucosa to the development of cancer remain poorly understood. This makes it logical to use animal models to understand the basis of gastric disorders.

Animals used for studying experimental *H. pylori* infection[6] have included monkeys, dogs, piglets, domestic cats, and rodents.[7–15] These models are not optimal, because they cannot be handled with ease or in large numbers, germ-free conditions must be used, or infection rates are rather low.

Published results about *H. pylori* infection in Mongolian gerbils appear promising. Naturally acquired gastritis among gerbils is rare.[16] Furthermore, natural infection of gerbils with *Helicobacter* species apparently does not occur.[17,18] These points suggest that Mongolian gerbils may be suitable for studying experimental *H. pylori* infection. Several experiments conducted in Japan have demonstrated that chronic *H. pylori* infection in Mongolian gerbils leads to the development of gastric carcinoma.[19–21]

Previously used *H. pylori* animal models have generally been used in small-scale experiments because they are expensive and time-consuming to care for, and some require special facilities. Furthermore, previous studies almost always used invasive tests to determine whether the inoculation was a success, requiring, regrettably, the animals to be killed, so large numbers of animals were needed for the research. Finding a reliable noninvasive method in the gerbil model of *H. pylori* infection is therefore appropriate.

Accordingly, various noninvasive tests that are useful and accurate in detection of *H. pylor*i infection in gerbils were evaluated. Previous studies cited many kinds of noninvasive tests developed for detecting *H. pylori* infection in humans. These include the urea breathing test (^{13}C-UBT),[22] the *H. pylori*-specific stool antigen assay (HpSA),[23] the RapidRun (urine-sampling) test,[24] and serological tests.[25] Little information is available, however, regarding noninvasive tests in a gerbil model.

The aim of our study was to evaluate the accuracy of the stool antigen test for detecting *H. pylori* infection in Mongolian gerbils.

Materials and methods

Animals and housing

Eight-week-old gerbils with body weight of 30–40g were purchased from the Kaohsiung Medical University Experimental Animals Center, Kaohsiung, Taiwan. Initially, four to five gerbils were housed per cage and maintained under standard laboratory conditions (room temperature, 23°–26°C; relative humidity, 55%–65%; 12/12-h light/dark cycle) with free access to a commercial rodent diet and tap water. However, by week 26 of testing, there was only one gerbil in each cage.

Helicobacter pylori *inoculation*

The *H. pylori* colony used in this study had been isolated from a patient with gastric ulcer. The strain produced vacuolating cytotoxin and contained the cytotoxin-associated gene (*cagA*). It was cultured on blood agar at 37°C under microaerobic conditions for 4 days, harvested, and then incubated in brucella broth (DIFCO Laboratories, Detroit, MI, USA) with 10% horse serum for 24h. The inoculum size was adjusted with sterile saline to produce McFarland 4 optical density at 540nm. First, five gerbils were inoculated with the above strain. These were killed 4 weeks later, and their gastric mucosa was collected for culture as per the above procedure. This harvested colony was used for inoculation of the other gerbils.

Fifty Mongolian gerbils were challenged with 10^9 colony-forming units (CFU) of *H. pylori* in 1.0ml of brucella broth by intragastric gavage after being fasted for 24h. They were then fed with chow (Oriental Yeast, Tokyo, Japan) and water, beginning 12h after *H. pylori* inoculation. The gerbils were fed twice within a 3-day interval. During this period, ten gerbils were fed only with 1.0ml of brucella broth as uninfected controls.

Diagnostic tests of H. pylori *infection*

The gerbils were killed at 26 weeks after inoculation. The gastric mucosa was examined by the following tests: culture, histology, rapid urease test (ProntoDry test),[25] and polymerase chain reaction (PCR). Gerbil stool samples were collected during weeks 4, 6, 8, 12, and 26 after inoculation and used for HpSA testing.

