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Review Article

Breast-feeding and *Helicobacter pylori* infection: systematic review and meta-analysis

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Abstract

Objective: To quantify the association between breast-feeding and *Helicobacter pylori* infection, among children and adolescents.
Design: We searched MEDLINE™ and Scopus™ up to January 2013. Summary relative risk estimates (RR) and 95 % confidence intervals were computed through the DerSimonian and Laird method. Heterogeneity was quantified using the I^2 statistic.
Setting: Twenty-seven countries/regions; four low-income, thirteen middle-income and ten high-income countries/regions.
Subjects: Studies involving samples of children and adolescents, aged 0 to 19 years.
Results: We identified thirty-eight eligible studies, which is nearly twice the number included in a previous meta-analysis on this topic. Fifteen studies compared ever *v.* never breast-fed subjects; the summary RR was 0·87 (95 % CI 0·57, 1·32; $I^2 = 34{\cdot}4$ %) in middle-income and 0·85 (95 % CI 0·54, 1·34; $I^2 = 79{\cdot}1$ %) in high-income settings. The effect of breast-feeding for ≥ 4–6 months was assessed in ten studies from middle-income (summary RR = 0·66; 95 % CI 0·44, 0·98; $I^2 = 65{\cdot}7$ %) and two from high-income countries (summary RR = 1·56; 95 % CI 0·57, 4·26; $I^2 = 68{\cdot}3$ %). Two studies assessed the effect of exclusive breast-feeding until 6 months (OR = 0·91; 95 % CI 0·61, 1·34 and OR = 1·71; 95 % CI 0·66, 4·47, respectively).
Conclusions: Our results suggest a protective effect of breast-feeding in economically less developed settings. However, further research is needed, with a finer assessment of the exposure to breast-feeding and careful control for confounding, before definite conclusions can be reached.

Keywords
Helicobacter pylori
Breast-feeding
Child
Adolescent

Helicobacter pylori infection has been classified a definite human carcinogen for almost two decades and is well accepted as the single most important risk factor for non-cardia gastric cancer[1,2]. Although the prevalence of infection has been decreasing in many of the more economically developed countries[3,4], it was estimated to be responsible for nearly one-third of the 2 million cases of cancer occurring worldwide due to infections in 2008[5].

H. pylori infection is acquired mainly during childhood and adolescence[6–8]; once obtained, and in the absence of a specific treatment, it can persist for decades[9]. Therefore, understanding the role of modifiable exposures that may be targeted to decrease the rate of *H. pylori* infection during childhood is of key importance to prevent its occurrence. Factors that promote interpersonal contact or are associated with poor hygienic conditions, including being born in a setting with a high prevalence of infection[10], having parents with a low education level[11], sharing a room with other subjects[12] or attending a child-care institution[13], have been consistently associated with *H. pylori* infection in the early years of life. Breast-feeding has long been recognized as protective against gastrointestinal and respiratory diseases[14,15], and a role in the infection with *H. pylori* may be postulated.

A previous systematic review including sixteen studies suggested a protective effect of breast-feeding in middle- and low-income countries[16]. However, the understanding of the relationship between breast-feeding

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and *H. pylori* infection may be improved by taking into account more detailed and accurate definitions of the exposure. Furthermore, a set of additional studies were published since the previous meta-analysis, allowing an update of the existing evidence on this topic.

Therefore, we conducted a new systematic review and meta-analysis to quantify the association between breast-feeding and *H. pylori* infection, among children and adolescents.

Methods

Search strategy

We searched MEDLINE™ and Scopus™ up to January 2013 to identify studies addressing the association between breast-feeding and *H. pylori* infection in childhood or adolescence. The PubMed™ and Scopus™ search expressions, and the systematic review flowchart, are presented in Fig. 1 according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) Statement[17]. The literature search was further complemented by backward citation tracking among the articles considered eligible for the systematic review.

Selection of the studies

The studies were assessed independently by two researchers (H.C. and B.P. or H.C. and A.B.) in three consecutive steps to determine their eligibility; disagreements were discussed and resolved by consensus or involving a third researcher (N.L.).

In step 1, the studies were evaluated considering only the information presented in the title and abstract. When the abstract was not available, the study was further assessed, except when the title provided enough information to unequivocally exclude it. The full texts of the articles selected for step 2 were read to evaluate their eligibility and adequacy for data extraction; in step 3 the studies were re-evaluated to determine their eligibility for meta-analysis.

