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Virulence of water-induced coccoid *Helicobacter pylori* and its experimental infection in mice

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Abstract

AIM: To explore the virulence and the infectivity of coccoid *Helicobacter pylori* (*H. pylori*) transformed from spiral form by exposure to sterile tap water.

METHODS: Three strains of *H. pylori*, isolated from gastric biopsy specimens of confirmed peptic ulcer, were converted from spiral into coccoid form by exposure to sterile tap water. Both spiral and coccoid forms of *H. pylori* were tested for the urease activity, and the adherence to Hep-2 cells. The presence of flagella was examined under electron microscopy. In the experimental animal infection, the spiral and coccoid forms of *H. pylori* originated from the same strain F49 were inoculated intragastrically into BALB/c mice respectively four times at a 3-day interval. Half of the mice from each group were sacrificed at Day 21 and Day 28 after the last inoculation. Histology and *H.pylori* colonization were detected by urease test of gastric mucosa, cultures of *H.pylori,* and electron microscopy and so on.

RESULTS: The urease activity and the ability of adherence to Hep-2 cells were found to be lower in coccoid *H.pylori* than that in its spiral form. For example, the transformation in strain F_{44} led to a significant decrease of the adherence rate and adherence index from 70.0±5.3 % to 30.2±3.5 % ($P<0.01$), and from 2.6±0.4 to 0.86±0.3 ($P<0.01$), respectively. The flagella of coccoid *H. pylori* were observed under electron microscope. In the experimental infection in mice, the positive rate of gastric mucosa urease test was 93.8 % (15/16) in the group infected by spiral *H.pylori* and 50 % (8/16) in the group infected by coccoid *H.pylori*, and the estimated coccoid *H.pylori* colony number was 1.75 *vs* 0.56. The positive rates of *H.pylori* culture were 87.5 % (14/16) in spiral *H. pylori* group and 68.8 % (11/16) in coccoid *H. pylori* group. There was no significant difference in either urease test or bacterial culture rate between the groups examined at Day 21 and Day 28 after inoculation. Electron microscopic examination of the samples taken from both groups showed the adherence of *H.pylori* in spiral, bacillary and coccoid shapes to the epithelial cells of gastric wall. Histological examination showed the occurrence of gastric mucosal injury as indicated by various degrees of erosion, ulcer, and inflammatory cell infiltration. Mucosal injury was slighter in the mice infected by coccoid *H.pylori.* No positive result was obtained in the control group that received intragastrical administration of sterile tap water.

CONCLUSION: Although the virulence of coccoid *H.pylori* induced by water decrease, coccoid *H.pylori* still remains a considerable urease activity and the adhering ability to epithelial cells. Furthermore, the flagella, an important component responsible for bacterial movement and infection, were still observed as a cellular structure of coccoid *H.pylori* under electron microscope. The coccoid *H.pylori* induced by water is capable of colonizing in gastric mucosa and causing gastrititis in mice.

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INTRODUCTION

Helicobacter pylori has been recognized as an important pathogen that causes chronic gastritis and peptic ulcer and likely as a risk factor associated with gastric carcinoma[1-9]. *H. pylori* infection is endemic. In despite of more than 10 years of intensive research, the precise mode and route of *H. pylori* transmission remain elusive. Four routes including fecal-oral, oral-oral, gastro-oral and iatrogenic transmission have been postulated[10-13]. The association between water consumption and *H.pylori* infection indicates that *H.pylori* may be transmitted through a waterborne route[14-16]. *H.pylori* exists in two forms: the spiral form and the coccoid form. Coccoid *H.pylori* is non-culturable but alive[17-20]. Some researches have shown that *H. pylori* can survive water microcosms in coccoid form[20,21]. The coccoid *H.pylori* in water has therefore been suspected to contribute an important part to the transmission of the bacteria. However, the virulence and infectivity of coccoid *H.pylori* in water has not been studied. To explore the pathogenicity of the coccoid *H.pylori* in water, three strains of spiral *H.pylori* were treated by prolonged exposure to sterile tap water and examined for the presence of flagella under electron microscopy and tested for their urease activity and their adherence to Hep-2 cells. A strain was inoculated into the BABL/C mice. The gastric mucosal samples were taken to assess the bacterial *in vivo* colonization and pathological effects by means of urease test, bacterial culture, electron microscopy, and light microscopy.

