# Utility of a stool antigen test to detect the incidence of helicobacter pylori infection and familial and community enviromental risk factors for this infection in pediatric age

*Utilita' dell'antigene fecale per rilevare l'incidenza dell'infezione da helicobacter pylori e i fattori di rischio familiari e di comunita' legati a questa infezione*

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*Key words: helicobacter pylori, antigen test, stool, risk factor, children*

## Abstract

**Background**: Helicobacter pylori (Hp) infection is mainly acquired during childhood; it is recognised as a cause of gastritis and peptic ulcer and it has been classified as a group A carcinogen by World Health Organization. The exact mode of transmission is as yet, not known.

**Aim** of our study has been to identify risk factors associated with Helicobacter pylori infection in a preschool and school population and to confirm if Hp antigen in faeces is useful as screening in epidemiological studies.

**Methods**: We interviewed, with questionnaire, 400 children (203 male; age range 3-10 years; mean age 6 years) of 3 different schools and stool samples were collected of all children too. 35 of 400 (8%) children underwent to upper gastrointestinal endoscopy because of a suspect of upper gastrointestinal disease.

**Results**: stool were collected from 400 school children and 35 of them shown positivity of Hp antigen test. A questionnaire about presence of nausea, vomit, recurrent abdominal pain, family size, parent's occupations and education, use of antibiotics, country of birth of child and parents, personal hygiene, breast feeding, presence of the animals was completed. 35 children with positive Hp stool antigen test and a suspicious of upper gastrointestinal disease (recurrent abdominal pain, diurnal or nocturnal abdominal pain, nausea, vomiting, iron deficiency) underwent to esophagogastroduodenoscopy (EGDS) that demonstrated antral gastritis and positive histology and urease rapid test.

**Conclusions**: the results of this study suggest that risk factors for Hp infection are low socioeconomics factors, hygiene and living conditions and that Hp antigen in faeces is useful as screening test.

## Riassunto

L'infezione da Helicobacter pylori (Hp) è per lo più acquisita in età pediatrica e riconosciuta come causa di gastrite e di ulcera peptica. Attualmente non è ancora perfettamente conosciuta la via di trasmissione.

Lo scopo dello studio è stato quello di identificare i fattori di rischio associati all'infezione da Hp in una popolazione di bambini in età scolare e prescolare e di confermare l'uso dell'antigene fecale Hp come screening negli studi epidemiologici.

Abbiamo intervistato, attraverso un questionario, 400 bambini (203 maschi di età compresa tra 3 e 10 anni – età media 6 anni) di tre differenti scuole ed abbiamo raccolto i campioni feci di tutti i bambini. 35 dei 400 bambini (8%) sono stati sottoposti ad esofagogastroduodenoscopia (EGDS) per il sospetto di una malattia del tratto intestinale superiore.

In 35 dei 400 campioni feci è stata rilevata la presenza dell'antigene fecale. Ogni famiglia ha compilato un questionario rispondendo a domande sulla presenza nei piccoli pazienti di nausea, vomito, dolori addominali ricorrenti e riguardo la numerosità della famiglia, l'impiego e il titolo di studio dei genitori, l'uso di antibiotici, il paese di

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nascita dei bambini e dei genitori, l'igiene personale, l'allattamento al seno e la presenza di animali in casa. I 35 bambini con antigene fecale positivo e con il sospetto di una patologia organica del tratto gastrointestinale superiore (dolori addominali ricorrenti, epigastralgia diurna o notturna, nausea, vomito, anemia sideropenica ), sono stati sottoposti a EGDS che ha dimostrato gastrite antrale e positività dell'esame istologico e del test rapido all'ureasi per l'HP.
I risultati dello studio suggeriscono che i fattori di rischio per l'infezione da Hp sono le condizioni socioeconomiche modeste, la scarsa igiene, il sovraffollamento familiare e che la ricerca dell'antigene Hp nelle feci è utile come test di screening.