We excluded studies according to the following *a priori* defined criteria: (i) studies with full text not written in English, French, Italian, Polish, Portuguese or Spanish; (ii) reports not involving humans (*e.g. in vitro* studies); (iii) review articles, editorials, methodological studies, case reports or comments; (iv) studies in which the sample selection was dependent on the risk of *H. pylori* infection and therefore not expected to represent the general population (e.g. children undergoing endoscopy for diagnostic procedures); (v) studies not providing data on the association between breast-feeding and *H. pylori* infection; (vi) studies assessing the *H. pylori* infection status only in adults; and (vii) duplicate reports of the same study (data could be extracted from one or more of the multiple reports to obtain the most complete information).

Data extraction

The following data were extracted from the original reports: (i) year of publication; (ii) country and region where the study was conducted; (iii) study design; (iv) sample characteristics (sample size and age distribution); (v) methods used to determine the *H. pylori* infection status; (vi) exposure to breast-feeding, namely regarding its duration and exclusiveness; and (vii) relative risk (RR) estimates, namely risk ratios, incidence rate ratios or odds ratios, preferably adjusted for the larger number of potential confounders, or the necessary information to compute them, along with the corresponding precision estimates. Specific estimates for exclusive and non-exclusive breast-feeding or different durations of exposure were extracted whenever available.

For the studies providing data for age groups including adults in addition to children and/or adolescents (e.g. 10–29 years), we computed the mid-point year and excluded the data when it was higher than 18 years.

The discrepancies in the data extracted independently by two reviewers (H.C. and B.P. or H.C. and A.B.) were discussed and resolved by consensus, or involving a third researcher (N.L.).

Meta-analysis

The DerSimonian and Laird method was used to compute summary estimates of the association between breast-feeding and *H. pylori* infection, and respective 95 % confidence intervals. Heterogeneity was quantified using the I^2 statistic[18].

Stratified analyses according to the characteristics of the populations and methodological specificities with potential impact on the internal or external validity of the results (economic development of the countries where the investigations were conducted[19], age of the participants, adjustment for the potential confounding effect of socio-economic status, method used to assess the *H. pylori* infection status, prevalence of *H. pylori* infection in the non-exposed participants, prevalence of breast-feeding) were conducted to identify factors associated with heterogeneous results.

Funnel plots and the Egger's regression asymmetry test were used for assessment of 'small studies effects'[20]. The statistical analysis was performed using the STATA® statistical software package version 9·2.

No review protocol was registered.

Results

We identified thirty-eight studies eligible for the systematic review[7,10–12,21,30,32–55] (Fig. 1 and Appendices 1 and 2). The studies involved samples of children and adolescents, aged 0 to 19 years, recruited in twenty-seven countries/regions, including four low-income[21,40,41,46,47], thirteen middle-income[22,24–28,30,32–34,37–39,42,44,49–53,55] and ten high-income countries/regions[7,10–12,23,29,35,36,43,45,48,49,54].

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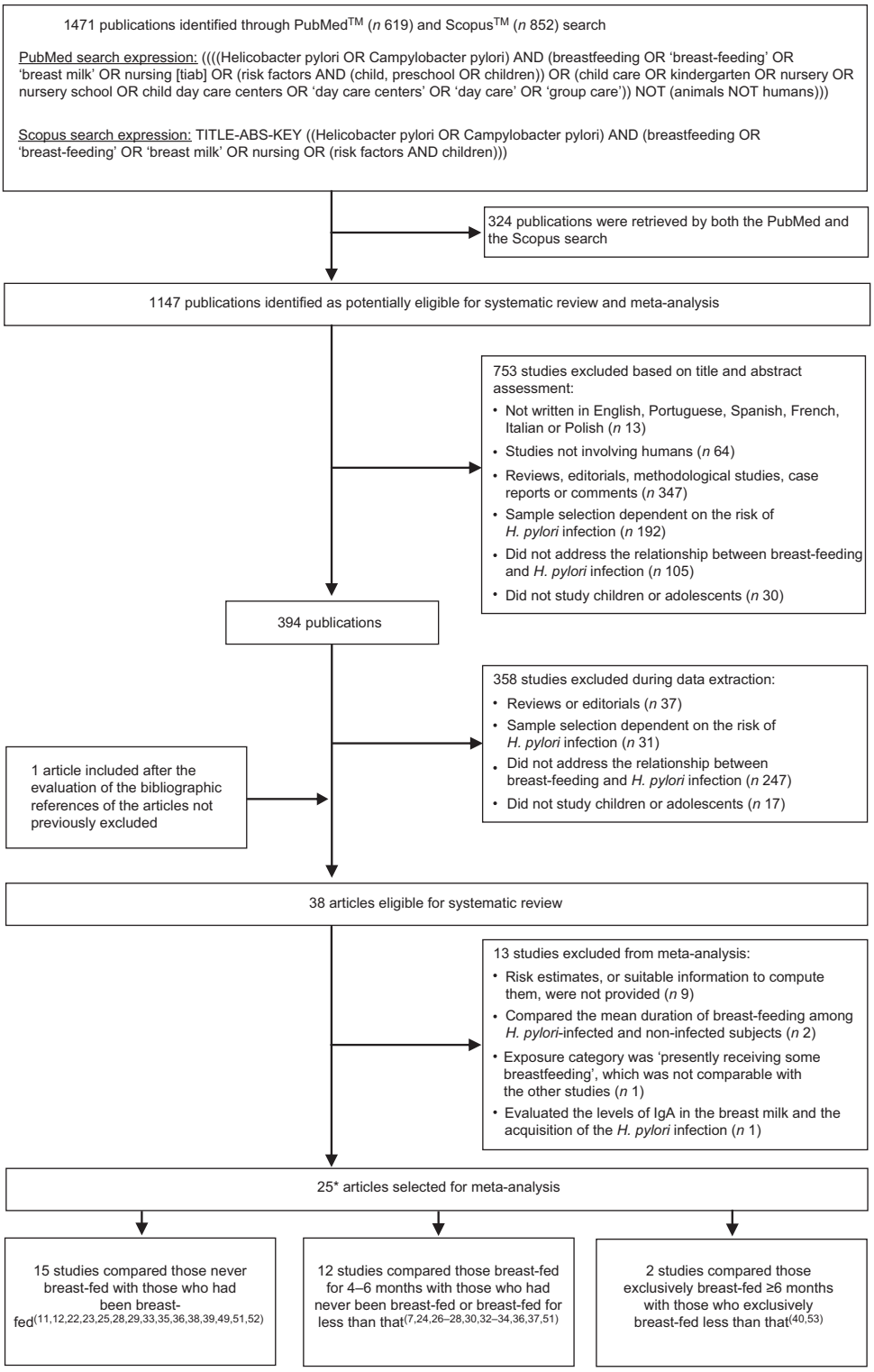

