THE ALL-AGE PREVALENCE OF *HELICOBACTER PYLORI* INFECTION AND POTENTIAL TRANSMISSION ROUTES. A POPULATION-BASED STUDY.

Ragnar K. Breckan [1,2], Eyvind J. Paulssen [2,3], Anne Mette Asfeldt [4], Jan-Magnus Kvamme[2,3], Bjørn Straume [5], and Jon Florholmen [2,3]

[1]Department of Gastroenterology, Division of Medicine, Nordland Hospital, Bodø, Norway

[2]Research group of Gastroenterology and Nutrition, Department of Clinical Medicine, UiT The Arctic University of Norway, Tromsø, Norway

[3]Department of Gastroenterology, University Hospital of North Norway, Tromsø, Norway

[4]Department of Microbiology, University Hospital of North Norway, Tromsø, Norway.

[5]Department of Community Medicine, UiT The Arctic University of Norway, Tromsø, Norway

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Correspondence to: Ragnar K. Breckan, Department of Gastroenterology, Division of Medicine, Nordland Hospital, N-8092 Bodø, Norway. E-mail: *rbreckan@online.no*. Tel +47 755 34 207; Cell +47 906 92 052; Fax +47 755 34 742

Abstract

Background and aims: Previous research on *H. pylori* epidemiology has mostly focused on adult populations. We have aimed to study *H. pylori* prevalence in all age groups including children and adolescents, and to identify potential routes of transmission.

Methods: Subjects from all age groups (children 0-11 years, adolescents 12-17 years and adults ≥18 years of age), recruited from both an urban and a rural community in Northern Norway, were invited to provide stool samples for the diagnosis of *H. pylori* antigen, and to fill in a questionnaire (adult and adolescents only) on gastrointestinal symptoms, lifestyle factors and biometric data.

Results: A total of 1 624 (35.3%) of the invited subjects, including 173 (39.3%) of the children, 46 (19.2%) of the adolescents, and 1 416 (36.1%) of the adults, responded to the invitation. *H. pylori* infection was nearly undetectable (0.6%) among the children, whereas the prevalence increased from 20% in adolescents towards a peak of 45% in the highest age group. Univariate analyses of possible risk factors of *H. pylori* infection showed significant associations to private well water, the use of outhouse toilet, and having farm animals in childhood, but the associations waned in multivariate analyses.

Conclusions: In our populations, with apparent high hygienic standards, the transmission of *H. pylori* infection may start not only in childhood, but also in adolescence, where potential transmission routes may be outdoor toilet use, private well water, and farm animals.

Introduction

Helicobacter pylori (*H. pylori*) is the cause of chronic gastritis, and is the most common chronic bacterial infection in humans (1). It has probably coexisted with humans for as long as mankind (2), yet its association with gastro-duodenal disease was first truly recognized when Marshall and Warren identified and cultured the bacterium in 1983 (3).

Among the large number of studies on *H. pylori* prevalence, only a few include children (4), and even fewer studies include adolescents (5). Most *H. pylori* prevalence studies have used serological testing (6), a method with known limitations. In recent years, studies using urea breath tests have been published (7), yet stool tests are increasingly being used (8),(9).

The transmission routes of *H. pylori* are still poorly understood (10).The transmission has been assumed to take place in early childhood (11), most likely from mother to child (12), (13), (14), although transient colonization may occur that does not lead to chronic infection (15). Animals have been proposed to be the source of the bacterium (16),(17), but several authors argue strongly against zoonotic transmission (18-20). Indeed, some studies suggest that having pets in the household may protect against *H. pylori* infection (18),(19). Drinking water supply have also been discussed as indirect transmission sources (20) - (21) as have toilet facilities (22). *H. pylori* has been found in very early childhood (29-31), yet most studies conclude that breast feeding is protective against this infection (32-34). Still, transmission routes can be classified into direct, i.e. oral-oral, gastro-oral, and fecal-oral, and indirect routes sucha as via food, animals, and drinking water (10), (23).