## Introduction

Helicobacter pylori infection usually starts in paediatric age, even before 5 years of age: occurs more frequently in developing countries than in developed countries and affects more than 50% of the world's human population (1).
Helicobacter pylori causes chronic active and persistent gastritis, peptic ulcer, gastric cancer and primary gastric B-cell lymphoma (MALToma) (2). While the Helicobacter pylori role in dyspepsia and extradigestive diseases (vascular, immunological and skin pathologies, sideropenic anaemia and delayed statural growth) (3) is controversial.
A high prevalence of infection has been observed among persons in settings where sanitary conditions are suboptimal or associated with low socio-economic status, suggesting that the bacteria are most likely spread from person to person through faecal-oral or oral-oral routes.[4]
In children the presence of the infection does not lead to clinically apparent disease and only endoscopy with biopsies can identify ulcer, esophagitis and other gastric pathology (5).
In the vast majority of individuals, Hp infection is acquired during childhood with those of the low socioeconomic means and having infected family members being at highest risk for early childhood acquisition (6). In developing countries, approximately 70% of children are infected with the bacterium by their 15th birthday (7). An ideal test for Helicobacter pylori infection in children is non invasive or minimally invasive, highly accurate, inexpensive and available.[8-9-10-11]
The aim of this study has been to identify risk factors associated with Helicobacter pylori infection in a pediatric population and to investigate if Hp antigen in faeces is useful as screening test in epidemiological studies on large population.

## Patients and methods

### Patients

At meeting in each of the 3 different schools (10 classes of elementar school and 9 classes of maternalschool) the children were given a questionnaire to answer at home with their parents. We interviewed 400 children about nausea, vomit, recurrent abdominal pain, family size, parent's occupations and education, use of antibiotics, country of birth of child and parents, personal hygiene, breast feeding, presence of the animals. A sample of faeces was collected from each of 400 children (203 male; mean age 3-10 years; range 6 years). 35 children with positive Hp stool antigen and a suspicion of upper gastrointestinal disease (recurrent abdominal pain, diurnal or nocturnal abdominal pain, nausea, vomiting, iron deficiency) underwent to esophagogastroduodenoscopy (EGDS) with biopsies. The recurrent addominal pains are abdominal pains of intensity to prevent the normal activity of child, recurrent at least once in a month during three months and with asymptomatic intercritical periods.
After a signed informed consent, all 35 children received an upper gastrointestinal endoscopy with gastric biopsy, that is the gold standard for Helicobacter pylori diagnosis. The biopsies were utilised for histologic examination and urease rapid test. The patients were considered Helicobacter pylori positive if they had the bacterium in histologic examination and in urease rapid test.

### Methods

The study was approved by the Ethics Committee.
Endoscopy was performed using Olympus videoscope XQ140 or GF100. Several biopsy specimens were obtained for histologic examination, urease rapid test and culture at two sites, the gastric body and the antrum.
Sections specimens were stained with hematoxylin and eosin and with Giemsa.
Rapid urease test (RUT) was obtained adding a biopsy specimen into an urea broth (NaCl, $KH_2PO_4$, NaOH); the result of the test is considered positive if there is a change of urea broth colour from yellow gold to pink red, because of increasing of pH induced by Helicobacter pylori.
An enzyme immunoassay (Premier Platinum HpSA) was used to detect Helicobacter pylori in the frozen stool, utilising polyclonal anti Helicobacter pylori antibody. Diluited faeces sample and a peroxidase conjugated to polyclonal antibody are added to the wells and incubated for one hour at room temperature. A wash is performed to remove unbound material. The substrate is added and incubated for ten minutes at room temperature. Colour develops in the presence of bound enzyme. Stop solution is added and the results are interpreted visually or spectrophotometrically.
We interviewed, with questionnaire, 400 children about presence of nausea, vomit, recurrent abdominal pain, family size, parent's occupations and education, use of antibiotics, country of birth of child and parents, personal hygiene, breast feeding, presence of the animals.

### Statistical Analyses

Statistical analyses were performed with the $\chi^2$ test (chi square test) and p value has been calculated.