Fig. 1 Systematic review flowchart according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) Statement[17]. *Four studies[27,32,35,50] provided data to quantify the association between both having been breast-fed and having been breast-fed for 4–6 months and *Helicobacter pylori* infection

Most studies had a cross-sectional design and eight were cohort studies[37,39,40,44,45,47,49,53].

Thirteen studies did not provide information to compute an RR estimate for the association between breast-feeding and *H. pylori* infection[10,21,41–48,50,54,55] (Appendix 1). From these, eight referred a lack of association between breast-feeding and *H. pylori* infection, although the point estimates were not provided[10,41,43–45,47,50,54]. One study

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reported that the 'percentage of breastfeeding in the population in Korat was 61·25 %; the selected group of seropositive children had 51 % of exclusive breastfeeding for more than 6 months and 21·5 % the seropositive children had a history of breastfeeding for less than 6 months'[55]. One study involved a sample of children aged from 1 to 99 months and defined exposure as '*presently* receiving some breastmilk'[46], which is not comparable with the definitions of breast-feeding used in the remaining reports. Two studies compared the mean duration of breast-feeding among *H. pylori*-infected and non-infected subjects[42,48], and therefore could not be considered for meta-analysis; only one study showed a shorter duration of breast-feeding among the participants who were infected. Thomas *et al.*[21] compared the levels of IgA in the breast milk of the mothers and the infection with *H. pylori* in the respective children; the five children from the mothers who produced the lowest levels of IgA were infected.

Twenty-five studies[7,11,12,22–30,32–40,49,51–53], from high-, middle- and low-income countries, provided data to quantify the association between breast-feeding and *H. pylori*. Among those, fifteen compared breast-fed *v.* non-breast-fed subjects[11,12,22,23,25,28,29,33,35,36,38,39,49,51,52] and twelve compared subjects breast-fed for 4–6 months *v.* never breast-fed or breast-fed for less than 4–6 months[7,24,26–28,30,32–34,36,37,51]. Two studies specifically addressed the exclusive breast-feeding until the age of 6 months[40,53] (Appendix 2).

H. pylori *infection according to history of breast-feeding (ever* v. *never)*

Having been breast-fed was not significantly associated with *H. pylori* infection in either high-income (summary RR = 0·85; 95 % CI 0·54, 1·34; $I^2 = 79·1$ %) or middle-income countries (summary RR = 0·87; 95 % CI 0·57, 1·32; $I^2 = 34·4$ %). The results were heterogeneous, possibly reflecting a large inter-study variation in the duration of breast-feeding, since the prevalence of breast-feeding and the age range of the participants varied widely across studies (Fig. 2).

The visual inspection of the funnel plot did not suggest the occurrence of publication bias (Fig. 3). This is corroborated by the Egger's asymmetry test ($P = 0·84$).