One set of mucosal specimens was used for culturing of *H. pylori,* which was done by rubbing them on the surface of a Campy-BAP agar plate [Brucella agar (Difco) + IsoVitalex (Gibco) + 10% whole sheep blood], which was then incubated at 35°C under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2) for 4–5 days. The culture of *H. pylori* was considered positive if one or more colonies of Gram-negative, oxidase (+), catalase (+), and urease (+) spirals or curved rods were present.

A biopsy-based ProntoDry test (Medical Instruments, Solothurn, Switzerland) was performed on another set of specimens. The results of the ProntoDry test were interpreted as positive if the color of the gel changed from yellow to pink or red within 24h at room temperature.

A third set of specimens was examined histologically. The specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin. An experienced pathologist (who was blinded to the experiment) examined the results of the assessed biopsy specimens. Density of *H. pylori* in the tissue was graded as normal (0), mild (1), moderate (2), or severe (3), based on the Sydney system.[26] Gastritis was graded as normal, mild, moderate, or severe, also based on the Sydney system. Histopathologic lesions of the glandular stomach were categorized as (1) active chronic gastritis (characterized by severe inflammatory cell infiltration and multiple lymphoid follicle formation throughout the pyloric region and part of the fundic region); (2) ulcer; (3) regenerative hyperplasia; (4) invagination of glands into the submucosa; (5) hyperplastic polyp; (6) intestinal metaplasia (diagnosed by the presence of goblet cells that were positive for Alcian blue/high-iron diamine staining); and (7) adenocarcinoma.

The *cagA* PCR fragment was obtained from the fourth set of specimens with the following primers 5′-GATATAGCCACTACCACCACCG-3′ and 5′-GGA

AATCTTTAATCTCAGTTCGG-3′. The *vacA* PCR fragment was obtained with the primers 5′-AGTAA CAGACTCATAT-3′ (nt 1708–1725) and 5′-AAGCTT GATTGATCACTCC-3′ (nt 4134–4116) for 35 amplification cycles of 94°C for 1 min, 41°C for 2 min, and 72°C for 2 min.

Stool samples were stored at –20°C until required for use and independently tested by a private laboratory. The investigators were blinded to the results of the other *H. pylori* tests. The stool specimens were analyzed for *H. pylori* antigen using an HpSA immunoassay as described by its manufacturer. A commercial kit, Premier platinum HpSA (Meridian Diagnostic, Cincinnati, Ohio, USA) was used. A portion of the fecal sample was mixed with 200 μl of the sample diluents. One drop of enzyme conjugates was added to the microwells, which were incubated for 1 h at room temperature and washed five times. The reaction was terminated with one drop of stool solution, and the results were read by spectrophotometry. An absorbance (450/630) of 0.160 was considered positive as recommended by the manufacturer.

Confirmation of H. pylori *infection*

Helicobacter pylori infection was confirmed when the culture was positive, or when two of the following three tests were positive: histology, PCR, and the ProntoDry test.

Statistical analysis

The sensitivity, specificity, and positive and negative predictive values were measured. A χ-squared test was used to compare these results. Statistical significance was defined as $P < 0.05$.

Results

Fifty gerbils were inoculated with *H. pylori*. The success rate of inoculation was 88% (44/50). All ten gerbils in the control group showed a negative result. Twenty-five gerbils showed a positive result in the culture test. Its sensitivity and specificity were 56.8% (25/44) and 100% (16/16). Thirty-nine gerbils showed a positive result on histological examination. Its sensitivity and specificity were 81.8% (36/44) and 81.3% (13/16). Forty-two gerbils showed a positive result on PCR. Its sensitivity and specificity were 93.2% (41/44) and 93.8% (15/16). Forty-one gerbils showed a positive result in the ProntoDry test. Its sensitivity and specificity were 88.6% (39/44) and 87.5% (14/16).