## Results

A questionnaire about presence of nausea, vomit, recurrent abdominal pain, family size, parent's occupations and education, use of antibiotics, country of birth of child and parents, personal hygiene, breast

feeding, presence of the animals was completed by the all children.
Clinical features of patients examined and their domestic social-economic, hygiene status and living conditions have been reported in graphics.Statistical analysis indicate that Hp infection is facilitated by low socioeconomics factors, hygiene and living conditions.
Stool were collected from 400 school children; 35 of them shown positivity of Hp antigen test and they underwent to upper gastrointestinal endoscopy because of a suspect of upper gastrointestinal disease (recurrent abdominal pain, diurnal or nocturnal abdominal pain, nausea, vomiting, iron deficiency). In these patients EGDS demonstrated antral gastritis and positive histology and urease rapid test.
The Hp positive patients were treated with the conventional therapy (12); after one month from the end of treatment, all those patients who have presented positive faecal antigen, repeat the Hp antigen in faeces, that confirm the eradication.

**Invasive test**
At endoscopic examination of the 35 affected children, hyperemia and frability of the gastric antrum was observed in 6 (17%) patients, micronodular appearance in 9 (26%) patients and a normal picture in 20 (57%) patients. The histologic examination of all infected patients has shown an active microerosive gastritis (neutrophilic infiltration) and chronic gastritis (lymphoplasmacytic infiltration).

## Discussion

In general HP prevalence increases with age and is universally related to social-economic, hygiene status and living conditions but definitive routes of transmission of infection are unclear, with evidence suggesting oral-oral, gastric-oral and fecal-oral routes (6) and reliable detection of acute Hp infection remains problematic.
Well recognized risk factors include low socio-economic markers such as croweded living conditions in childhood, a large number of siblings, unclean water and ethnicity. A positive infection status of family members, particularly in the mother and older siblings, also increases risk of infection (4).
About the role of childhood nutrition that may be related to acquisition of infection, Rothenbacher et al suggest that breast feeding in infancy does not protect against Hp infection among pre-school children in industrialized countries (13-14).
In our study the analysis performed on the data indicated that social status and habits seems to have an impact on Hp frequency, e.g. mother's occupation or keeping pets at home No particular or clear correlations are visible showing the results of the questions addressed to common symptoms of digestive disorder.

## Conclusions

The data in have been collected using a form filled by about 400 children of a School located in Rome.

Hereafter the questions.

**A** You have been feeling abdominal pain for:
1) more than 3 months
2) less than 3 months

**B** You feel abdominal pain:
1) when eating
2) during the day but not when eating
3) during the night (subject awaken)

**C** Where the pain is located on the belly:
1) center/up (epigastric)
2) bottom (umbilical)

**D** The pain is blocking for normal daily activies of the subject:
1) Yes
2) No

**E** Subject does not feel any abdominal pain:
1) Yes
2) No

**F** in the last 3 months the subject experienced nausea but no regurgitation:
1) Yes
2) No

**G** in the last 3 months the subject experienced pyrosis:
1) Yes
2) No

**H** in the last 3 months the subject has regurgitated:
1) Yes
2) No

**I**
☐ Male
☐ Female

**II** Father birthplace

**III** Mother birthplace

**IV** Father degree:
1) Primary school (up to 5 years of study)
2) Secondary school (up to 6-8 years of study)
3) Diploma (9-13 years of study)
4) University (more than 13 years of study)

**V** Mother degree:
1) Primary school (up to 5 years of study)
2) Secondary school (up to 6-8 years of study)

3) Diploma (9-13 years of study)
4) University (more than 13 years of study)

**VI** Father occupation:
1) Professional (Professor, Manager, Sales Dir., etc.)
2) Employee
3) Artisan
4) Laborer (no specialization)
5) Other (jobless, retired, etc.)

**VII** Mother occupation:
1) Professional (Professor, Manager, Sales Dir., etc.)
2) Employee
3) Artisan
4) Laborer (no specialization)
5) Other (jobless, retired, etc.)