H. pylori *infection according to duration of breast-feeding*

Only two studies provided data to evaluate the association between being breast-fed for 4 months or more *v.* never breast-fed or breast-fed for less than 4–6 months in high-income settings[7,36]. The overall RR estimate was 1·56 (95 % CI 0·57, 4·26; $I^2 = 68·3$ %; Fig. 4). The single study that provided an RR estimate adjusted for confounders yielded an OR of 2·57 (95 % CI 1·19, 5·55).

The combined results of the ten studies conducted in middle-income settings showed a summary RR of 0·66 (95 % CI 0·44, 0·98; $I^2 = 65·7$ %). The summary RR was non-significant when considering the adjustment for potential confounding effect of socio-economic factors (adjusted: summary RR = 0·77; 95 % CI 0·48, 1·20; $I^2 = 40·5$ %; unadjusted: summary RR = 0·58; 95 % CI 0·30, 1·10; $I^2 = 76·3$ %), or the prevalence of *H. pylori* infection among the non-exposed subjects (using the median as cut-off; ≤43 %: summary RR = 0·67; 95 % CI 0·35, 1·27; $I^2 = 75·0$ %; >43 %: summary RR = 0·66; 95 % CI 0·38, 1·15; $I^2 = 60·7$ %). The three studies that used diagnostic tests based on the detection of stool antigens yielded lower RR estimates (summary RR = 0·33; 95 % CI 0·15, 0·73; $I^2 = 64·3$ %), as did the seven studies with younger subjects (using the median as cut-off; ≤7 years: summary RR = 0·50; 95 % CI 0·32, 0·78; $I^2 = 56·7$ %; >7 years: summary RR = 1·09; 95 % CI 0·77, 1·55; $I^2 = 0·0$ %). The only cohort analysis showed a non-significant positive association between breast-feeding and *H. pylori* infection (RR = 2·54; 95 % CI 0·29, 22·40), although only six out of 110 children seroconverted during the 2-year follow-up period since birth[37].

The visual inspection of the funnel plot and the results of the Egger's asymmetry test ($P = 0·82$) did not suggest publication bias (Fig. 3).

H. pylori *infection according to history of exclusive breast-feeding*

Two studies[40,53], conducted in Ethiopia (low-income country) and in Chile (middle-income country), assessed the effect of exclusive breast-feeding for more than 6 months; the RR was 0·91 (95 % CI 0·61, 1·34) and 1·71 (95 % CI 0·66, 4·47) in the low- and middle-income setting, respectively.

Discussion

The available evidence on the relationship between breast-feeding and *H. pylori* infection is compatible with a protective effect in the less economically developed settings. However, only a few studies accounted for the potential confounding by socio-economic factors or assessed the effects of breast-feeding duration or exclusivity, precluding definite conclusions on this topic.

The present study updated a previous systematic review and meta-analysis conducted by Chak *et al.*[16] and the interpretation of our findings needs to take into account the evidence that was published since then, as well as the differences in the completeness of the search strategy and options for data synthesis. The present systematic review included eighteen studies[7,22–26,29,30,32,34,37,38,50–55] that were not considered in the paper published by Chak *et al.*[16]; most of the studies were published since then and two[25,37] were written in languages probably not considered in the previous review. However, due to our methodological options, six studies[31,46,56–59] included in