A marked difference in prevalence between developed and undeveloped countries has been reported (24), as well as between affluent and less affluent regions of Europe (25). In

addition, differences in the prevalences of *H. pylori* between Western and Asian countries, and the apparent decline in *H. pylori* occcurence observed the last years in Western countries, may indicate that life style differences and changes may have impact on the prevalence (26), (27).

We have previously reported the *H. pylori* prevalence in adults in our study population to be 32.9% (24.5% after age adjusment) (40). In the present study, we have aimed first to explore the prevalence of *H. pylori* across all age groups, based on data from both a rural and and an urban population in a population-based setting, using a stool antigen-based, monoclonal immunoassay amplification method. Furthermore, we wanted to screen for variables with a possible association to *H. pylori* infection in order to unmask routes of transmission. For comparison, we have also included an overview of population-based publications of *H. pylori* prevalence around the world (Tables 3 and 4).

Material and Methods

Study population

Participants were recruited from the populations of Bodø city center and from the more rural municipality of Sørreisa in Northern Norway, as parts of the Bodø Helicobacter Study (28)) and the Sørreisa Gastrointestinal Study (29), respectively, in the period from autumn 2004 to spring 2005. The recruitment process for adults has been previously described (28), (29). Thus, the recruited study subjects were 173 children, 46 adolescents and 1416 adults. The children (0-11 years old) and the adolescents (12-17 years), were recruited only from the urban area of Bodø, whereas adults (>= 18 years) were recruited from both study sites. A total of 3927 adults were invited to participate by completing a questionnaire and to provide a stool sample for *H. pylori* antigen detection. The two populations had similar socioeconomic

background, and a homogenous ethnicity. There has been no systematic screening for, or treatment of, H. pylori in Northern Norway.

In addition, a population-representative group of 440 children between 0 and 11 years of age, and 240 adolescents 12-17 years old in Bodø were invited. The children's guardians were asked to provide a stool sample from the child, whereas the adolescent subjects were asked to fill in a simplified questionnaire (see below) in addition to provide a stool sample, as for the adult subjects.

Assessment of Helicobacter pylori *infection*

The presence of *H. pylori* was assessed by detection of bacterial antigen in stool samples with a monoclonal immunoassay amplification method ("Hp Star", Dako Cytomation, Glostrup, Denmark) strictly according to the manufacturer's instructions. This method has been shown to have a sensitivity and specificity of more than 90% in adults as well as children (8), (9), and a corresponding sensitivity and specificity of 98% and 94%, respectively, in adult patients in our region (30).

Questionnaires

The self-administered questionnaires for adults have been described earlier and included questions regarding gastrointestinal complaints, lifestyle factors and biometric data (29). In addition, questions regarding potential transmission routes were included. Based on previous litterature, five variables representing possible transmission routes of *H. pylori* infection prior to the age of 18 years were addressed: the type of toilet available (22), (31), the source of drinking water (20), (21),(32), (33), breastfeeding or not during infancy (34), (35), (36), contact with pets (19), (18), and) contact with farm animals (17), (37). The simplified questionnaire for adolesents contained questions from the adult questionnaire regarding

gastrointestinal complaints, lifestyle factors and biometric data.in a simplified version. No questionnaire was used for the children.

Statistical analyses

The SPSS statistical software version 23.0 (IBM Corporation, Armonk, NY, USA) was used for statistical analysis. Comparisons between groups and subgroups were done with Mann-Whitney U, Pearson χ^2, or Fischer's exact tests where appropriate. Two-sided p-values <0.05 were considered statistically significant. Binomial logistic regression analyses were used to evaluate risk factors for *H. pylori* transmission. Variables that in univariate analyses had p-values <0.20 were included in a multivariate model (forced entry). Model summary was estimated with Nagelkerke's R^2. Results are presented as odds ratio (OR) with 95% confidence intervals (95% CI)

Ethics

The study was approved by the Regional Committee for Medical Research Ethics. Licence to register participants was granted by the Norwegian Data Inspectorate. Each participant or participant's guardian provided written informed consent.

