INTRODUCTION

H. pylori is an important etiological agent of gastritis, peptic ulcer disease and gastric carcinoma (Ansorg et al., 1991; Parsonnet, 1994; Logan et al., 2001). There are several techniques available for the detection of H. pylori, including the rapid urease test (RUT), culture and histological analysis. However, all these assays require gastric biopsy specimens from endoscopy, which is invasive (Fabre et al., 1994; Heatley, 1995; Kisa et al., 2002).

A serological assay is a non-invasive alternative for the diagnosis, screening and epidemiological study of H. pylori infection (Talley and Noack, 1993; van de Wouw et al., 1995; Logan and Walker, 2001). Enzyme-linked immunosorbent assay (ELISA) is a non-invasive, simple and cheap method for detecting H. pylori infection (van de Wouw et al., 1996; Logan and Walker, 2001; Hanvivatvong et al., 2002). It has a sensitivity and specificity in predicting H. pylori infection in untreated patients as accurate as invasive tests (Talley et al., 1991; 1993; van de Wouw et al., 1995, 1996; Logan and Walker, 2001).

It has been suggested that the serological test for H. pylori be locally validated relative to other methods because assays vali-
dated in one region may not be valid for other regions (Malfertheiner et al, 2002; Hoang et al, 2004). In Thailand, invasive methods, such as the RUT and histological examination, are more widely used for the diagnosis of this fastidious microorganism than culture and other non-invasive methods (Arnantapunpong, 1999; Phiphitaporn, 1999; Pankongngam, 2001). Although culture is the gold standard for diagnosing \textit{H. pylori} infection, it is time-consuming and has a lower sensitivity than other methods (Hazell et al, 1989; Heatley, 1995). Both RUT and histological examination require biopsies, making them invasive. Therefore, a non-invasive method, such as the ELISA test, is a potential alternative for detecting \textit{H. pylori} in Thai dyspeptic patients.

The seroprevalence of \textit{H. pylori} infection in asymptomatic people varies between nations, ages, races, socioeconomic groups, regions and time of study (Andersen et al, 1996). The seroprevalence of \textit{H. pylori} infection in the Thai population in some regions has been reported to be higher than in industrialized countries (Perez-Perez et al, 1990). Therefore, the epidemiology of \textit{H. pylori} infection in Thailand’s regions should be investigated in order to understand the local risk factors for this microorganism.

In this study we compared a commercial ELISA (Pyloriset EIA-GIII, Orion, Diagnostic, Espoo, Finland) which has been shown to have a high sensitivity in other countries (Talley et al, 1991; van de Wouw et al, 1995; Logan and Walker, 2001) with culture, RUT and histological examination methods in Thai dyspeptic patients in Khon Kaen, Thailand for the diagnosis of \textit{H. pylori} infection. In order to know the epidemiology of \textit{H. pylori} infection in different regions in Thailand, we assessed the seroprevalence of \textit{H. pylori} infection among healthy blood donors at Srinagarind Hospital’s Blood Bank, Khon Kaen University, using this same commercial ELISA kit.

**MATERIALS AND METHODS**

**Patients and blood donor subjects**

One hundred and thirty-seven consecutive patients with dyspeptic symptoms who underwent upper gastrointestinal endoscopy were included in this study. They were recruited from the Endoscopy Unit of Srinagarind Hospital, Faculty of Medicine, Khon Kaen University between March 2003 and January 2005. The subjects were diagnosed as having non-ulcer dyspepsia (NUD), peptic ulcer dyspepsia (PUD), gastric carcinoma (GCA) and other gastrointestinal diseases (GERD, MALT, duodenitis, etc). The group was comprised of 58 males and 79 females with an age range of 18 to 88 years (mean 48.5 years). We excluded patients who had antibiotic therapy, bismuth treatment, proton pump inhibitors, or histamine-blockers within the previous month.

For the blood donor subjects, a total of 100 consecutive donors who were asymptomatic regarding dyspepsia attending Srinagarind Hospital’s Blood Bank, Khon Kaen University between March 2003 and January 2005 were randomly included. The group was comprised of 64 males and 36 females with an age range of 17 to 51 years (mean 29.8 years).

The study was approved by the Ethics Committee of Khon Kaen University and performed in accordance with the Declaration of Helsinki. Informed written consent was obtained from all subjects before being included in the study.

**Biopsy specimens**

Three gastric mucosal biopsy specimens from the antrum and the corpus were obtained from each patient and divided into three parts. Both antral and corpus specimens were used for culture, the rapid urease test (RUT) and histological examination.

**Culture**

The culture was performed according to Hazell et al (1989) with modification. Briefly,
each antral and corpus specimen was immediately placed in transport media and brought to the laboratory within 2 hours, and stored under cold conditions. The biopsy specimens were homogenized in 200 µl of normal saline and cultured on 7% human blood agar (Difco, Detroit, Michigan, USA) containing the supplement SR147 (5 mg/l Trimethoprim, 10 mg/l Vancomycin, 5 mg/l Amphotericin B, 5 mg/l Cefsulodin (SR147, OXOID). The plates were incubated at 37°C under microaerophilic conditions (5% O₂, 10% CO₂, 85% N₂) and examined after 4 and 7 days of incubation. Characteristic colonies of H. pylori were confirmed by Gram staining, oxidase, catalase and urease tests.

