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PCR-based diagnosis of *Helicobacter pylori* infection in Polish children and adults

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Infection and associated disease caused by *Helicobacter pylori* are common in Poland, as in much of Eastern Europe, although the genotypes of strains have not been much studied, especially in terms of traits that might be important in disease. This study developed a sensitive and efficient polymerase chain reaction (PCR) test for the presence of *H. pylori* in gastric biopsy samples with *ureA* gene-specific primers and primers for the virulence-associated *cag* pathogenicity island (PAI). These tests were used with biopsy samples from 246 symptomatic children (age range 1–17 years) and 82 adults (age range 18–53 years) in Warsaw. An assessment was also made of the success of metronidazole-based therapy intended to eradicate infection. *H. pylori* was detected by *ureA*-specific PCR in 83 (76.9%) children and in 41 (87.2%) adults with histologically proven gastritis, and in 28.4% and 29.2%, respectively, of the 38 children and 7 adults with little or no evidence of gastritis. In general, *H. pylori* was detected more often by PCR than by culture (70.3% compared with 52.8% in children and 62.8% compared with 38.6% in adults), although in several cases a negative PCR was associated with a positive culture result. The rate of *H. pylori* infection increased with age from 5.4% in children up to 5 years old to 29.2% to age 10 and 65.4% to age 18. The tests detected the *cagPAI* in 97 (75%) and 44 (85%) of the *H. pylori*-infected children and adults, respectively. Some *H. pylori*-infected patients with a *ureA*+ PCR result contained the 'empty site' of the *cagPAI* and only four patients were infected with mixed *cag*+ *cag*– strains. PCR with *cagPAI* and 'empty site' of the *cagPAI* represents a novel tool for fast screening of mixed *cag*+ *cag*– infection. These results confirm and further illustrate that direct PCR of biopsy specimens can be useful for detection of infection and genotyping of resident strains, and that *H. pylori* infection is very common among children as well as adults in Poland. They also show that Polish strains vary with regard to the presence or absence of the *cagPAI*, and suggest that the proportion of strains that are *cag*+ is higher in Poland than in Western European countries, which may reflect the relatively higher risk of infection in this society.

Introduction

Helicobacter pylori is a micro-aerophilic gram-negative bacterium that establishes chronic infections in man throughout the world [1, 2]. It is implicated as a primary cause of gastritis and duodenal and gastric ulcers, and as a critical early risk factor for gastric cancer, with most infections being asymptomatic [1–6]. The incidence of infection is much higher in developing countries and probably higher in Eastern

Europe than in Western Europe or North America [1, 2, 5, 7–12]. This is considered to reflect transmission primarily by faecal–oral and oral–oral routes or through recently contaminated food and water, or both [9].

H. pylori is an extremely diverse species, and several genetic markers that are present in only a subset of Western European or American strains and that are virulence-associated epidemiologically have been identified [1, 10, 13]. Prominent among them is the *cag* pathogenicity island (*cagPAI*), a 37-kb DNA segment many of whose encoded functions seem to help elicit synthesis of the pro-inflammatory cytokine interleukin-8 (IL-8) and thus, probably, do much to stimulate

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inflammatory responses that contribute significantly to the risk or severity of disease [1, 10, 14, 15].

Tests in many countries have shown that eradication of *H. pylori* from patients with peptic ulcer generally results in permanent cure of disease.

The worldwide distribution and high prevalence of *H. pylori* and its importance in disease have stimulated interest in efficient detection of infection, especially by strains of potentially more virulent genotypes. Traditionally, diagnosis was dependent on culture from gastric biopsy samples or visualisation during histological examination. Although culture is important, the bacterium is slow-growing and fastidious, and it is possible that some *H. pylori* strains will not form colonies on currently available media [16]. Histological detection also has drawbacks, especially in sensitivity of detection of current infection [17]. Accordingly, several laboratories have developed polymerase chain reaction (PCR)-based detection in the expectation that it would be far faster and perhaps more sensitive, efficient and reliable than other detection methods.