Results

Participation

Of the adult subjects invited to participate, 1681 individuals (42.8%) completed the study questionnaire and 1416 (36.1%) provided stool samples for *H. pylori* antigen detection as previously described (29), (28). Among the adolescents, 45 (18.8 %) completed the

questionnaire, and 35 (14.6%) provided a valid stool sample. Among the children, 173 (39.3%) valid stool samples were collected (Table 1).

All-age prevalence of H. pylori *infection*

The prevalence of *H pylori* infection across the age groups is reported in Figure 1. There was a slightly lower prevalence of *H pylori* carriers in Bodø as previously described (28) (data not shown). *H pylori* infection was nearly absent in children (0.6 %). Among the adolescents the prevalence was 20%. Four adolescents reported gastrointestinal complaints weekly or monthly, of whom two were *H. pylori* positive and two negative.

In adults, the prevalence increased gradually with age, except for in the age group above 70 years, where a decline among men was observed (Figure 1). The frequency of gastroinestinal complaints have in part been described previously (28), (38). In the present study, 95% of the adults had no or negligible abdominal complaints (data not shown).

Transmission factors

Among the risk factors tested, univariate analyses showed significant associations of *H. pylori* infection to private well, outhouse toilet, and farm animals (Table 2). However, these significances were lost in multivariat analyses (data not shown), where age persisted as the only significant risk factor

Discussion

In this population-based study of *H pylori* prevalence, the risk of being infected with the bacterium was nearly undetectable in childhood, whereas an age-dependent increase from adolescence onwards was observed, which peaked at age 60-70 years. A broad screening for

potential transmission routes showed that ever having a private water source, an outhouse toilet in childhood, but not breast-feeding in childhood, was significantly associated to *H. pylori* positivity in univariate analyses. However, no independent risk factor could be identified in the multivariate analyses. Our data indicate that the transmission routes in the 21st century may be related to life-style factors in adolescence.

We used a fecal antigen test with both a high sensitivity and specificity (30), which should be well suited for population studies. Previous studies, although few, using fecal antigen-based tests in children, report prevalence numbers between 7.1% and 47% (39) -(40).

Our results equal those of the serology-based Dutch study, in which the prevalence was 0.5% in children with two Dutch parents, versus 2.6% in children with at least one non-Dutch parent (41). An overview of the world-wide prevalences of *H. pylori* infection in healthy children is shown in Table 3. In Europe, prevalences in children have been reported up to 66% (42), and in the US up to 29.1% (18). High prevalences have also been reported from other parts of the world, such as up to 51.4% in Africa (43), up to 64.2% in Asia (44), and up to 54.2% in South America (45).

We observe a marked increase in the prevalence of *H. pylori* from childhood to adolescence. Prevalence studies from this age are harder to find in the literature. In Siberia, the seroprevalence in youths aged 14-17 was 56.3% (5), comparable to 44.9% in a European multinational study in the age group 12-17 (46). In a follow-up study from Turkey in the age group 9-18, the prevalence based on Urea Breath Test was around 50% (47). In a study from the US in 1996, the seroprevalence in the 10 to 19 year olds was 26-29% (18).

In our adult population, the crude prevalence of *H. pylori* was 32.9%, and the age-adjusted prevalence was 24.5% (28), both beeing somewhat lower than reported from most of the rest

of the world as outlined in Table 4. The prevalence of *H. pylori* in the adult population is decreasing. Previous population studies from our region has showed a reduction from 42% in 1987 (48) to some 25% 17 years later (29). The same tendency is documented from other regions (49).

The world-wide differences in the prevalences are hard to explain, but our study and the previously mentioned Dutch study (41) indicate that the traditional transmission routes are compromised in populations with improved sosio-economic and sanitary standards. This is also confirmed in a report from Russia (49). The previously proposed mother-to-child transmission routes, such as breast-feeding (50), is most likely reduced to a minimum in the Western population.