MATERIALS AND METHODS

Animals

Female BALB/c mice were purchased from Shanghai

Experimental Animal Center, Chinese Academy of Sciences and raised under SPF conditions. Those of 8 weeks old, weighing 20-22 g were used for bacterial inoculation. Sterile food and tap water were given ad libitum.

Cells

Human epithelial cell line Hep-2 cells were maintained in 1 640 medium supplemented with 10 % fetal calf serum, 200 IU/ml penicillin and 50 μg/ml streptomycin at 37 ℃ in 5 % CO_2-95 % air, and re-cultivated twice a week.

Bacterial strains

Three strains (F44, F45 and F49) of *H.pylori* were isolated in this laboratory from gastric biopsy specimens of confirmed peptic ulcer patients. The isolates were spiral in shape, positive for catalase, oxidase, urease, and cagA and vacA gene. Stock cultures were maintained in defatted milk at -80 ℃.

H. pylori cultivation and coccoid form induction

The stored strains of *H.pylori* were cultured on Brucella agar with 5 % sheep blood at 37 ℃ for 2-3 d under microaerophilic conditions (5 % O_2; 10 % CO_2; 85 % N_2). After being subculturied, the bacteria were harvested and suspended in sterile tap water and the suspensions were incubated at 4 ℃ for a few days (about 3-4 d) until 100 % transformation to coccoid form was achieved and confirmed under light microscopy. The transformed bacteria were inoculated on the Brucella agar media supplemented with 5 % sheep blood for reversion trial culture. The stock suspensions were stored at 4 ℃ until use.

Electron microscopy

H. pylori flagella were examined under A Hu-12A transmission electron microscope. To prepare the bacterial samples, *H. pylori* suspensions were centrifuged, and the pellets were embedded in Epoxy 618. The ultra-thin sections were cut and negatively stained by 1 % phosphotungstic acid.

Assessment of cell adherence

Hep-2 cells were grown to confluence on glass coverslips in culture flask, and 0.5 ml of the suspension of *H.pylori* (10^8 cfu/ml) was added to culture medium (5 ml) for an additional 3.5 h culture at 37 ℃ in 5 % CO_2-95 % air. Cultures on the coverslips were washed and stained with Wright-Giemsa. One hundred Hep-2 cells were examined under light microscope for the counts of both the cells adhered by bacteria and the bacteria adhering to each cell. The adherence rate and adherence index were then calculated by the formula (described in the Results).

Animal infection experiment

Forty-two BALB/c mice were randomly divided into 3 groups. By oral gavage, groups 1 and 2 (16 mice each) were given 0.4 ml (10^9 cfu/ml) of suspensions of F49 strain spiral *H.pylori* and coccoid *H.pylori* (in water for 40 days), respectively, four times at a 3-day interval. The control group (10 mice) received 0.4 ml sterile tap water. At Day 21 and 28 after inoculation, half of the mice from each group were sacrificed, respectively. Before killing, the mice were fasted for 36 hours with free access to water. At sacrifice, stomachs were removed, opened and washed with sterile saline and longitudinally divided into 3 sections in same size, which were used respectively for fast urease test and bacterial culture, electron microscopy, and histological examination.

Urease activity assay

Urease activity fast assay kit was purchased from Sanqiang Company (Sanming, Fujian). The assays were made according to the manufacturer' s instructions. Diluted *H. pylori* cultures (10^{10} cfu/ml, 5 μ), or tissue fragments (3×3 mm) obtained from the pylorus part of one-third of the mouse gastric mucosa were added to the test wells to react with the commercial reagents. To evaluate the urease activity, the colors developed in the assay were scored into five grades (++++, +++, ++, + and -) for bacterial cultures and four grades (+++, ++, + and -) for tissue fragments.