This report describes the implementation of PCR-based detection of *H. pylori* in gastric biopsy samples and thus of active infection in a large cohort of children and adults in Warsaw, Poland, and the preliminary use of PCR to characterise polymorphic virulence determinants. PCR with *ureA*-specific primers was considerably more sensitive, faster and less laborious than culture for detection of *H. pylori* in biopsy specimens. PCR detection of the *cagPAI* and 'empty site' of *cagPAI* served as a sensitive tool for estimating the prevalence of this virulence-associated factor in strains isolated in the study population and determination of the number of mixed infections with *cag+* *cag-* strains.

Material and methods

Clinical specimens

All patients enrolled in this study attended the Out-patient Gastroenterology Department at The Children's Memorial Health Institute (CMHI) with dyspepsia or upper gastrointestinal tract disorders during the period 1993–1996, and gave informed consent for use of their biopsy material for clinical research. The research protocol was approved by the Human Studies Committee of CMHI in accordance with Polish national guidelines for protection of patients' rights. At least three biopsy samples were taken from the gastric antrum and additional biopsy samples were taken from the duodenum in some cases. They were then processed routinely for histopathology, bacteriology and PCR tests. Specimens for culture were placed immediately in phosphate-buffered saline (0.9%) on ice, and transported to the laboratory. A total of 246

children (122 girls, 124 boys; average age, 13.5 years, range 1–17 years) and 74 adults (average age 36.8 years) was studied. Eradication therapy generally consisted of metronidazole, amoxycillin (20 mg/kg/day for 10 days) and bismuth salts (Ventrisol: 7–8 mg/kg/day for 6 weeks) and its efficacy was checked by culture and PCR in 44 children.

Bacterial strains

Gastric biopsy samples taken during endoscopy were homogenised and cultured on Wilkins Chalgren medium with horse blood 7%, vancomycin 10 mg/L, trimethoprim 5 mg/L, cefsulodin 5 mg/L and cycloheximide 100 mg/L. Plates were incubated in a micro-aerophilic atmosphere with CO₂ 5–8% at 37°C for 5–7 days. Species identification was based on colony and cell morphology and on biochemical tests for oxidase, catalase and urease by use of API CAMP and Rapidec *pylori* (bioMérieux) tests [16]. All cultured strains were stored in brain heart infusion broth with glycerol at –70°C. *H. pylori* ATCC43504 and *H. pylori* 26695 *cagA+* were used as reference strains. The latter was used for full genome sequencing [18] and both contained the *cagPAI*.

Histopathology

Several sections cut from each embedded biopsy sample were stained with haematoxylin and eosin and examined to assess the histopathological status of the mucosa. Histological examination was performed in the Department of Pathomorphology, CMHI. Every biopsy specimen was assessed by the pathologist in terms of chronic, acute or a special form of gastritis. In chronic gastritis the predominant cell infiltrate consisted of lymphocytes and plasma cells and was scored as mild, moderate or severe. The presence of neutrophil granulocytes in the lamina propria or intra-epithelial sites, or both, determined its activity, and loss of gastric gland indicated atrophy. In acute gastritis, the predominant cell infiltrate consisted of neutrophils either in the lamina propria or in intra-epithelial sites or both [17].

Preparation of DNA from biopsy specimens

Biopsy specimens suspended in 0.5 ml of NaCl 0.9% were centrifuged at 2300 *g* for 5 min and the pellet was resuspended in 300 µl of extraction buffer (20 mM Tris-HCl, pH 8.0; Tween 20 0.5%) and 3 µl of proteinase K (200 µg/ml) were added. Samples were incubated for 1–2 h at 55°C to ensure complete lysis. Proteinase K was inactivated by heating at 98°C for 10 min [19]. DNA was extracted twice with a mixture of phenol:chloroform:isoamyl alcohol (25:24:1) and twice with chloroform:isoamyl alcohol (24:1). DNA was precipitated with one-tenth volume of 3 M sodium acetate, pH 5.5, and absolute ethanol [19]. Samples

were incubated for 1 h at -70°C and then were centrifuged at 13 100 *g* for 30 min at 4°C . The DNA pellet was washed in ethanol 70%, dried and resuspended in 40 μl of TE buffer (10 mM Tris, 1 mM EDTA). The quality and quantity of DNA were determined spectrophotometrically at 260 nm and 280 nm and by electrophoresis of samples in an agarose 1% gel with diluted preparations of λ phage DNA of known concentrations.