A comprehensive search for potential risk factors for transmission routes in our study indicated that having a private water source or an outhouse toilet in childhood, as well as contact with farm animals, were all associated to *H. pylori* infection, but these were not age-independent factors. In multivariate analyses these associations were lost (data not shown), probably because of the non-independent nature of there variables.

The prevalence of *H. pylori* in children as described above indicates the low rates of bacterial transmission in the 21st century. Our data indicate that *H. pylori* may stem from animal sources either via drinking water or physical contact with the animals, or from other humans via drinking water due to sub-optimal sewage standards. In addition, our data indicate that the transmission routes in this century may be related at least somewhat to behavioural life-style factors in adolescence.

Thus, the main source of *H. pylori* and the transmission routes remains so far unsettled, although the most important reservoir for the bacterium is the human stomach (23). *H. pylori* has been detected in animals (16, 17, 46, 61, 62), and animal faeces has been proposed as an important source for *H. pylori* (28, 63, 64), but the data regarding transmission from animals to humans are not fully consistent (19). It is still broadly assumed that transmission is essentialy inter-human and intra-familial (51), but our findings indicate that the intra-familial hypothesis may be less important in our region, considering the very low prevalences in children and adolescents.

The world-wide decline in the prevalence (66-68) is most likely associated to improved hygienic standards. This is especially true in mother and child care, which may be the most important factor to explain low prevalence of *H. pylori* infection in the lower age groups. Thus, it may seem that the transmission is postponed from childhood to adolescence.

Furthermore, our data indicate that in adolescents with high sosio-economic and health standards, there may be so far unknown life-style or environmental factors contributing to the transmission of H. pylori. We have not regarded sexual transmission as an important factor for the spreading of H. pylori in children and adolescents. However, oral-oral transmission may be much more relevant as we believe such activity commences some time earlier. In Northern Norway, outdoor leisure activities all year round (scouting, hiking, fishing, hunting, skiing, overnight camping etc.) are widespread activities among older children and teenagers, which may involve intake of contaminated water, melted snow and plant material etc. Thus, transmission via animal waste may be possible.

It must be emphasized that a cohort effect cannot be ruled out, and if so, the mothers' prevalences must differ significantly in the two cohorts 0-11 years (children born

approximately 1994-2005), and 12-17 years (teenagers born approximately 1988-1993), as indicated in Figure 1. A follow-up study of these cohorts would help to clarify this.

The strength of this study is the population-based prevalence measurements including all age groups, from both an urban and a rural community. Moreover, we have based our data on an antigen-based *H. pylori* test, with known high sensitivity and specificity. Yet there are areas with some weakness. Firstly, the participation from the adolescence group was low. The prevalence data of *H. pylori* infection from this group thus remains uncertain, but the lowest possible prevalence would still be 3% if all the 205 non-participating subjects had tested negative. In comparison, the prevalence in the studied children was 0.6% and in the next age group 18-30 years 7-8%. Therefore, we have evidence from this study that *H. pylori* infection first occurs in adolescence. Another weakness of our study is that more comprehensible data would have been available if a family-based prevalence study had been performed in the *H. pylori* positive subjects.

In conclusion, in this population-based, urban and rural, all-age prevalence study of *H. pylori* infection, the risk of having the infection was low. It was mostly absent in children, whereafter an age-dependent increase from the adolescence on was observed with a peak around 70 years of age. No clear transmission source of *H. pylori* was found, but our data indicate an animal source may play a pivotal role.

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Disclosures

Declaration of interests: the authors have no competing interests. The authors alone are responsible for the content and writing of the paper.

Legends to figures.

Figure 1: Population-based prevalence (%) in the the communities of Bodø and Sørreisa.

The figure shows the age distribution as well as gender differences of *H. pylori* prevalence (*Hp +ve*: *H. pylori* antigen positive) in the combined study population (Bodø: children, adolescents and adults, and Sørreisa: adults only).