The histological findings of the gerbil gastric mucosa are shown in Fig. 1 and listed in Table 1. In our study, gastric ulcer, atrophic gastritis, and focal intestinal metaplasia began to develop 5–6 months after *H. pylori* inoculation. Epithelial hyperplasia occurred, and intestinal metaplasia was observed in the mucosa of the

Table 1. Histological findings of gerbils at 26 weeks after inoculation

	Active chronic gastritis (%)	Ulcer (%)	Density of *H. pylori* (%)				Intestinal metaplasia (%)
			0	1	2	3	
26 weeks							
Control ($n = 10$)	0	0	100	0	0	0	0
Inoculated ($n = 50$)	88	80	12	16	20	52	62

The gastric mucosa in gerbils did not show obvious damage in the control group, but gerbils showed obvious mucosal changes 26 weeks after inoculation. All 44 successfully infected gerbils showed active chronic gastritis. Intestinal metaplasia and ulcer were also found. Density of *H. pylori* was low in 36% (18/50) of gerbils and high in 52% (26/50)

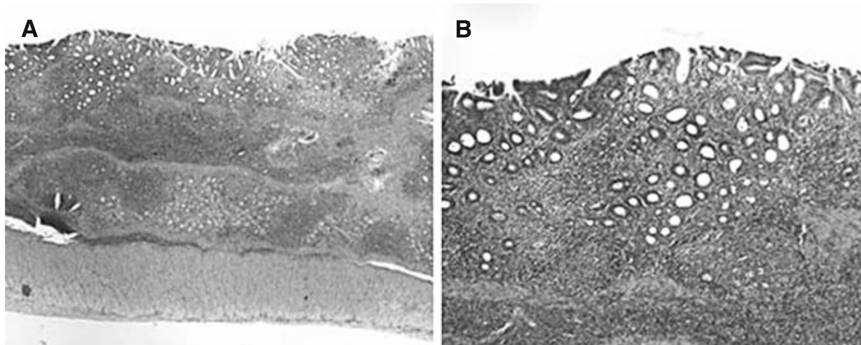

Fig. 1A,B. Active chronic gastritis observed in Mongolian gerbils 26 weeks after *Helicobacter pylori* inoculation. The gastritis is characterized by severe neutrophil and mononuclear cell infiltration in both the mucosa and submucosa (hematoxylin and eosin; original magnification, **A** 65×, **B** 130×)

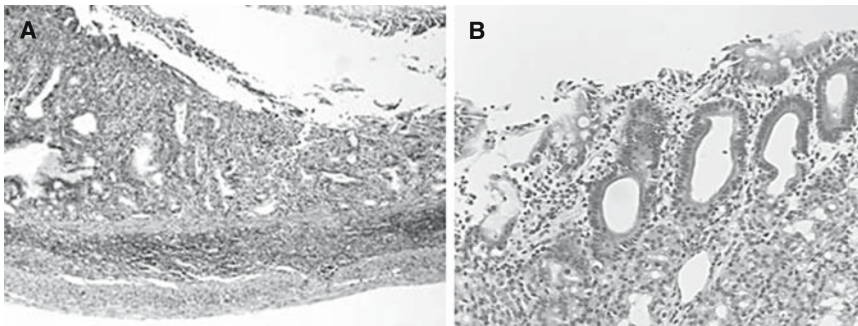

Fig. 2A,B. Ulcer and intestinal metaplasia were observed in Mongolian gerbils 26 weeks after *H. pylori* inoculation. The normal mucosal architecture was almost completely lost. Ulcer (**A**) and intestinal metaplasia (**B**) were observed (hematoxylin and eosin; original magnification, **A** 65×, **B** 130×)