Bacterial examination

After collected for urease assay, the remaining one-third gastric mucosa samples were grounded into homogenate, daubed on Brucella agar with 5 % sheep blood, and incubated at 37 ℃ for 3-4 d under microaerophilic conditions. Colonies were taken and identified under light microscopy, urease activity test and cagA gene amplification by PCR. In addition, two samples from groups 1 and 2 respectively, witch were bacteriologic positive and trimmed to 1 mm^3, were embedded in Epoxy 618, then the ultra-thin sections were cut, stained by uranyl acetate and lead citrate and examined under a Hu-12A transmission electron microscope.

Light microscopic histological examination

The gastric mucosal samples were embedded in paraffin, cut in 5 μm sections, stained with hematoxylin-eosin, and examined under light microscope.

Statistical analysis

Data was analyzed using the Student *t* test. The statistically significant difference was suggested by a value of $P<0.05$, and the very significant difference by $P<0.01$.

RESULTS

In vitro virulence of water-induced coccoid H.pylori

Flagella Three strains (F44, F45 and F49) of *H.pylori* were seen under light microscope in a typical spiral shape before their exposure to water. After 3-4 d incubation in sterile tap water at 4 ℃, no spiral but only coccoid shaped bacteria were observed. Reversion trial showed that water-induced coccoid *H. pylori* failed to grow on Brucella agar supplemented with 5 % sheep blood. Electron microscopy showed that the flagella remained a part of the bacterial cell structure (Figure 1).

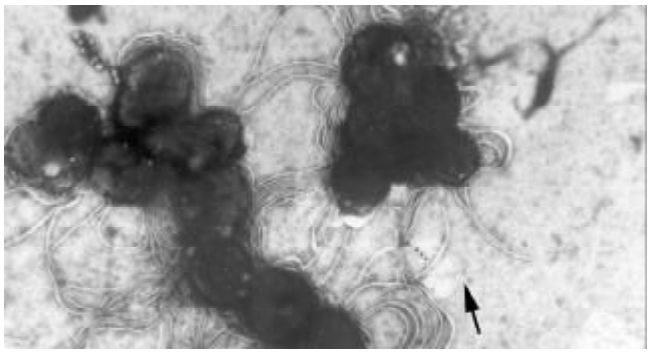

Figure 1 The flagella of coccoid *H.pylori* under transmission electron microscope ×6 000.

Urease activity Table 1 shows the urease activity assayed for three strains (F44, F45 and F49) of *H.pylori* both in spiral form (normal culture) and the respective coccoid form (subjected to water treatment). Strong urease activity (++++) was confirmed in the spiral *H. pylori* of all the three strains tested. The urease activity of the water-induced *H. pylori*, i.e., the

coccoid form of these three strains, significantly reduced, but still existed on a detective level (+ to ++).

Table 1 Urease activity of *H.pylori*

Strain	Urease activity	
	Spiral form	Coccoid form
F44	++++	+
F45	++++	++
F49	++++	+

Adhering ability All the three strains of *H. pylori* in both spiral form and water-induced coccoid form were tested for their adhering ability to Hep-2 cells. According to the following formula, the rate and the index of adherence were calculated.
The rate of adherence=the amount of cell adherenced by bacteria/100×100 %;
The index of adherence=the amount of bactria adhereing to cells/100;
Five groups of each 100 Hep-2 cells were counted for the number of cells adhered by bacteria and the total number of bacteria adhering to the cells. The percentages of cells adhered by bacteria (adherence rate) and the average bacteria number (adherence index) adhered to each cell are presented in Table 2. The adherence was observed in all groups tested. Student *t* test showed a very significant difference between the spiral and coccoid forms of *H. pylori* in either adherence rate or adherence index.