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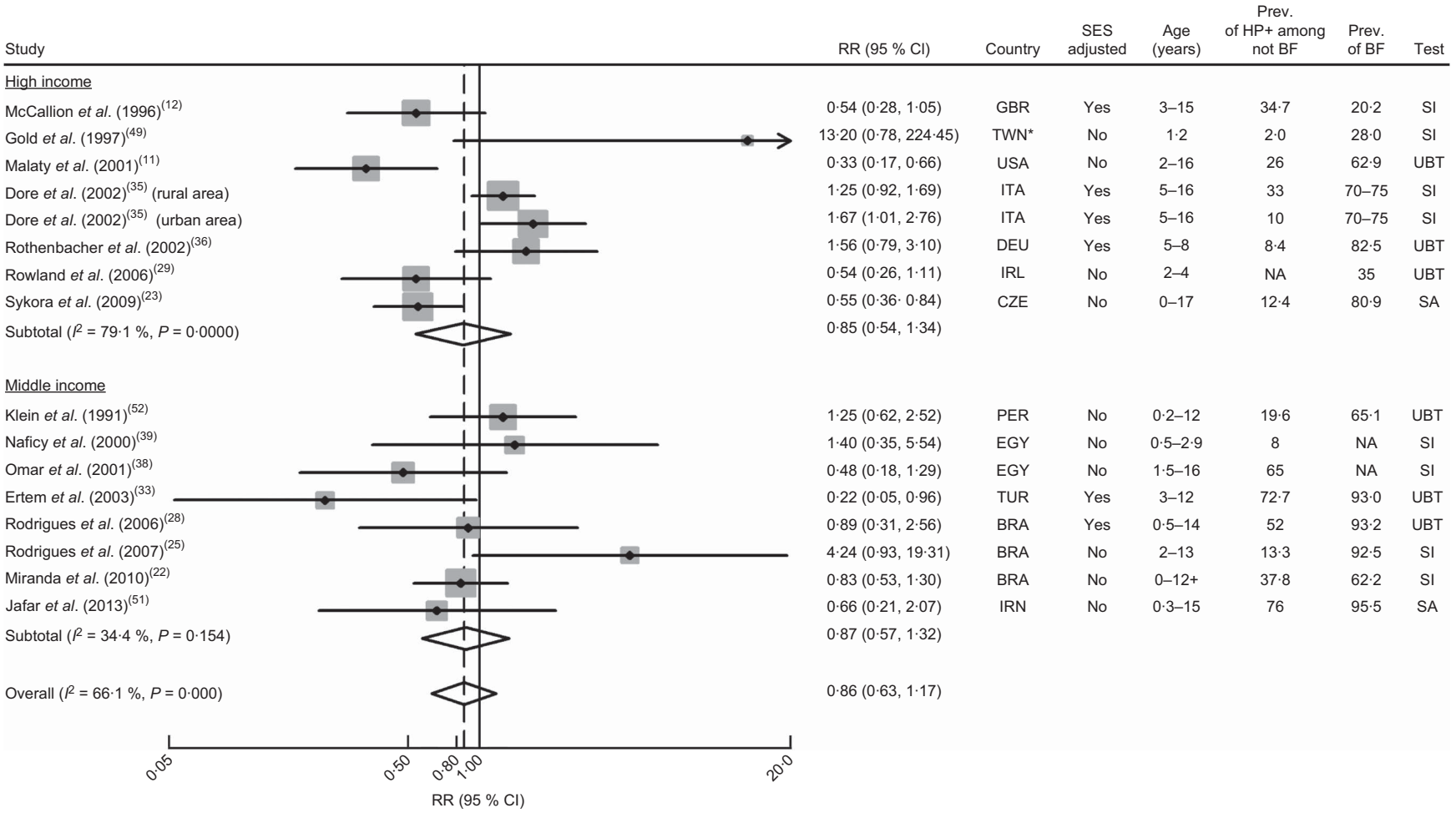

Fig. 2 Meta-analyses of studies evaluating the association between ever being breast-fed and *Helicobacter pylori* infection. Relative risk (RR) estimates and 95 % confidence intervals of *H. pylori* infection according to economic development of the countries where the investigations were conducted[19]. For each study, the black diamond indicates the best estimate, the size of the grey square indicates the study's weight in the analysis (weights are from random-effects analysis) and the horizontal line represents the 95 % CI. The centre of the open diamond indicates the summary estimate of the RR and its width represents the 95 % CI of the summary RR estimate. General abbreviations: SES, socio-economic status; Prev., prevalence (%); HP + , *H. pylori*-infected; BF, breast-feeding; NA, not available. Abbreviations for countries: BRA, Brazil; TWN, Taiwan, Republic of China; CZE, Czech Republic; DEU, Germany; EGY, Egypt; GBR, United Kingdom; IRL, Ireland; IRN, Islamic Republic of Iran; ITA, Italy; PER, Peru; TUR, Turkey. Abbreviations for tests: SA, test based on the detection of stool antigens; SI, test based on serum immunology; UBT, urea breath test. *In the World Bank statistics, Taiwan, Republic of China, is not listed as a separate country. However, for most indicators, Taiwan's data are not added to the data for China, but it is added to the world aggregate and the high-income countries aggregate. Therefore, Taiwan was included along with other high-income settings

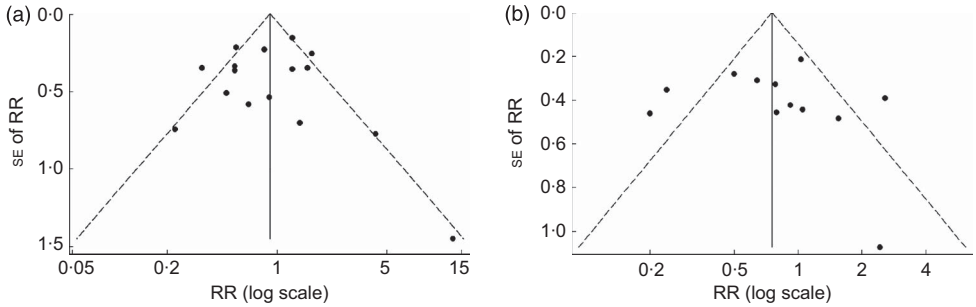

Fig. 3 Funnel plot of studies evaluating the association between breast-feeding and *Helicobacter pylori* infection: (a) ever breast-feeding *v.* never; (b) breast-feeding for 4–6 months *v.* less than that. Studies were plotted with their relative risk (RR) estimate on the *x*-axis (log scale) and the corresponding standard error of the RR along the *y*-axis; pseudo 95 % confidence limits are represented by dashed lines