**VIII** Number of persons living at home with the subject

**IX** Milk feed type:
0) Breast
1) Artificial

Milk feed duration
<# months>

**X** Pets at home
0) No
1) Yes

**XI** The subject had antibiotics in the past:
0) never
1) 1-2 cycles in the last year
2) 3-4 cycles in the last year
3) >4 cycles in the last year

**XII** The subject attended kindergarten:
0) No
1) Yes

**XIII** The subject use to have meals out of home:
0) No
1) Yes

**XIV** The subject clean his/her hands before to eat:
0) Never or very seldom
1) Sometimes
2) Always or usually

**XV** The subject has eaten reusing dishes without cleaning:
0) No
1) Yes

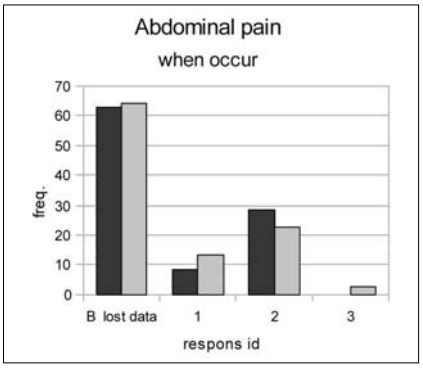
**XVI** The subject clean his/her hands after using the bathroom:
1) Never or very seldom
2) Sometimes
3) Always or usually

**XVII** The subject had an antibiotic therapy to eradicate HP:
1) Yes
2) No

The survey has been conducted on a population of 403 people attending school, 37 were Hp. The results are illustrated in the following images.

**Legenda**
Freq= percentage (%)
■ **POSITIVE**
□ **NEGATIVE**


A statistics

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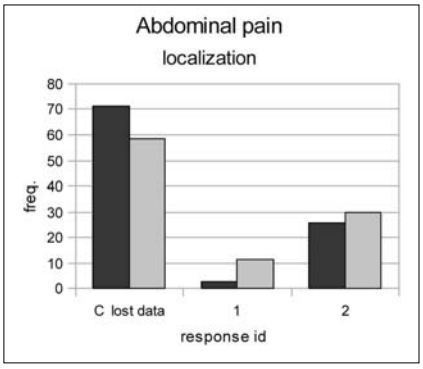


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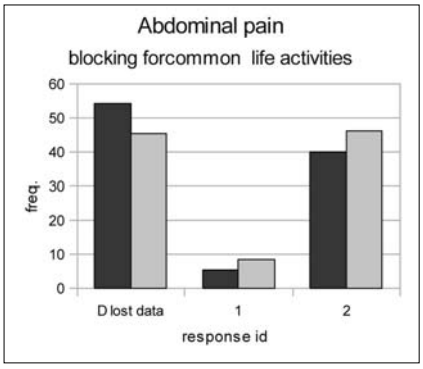


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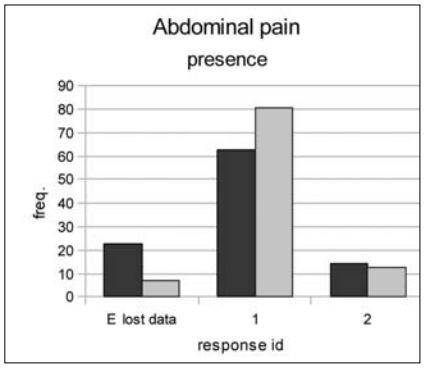


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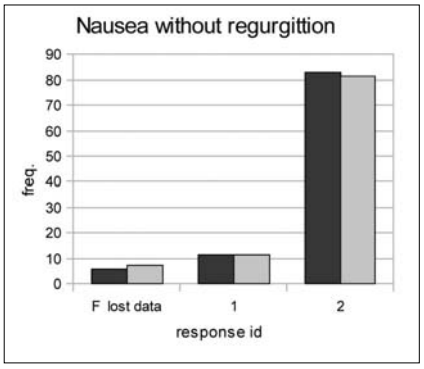


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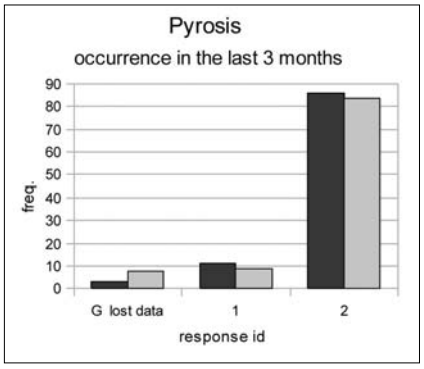