Table 2 Adherence of *H.pylori* to Hep-2 cells

	Adherence rate (%)[a]			Adherence index[b] (Bacteria numbers per cell)		
	F44	F45	F49	F44	F45	F49
Spiral form	70.0±5.3	73.0±5.1	72.6±4.5	2.6±0.4	3.1±0.5	2.9±0.4
Coccoid form	30.2±3.5	35.7±4.1	31.4±4.0	0.86±0.3	0.91±0.3	0.88±0.4
t value	12.3	11.2	12.8	7.2	7.8	7.4
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[a]Adherence rate=amount of cells adhered by bacteria/100×100 %; [b]Adherence index=total amount of bacteria adhering to 100 cells/100; Five groups of 100 cells on the same coverslip were counted for the bacteria adhered. Data are presented as mean ±SD. *t* and *P* values were obtained using Student *t* test.

Coccoid H. pylori infection in mice

Baterial colonization *H.pylori* colonization in the gastric mucosa of inoculated mice was determined by the urease assay and bacterial culture of the of tissue samples. The bacterial cultures were found to be characteristic of spiral *H.pylori* as proved by the spiral shaped structure under light microscope, the positive urease activity, and the positive amplification of cagA gene fragments (data not shown). Data shown in Table 3 were the rates of positive findings in each group of mice. The positive rates of urease test of gastric mucosa, which was infected by spiral *H.pylori* and coccoid *H.pylori*, were 93.8 % (15/16) and 50 %(8/16), respectively. The positive rates of cultures of *H.pylori* were 87.5 %(14/16) and 68.8 %(11/16) respectively. Neither urease assay nor bacterial culture was found positive in the mice of the control group. Sampling at Day 21 and Day 28 after inoculation found almost no difference in both tests. In the semi-quantitative study, the color development in fast urease assay was scored. The colors distinguished at grades -, +, ++, and +++, which were associated with the existence of the *H. pylori* in 0, 1-10,11-30, and >30 per microscope filed, respectively, according to the guide of test kit, were accordingly assigned by 0, 1, 2, and 3 point(s). Table 4 presents the number of mice scored at the same points in this assay and the average points of each group. Again, the score in group 1 was much higher than in group 2 (1.75 *vs* 0.56), while the score in control group was zero. In addition, electron microscopy showed the adherence of bacteria to the gastric mucosal samples taken from both group 1 and group 2 (Figure 2A and B). These bacteria were in spiral, bacillary or coccoid shapes,and some of them had invaded into the gastric epithelial cells. No similar bacterium adherence and invasion was observed in the samples from control group.

Table 3 Tests of bacteria in gastric mucosa samples

Group	Total no.	Fast urease test			Culture of bacterial		
		Positive/total		Positive rate(%)	Positive/total		Positive rate(%)
		D21	D28		D21	D28	
1	16	7/8	8/8	93.8	7/8	7/8	87.5
2	16	4/8	4/8	50.0	5/8	6/8	68.8
Control	10	0/5	0/5	0	0/5	0/5	0

Group 1 was inoculated with spiral *H. pylori*, group 2 was inoculated with water-induced coccoid *H. pylori*, and control group received sterile tap water.

Table 4 Scores for urease tests of tissue samples

Groups	Color development				Urease activity
	-	+	++	+++	(mean score)
1	1[a]	5	7	3	1.75[b]
2	8	7	1	0	0.56
Control	10	0	0	0	0

[a]Numbers of mice; [b]Refers to text for scoring and calculation.