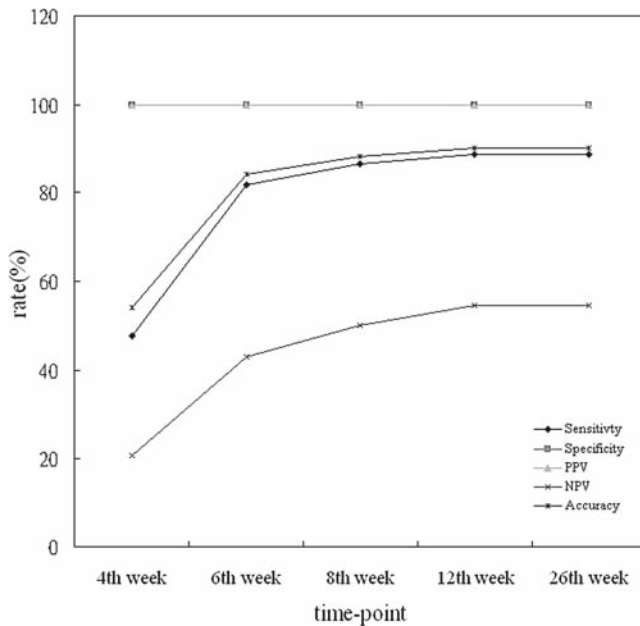

Fig. 3. Results of the *H. pylori*-specific stool antigen assay (HpSA) test among samples collected at different time points. The final results of HpSA test were sensitivity, 88.6%); specificity, 100%; positive predictive value (*PPV*), 100%; negative predictive value (*NPV*), 54.5%; and accuracy, 88%. The week 4 group showed significantly lower sensitivity and accuracy than the other groups ($P < 0.005$)

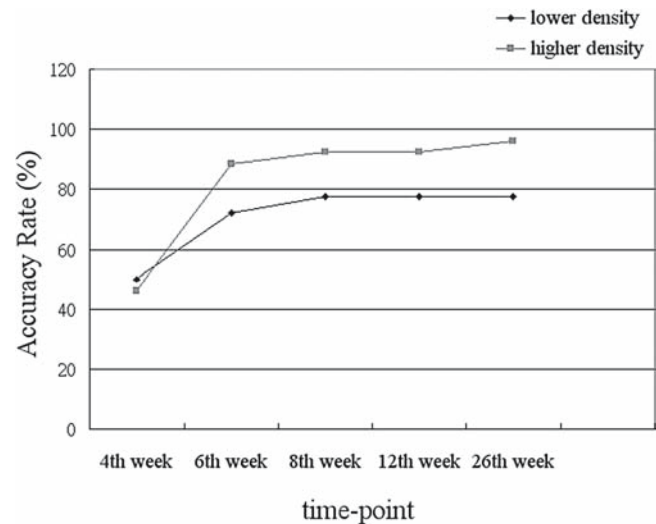

Fig. 4. Results of the HpSA test comparing different density groups of *H. pylori.* The final accuracy rates of the HpSA test in low- and high-*H. pylori* density gerbils were 77.7% and 96.2%, respectively, a significant difference ($P < 0.05$). Obviously different levels of accuracy were noted beginning the sixth week

pyloric region, especially in the area close to the ulcer. The severity of active gastritis, ulcer, and intestinal metaplasia were obvious by 26 weeks after inoculation (Fig. 2). No evidence of cancer was found at this time point. Five gerbils gave false-negative results by the histological method, so we asked our pathologists to review more specimens blindly and confirmed the density results. All five gerbils were classified as with grade 1 density. Of the successfully inoculated gerbils, 36% (18/50) showed a low density of *H. pylori* and 52% (26/50) showed a high density.

The results of the HpSA test of specimens collected at different time point groups are shown in Fig. 3. The final sensitivity and specificity values were 88.6% and 100%. The positive and negative predictive values were 100% and 54.5%, respectively. The accuracy of the HpSA test was 88%. These results were acceptable and similar to results in humans.[23]

When we compared the results among groups collected at different time points, we found that the accuracy rates were significantly different (54% vs. 84%, $P < 0.05$) between the week 4 and week 6 groups. We found similar differences in sensitivity (47.7% vs. 81.8%). The accuracy and sensitivity tended to become higher as the time since inoculation increased, but no significant difference was found among the week 6, 8, 12, and 26 groups.

We divided the gerbils into two groups according to the density of *H. pylori.* The final accuracy rates of the HpSA test in the low- and high-*H. pylori* density gerbils were 77.7% and 96.2%, respectively, which showed a significant difference ($P < 0.05$) (Fig. 4). An obvious difference in accuracy between the two groups was noted from the sixth week. There seemed to be a trend of the

accuracy becoming higher with increasing time after inoculation in the high-density group, but no similar trend was observed in the low-density group.