Amplification of H. pylori gene encoding urease A fragment

PCR to detect *H. pylori* was performed with primers that had been developed and validated by Clayton *et al.* [20]. PCR with HPU 1-5' GCCAATGGTAAATTAGTT 3' and HPU 2-5' CTCCTTAATTGTTTTTAC 3' yields a 411-bp fragment on amplification [20]. PCR was performed in a volume of 50 μl in a Gen Amp 9600 thermocycler (Perkin Elmer). The mixture contained: incubation buffer 10 \times (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 2.5 mM MgCl_2 ; gelatin 0.001%; four deoxyribonucleotides, 100 μM each; primers, 50 pmol each; Taq polymerase (Boehringer) 2 U and 0.5–1 μg of target DNA. The following cycle conditions were used: denaturation 94°C , 4 min, followed by 35 cycles of denaturation (94°C , 1 min), annealing (45°C , 1 min), extension (72°C , 1 min) and a final extension step (72°C , 5 min). PCR on genomic DNA from *H. pylori* strain ATCC43504 and glass-distilled pure water were used as positive and negative controls, respectively.

Sensitivity of the PCR assay

To assess the sensitivity of the *ureA* PCR, a suspension of ATCC 43504 containing *c.* 5×10^5 cfu/ml was diluted and the number of bacteria was calculated from the McFarland standard, and samples of sequential 10-fold dilutions were also spread quantitatively on Wilkin's-Chalgren medium and cultured for 5–7 days. DNA was extracted from known numbers of ATCC 43504 cells and used in the PCR as described above to determine the smallest number of cells (cfu/ml) that could be detected by PCR (primers HPU1, HPU2).

Control of PCR-negative results

Host globin gene amplification was performed on DNA extracts from biopsy samples in each case in which *ureA*-PCR gave a negative result to test for possible inhibitors of PCR. This entailed use of the β -globin-specific primers: BGLO 1-5' ACACAAGTGTGTTCACTAGC-3' and BGLO 2-5' CAACTTCATCCACGTT-CACC-3' as a control of possible inhibition of reaction in lysates and DNA samples obtained from biopsy specimens. Amplification of the 110-bp fragment of the gene encoding β -globin signified the lack of inhibitors in the sample [21]. The reaction was performed in a volume of 50 μl in a Gen Amp 9600 thermocycler. The

mixture contained: incubation buffer 10 \times (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 2.5 mM MgCl_2 ; gelatin 0.001%); four deoxyribonucleotides, 100 μM each; primers, 50 pmol each; Taq polymerase (Boehringer) 2 U; and 0.5–1 μg of target DNA or 5 μl of lysate. The conditions for the PCR reaction were: denaturation (95°C , 5 min) followed by 35 cycles of denaturation (94°C , 1 min), annealing (45°C , 1 min); extension (72°C , 1 min) and final extension (72°C , 7 min).

Detection of the cagPAI region and the 'empty site' of the cagPAI by PCR

All DNA extracts from biopsy specimens that were PCR-positive for the urease subunit A gene of *H. pylori* were additionally tested for the presence of *cagPAI*. The chosen primers – Luni 1-5'-ACAT-TTTGGCTAAATAAACGCTG-3' and L *cagI*-5'-TCTCCATGTTGCCATTATGCT-3' – are specific for the left end of virulence-associated 37-kb DNA fragment [15]. Amplification of *cagPAI*-positive *H. pylori* samples yields a 360-bp fragment. The PCR was performed in a volume of 50 μl in a Gen Amp 9600 thermocycler. The mixture contained: incubation buffer 10 \times (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 2.5 mM MgCl_2 ; gelatin 0.001%); four deoxyribonucleotides, 100 μM each; primers, 50 pmol each; Taq polymerase (Boehringer) 2 U; and 0.5–1 μg of target DNA.

