

DIAGNOSTIC VALUE OF MICROSCOPIC EXAM AND UREASE TEST IN HELICOBACTER SPECIES INFECTIONS

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Abstract: Authors have followed-up the presence of *Helicobacter pylori* and *Helicobacter heilmannii* in 4818 gastric mucosa biopsies in children with upper dispeptic manifestations, by microscopic examination of Gram fuchsin stained smear and Giemsa stained histological sections and by pre-formed urease test. Prevalence of these infections was 45.4% and 0.8% respectively, being assessed the endoscopic and histopathological aspects of studied cases. Classification on age groups of these etiologies showed the predominance of positive cases after 10 years age. None of the investigated cases showed simultaneous presence of the two species.

INTRODUCTION

The evidence, in gastric mucosa biopsies, by R. Warren and B. Marshall (1982), using microscopical examination and culture of some spiral bacteria afterwards identified as *H. pylori*, is one of the most important medical discoveries of the last century. In 1983, the two authors published the first results, and established for the first time the correlation between the presence of bacteria and chronic gastritis (Warren, Marshall, 1983). By further studies it is now clear that this bacteria is involved in gastroduodenal diseases as peptic ulcer, gastric cancer and lymphoma (Glupeczynski, 1998).

Systematic investigation of gastric mucosa biopsies for evidence of *Helicobacter pylori* allowed description of another spiral bacteria of animal origin associated with dyspepsia in adults and children, afterwards known as *Helicobacter heilmannii*.

The frequency of antral gastritis with *H. heilmannii* is relatively low compared with that produced by *H. pylori*, being recorded approximately 300 cases in literature (Debongnie, 1996).

In 1992 we were the first in the country to report a *H. heilmannii* infection in children (Coman et al., 1993). At present, after 12 years from the introduction of oesophago-gastroduodenal endoscopic exam in the "Sf. Maria" Emergency Pediatric Hospital from Iași, we have a number of cases which allowed us to make some bacteriological, endoscopic and histopathological consideration about this infection in children.

THE AIM OF THE STUDY

The aim of the study was to evaluate the prevalence of the infections produced by *Helicobacter* species in children gastrointestinal pathology.

MATERIAL AND METHOD

During december 1991 - november 2003, 4818 children aged 3 to 16 years with dispeptic manifestation, hospitalized in "Sf. Maria" Emergency Pediatric Hospital from Iași, Romania have been examined in diagnosis purpose by endoscopic exam. From each patient, at least 3 gastric mucosa biopsies (antral or fundic) were obtained by endoscopy, from which one has been introduced in a transport medium (20% glucose solution) for microscopic examination, one has been inserted in a vial with buffered liquid medium with urea and pH indicator (phenol red) for rapid urease test, and the third has been fixed in absolute ethilic alcohol or paraformaldehyde for histological sections.

A positive reaction of urease test has been indicated by the colour change of pH indicator from yellow to pink (figure 1). The examination was made at differents intervals of time (30 minutes, 1 hour, 3 hours and 24 hours); incubation was done at 37°C.



Figure 1 – Urease test: positive (left) and negative (right)

From the biopsy placed in 20% glucose solution a smear has been made, stained 5 minutes with Gram fuchsine and microscopic examined with an oil immersion lens, to detect the presence of *H. pylori* (figure 2A) or *H. heilmannii* (figure 2B) in gastric biopsy.

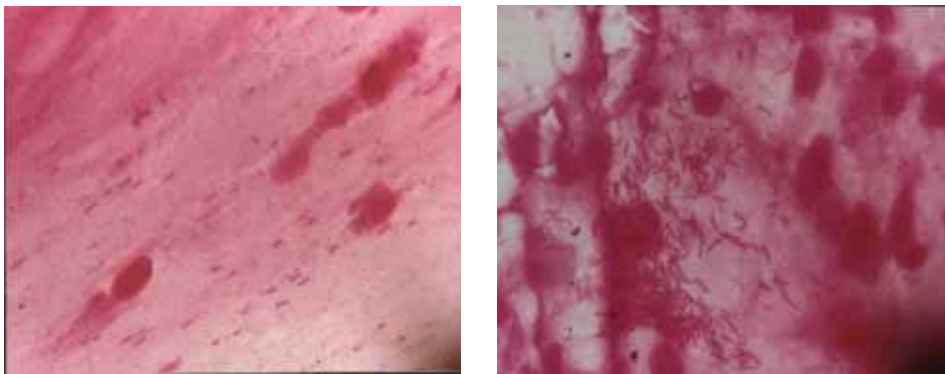


Figure 2 - *Helicobacter pylori* (LEFT) and *Helicobacter heilmannii* (RIGHT) in antral mucosa biopsy smear (900X)

On histological sections stained with hematoxylin and eosin the gastritis lesions were evaluated by comparison with the Sydney System (Price, 1991), and, in addition to that, a modified Giemsa staining technique has been used for bacterioscopic exam.