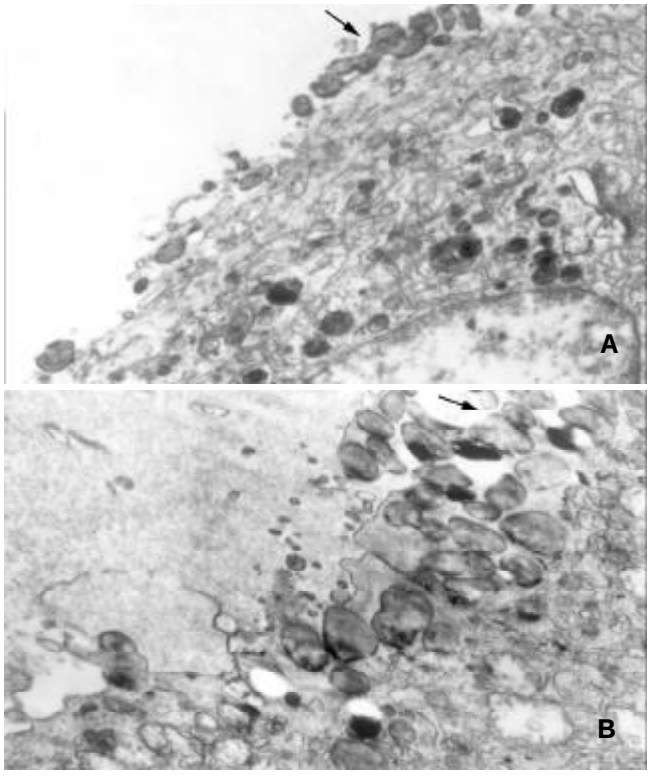

Figure 2 *H.pylori* colonization in mouse stomach under transmission electron microscope. A. infection of spiral *Hp*. ×7 000; B. infection of coccoid *Hp*. ×9 000.

Histolopathological alteration Inflammatory pathological features were observed in both group1 and group 2 samples under light microscope (Table 5 and Figure 3). Fifteen mice of group 1 and ten mice of group 2 developed inflammatory cell infiltration and different degrees of erosion or ulcer. The frequency and intensity of the erosion in group 1 was higher than in group 2. Two out of sixteen mice in group 1 even developed mucosal ulcers. Mucosal injury was slighter in the mice infected by coccoid H.pylori. None of these features was found in the control group.

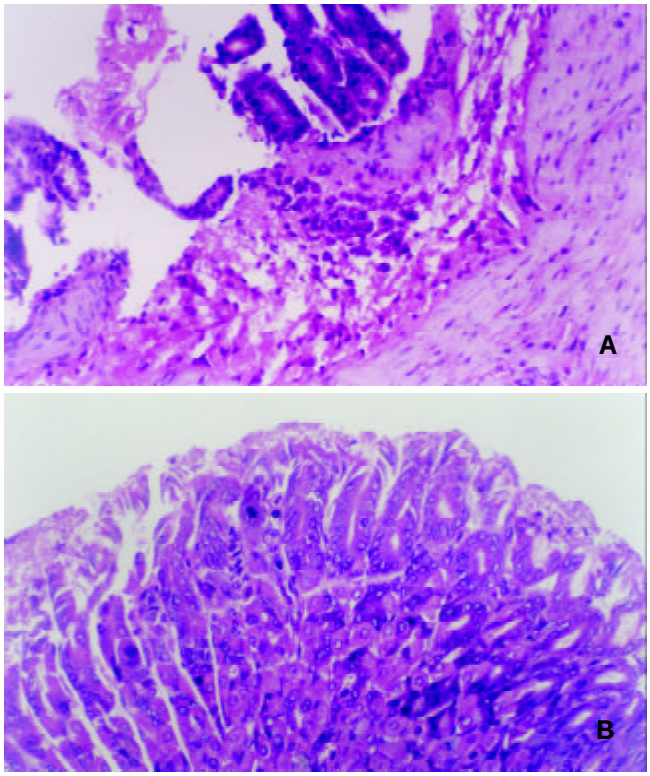

Figure 3 Light microscopy for gastric mucosa of mice. H&E×200. A. infection of spiral *Hp*; B. infection of coccoid *Hp*.

Table 5 Pathogogical alterations of gastric mucosa from mice

Groupe	Histopathological event (no. of mice)				
	Normal	Gentle erosion	Deep erosion	Ulcer	Total
1	1	7	6	2	16
2	6	9	1	0	16
Control	10	0	0	0	10

DISCUSSION

Increasing reports showed that *H. pylori* had been detected from water by immunomagnetic separation, bacterial culture or polymerase chain reaction (PCR) technique[22-27] and that consumption of water was closely related to *H.pylori* infection[14-16]. Water borne route is therefore thought to be an important route of *H. pylori* transmission. *H.pylori* has been found to be able to convert from spiral form to coccoid form under certain adverse circumstances such as increased oxygen tension, extended incubation and exposure to antibiotics or water[17,28-32]. Some researches suggested that *H. pylori* in coccoid form can survive the water for a long time. However, it remains unknown whether coccoid *H. pylori* can attack and colonize in stomach., resulting in the diseases of digestive system. Going deep inside to the behavior of coccoid *H. pylori* will thus be very beneficial to our understandings on the transmission of *H. pylori* infection and its association with many severe human diseases like gastritis, ulcer and peptic carcinomas.