Primers Luni 1-5'ACATTTTGGCTAAATAAACGCTG-3' and Rnew1-5'TCATGCGAGCGGCGATGTG-3' determined the amplification of the 360-bp fragment of DNA representing the 'empty site' of the whole pathogenicity island in which other chromosomal genes (HP05419 and HP0549 in the TIGR database [18]) that had been separated by the 37-kb segment were now adjacent to one another [15]. The same quantities of mixture reagents were used for PCR amplification. The primer sequences were kindly provided by Prof. D. E. Berg (Washington University, St Louis, USA). The conditions for the PCR were: denaturation (94°C , 4 min), followed by 35 cycles of denaturation (94°C , 1 min), annealing (57°C , 1 min) and extension (72°C , 1 min) and final extension (72°C , 5 min). The positive control was amplification of DNA from *H. pylori* 26695 *cagA*⁺ strain (provided by Prof. D. E. Berg). Distilled water provided a negative control.

Detection of PCR products

Amplification products were detected by electrophoresis in agarose 2% gels, stained with ethidium bromide 0.5 $\mu\text{g}/\text{ml}$ and visualised under UV illumination (Pharmacia). Electrophoresis was performed in TBE buffer (89 mM Tris-borate acid, 0.5 M EDTA, pH 8.0) in SUBDNA apparatus (Kucharczyk, Poland). Gels were photographed with a Polaroid camera.

Prevention of contamination

Published recommendations [22] for elimination of PCR false results were followed strictly. Different work stations for DNA isolation, amplification and PCR product detection were separated physically. Different rooms were individually equipped. Decontamination was based on application of UV light between sessions and overnight.

Primers

All primers were synthesised by Universal DNA, Tigard, OR, USA.

Results

Endoscopic and histopathological analysis

The histopathology of antral biopsy samples taken during upper gastrointestinal endoscopy of 246 children (average age 13.5 years) and 74 adults (average age 36.8 years), each of whom had been recommended for endoscopy because of dyspepsia, was investigated. Among the 246 children, 107 (43.5%) had evidence of gastritis, two had duodenitis with gastritis, one had duodenitis without evident gastritis and one had a duodenal ulcer with gastritis. Of the 74 adults tested, 47 (63.5%) had gastritis, one had duodenitis and two had gastritis with duodenitis. No significant pathology was detected in the biopsy samples from any of the other 134 children and 24 adults scored, although just one biopsy sample was analysed from each patient.

Detection of *H. pylori* infection

The presence or absence of *H. pylori* infection was assessed by two complementary detection methods, each on a different gastric biopsy specimen: PCR with *ureA* gene-specific primers and culture on Wilkins Chalgren medium (Table 1). Reconstruction tests with the type strain ATCC 43504 indicated that PCR could detect as few as 50 *H. pylori* cells per reaction (Fig. 1).

H. pylori was detected by *ureA*-specific PCR in 83 (76.9%) of the 108 children and in 41 (87.2%) of the 47 adults with histological gastritis and in 38 (28.4%) of 134 children and 7 (29.16%) of 24 adults in whom no gastric pathology was detected. *H. pylori* was detected by culture in 89 of the 124 children and 46 of the 51 adults found to be positive by PCR, and also in seven patients in whom *H. pylori* was not detected by PCR. In these negative samples, additional PCR with BGLO1 and BGLO2 primers was performed and the absence of 110-bp amplified fragments of the β -globin gene revealed the presence of inhibitors.

The frequency of *H. pylori* infection in children estimated by taking together positive culture and PCR results from 36.8% to 52.8% for children and from 63.5% to 70.3% for adults. Overall, sensitivities of PCR and culture were 98.3% and 81.1%, respectively.

Amongst the 130 children with *H. pylori* infection 5.4% were <5 years old, 29.2% were <10 years old and 65.4% were \leq 18 years old.