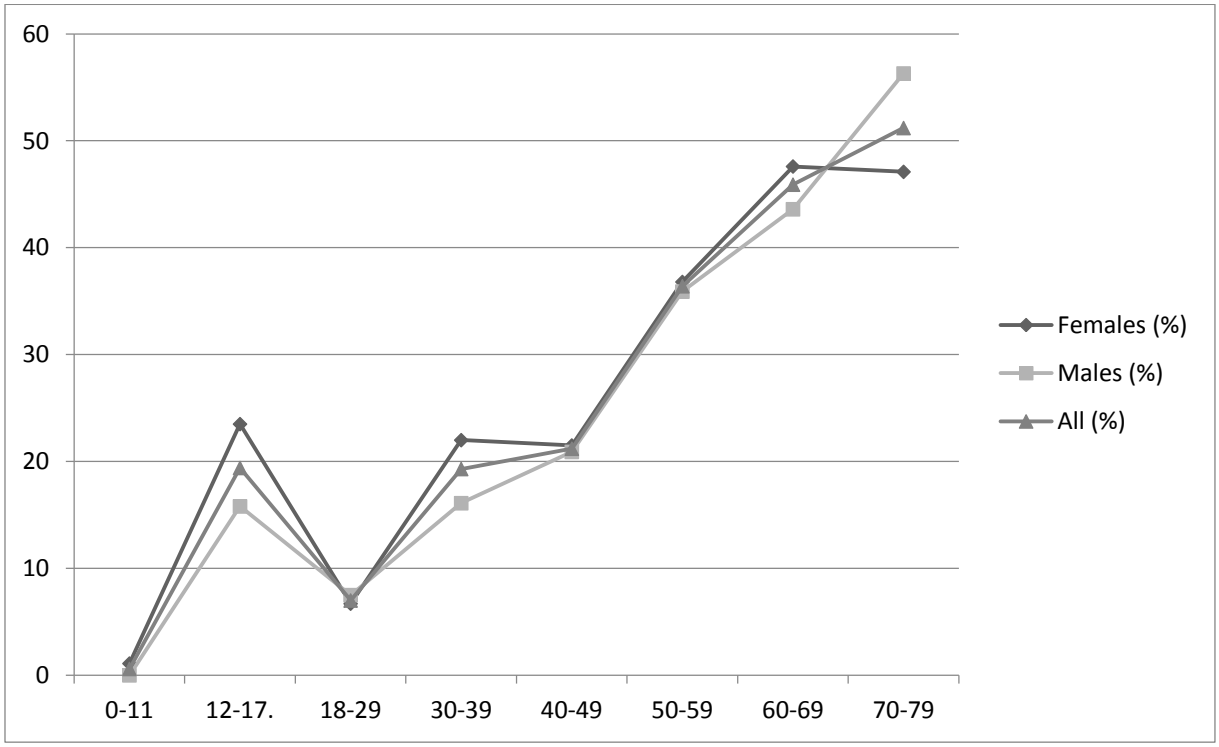

Table 1. Response rates among children and adolescents in the Bodø Helicobacter Study.

Age group	Invited		Stool samples		Questionnaires	
	N	M/F	n (%)	M/F	n (%)	M/F
0-11 years	440	220/220	173 (39.0)	92/81	n/a	n/a
12-17 years	240	120/120	35 (14.6)	18/17	45 (18.8)	22/23

Over-all and gender distribution of response rate (n, percent) to the invitation to fill in a questionnaire (adolescents only) and provide stool samples for the detection of *H. pylori* in the Bodø Helicobacter Study. An equal number of males (M) and females (F) were invited.

Table 2. Odds ratios of selected cildhood risk factors for *H. pylori* infection in the Bodø and Sørreiseia Helicobacter Study.