Commercial rapid urease test (RUT, Pronto Dry test)

The RUT was performed according to the manufacturer’s instructions (Medical Instruments Corporation, Solothurn, Switzerland). Briefly, one antral and one corpus specimen were directly inoculated onto the commercial RUT agar gel. The results were observed and recorded within 24 hours: a positive result was indicated when the color changed from yellow to pink.

Histological examination

One antral and one corpus biopsy were fixed in 10% buffered formalin, processed, then embedded in paraffin. Four sections, 3-4 micron thick were stained with modified Warthin-Starry stain for identification of H. pylori (Cohen and Laine, 1997; Li et al, 2004). The presence of spiral organisms on any of the slides was considered positive for H. pylori.

Commercial ELISA test (Pylori EIA-G III)

Serum samples taken from those dyspeptic patients and 100 healthy blood donors were assayed for IgG antibodies against H. pylori. The tests were performed according to commercial ELISA kit [(Pyloriset EIA-GIII), Orion, Diagnostica, Espoo, Finland]. A positive result was defined as a titer equal to or greater than 20 U/ml, according to the manufacturer’s recommendations.

Analysis of test results

Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) for the ELISA test for H. pylori were compared with true positive criteria. The chi-square test was used for statistical analysis of H. pylori infection in males and females. P<0.05 was considered statistically significant.

The criteria for a true positive for H. pylori infection were when it showed 1) a positive culture or 2) negative culture but positive RUT and histological examination (Pajares-Garcia, 1998; Liao et al, 2003).

RESULTS

Comparison of culture, RUT, histological examination and ELISA for the diagnosis of H. pylori

Of the gastric biopsy specimens in 137 dyspeptic patients, H. pylori was found by culture, RUT, histological examination, and ELISA in 50 (36.5%), 64 (46.7%), 71 (51.8%), and 75 (54.7%), respectively. Regarding true positive test criteria, H. pylori infection was found in 60 (43.8%) of those patients (Fig 1).

![Fig 1–Positive detection rates for H. pylori infection in 137 dyspeptic patients detected by culture, RUT, histological examination and ELISA method. The true positive is the culture positive or both the urease and the histological examination positive.](image-url)
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Numbers and percentages of H. pylori infections detected by the four diagnostic methods.

<table>
<thead>
<tr>
<th>Culture</th>
<th>RUT</th>
<th>Histological examination</th>
<th>ELISA</th>
<th>Number (%)</th>
<th>Evaluation of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>43 (31.3)</td>
<td>TP</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>3 (2.2)</td>
<td>TP</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>9 (6.6)</td>
<td>TP</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>4 (2.9)</td>
<td>TP</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>1 (0.7)</td>
<td>TP</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>1 (0.7)</td>
<td>FP</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>3 (2.2)</td>
<td>FP</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>3 (2.2)</td>
<td>FP</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>12 (8.8)</td>
<td>FP</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>15 (10.9)</td>
<td>FP</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>43 (31.3)</td>
<td>TN</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>137 (100.0)</td>
<td>60 (43.8)a</td>
</tr>
</tbody>
</table>

TP = True positive, TN = True negative, FP = False positive
TP, culture positive or both urease and histological examination positive
a Total numbers (%) of TP

Table 2
Sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV), and accuracy of the commercial ELISA test for IgG antibodies to H. pylori at different cut-off values among 137 patients.

<table>
<thead>
<tr>
<th>Cut-off values (U/ml)</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>PPV %</th>
<th>NPV %</th>
<th>Accuracy %</th>
</tr>
</thead>
<tbody>
<tr>
<td>20a (56 / 60)</td>
<td>93.3 (56 / 77)</td>
<td>75.3 (56 / 75)</td>
<td>74.7 (56 / 62)</td>
<td>93.5 (114 / 137)</td>
<td>83.2 (114 / 137)</td>
</tr>
<tr>
<td>25 (55 / 60)</td>
<td>91.7 (59 / 77)</td>
<td>76.6 (55 / 73)</td>
<td>75.3 (59 / 64)</td>
<td>92.2 (114 / 137)</td>
<td>83.2 (114 / 137)</td>
</tr>
</tbody>
</table>

a Recommended by the manufacturer

Forty-three (31.3%) samples were positive by all four diagnostic methods, 16 (11.7%) by three methods, 5 (3.6%) by two methods, and 30 (21.9%) by only one method (Table 1). The sensitivity, specificity, PPV, NPV, and accuracy of the ELISA test against IgG antibodies at antibody levels higher than 20 U/ml as recommended by the manufacturers and higher than 25 U/ml were compared (Table 2).

Seroprevalence of H. pylori infection in blood donors

We determined the seroprevalence of H. pylori specific serum IgG among 100 healthy blood donors in Khon Kaen, Thailand. The prevalence of seropositivity among the 100 blood donors was 57%. The seroprevalences of H. pylori positivity were 60.9% (36/64) in male and 50% (18/39) in female blood donors.
Although the infection rate in males was higher than in females, there was no statistically significant difference (p>0.05). The median titer level was 36 U/ml (Table 3). The seropositive rates were 54.4% (31/37) and 60.5% (26/43) in the 10-30 and 30-60 year age groups.