We traced the results of the HpSA test in all gerbils and found that no gerbil exhibited a change from positive to negative. Thus, there was no spontaneous eradication of *H. pylori* in infected gerbils.

Discussion

In our study, the severity of active gastritis, ulcer, and intestinal metaplasia symptoms were obvious 26 weeks after inoculation. This demonstrated that *H. pylori* infection was chronic in our gerbil model, and the success rate was high (88%).

Our study showed significantly less sensitive results in the week 4 group than in the other groups, which is similar to results observed in humans.[24] Although the rate of accuracy seemed to become higher as the period after inoculation increased, the differences were not significant between the week 6, 8, 12, and 26 groups. According to the above findings, we suggest that the optimal time point for confirming colonization of *H. pylori* in gerbils is 6 weeks after inoculation. Because the accuracy and sensitivity are still higher at later periods, a second test should be performed if a negative result is obtained the first time.

In a previous study using a BALB/c mice model,[27] HpSA also yielded results with high accuracy (94.6 %) in a mouse model, but the cutoff value used was higher (0.20), and the optimal testing time point indicated by the data was 7 days after inoculation, which is earlier than that indicated by our data. The differences might reflect differences in the intestinal flora between gerbils and mice. Another possible explanation might be that *H. pylori* needs a longer period to colonize a gerbil's stomach. Moreover, this mouse study did not discuss the influence of *H. pylori* density on the HpSA results.

We found no spontaneous eradication of *H. pylori* in gerbils during this period, perhaps because the Mongolian gerbil is very suitable for *H. pylori* to colonize. On the other hand, this result might be related to the *H. pylori* colony that we used. This strain was cultured from the mucosa of *H. pylori*-fed gerbils collected 8 weeks after inoculation.

The detection methods currently used in animal models rely on a combination of culture, histological examination, detection of specific antibodies, and rapid urease-based assays.[28–31] All of these techniques have some disadvantages. Culturing of these fastidious, slow-growing organisms is often difficult because they require a rich medium and special culture conditions. The urease assay as a *Helicobacter* detection method has a limit of detection of 10^6 cells per assay.[32] Similarities in morphology between *Helicobacter* spp. and other spiral-shaped bacteria can lead to misinterpretations in histological examination.[31,32] Although PCR is a very powerful technique in terms of sensitivity and specificity, it is of limited use for accurate quantification.[32,33] Moreover, all of these techniques also have the same disadvantage that the gerbils must be killed. The stool-based test does not necessitate that the gerbils be killed, so it can be repeated in the same gerbil, and infected gerbils can be observed for a longer period. This is very important when surveying for gastric carcinogenesis because the survey is usually very time-consuming.

The more frequently used noninvasive tests in humans are ^{13}C-UBT and serological tests because they are convenient and safe. We compared the HpSA and ^{13}C-UBT tests (unpublished data). Our data showed that HpSA had a higher sensitivity than ^{13}C-UBT, in contrast to reports on human populations. This discrepancy may be because we did not have an accurate cutoff value of ^{13}C-UBT in gerbils and because of the difficulty in collecting samples from gerbils. A common drawback of serological analysis is that it measures antibodies to *H. pylori*, which persist long after the bacterium is eradicated (by antibiotics), making it possibly unsuitable for surveying the effect of eradicating *H. pylori* on pathogenesis in gerbil stomachs. The stool antigen test is suitable, however, for such studies, because it is able to detect current infection. Therefore, we choose a stool-based test as a noninvasive test for gerbils.