Risk factors	OR	95% CI	p-value
Various			
No breast milk	1.07	0.65 – 1.75	0.796
Only private well	1.39	1.08 – 1.78	0.010
Only outhouse toilet	1.21	1.02 – 1.43	0.032
Animal contact			
Farm animals	1.65	1.17 – 2.34	0.005
Regular pets	1.25	0.90 – 1.75	0.189

The table shows odds ratios (OR) with 95% confidence intervals (95% CI) for selected childhood risk factors for the presence of *H. pylori*. Each factor is adjusted for age group, gender and study location (Sørreisa vs. Bodø).

Table 3. Selected population-based studies of *H. pylori* prevalence in children according to continent

Author (publ year)	Ref #	Test used	Age group (years)	N	Country	Hp +ve (%) overall
AFRICA						
Siai 2008	(43)	S	6-7	1 055	Tunis	51.4
Hestvik 2010	(52)	S	0-12	427	Uganda	44.3
Ravelomanana 2013	(53)	S	Children	434	Madagascar	39.6
ASIA						
Huang 2004	(54)	F	0.6 - 10	114	Borneo	39
Ozen 2006	(47)	U	3-12	327	Turkey	53-57
Jafarzadeh 2007	(55)	S	1-15	386	Iran	46.6
Tam 2008	(56)	U	6 - 19	2 480	Hong Kong, China	13.1
Chi 2009	(57)	U	13 - 15	106	Taiwan	54.7
Yucel 2009	(58)	F	2-12	165	Turkey	30.9
Jafri 2010	(59)	S	1-15	1.976	Pakistan	53.5
Sherpa 2012	(60)	F	<10 - >60	383	Nepal	78.1< 10 yrs
Ghasemi-Kebria 2013	(61)	S, P	1 - 15	194	Iran	50.5
Jafar 2013	(44)	F	0.3 - 15	458	Iran	64.2
Okuda 2015	(62)	F,S,U	0 - 11	835	Japan	1.8
Cinar 2015	(63)	UBT	>6 - 16	500	Turkey	49
EUROPE						
Herbarth 2001	(4)	U	6-7	3 347	Germany	6.5 - 5.7
Rothenbacher 2002	(64)	F	1	56	Germany	11
Reshetnikov 2003	(5)	S	14-17	423	Russia	56.3
Rowland 2006	(65)	U	2-4	327	Ireland	8.6
Mourad-Baars 2007	(41)	S	2-4	1 258	the Netherlands	1,2
Tkachenko 2007	(49)	S	<5 - 19	307	Russia 1995	44
			<5 - 19	370	Russia 2005	13
Sykora 2009	(39)	P	0 - 15	1 650	Czech Republic	7.1
Strebel 2010	(66)	U	7 - 8	3.674	Germany	5.9
Bauer 2011	(67)	U	14	1.905	Germany	6,5
Oleastro 2011	(68)	F	0-15	844	Portugal	19.9-51.5
Bastos 2013	(42)	S	13	1.312	Portugal	66.2
Lasewicz 2014	(69)	S	0.5 - 18	3546	Poland	32.0
den Hollander 2015	(70)	P, S	4 - 8	4467	the Netherlands	10
This paper 2016?		F	0 - 12		Norway	0.6

NORTH AMERICA

Staat 1996	(18)	S	6 - 19	2 581	USA	16.7 - 29.1
Malaty 2002	(71)	S	1 - 3	224	USA	8
Krueger 2015	(72)	S	3 - 13	1 806	USA	7.1

SOUTH AMERICA

Goodman 2000	(73)	U	2-9	684	Colombia	40.1
Dattoli 2010	(74)	S	4-11	1.104	Brazil	29
Carter 2012	(45)	U	6-12	96	Bahamas	54.2
Duque 2012	(75)	U	6-13	718	Mexico	38

The table shows relevant publications of child prevalences of *H. pylori.*

Abbreviations are *Hp +ve: H. pylori* positive. F: faecal (stool) test;, H: histology, P: polymerase chainreaction (PCR) in gastric biopsy, R: rapid urease test (RUT) in gastric biopsy, S: serology, U: Urea breath test