Efficacy of eradication

Forty-four of the paediatric patients were treated with metronidazole, amoxycillin (20 mg/kg/day for 10 days) and bismuth salts (Ventrisol 7–8 mg/kg/day for 6 weeks) to eradicate the *H. pylori* infections, and were then followed by periodic endoscopy. In 19 of these 44 patients, *H. pylori* was judged to have been eradicated by treatment, based on negative scores by follow-up endoscopy after 3–4 months for PCR, culture and histological examination. Eradication was associated with decreased inflammation and a more normal-looking gastric mucosa by 3 months.

In 20 of the children, therapy was deemed to have failed on the basis of positive culture and PCR and no improvement in the condition of the gastric mucosa. In five children who still remained PCR positive, a lower intensity of inflammation was observed.

Table 1. Detection of *H. pylori* by culture and PCR in 246 children and 74 adults with different disease states

Disease state (n)	Number (%) of patients who were			
	Cult+, PCR+	Cult+, PCR–	Cult–, PCR+	Cult–, PCR–
Children				
G (108)	74 (30.1)	3 (1.2)	9 (3.7)	22 (8.9)
G+DUO (2)	1 (0.4)	0	0	1 (0.4)
DUO (1)	1 (0.4)	0	0	0
UD+G (1)	1 (0.4)	0	0	0
N (134)	12 (4.9)	3 (1.2)	26 (10.6)	93 (37.8)
Total (246)	89 (36.2)	6 (2.4)	35 (14.3)	116 (47.1)
Adults				
G (47)	40 (54.05)	1 (1.35)	1 (1.35)	5 (6.75)
G+DUO (2)	1 (1.35)	0	1 (1.35)	0
DUO (1)	1 (1.35)	0	0	0
N (24)	4 (5.4)	0	3 (4.05)	17 (23)
Total (74)	46 (62.2)	1 (1.35)	5 (6.8)	22 (27.7)

G, gastritis; G+DUO, gastritis + duodenitis; UD+G, duodenal ulcer + gastritis; DUO, duodenitis; N, normal mucosa; Cult, culture.