The putative pathogenic determinants of *H.pylori* have been divided into two major groups[35]: maintenance factors, which allow the bacterium to colonize and remain within the host, and virulence factors, which contribute to the pathogenetic effects of the bacterium. Flagella, urease activity and adherence to epithelial cells of *H.pylori* are important maintenance factors[34-37]. If coccoid *H.pylori* in water remains infective, they must possess maintenance factors in order to colonize and remain in stomach. In this study, it is shown that both urease activity and adherence to Hep-2 cells of coccoid *H. pylori* decreased as compared with the spiral forms, suggesting a reduction of virulence related to colonization of *H.pylori* when the transformation to coccoid form occurs. However, as shown by the microbial assays, coccoid *H.pylori* induced by water still remains a considerable urease activity and the adhering ability to epithelial cells. Furthermore, the flagella, an important component responsible for bacterial movement and infection, were still observed as a cellular structure of coccoid *H.pylori* under electron microscope. This adds to the potential of *in vivo* infection of the coccoid *H.pylori* induced by water.

In the animal experiments described here, some mice (10/16) inoculated with water-induced coccoid *H.pylori* developed significant pathological changes such as mucosal erosion and inflammantory cell infiltration in gastrical mucosa, as were shown by histopathological examinations. The evidences of the coccoid *H.pylori* being the pathogen of the mucosal injury were further provided by bacteriological examinations. In this aspect, a 50 % positive rate and a considerable intensity of urease test were detected in the mucosal samples of mice inoculated with water-induced coccoid *H.pylori*, and the positive *H.pylori* cultures of these samples reached a percentage of 68.8 %. In addition, electron microscopy for these samples showed the presence of spiral bacteria in gastric mucosa. All these findings reveal the ability of water-induced coccoid *H. pylori* in their colonization on mouse gastric wall and their injury to the mucosal tissues.

It might be reasonably queried whether there still exists an undetectable trace amount of spiral *H.pylori* among the huge quantity of their coccoid variance, which could be intrinsically responsible for the virulence and infectivity of the bacteria in some studies including ours. The facts that the bacteria were kept in in-nutritious water for up to 40 days and that the water-treated bacteria were assayed for *in vitro* virulence in real time, eliminated the possibility of an expansion of the spiral population or in-water reversion of the coccoid variance to its spiral origins. The failure of the trial reversion in supplemented Brucella medium further supported the concept of a direct virulence of the coccoid *H. pylori*. Now that the spiral shaped bacteria were observed in the mucosal tissues of mice inoculated with coccoid *H. pylori*, it seemed that the reversion took place *in vivo*. However, whether the reversion is a key precondition for the infection remains unclear. In despite of our ignorance in the process and mechanisms of the inter-transformation of *H.pylori*, conclusions can be drawn from our current study that water-induced coccoid form of *H.pylori* remains virulent and infective to gastric wall in mice. Water borne route transmission of *H.pylori* needs more attention.

REFERENCES

1 **Gao GL**, Pan BR, Yang SF, SongG, Xu XQ, Liu Y. The value of *Helicobacter pylori* in gastro-duodenal diseases. *Xin Xiaohuabingxue Zazhi* 1994; **2**: 232-233

2 **Li ZX**, Zhang WD, Zhou DY, Zhang YL, Guo XP, Yang HT. Relationship between *Helicobacter pylori* and duodenalulcer. *Xin Xiaohuabingxue Zazhi* 1996; **4**: 153-155