There are also limitations to HpSA in collecting samples. We were unable to determine the timing of gerbil defecation exactly. Sensitivity and accuracy also decreased if the stool sample was stored for more than 4–6 h after defecation.[23] Thus, stool samples were usually collected every 4–6 h to overcome these limitations. Also, mouse-chowing might interfere with the HpSA result.[23] In addition, there is no well-known cutoff value for HpSA in gerbils. In our study, we used the cutoff value of 0.16 as recommended by the manufacturer, which might be a limitation of this study. The amount of *H. pylori*'s antigen in a gerbil's stool might be less than in human. So the cutoff value might need to be modified. In one study using BALB/c mice models,[27] the HpSA cutoff value was set at 0.20. In further studies, one goal might be to survey the optimal HpSA cutoff value in the gerbil model.

In addition, we also obtained less accurate results in the low-density group. About 40% of infected gerbils belonged to the low-density group. This might influence the results of HpSA testing and be a possible source of bias.

In summary, stable and reliable *H. pylori* infection was established in a Mongolian gerbil model by inoculation with recultured *H. pylori* obtained from infected gerbils. The HpSA test was found to be sensitive and

accurate in *H. pylori*-infected gerbils. The optimal timing for detecting *H. pylori* infection is around 6 weeks after inoculation. This technique is suitable for long-term studies of gastric carcinogenesis and avoids the need to kill many gerbils. Accordingly, this stool antigen test may be a reliable and economical method for confirming *H. pylori* infection in gerbils.