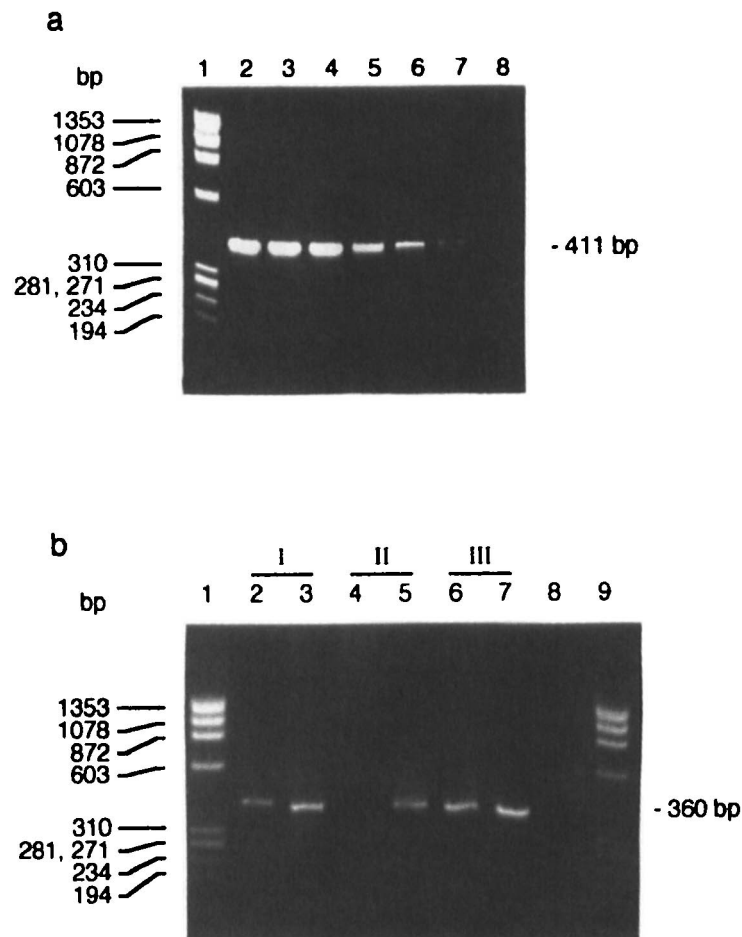


Fig. 1. PCR sensitivity and PCR detection of the *cagPAI* region and 'empty site' of the *cagPAI*. (a) Sensitivity of PCR assay (HPU1 and HPU2) for detection of *H. pylori*. (b) Detection of PCR products characterising presence of *cagPAI* or 'empty site' of *cagPAI* for three samples (I rows 2, 3; II rows 4, 5; III rows 6, 7). Lanes A1, B1, 9 X174RFI DNA *Hae*III marker; A2–8, results of amplification corresponding to dilutions of *H. pylori* ATCC 43504 control strain from 50×10^6 to 5 cfu/ml; B2, 4, 6, amplification of the left end of the *cagPAI* (Luni 1 and Lcag1 primers); B3, 5, 7, amplification of the 'empty site' of the whole *cagPAI* (Luni 1 and Rnew 1); B8, negative PCR control.

Direct detection of *cagPAI* in gastric biopsy specimens

PCR was used to test for the presence of the *cagPAI* with primers specific for the left end of this virulence-associated 37-kb DNA segment in DNA extracted from each biopsy specimen that had been scored as *H. pylori*-positive by *ureA* PCR (Table 2). As much as 74.6% of the 130 *ureA* PCR-positive biopsy specimens from children and 84.6% of 51 biopsy samples from adults were found to be *cag*+ by this test. Complementary PCR was also performed with primers specific for the 'empty site' of the *cagPAI* region in which other chromosomal genes that had been separated by the 37-kb *cagPAI* were now adjacent to one another [15]. Thirty-three and eight of the biopsy specimens from children and adults respectively, were scored as definitively *cag*– as they yielded a product with 'empty site'-specific primers. These included four patients whose samples were mixed (*cag*– and *cag*+ in the *cagPAI*-specific PCR) by these tests. No ambiguous cases (neither *cag*– nor 'empty site') were observed.

Among those isolates that were PCR *ureA*–, but culture positive, the study could not evaluate the presence of the *cagPAI* because these isolates were negative for the β -globin gene, which is a marker for the presence of inhibitors. As many as 72 (84%) of the 86 *H. pylori*-positive children with evident gastritis were found to be *cag*+, whereas 22 (53.7%) of the 41 cases that were positive for *H. pylori*, but in whom no significant gastritis was observed, were found to carry the *cagPAI*. In *ureA* PCR-positive biopsy samples from 42 adults with evident gastritis, 37 (88%) were found to be *cag* positive, whereas 4 (57.1%) of 7 without gastritis were carrying the *cagPAI*.

Discussion

H. pylori infection in the stomach is an important aetiological factor in chronic active gastritis and in the development of peptic ulcer disease [1–6]. Seropositivity for *H. pylori* has been shown to correlate with the incidence of gastric cancer in many countries

Table 2. Identification by PCR of *cagA*PAI in *H. pylori*-positive biopsy specimens from 130 children and 52 adults

Disease state (n, %)	Number (%) of samples that were		
	<i>cagA</i> +	<i>cagA</i> −	<i>cagA</i> + <i>cagA</i> −
Children			
G (86, 66.1)	72 (55.3)	12 (9.2)	2 (1.5)
UD+G (1, 0.8)	1 (0.8)	0	0
DUO (1, 0.8)	1 (0.8)	0	0
DUO+G (1, 0.8)	1 (0.8)	0	0
N (41, 31.5)	22 (16.9)	18 (13.9)	1 (0.8)
Total (130)	93 (74.6)	30 (23.1)	3 (2.3)
Adults			
G (42, 80.8)	37 (71.2)	4 (7.7)	1 (1.9)
DUO (1, 1.9)	1 (1.9)	0	0
DUO+G (2, 3.8)	2 (3.8)	0	0
N (7, 13.5)	4 (7.7)	3 (5.8)	0
Total (52)	44 (84.6)	7 (13.5)	1 (1.9)

G, gastritis; G+DUO, gastritis + duodenitis; UD+G, duodenal ulcer + gastritis; N, normal mucosa; DUO, duodenitis.