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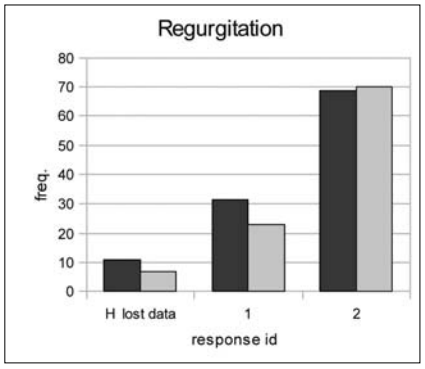


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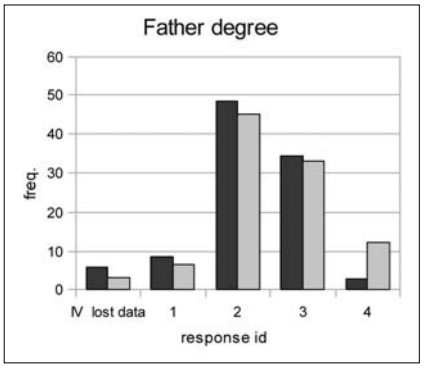


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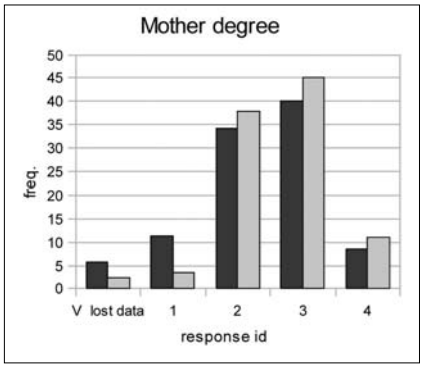


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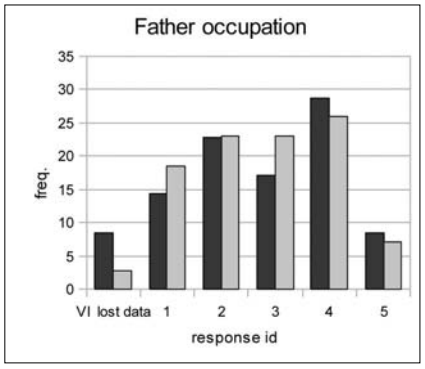


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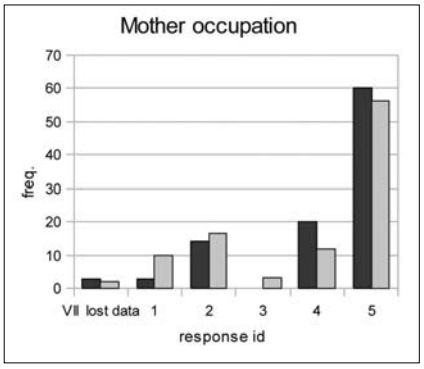


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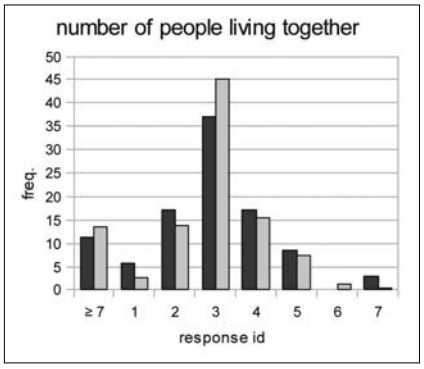


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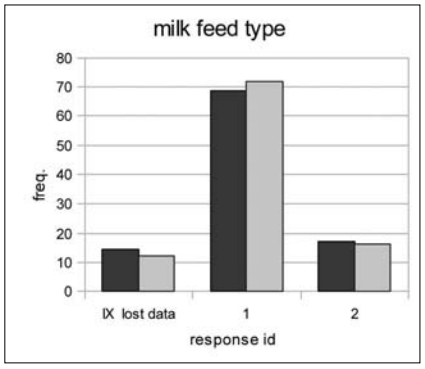


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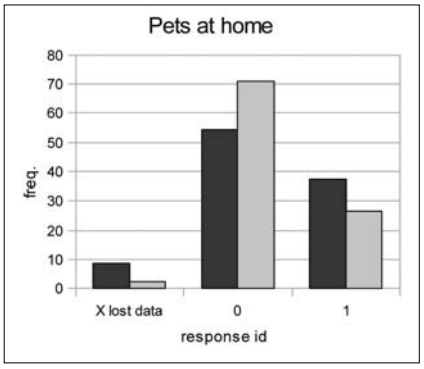