the previous meta-analysis were not included in our meta-analyses. The study by Suoglu *et al.*[58] was not eligible because the sample selection was not independent of the *H. pylori* status. The study by Braga *et al.*[59] included a sample that partially overlapped with the sample of the study by Rodrigues *et al.*[28] and only the latter was considered in our review. The study by Mahalanabis *et al.*[46] defined the exposure as 'presently receiving some breast milk' although it also included children old enough for not being breast-fed for a long time and was therefore excluded from our analyses. We also opted for not including the studies evaluating the *H. pylori* infection status in adulthood[31,56,57], as the larger the lag between the exposure to breast-feeding and the assessment of infection status, the more likely it is that the RR estimates reflect the effect of other factors in addition to breast-feeding, namely taking into account that the incidence rates may remain high throughout adolescence[60].

The inclusion of a larger number of studies allowed a finer assessment of the exposure of breast-feeding. Chak *et al.*[16] provided a summary OR estimate combining the results of all eligible studies, regardless of the breast-feeding definition, and conducted stratified analysis according to the duration of breast-feeding (≥4 months *v.* <4 months or not specified). We opted for conducting two sets of analyses: (i) according to the breast-feeding status (ever breast-fed *v.* never breast-fed); and (ii) according to the duration of breast-feeding (≥4–6 months *v.* <4–6 months). Despite our efforts to combine the results from more homogeneous groups of studies, the inter-study variability in the estimates remained high. Among the studies that assessed the *H. pylori* status among those ever breast-fed and those who were never, the heterogeneity of the results is likely to be explained primarily by the differences implicit in the definition of ever having been breast-fed, which may include children breast-fed for one week or one year; however, the original reports did not provide information to account for these methodological aspects in our analyses. This depicts the need for standardized breast-feeding definitions to be used for the collection and description of data on this topic[61]. In 1988, the Interagency Group for Action on Breastfeeding[62] recognized that the term 'breast-feeding' is not enough to accurately describe its numerous variations. Specifically, it is required to distinguish between full and partial breast-feeding, and between the different levels of partial breast-feeding[62].

Our results suggest that having been breast-fed for 4–6 months is associated with a lower risk of *H. pylori* infection only in middle-income countries. We may hypothesize that in the latter settings children who are being breast-fed may present a substantially better nutritional status and therefore present more resistance to infections. Also, children whose mothers had breast milk with higher levels of anti-*H. pylori* IgA had a lower risk of *H. pylori* infection, compared with those whose mothers had lower levels[21]. Furthermore, breast-feeding may protect against the acquisition of the infection by acting as a natural antibiotic, as bovine lactoferrin was shown to inhibit the growth of *H. pylori*[63–65]; lactoferrin is much more abundant in breast milk than it is in cow's milk. Another component of breast milk, κ-casein, was shown to play a role in the inhibition of *H. pylori* adhesion to gastric mucosa[66].

Although similar results were obtained when considering crude RR estimates with those adjusted for the potential confounding by socio-economic factors, this is a methodological aspect of major importance and a sound assessment of this relationship requires the control of these confounders. The relationship between low socio-economic status and *H. pylori* infection is well known[67,68]. Breast-feeding is also influenced by these factors, although the relationship may vary with time and across settings with different economic and cultural background[69–71].

There was no association between breast-feeding and *H. pylori* infection when only the studies including older children were considered for analysis, which may reflect a lack of longer-term effects of breast-feeding, or that more important risk factors exert their effects after the cessation of breast-feeding.