[1, 23]. Early detection of infection may be a crucial factor in preventing the more serious consequences of *H. pylori* infection of gastric mucosa [1, 2, 5, 6, 9].

The diagnostic techniques adapted to identify *H. pylori* infection can be divided into invasive and non-invasive methods. The non-invasive assays that do not require upper gastrointestinal endoscopy to biopsy specimens include serological testing for the presence of antibodies against *H. pylori*, or the urea breath tests (UBT) with ^{13}C - or ^{14}C -labelled urea [24, 25]. Serological methods, well suited for epidemiological purposes, have some limitations in the differentiation of current active *H. pylori* infection from past *H. pylori* infection and in evaluating the efficacy of treatment directed at eradication of the organism [26, 27]. The sensitivity and specificity of the UBT are comparable with culture and histology, but costs and time-consuming procedures are reported as disadvantages [28]. The biopsy specimens can be analysed for the presence of *H. pylori* directly by histological staining techniques, culture, microscopic examination and the rapid urease test. The latter two are reported as having low sensitivity and specificity [19]. Although culture rather than histopathological examination is considered to be the gold standard [29], culture is difficult and false negative results cannot be excluded. Failure of culture can be explained by lack of experience, incorrectly prepared media, or unsuitable incubation conditions, and prolonged use of inappropriate transport medium for biopsy specimens. Major advantages of culturing for *H. pylori* include the possibility of antimicrobial susceptibility testing and detailed characterisation of the isolates.

The goal of this study was to evaluate the presence of *H. pylori* infection in a large cohort of Polish symptomatic children and adults by specific detection of *H. pylori* urease A gene (*ureA*) by PCR. In addition, the PCR technique was used to characterise the virulence-associated *cagA*PAI, correlated as it is

with higher risk for the development of more serious consequences of infection.

Previous comparative studies of the sensitivity of PCR and culture in the detection of *H. pylori* were mostly based on < 50 samples [19, 20, 30–32], or 50–100 samples [3, 26, 28, 34] and showed similar sensitivity of both methods [28], or higher sensitivity of PCR than culture [3, 19, 28, 31–33]. In the present cohort study of 246 children and 74 adults, the overall sensitivity of PCR (98.3%) was higher than that of culture (81.1%). It was possible to detect 50 cells of *H. pylori* in a clinical sample, giving a sensitivity range comparable with that of Clayton *et al.* [20], Hammar *et al.* [19] and others [24, 28, 30, 31, 33, 34]. The higher sensitivity of *ureA*-specific PCR compared with culture allowed the identification of *H. pylori* infection in an additional 35 children and five adults. Lower levels of *H. pylori* colonisation in the gastric mucosa might be responsible for culture failure. Moreover, 26 children and three adults presented with normal mucosa, suggesting fresh infection. These results seemed to be in agreement with those of Mapstone *et al.* [30] and Clayton *et al.* [20], who found that some patients with normal mucosa were colonised with *H. pylori*, as well as patients with chronic gastritis who had not been infected with *H. pylori*. By strictly following the rules for sample transportation times, the present study reduced the risk of making the target micro-organism non-culturable or dead [26]. The higher sensitivity of *ureA*-specific PCR also allowed the identification of infection in five patients on specific therapy and in whom negative cultures suggested successful eradication. It can be speculated that the lack of positive culture in these patients might reflect the relatively low number of bacteria persisting after therapy and without PCR assay such patients would be deemed to be healthy subjects and could be at high risk of recurrence of infection by the remaining bacteria, reactivation of chronic gastritis and increasing liability to ulcer disease. The results of the present study suggest that

the sensitivity of *ureA*-specific PCR makes it valuable in patient management, not only for precise diagnosis but also for evaluation of treatment efficacy.