### DISCUSSION

Alternative serodiagnostic techniques for detection of *H. pylori* infection include bacterial agglutination, passive hemagglutination, complement fixation, indirect immunofluorescence, and ELISA (Lozniewski et al, 1996; Malfertheiner, 1999; Monteiro et al, 2001; Hoang et al, 2004). ELISA is one of the most extensively employed tests, because it is relatively inexpensive, quick, easy to perform, suitable for screening large populations, and capable of detecting class-specific immunoglobulins (Talley et al, 1991; van de Wouw et al, 1995; Logan and Walker, 2001). ELISA has been widely used in epidemiological studies, including retrospective studies to determine the prevalence or incidence of infection (Logan and Walker, 2001).

In western countries, ELISA kits have been shown to have high sensitivities and specificities (80-95%) (Logan and Walker, 2001), whereas in developing countries, including Thailand, commercial ELISA kits have high sensitivities but low specificities (Perez-Perez et al, 1990; Lin et al, 1996; Hanvivatvong et al, 2002). A higher sensitivity and specificity were reported when using the antigen prepared from local *H. pylori* isolates than the commercial ELISA reagent kits (Perez-Perez et al, 1990; Bodhidatta et al, 1993).

A previous study in Bangkok evaluated the five commercial ELISAs for detection of antibodies against *H. pylori* in dyspeptic patients, and found high sensitivities (86.3-97.9%) but low specificities (57.9-72.2%). In this study, we found the commercial ELISA test (Pyloriset EIA-G III) had a high sensitivity (93.3%) and an even higher specificity (75.3%) than in a previous report (72%) (Hanvivatvong et al, 2002).

The differences of the commercial ELISA tests in sensitivity and specificity may be the result of many factors, including the antigens used, the population group studied, and/or the reference method used. Lack of specificity may result from cross reaction with other organisms and human gastric mucosa (Newell, 1987; Negrini et al, 1996). The high sensitivity makes this ELISA kit reliable as a screening test and an alternative to endoscope in dyspeptic patients. There were 19 (13.9%) false positive samples detected by ELISA classified according to positive criteria. These results showed a slightly lower specificity (75.3%) and PPV (74.7%) compared to culture, histology and RUT (data not shown).

When compared to a previous report in Bangkok using a similar commercial ELISA kit based on IgG detection (Pyloriset EIA-G III) to evaluate *H. pylori* infection in serum (Hanvivatvong et al, 2002), our result showed a higher specificity (75.3%) than theirs.
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(57.9%). This may be due to differences in detection time and in the population group investigated.

The seroprevalence of H. pylori infection in a population of blood donors in Khon Kaen was high (57%) among the median age group (29.8 years old). Our results agree with a previous report in Thailand that showed the seroprevalence in the Thai community of Nakhon Ratrasima increased to 55% among those 20-30 years of age, and peaked (75%) among the 30-40 year old age group (Perez-Perez et al, 1990). In our study, seropositivity in Khon Kaen blood donors age 10-30 was 54.4% and age 30-60 was 60.5%, indicating that with increasing age there is increased risk of acquiring H. pylori infection, though our number are lower than a previous report in the 30-40 year old age group (Perez-Perez et al, 1990).

The high seroprevalence rate indicates transmission of H. pylori commonly occurs in the Thai population. This may influence the accuracy of the ELISA test in diagnosing H. pylori infection in Thai dyspeptic patients because of false positives and low specificity. Another caution in using the ELISA test for the diagnosis of H. pylori infection is the possibility that the immune response (IgG) against H. pylori may persist for a long time (Parsonnet et al, 1997). Some previous reports showed the immune response was completely eliminated (100%) 3-12 months after H. pylori eradication (Laheij et al, 1998; Koizumi et al, 2003). Therefore, ELISA is useful for monitoring the outcome of H. pylori eradication therapy. However, it may be difficult to distinguish between past and present infection in the dyspeptic patients who did not eradicate this microorganism.

Regarding the rather high seroprevalence of H. pylori in the Khon Kaen population, some previous studies have shown the ELISA test for H. pylori detection should be adjusted for the population studied (Bener et al, 2002; Hoang et al, 2004). Therefore, we tried to use higher cut-off levels (25 U/ml instead of 20 U/ml). However, the specificity and PPV were slightly higher (76.6%) (Table 2), the sensitivity and NPV were slightly lower, but the accuracy remained unchanged. Therefore, increasing cut-off levels in the ELISA test was useful for increasing specificity but this should not be used for screening.

We conclude that this commercial ELISA test can be used as a screening test for the detection of H. pylori infection because of its high sensitivity and accuracy. The high seroprevalence of H. pylori infection in Khon Kaen blood donors is similar to that found in some rural areas in Thailand, indicating that this microorganism may be commonly transmitted among the Thai population. In future, large-scale population-based studies should determine the source of transmission for H. pylori.

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