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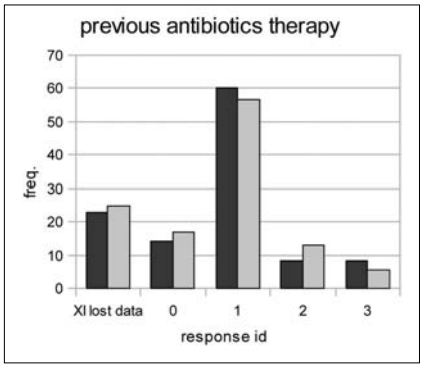


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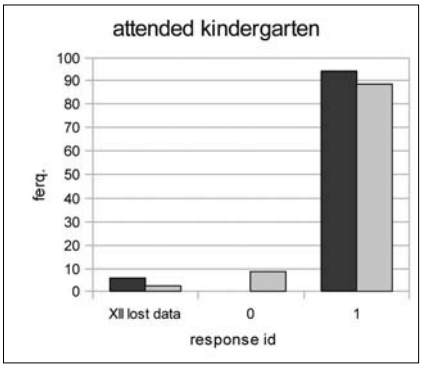


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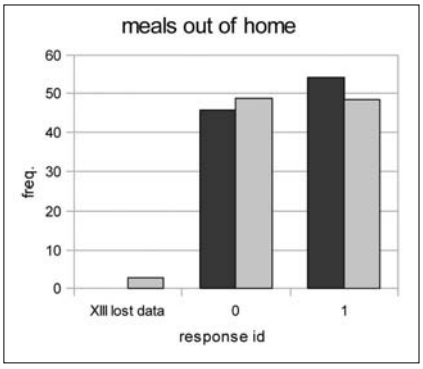


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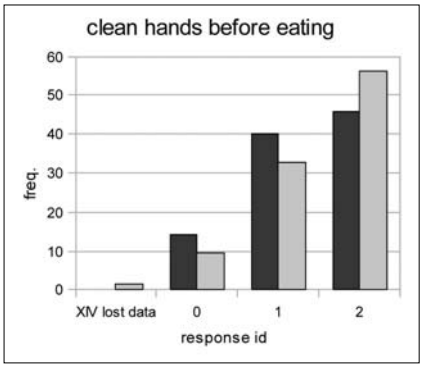
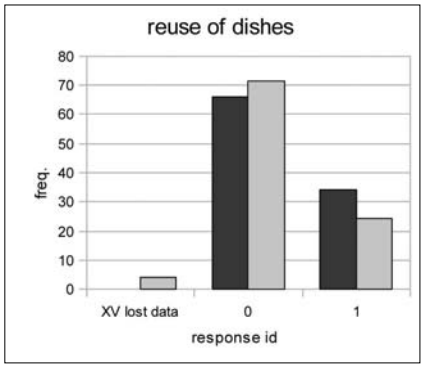