In general, *H. pylori* was detected more often by PCR than by culture (70.3% compared with 52.8% in children and 62.8% compared with 38.6% in adults). Although DNA extracts were used – which are more efficient PCR targets than boiled biopsy lysates and are associated with decreased numbers of inhibitory agents after phenol-chloroform extraction [32, 35] – negative results for β -globin gene were found in seven *H. pylori* negative samples, suggesting the presence of inhibitors. These exceptions in which PCR did not detect infections that were detected by culture may be attributable to sequence divergence in a PCR binding site or patchiness of *H. pylori* growth in infected carriers, but no additional tests with more biopsy samples or other primers were performed to investigate these possibilities.

H. pylori was detected by *ureA*-specific PCR in 83 (76.9%) of 108 children and in 41 (87.2%) of 47 adults with histological evidence of gastritis, and also in 38 (28.4%) of 134 children and 7 (29.5%) of 24 adults with normal gastric mucosa. Among 124 children and 51 adults found to be positive by PCR, 89 and 46 patients, respectively, also had positive cultures. Thus, there was generally quite good concordance between these tests, although PCR with *ureA*-specific primers was considerable more sensitive, faster and less laborious than culture. Serological examinations showed that 45% [36] – 65.5% (personal communication with Dr U. Wojda, Department of Clinical Microbiology, CMHI) of symptomatic children aged 2–18 and 9–18 years, respectively, are seropositive. The present study indicated that the rate of *H. pylori* infection increases from 5.4% at 5 years of age to 29.2% at 10 years and to 65.4% at age 18 years. Thus, it is postulated that PCR can be a useful additional diagnostic test for *H. pylori*.

It has been suggested that positive *cagA* status of *H. pylori* infection is epidemiologically associated with the development of peptic ulceration and with an increased tendency to develop atrophic gastritis, intestinal metaplasia and gastric cancer [37, 38]. However, until now it has been recognised that some genes throughout the 37-kb *cagPAI* (both within *cagI* and *cagII*) may participate in the induction of IL-8 synthesis and resultant inflammation, as it has been shown that they are needed to elicit synthesis of IL-8 in cultured epithelial cells [15].

This study demonstrated that PCR with *cagPAI*- and 'empty site' of *cagPAI*-specific primers is an accurate and rapid assay for identifying *H. pylori* strains carrying *cagPAI* (detection of the left end of the whole 37-kb pathogenicity island), lacking *cagPAI* (detection of the sequences adjacent to the 37-kb

pathogenicity island) and mixed infection by strains carrying both the *cagPAI* and 'empty site' of the *cagPAI* (Fig. 1). As many as 74.6% and 84.6% PCR-*ureA*-positive biopsy specimens taken from children and adults were infected with *cagPAI*+ *H. pylori* strains. Other PCR *ureA*+ biopsy specimens were negative for *cagPAI* and contained 'empty site'; four patients were found to be infected with a mixture of *cag*+ and *cag*– variants, reflecting a very low level of the mixed *cag*+ *cag*– infections. In these cases, two different strains maybe present, but this result does not rule out the possibility of *cag*+ and *cag*– variants of the same strain. This issue needs further study. Also, a PCR just for *cagA* or left or right end of *cagPAI* does not exclude the possibility that the *cag* pathogenicity island is completely deleted, but cases where the *cagI* segment may be deleted and *cagII* may be still present (or *vice versa*) are very rare [15].

In cases of gastritis, infection with *cagPAI*+ *H. pylori* was found in 84.0% and 88% of children and adults, respectively. Half of the patients revealed the presence of *H. pylori* *cagPAI* in biopsy samples where no mucosal changes were seen. This is in accord with the generally lower virulence of *cag*– strains.

Data obtained from the present study indicate that the frequency of *H. pylori* infection in Polish children, is two to three-fold higher than in Belgium, where only 26.3% of symptomatic children were *H. pylori* culture positive [29]. Moreover the number of *H. pylori*-infected young asymptomatic Polish individuals is seven times higher [29, 39]. It is suggested that in addition to their importance in diagnosis, such data will provide a critical baseline for future projection of epidemiological trends. The high incidence of *cag*+ *H. pylori* strains in children suggests that this population may be at risk of developing more serious pathologies of the gastric mucosa [40].

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