RESULTS AND DISCUSSIONS

Among the 4818 children with dispeptic manifestation, examined by endoscopy, 2187 cases with *H. pylori* infection and 38 cases with *H. heilmannii* infection were found. Comparatively with *H. pylori* infection that was found with a 45.4% frequency, *H. heilmannii* gastritis had a lower prevalence (0.8%)(figure 3). Our study confirmed previous reports indicating that the prevalence of *H. heilmannii* infection in population samples examined by endoscopy is inferior to the value of 2% (Debongnie, 1996), comparatively with the *H. pylori* infection frequency - more than 70% in developing countries (Dunn et al., 1997, Glupczynski, 1998). Also, pediatric studies showed that in developing countries the prevalence of *H. pylori* infection comes near to 50% (Le Luyer, 1994; Coman et al., 1996), but in the case of *H. heilmannii* gastritis two pediatric studies reported values of 0.3% and 1.9% respectively (Debongnie, 1996).

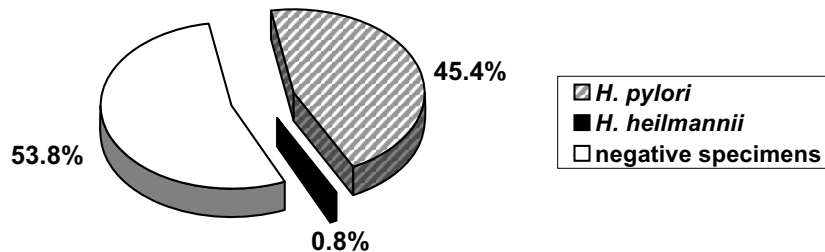


Fig. 3 – Prevalence of infections produced by *Helicobacter species*

On a number of 121 cases of *H. pylori* infection, compared to the 38 positive cases with *H. heilmannii*, distribution on age, sex and origin was approximately the same (table 1); receptivity to infection was evident starting with 3 years of age, children between 11 and 16 years being more affected.

Table 1 – Distribution of the positive cases on age, sex and origin

CRITERION		NUMBER OF CASES (%)	
		<i>H. pylori</i>	<i>H. heilmannii</i>
AGE:	3-6 YEARS-OLD	4 (3.3%)	4 (10.5%)
	7-10 YEARS-OLD	16 (13.2%)	9 (23.7%)
	11-16 YEARS-OLD	101 (83.5%)	25 (65.8%)
SEX:	MALE	48 (39.7%)	17 (44.7%)
	FEMALE	73 (60.3%)	21 (55.3%)
ORIGIN:	URBAN AREA	82 (67.8%)	20 (52.6%)
	VILLAGE AREA	39 (32.2%)	18 (47.4%)

Diagnosis of *H. pylori* gastritis can be established by several methods: evidence by microscopical examination of smear or histological sections; inoculation on culture media; detection of pre-formed urease (enzyme that is characteristically produced in important amount by this species) in biopsies or by breath test with labelled urea; evidence of specific immune response by detection of immunoglobulin (Ig) by quantitative and qualitative methods. Sensitivity of these methods is somehow similar (Megraud, 1996), but none of them is recognized as reference method. The combination of several methods allows obtaining the best results (Glupczynski, 1998).

Because we don't have the possibility to perform culture for all the samples - only on a small number - the diagnosis was established by the following methods: histological examination, Gram stained smear and urease test (at least two positive tests).

Diagnosis of *H. heilmannii* gastritis is bacteriological. Evaluation is performed according to the endoscopic and biopsy results, bacterioscopic examination being for the moment the only diagnostic method. Repeated experiments of several authors aiming to grow this bacterium were unsuccessful (Debonnie, 1996); the only encouragement was the possibility to grow in vivo by intragastric inoculation in mouse or rat (Solnick, Schauer, 2001). Non-invasive diagnostic methods, as antibody detection, lack specificity, which is a major concern in epidemiologic studies for this infection.

Microscopic examination of the Gram fuchsin stained smear of gastric biopsy has proved to be the most sensitive method in detection of *H. heilmannii*. Current algorithm used in invasive diagnosis for *H. pylori* gastritis by urease test, microscopic examination of histologic sections and growth can fail to detect all cases of *H. heilmannii* infection (Coman et al., 2000).