Table 4. Selected population-based studies of *H. pylori* prevalence in adults according to continent

Author (publ year)	Ref #	Test used	Age group (years)	N	Country	*Hp +ve*) in age groups			
						0-20	15-40	40-60	>60
AFRICA									
Baingana 2014	(76)	F	15 - 44	447	Uganda				
ASIA									
Huang 2004	(54)	F	0.6-89	295	Borneo	39-38	29-39	30-	> 50
Alborzi 2006	(77)	F	0.75-15	593	Iran				
			20-60	200					
Nouraie 2009	(6)	S	18-65	2 561	Iran		57-73	79-75	
Cheng 2009	(78)	U	32-59	1.232	China				
Sasidharan 2009	(79)	S	adults	5.370	Malaysia				
Nakajima 2010	(80)	S	adults	142 in 1998	Japan				
				242 i 2005					
Sherpa 2012	(60)	F	<10 - >60	383	Nepal	78.1< 10 yrs			
Hu 2013	(81)	S	adults	3.995	China				
Ozaydin 2013	(82)	U	> 17	4622	Turkey				
Lim 2013	(83)	S	16 - >70	10.796	South Korea				
Chen 2014	(84)	U	12 - 89	796	Taiwan				
Ueda 2014	(85)	F, S, urine	adults	14 716	Japan				
Zhu 2014	(86)	U	30 - 69	5417	China				
Zhang 2014	(87)	P, S	> 60	2006	China				
AUSTRALIA									
Pandeya 2011	(88)	S	18-79	1.355	Australia		1	6 - 21	40 - 32
EUROPE									
Bernersen 1992	(48)	B	20 - 69	619	Norway				
Murray 1997	(89)	S	12-64	4 742	N Ireland	24	38-48	60-68	73
Bures 2006	(7)	U	5-100	2 509	Czech Rep	31-28	39	50	56
Leja 2012	(90)	S	Adults	3.564	Latvia				
Bures 2012	(91)	U	5-98	1.837	Czech Rep	4.8?	15-25	38-44	37-38
van Blankenstein 2013	(92)	S		1.550	the Netherlands				
Bastos 2013	(93)	S	18 - 92	2.067	Portugal				
Mana 2013	(94)	U	12 - 25	509	Belgium				

den Hollander 2013	(95)	P,S	24 - 35	6837	the Netherlands				
Michel 2014	(96)	P, S	1 - 82	1797	Germany				
Luzza 2014	(97)	P, S, U	3 - 97	595	Italy	< 20	20 - 60	60 - 80	80 - 85
Lasewicz 2014	(69)	S	0.5 - 89	6565	Poland				
		S	19 - 89	3581					
This paper		F	0-80	706	Norway	0.6*	7-12	24-30	45-44
NORTH AMERICA									
Graham 1991	(98)	S, U	15-80	485	USA	10	10-12	38-45	53-74
Everhart 2000	(99)	S	20-70+	7 465	USA		17-28	29-40	49-57
Gillum 2004	(100)	S	40-74	31	USA			28-41	50-55
Melius 2012	(101)	U	5 - 88	166	USA				
Krueger 2015	(72)	S	2 - >52	7493	USA	17.0		27.4	38.5
SOUTH AMERICA									
Rodrigues 2005	(102)	U	0.5-60+	610	Brazil	48-73	77-82	82-94	85
Porras 2013	(103)	U	21 - 65	1.852	Latin America				
Sivapalasingam 2014	(104)	U	< 3 - 40+	1065	Bolivia	40-82	92		95
Poveda 2014	(105)	S		274	Chile				
Alvarado-Esquivel 2014	(106)	S	16-91	345	Mexico		42	68	81

The division in age groups is not exact due to different use of these groups.

Abbreviations are *Hp +ve: H. pylori* positive. F: faecal (stool) test, H: histology, P: polymerase chain reaction (PCR) in gastric biopsy, R: rapid urease test (RUT) in gastric biopsy, S: serology, U: Urea breath test.

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