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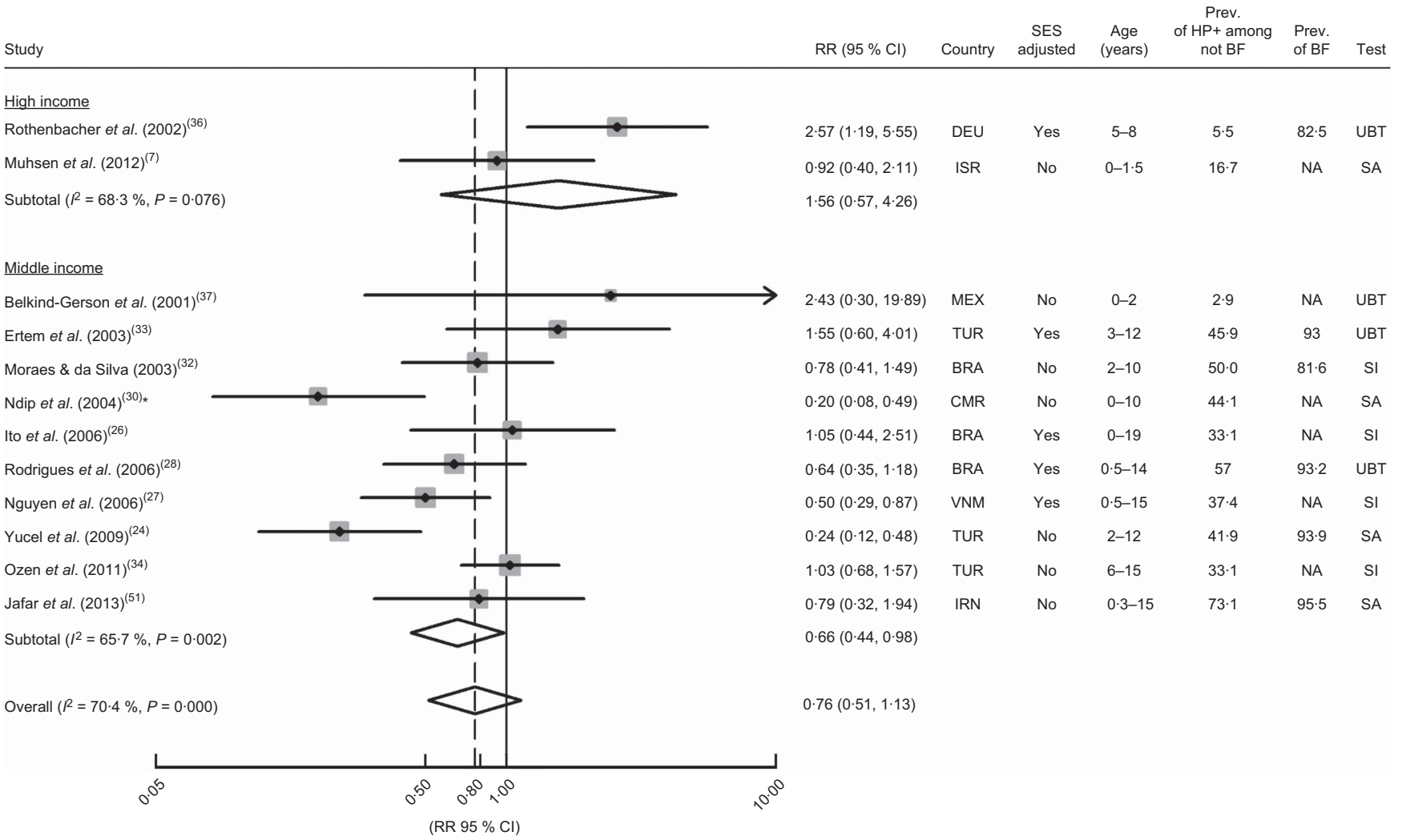

Fig. 4 Meta-analyses of studies evaluating the association between the duration of breast-feeding (<4–6 months *v.* >4–6 months) and *Helicobacter pylori* infection. Relative risk (RR) estimates and 95 % confidence intervals of *H. pylori* infection according to economic development of the countries where the investigations were conducted[19]. For each study, the black diamond indicates the best estimate, the size of the grey square indicates the study's weight in the analysis (weights are from random-effects analysis) and the horizontal line represents the 95 % CI. The centre of the open diamond indicates the summary estimate of the RR and its width represents the 95 % CI of the summary RR estimate. General abbreviations: SES, socio-economic status; Prev., prevalence (%); HP+, *H. pylori*-infected; BF, breast-feeding; NA, not available. Abbreviations for countries: BRA, Brazil; CMR, Cameroon; DEU, Germany; IRN, Islamic Republic of Iran; IRS, Israel; MEX, Mexico; TUR, Turkey; VNM, Vietnam. Abbreviations for tests: SA, test based on the detection of stool antigens; SI, test based on serum immunology; UBT, urea breath test. *Study comparing those who were breast-fed for more than 6 months with those breast-fed for less than 2 months. A sensitivity analysis excluding this study yielded an overall RR estimate of 0·80 (95 % CI 0·54, 1·19; $I^2 = 65{\cdot}8$ %)

Conclusion

In conclusion, our results suggest a protective effect of breast-feeding in economically less developed settings. However, further research is needed, with a finer assessment of the exposure to breast-feeding and infection status, as well as a careful control of confounding, before definite conclusions can be reached.