A statistics

Tabella 1

| ID | H0 hypotesis | P-value | H0 result | Conclusion |
|---|---|---|---|---|
| A | Abdominal pain in the last 3 months is not significant: Probability 0=0,5 1=0,5 | Chi-sqr test single sample. 0,001 | REJECTED | Adbominal pain in the last 3 months is significant for HP incidence. |
| B | Abdominal pain is felt independently when eating, daily, or in the ni.ght | Chi-sqr test test single sample. 0,000 | REJECTED | Abdominal pain timeline is not random. |
| C | Abdominal pain center is located randomly center up or bottom. | Chi-sqr test test single sample. 0,000 | REJECTED | Abdominal pain (if present) localization is mainly bottom. |
| D | Pain blocking for common life activites is random. | Chi-sqr test test single sample. 0,001 | REJECTED | Pain is not blocking for common life activities in case of Hp infection. |
| E | Adbominal pain incidence is random. | Chi-sqr test test single sample. 0,000 | REJECTED | Abdominal pain is not significant for Hp infection. |
| F | Nausea without regurgitation incidence is random. | Chi-sqr test test single sample. 0,000 | REJECTED | Nausea without regurgitation is not significant for Hp infection. |
| G | Pyrosis in the last 3 months is random. | Chi-sqr test test single sample. 0,000 | REJECTED | Pyrosis is not significant for Hp infection. |
| H | Regurgitation in the last 3 months. | Binomial test single sample. 0,043 | REJECTED | Regurgitation is not significant for Hp infection. |
| I | Gender is random. | Binomial test single sample. 1,000 | ACCEPTED | Test non significant. |
| II | Father birthplace | | | Not investigated |
| III | Mother birthplace | | | Not investigated |
| IV | Father's degree is random | Chi-sqr test single sample. 0,000 | REJECTED | More impact on secondary school/diploma. This data has to be interpreted compared with the distribution of the population. |
| V | Mother's degree is random. | Chi-sqr test single sample. 0,000 | REJECTED | More impact on secondary school/diploma. This data has to be interpreted compared with the distribution of the population. |
| VI | Father' occupation is random | Chi-sqr test single sample. 0,247 | ACCEPTED | Test non significant. |
| VII | Mother' occupation is random. | Chi-sqr test single sample. 0,000 | REJECTED | "Others" category (jobless, retired, etc.) has a significant impact on Hp infection. |
| VIII | Number of people living together is random. | Chi-sqr test test single sample. 0,004 | REJECTED | This data has to be interpreted compared with the distribution of the population. |
| IX | Milk feed type is random. | Chi-sqr test single sample. 0,000 | REJECTED | Breast feed has higher impact. This data has to be compared with the percentage of breast feeding in the population. |
| X | Pets at home incidence is random. | Chi-sqr test single sample. 0,000 | REJECTED | Pets at home is a risk factor for Hp infection. |
| XI | Antibiotics in the past incidence is random. | Chi-sqr test single sample. 0,000 | REJECTED | 1-2 cycles in the last year. |
| XII | Attended kindergarten impact is random. | Binomial test single sample. 0,000 | REJECTED | Kindergarten attendees percentage is fairly higher.This data has to be interpreted compared with the distribution of the population. |
| XIII | Meals out of home incidence is random. | Chi-sqr test single sample. 0,735 | ACCEPTED | Test non significant. |
| XIV | Clean hands before to eat incidence is random. | Chi-sqr test single sample. 0,530 | ACCEPTED | Test non significant. |
| XV | Reusing dishing incidence is random | Binomial test single sample. 0,910 | ACCEPTED | Test non significant. |
| XVI | Clean hands after using bathroom incidence is random. | Chi-sqr test single sample. 0,02 | REJECTED | People not cleaning hands after using bathroom incidence is higher. |
| XVII | Former therapy to eradicate Hp has random incidence | N/A | N/A | NO asymptotic significance. |

Analysis performed with SPSS 18 ver. 18.0.0 (30/07/2009)

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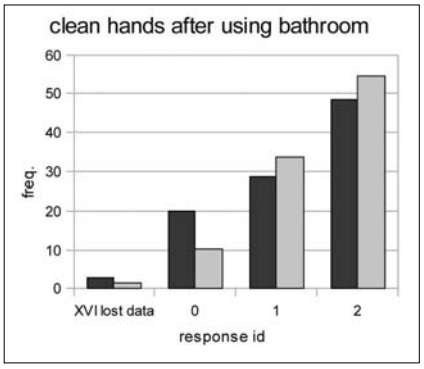


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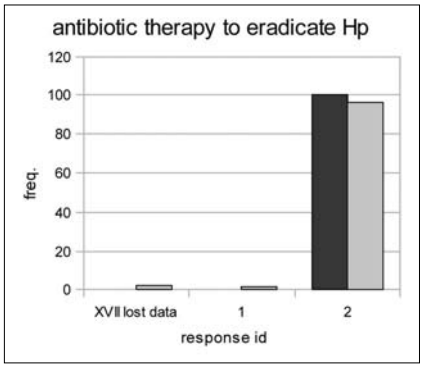


A statistics

**Test significance matrix**

The global incidence of Hp infection is emerging from the data collected is 10,109%. In the analysis of **Tab.1** **=0,05** is assumed as P-value threshold to evaluate significance.

the data of the positive patients have been analyzed with SPSS 18 to verify the significance, result are illustrated in **Tab.1**.

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