In our study, we have detected by histological examination only 7 cases of *H. heilmannii* infection out of the 23 cases investigated by this method (table 2). In the case of *H. pylori* infection, histological examination allowed detection of 63 from 121 cases of *H. pylori* infection defined by the simultaneous positivity of bacterioscopy on the Gram fuchsin stained smear and urease test. The explanation could be due to the transport conditions assured for the biopsy specimen used in smears carried out, that allowed the preservation of mucus layer, the election place of these bacteria (Debonnie, 1996).

The urease test was positive after 30 minutes on more than 50% of specimens with *H. pylori*. In the case of *H. heilmannii* infection the urease test has proved to be especially variable or tardy positive (table 2), *H. heilmannii* is known to have a lower urease activity compared to *H. pylori* (Debonnie, 1996; Coman et al., 1996; 2000).

Table 2 – The results of laboratory tests in the diagnosis of gastritis with *Helicobacter* species

DETECTION METHOD		NUMBER OF CASES (%)	
		<i>H. pylori</i>	<i>H. heilmannii</i>
MICROSCOPIC EXAMINATION:	SMEAR	121	38
	HISTOLOGIC SECTION	63/ 112 *	7/ 23 *
UREASE TEST:	30 MIN.	73 (60.3%)	7 (18.4%)
	1 HOUR	19 (15.7%)	8 (21.1%)
	3 HOURS	18 (14.9%)	4 (10.5%)
	24 HOURS	11 (9.1%)	12 (31.6%)
	NEGATIVE	0 (0%)	7 (18.4%)

* biopsies with histologic exam performed

The presence of *Helicobacter* species in gastric mucosa biopsies was associated with endoscopic changes (table 3). In both etiologies, the nodular aspect of the antral mucosa was evident in > 50% of cases, this gastritis type being encountered only in children (Le Luyer, 1994; Debonnie, 1996; Dunn et al., 1997).

Table 3 - *H. pylori* and *H. heilmannii* gastritis – endoscopic aspects

ENDOSCOPIC ASPECTS	NUMBER OF CASES (%)	
	<i>H. pylori</i>	<i>H. heilmannii</i>
Diffuse congestion of gastric mucosa	56 (46.3%)	17 (44.8%)
Congestive antral mucosa and with nodular aspect	60 (49.6%)	20 (52.6%)
Congestive antral mucosa, nodular aspect and ulceration on greater curve	0 (0%)	1 (2.6%)
Congestive antral mucosa, nodular aspect and duodenal ulcer	5 (4.1%)	0 (0%)

In *H. pylori* infection, lesions were exclusively of chronic gastritis, acute lesions being evident only in one case of *H. heilmannii* infection; both in *H. pylori* and *H. heilmannii* gastritis the active form predominates with presence of polymorphonuclear cells (table 4). The above mentioned aspects were quoted by other authors (Heilmann, Borchard, 1991; Debongnie, 1996; Fox, 2002).

Table 4 - *H. pylori* and *H. heilmannii* gastritis – histopathological aspects

HISTOPATHOLOGICAL LESIONS	NUMBER OF CASES (%)	
	<i>H. pylori</i>	<i>H. heilmannii</i>
Acute gastritis	0 (0%)	1 (4.3%)
Chronic gastritis, mild form		
- active	49 (43.7%)	4 (17.4%)
- inactive	3 (2.7%)	6 (26.1%)
Chronic gastritis, severe form		
- active	33 (29.5%)	2 (8.7%)
Chronic gastritis with lymphatic follicles		
- active	24 (21.4%)	8 (34.8%)
- inactive	3 (2.7%)	2 (8.7%)
Bad quality histological sections	9	8
Exam not performed	0	7

In our study, we didn't found simultaneous infection with both *H. pylori* and *H. heilmannii*. In literature only 9 cases are reported, this low frequency suggesting a possible antagonism between the two species (Debongnie, 1996).

CONCLUSIONS

Microscopical examination of the Gram fuchsin stained gastric mucosa smear proved to be the most sensitive and quick method for detection of *H. pylori* and *H. heilmannii* species.

Distribution on age, sex and origin of infections caused by the two *Helicobacter* species is similar.

In > than 50% cases of *H. pylori* or *H. heilmannii* infections endoscopic examination showed nodular gastritis, characteristic aspect in children.

Histopathologic examination showed the active form of gastritis in most cases with *H. pylori* (94.6%) and *H. heilmannii* (60.9%).

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