3 **Liu WZ**, Zheng X, Shi Y, Dong QJ, Xiao SD. Effect of *Helicobacter pylori* infection on gastric epithelial proliferation in progression from normal mucosa to gastriccarcinoma. *World J Gastroenterol* 1998; **4**: 246-248

4 **Blaser MJ**, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH. Stemmermann GN, Nomura A. Infection with *Helicobacter pylori* strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer* 1995; **55**: 2111-2115

5 **Lu XL**, Qian KD, Tang XQ, Zhu YL, Du Q. Detection of *H.pylori* DNA in gastric epithelial cells by in situ hybridization. *World J Gastroenterol* 2002; **8**: 305-307

6 **Liu WZ**, Zheng X, Shi Y, Dong QJ, Xiao SD. Effect of *Helicobacter pylori* infection on gastric epithelial proliferation in progression from normal mucosa to gastric carcinoma. *World J Gastroenterol* 1998; **4**: 246-248

7 **Wang RT**, Wang T, Chen K, Wang JY, Zhang JP, Lin SR, Zhu YM, Zhang WM, Cao YX, Zhu CW, Yu H, Cong YJ, Zheng S, Wu BQ. *Helicobacter pylori* infection and gastric cancer: evidence from a retrospective cohort study and nested case-control study in China. *World J Gastroenterol* 2002; **8**: 1103-1107

8 **Xue FB**, Xu YY, Wan Y, Pan BR, Ren J, Fan DM. Association of *H. pylori* infection with gastric carcinoma: a Meta analysis. *World J Gastroenterol* 2001; **7**: 801-804

9 **Cai L**, Yu SZ, Zhang ZF. *Helicobacter pylori* infection and risk of gastric cancer in Changle County, Fujian Province, China. *World J Gastroenterol* 2000; **6**: 374-376

10 **Song Q**, Spahr A, Schmid RM, Adler G, Bode G. *Helicobacter pylori* in the oral cavity: high prevalence and great DNA diversity. *Dig Dis Sci* 2000; **45**: 2162-2167

11 **Yoshimatsu T**, Shirai M, Nagata K, Okita K, Nakazawa T. Transmission of *Helicobacter pylori* from challenged to nonchallenged nude mice kept in a single cage. *Dig Dis Sci*, 2000; **45**: 1747-1753

12 **Leung WK**, Siu KL, Kwok CK, Chan SY, Sung R, Sung JJ. Isolation of *Helicobacter pylori* from vomitus in children and its implication in gastro-oral transmission. *Am J Gastroenterol* 1999; **94**: 2881-2884

13 **Liu WZ**, Xiao SD, Jiang SJ, Li RR, Pang ZJ. Seroprevalence of *Helicobacter pylori* infection in medical staff in Shanghai. *Scand J Gastroenterol* 1996; **31**: 749-752

14 **Baker KH**, Hegarty JP. Presence of *Helicobacter pylori i*n drinking water is associated with clinical infection. *Scand J Infect Dis* 2001; **33**: 744-746

15 **Klein PD**, Graham DY, Gaillour A, Opekun AR, Smith EO. Water source as risk factor for *H.pylori* infection in Peruvian children. *Lancet* 1991; **337**: 1503-1506

16 **Bunn JE**, MacKay WG, Thomas JE, Reid DC, Weaver LT. Detection of *Helicobacter pylori* DNA in drinking water biofilms: implications for transmission in early life. *Lett Appl Microbiol* 2002; **34**: 450-454

17 **Bode G**, Mauch F, Malfertheiner P. The coccoid forms of *Helicobacter pylori*. Criteria for their viability. *Epidemiol Infect* 1993; **111**: 483-490

18 **Cellini L**, Allocati N, Di Campli E, Dainelli B. *Helicobacter pylori*: A fickle germ. *Microbiol Immunol* 1994; **38**: 25-30

19 **Benaissa M**, Babin P, Quellard N, Pezennec L, Cenatiempo Y, Fauchere JL. Changes in *Helicobacter pylori* ultrastructure and antigens during conversion from the bacillary to the coccoid form. *Infect Immun* 1996; **64**: 2331-2335