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References

1. Marshall BJ, Narren JR. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983;1:1273–5.
2. Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, et al. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. BMJ 1991;302:1302–5.
3. Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med 1991;325: 1132–6.
4. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al. *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med 1991;325:1127–31.
5. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process—First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res 1992;52: 6735–40.
6. Engstrand L. Potential animal models of *Helicobacter pylori* infection in immunological and vaccine research. FEMS Immunol Med Microbiol 1995;10:265–70.
7. Shuto R, Fujioka T, Kubota T, Nasu M. Experimental gastritis induced by *Helicobacter pylori* in Japanese monkeys. Infect Immun 1993;61:933–9.
8. Radin MJ, Eaton KA, Krakowka S, Morgan DR, Lee A, Otto G, et al. *Helicobacter pylori* gastric infection in gnotobiotic beagle dogs. Infect Immun 1990;58:2606–12.
9. Lambert JR, Borromeo M, Pinkard KJ, Turner H, Chapman CB, Smith ML. Colonization of gnotobiotic piglets with *Campylobacter pyloridis*—an animal model? J Infect Dis 1987;155:1344.
10. Krakowka S, Morgan DR, Kraft WG, Leunk RD. Establishment of gastric *Campylobacter pylori* infection in the neonatal gnotobiotic piglet. Infect Immun 1987;55:2789–96.
11. Karita M, Li Q, Cantero D, Okita K. Establishment of a small animal model for human *Helicobacter pylori* infection using germfree mouse. Am J Gastroenterol 1994;89:208–13.
12. Karita M, Kouchiyama T, Okita K, Nakazawa T. New small animal model for human gastric *Helicobacter pylori* infection: success in both nude and euthymic mice. Am J Gastroenterol 1991;86: 1596–603.
13. Fox JG, Batchelder M, Marini R, Yan L, Handt L, Li X, et al. *Helicobacter pylori*-induced gastritis in the domestic cat. Infect Immun 1995;63:2674–81.
14. Dubois A, Berg DE, Incecik ET, Fiala N, Heman-Ackah LM, Perez-Perez GI, et al. Transient and persistent experimental infection of nonhuman primates with *Helicobacter pylori*: implications for human disease. Infect Immun 1996;64:2885–91.
15. Dubois A, Fiala N, Heman-Ackah LM, Drazek ES, Tarnawski A, Fishbein WN, et al. Natural gastric infection with *Helicobacter pylori* in monkeys: a model for spiral bacteria infection in humans. Gastroenterology 1994;106:1405–17.
16. Bingel SA. Pathologic findings in an aging Mongolian gerbil (*Meriones unguiculatus*) colony. Lab Anim Sci 1995;45:597–600.
17. Worthington JM, Fulghum RS. Cecal and fecal bacterial flora of the Mongolian gerbil and the chinchilla. Appl Environ Microbiol 1988;54:1210–5.
18. Bartizal KF, Wostmann BS, Wagner M. Distribution and effects of a defined six-member murine-derived microflora in gnotobiotic gerbils. Appl Environ Microbiol 1984;47:746–51.
19. Tokieda M, Honda S, Fujioka T, Nasu M. Effect of *Helicobacter pylori* infection on the *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced gastric carcinogenesis in Mongolian gerbils. Carcinogenesis 1999;20:1261–6.
20. Sugiyama A, Maruta F, Ikeno T, Ishida K, Kawasaki S, Katsuyama T, et al. *Helicobacter pylori* infection enhances *N*-methyl-*N*-nitrosourea-induced stomach carcinogenesis in the Mongolian gerbil. Cancer Res 1998;58:2067–9.
21. Shimizu N, Inada K, Nakanishi H, Tsukamoto T, Ikehara Y, Kaminishi M, et al. *Helicobacter pylori* infection enhances glandular stomach carcinogenesis in Mongolian gerbils treated with chemical carcinogens. Carcinogenesis 1999;20:669–76.
22. Mitchell H, Megraud F. Epidemiology and diagnosis of *Helicobacter pylori* infection. Helicobacter 2002;7(Suppl 1):8–16.
23. Wu IC, Ke HL, Lo YC, Yang YC, Chuang CH, Yu FJ, et al. Evaluation of a newly developed office-based stool test for detecting *Helicobacter pylori*: an extensive pilot study. Hepatogastroenterology 2003;50:1761–5.
24. Wu IC, Wu DC, Lu CY, Kuo CH, Su YC, Yu FJ, et al. Comparison of serum and urine ELISA methods for the diagnosis of *Helicobacter pylori*—a prospective pilot study. Hepatogastroenterology 2004;51:1736–41.
25. Kuo CH, Wu DC, Lu CY, Su YC, Yu FJ, Lee YC, et al. The media of rapid urease test influence the diagnosis of *Helicobacter pylori*. Hepatogastroenterology 2002;49:1191–4.
26. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 1996;20:1161–81.
27. Wang YL, Sheu BS, Yang HB, Huang AH. A noninvasive *H. pylori* stool antigen assay to detect *H. pylori* infection of in vivo BALB/c mice models. Hepatogastroenterology 2001;48:724–6.
28. Goodwin CS, Blincow E, Peterson G, Sanderson C, Cheng W, Marshall B, et al. Enzyme-linked immunosorbent assay for *Campylobacter pyloridis*: correlation with presence of *C. pyloridis* in the gastric mucosa. J Infect Dis 1987;155:488–94.
29. von Wulffen H, Heesemann J, Butzow GH, Loning T, Laufs R. Detection of *Campylobacter pyloridis* in patients with antrum gastritis and peptic ulcers by culture, complement fixation test, and immunoblot. J Clin Microbiol 1986;24:716–20.
30. Weiss J, Mecca J, da Silva E, Gassner D. Comparison of PCR and other diagnostic techniques for detection of *Helicobacter pylori* infection in dyspeptic patients. J Clin Microbiol 1994;32:1663–8.
31. Xia HX, Keane CT, O'Morain CA. Pre-formed urease activity of *Helicobacter pylori* as determined by a viable cell count technique—clinical implications. J Med Microbiol 1994;40:435–9.
32. Ho SA, Hoyle JA, Lewis FA, Secker AD, Cross D, Mapstone NP, et al. Direct polymerase chain reaction test for detection of *Helicobacter pylori* in humans and animals. J Clin Microbiol 1991;29: 2543–9.
33. Kanangat S, Solomon A, Rouse BT. Use of quantitative polymerase chain reaction to quantitate cytokine messenger RNA molecules. Mol Immunol 1992;29:1229–36.
34. Ursi JP, Ursi D, Ieven M, Pattyn SR. Utility of an internal control for the polymerase chain reaction. Application to detection of *Mycoplasma pneumoniae* in clinical specimens. APMIS 1992;100: 635–9.