20 **Shahamat M**, Mai U, Paszko-Kolva C, Kessel M, Colwell RR. Use of autoradiography to assess viability of *Helicobacter pylori* in water. *Appl Environ Microbiol* 1993; **59**: 1231-1235

21 **Mizoguchi H**, Fujioka T, Nasu M. Evidence for viability of coccoid forms of *Helicobacter pylori*. *J Gastroenterol* 1999; **34** (Suppl 11): 32-36

22 **Winiecka-Krusnell J**, Wreiber K, von Euler A, Engstrand L, Linder E. Free-living amoebae promote growth and survival of *Helicobacter pylori*. *Scand J Infect Dis* 2002; **34**: 253-256

23 **Lu Y**, Redlinger TE, Avitia R, Galindo A, Goodman K. Isolation and genotyping of *Helicobacter pylori* from untreated municipal wastewater. *Appl Environ Microbiol* 2002; **68**: 1436-1439

24 **Mazari-Hiriart M**, Lopez-Vidal Y, Castillo-Rojas G, Ponce de Leon S, Cravioto A. *Helicobacter pylori* and other enteric bacteria in freshwater environments in Mexico City. *Arch Med Res* 2001; **32**: 458-467

25 **Horiuchi T**, Ohkusa T, Watanabe M, Kobayashi D, Miwa H, Eishi Y. *Helicobacter pylori* DNA in drinking water in Japan. *Microbiol Immunol* 2001; **45**: 515-519

26 **Mazari-Hiriart M**, Lopez-Vidal Y, Calva JJ. *Helicobacter pylori* in water systems for human use in Mexico City. *Water Sci Technol* 2001; **43**: 93-98

27 **Hegarty JP**, Dowd MT, Baker KH. Occurrence of *Helicobacter pylori* in surface water in the United States. *J Appl Microbiol* 1999; **87**: 697-701

28 **Catrenich CE**, Makin KM. Characterization of the morphologic conversion of *Helicobacter pylori* from bacillary to coccoid forms. *Scand J Gastroenterol* 1991; **181** (Suppl): 58-64

29 **Cellini L**, Allocati N, Angelucci D, Iezzi T, Di Campli E,, Marzio L, Dainelli B. Coccoid *Helicobacter pylori* not culturable *in vitro* reverts in mice. *Microbiol Immunol* 1994; **38**: 843-850

30 **Costa K**, Bacher G, Allmaier G, Dominguez-Bello MG, Engstrand L, Falk P, de Pedro MA, Garcia-del Portillo F. The morphological transition of *Helicobacter pylori* cells from spiral to coccoid is preceded by a substantial modification of the cell wall. *J Bacteriol* 1999; **181**: 3710-3715

31 **Xu ZM**, Zhou DY, Pan LJ, Song S. Transformation and reversion of *Helicobacter pylori in vitro*. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 215-217

32 **Shirai M**, Kakada J, Shibata K, Morshed MG, Matsushita T, Nakazawa T. Accumulation of polyphosphate granules in *Helicobacter pylori* cell under anaerobic conditions. *J Med Microbiol* 2000; **49**: 513-519

33 **Dunn BE**, Cohen H, Blaser MJ. *Helicobacter pylori*. *Clin Microbiol Rev* 1997; **10**: 720-741

34 **Eaton KA**, Brooks CL, Morgan DR, Krakowka S. Essential role of urease in pathogenesis of gastritis induced by *Helicobacter pylori* in gnotobiotic piglets. *Infect Immun* 1991; **59**: 2470-2475

35 **Smoot DT**, Mobley HL, Chippendale GR, Lewison JF, Resau JH. *Helicobacter pylori* urease activity is toxic to human gastric epithelial cells. *Infect Immun* 1990; **58**: 1992-1994

36 **Boren T**, Falk P, Roth KA, Larson G, Normark S. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. *Science* 1993; **262**: 1892-1895

37 **Ottemann KM**, Lowenthal AC. *Helicobacter pylori* uses motility for initial colonization and to attain robust infection. *Infect Immun* 2002; **70**: 1984-1990

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