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Appendix 1 Main characteristics and results of the studies included in the systematic review but excluded from the meta-analysis

Authors, year of publication, reference	Country, region	Study population	Age (years): range; mean (SD)	Assessment of *Helicobacter pylori* status		Description of the main findings	Reason for exclusion
				Sample	Method		
Hestvik *et al.* (2010)[41]	Uganda, Kampala	'In the Mulago II parish children aged 0–12 years were recruited consecutively by door-to-door visits, an equal number of children in each age category […]'	0–12; 4·8 (3·6)	Stool	Immunoassay	'There were no significant differences in prevalence by breastfeeding duration (shorter or longer than 24 weeks) […]'	An RR or suitable information to compute it was not provided
Bhuiyan *et al.* (2009)[47]	Bangladesh, Mirpur	'695 pregnant mothers were screened and 321 newborn children were enrolled. From this cohort, 238 children […] who had completed follow-up and from whom complete set of serum and stool specimens were available'	Baseline: newborns Follow-up: 2 years	Blood Stool	ELISA	'There was no difference in the prevalence of *H. pylori* infection among children who were exclusively breast-fed in comparison with those who received mixed feedings when analyzing samples collected during the first 6 months of life (P = NS) (data not shown). Similarly, we could not find any relationship between *H. pylori* infection in children breast-fed after age 6 months and those who were not'	An RR or suitable information to compute it was not provided
Siai *et al.* (2008)[42]	Tunisia, Cap-Bon	'Among the 10,703 first-grade pupils identified in the healthcare centers' databases, 1055 were randomly selected for inclusion (the first, 10th and 20th children on the health-center lists)'	6–7; ND (ND)	Blood	ELISA (IgG)	'Statistically, there was no difference between infected and non infected children in terms of the following variables: […] duration of breastfeeding […]. Mean duration of breastfeeding: 12·48 months among *H. pylori* positive subjects; 11·85 months among *H. pylori* negative subjects'	It compares the mean duration of breast-feeding among *H. pylori*-infected and non-infected subjects
Przybyszewska *et al.* (2006)[43]	Poland, Cracow	'From 1999 until 2001 the study was carried out on randomly selected healthy children aged 6 months to 4 years, attending Healthy Child Centres in Cracovia for their immunization or physical assessment of their health'	0·5–4·3; 2·7 (0·98)	Expired air	UBT	'Also Hp infection was not dependent on […] breastfeeding […]'	An RR or suitable information to compute it was not provided

Appendix 1 *Continued*

Authors, year of publication, reference	Country, region	Study population	Age (years): range; mean (SD)	Assessment of *Helicobacter pylori* status		Description of the main findings	Reason for exclusion
				Sample	Method		
Kivi *et al.* (2005)[10]	Sweden, Stockholm	'The present cross-sectional study in an extension of a previous serological survey in 11 Stockholm schools, conducted between February and April 1998, investigating risk factors for *H. pylori* infection in children'	10–14; 12* (ND)	Blood	ELISA (IgG)	'No associations with index child infection were found for […] breastfeeding […]'	An RR or suitable information to compute it was not provided
Vivatvakin *et al.* (2004)[55]	Thailand, central, northern, north-eastern and eastern	'[…] sera of Thai children who visited the Out Patients Clinics […] were collected. […] The exclusion criteria were the children who had either blood or plasma transfusion and history of recurrent abdominal pain'	0–16; ND (ND)	Blood	ELISA and Western blot (IgG)	'Percentage of breastfeeding in the population in Korat was 61·25 %. The selected group of seropositive children had 51 % of exclusive breastfeeding for more than 6 months and in only 21·5 % the seropositive children had a history of breastfeeding less than 6 months'	An RR or suitable information to compute it was not provided
Glynn *et al.* (2002)[44]	Bolivia, ND	'[…] we conducted a serosurvey (survey I) to establish baseline *H. pylori* seroprevalence rates […]. All children aged 6 months through 9 years were eligible to enroll in the health day activities, including testing for *H. pylori* […] we returned to the same 17 villages and conducted a second serosurvey (survey II). […] we restricted the enrollment in survey II to children aged ≤6 years'	0·5–9; ND (ND)	Blood	ELISA (IgG)	'Behaviors associated with breast-feeding, including breast-feeding from multiple women or breast-feeding from a woman who was simultaneously nursing other children, also were not significantly associated with seroconversion'	An RR or suitable information to compute it was not provided

Appendix 1 *Continued*

Authors, year of publication, reference	Country, region	Study population	Age (years): range; mean (SD)	Assessment of *Helicobacter pylori* status		Description of the main findings	Reason for exclusion
				Sample	Method		
Daugule *et al.* (2001)[50]	Latvia, Riga	'Consecutive children […] without symptoms from the gastrointestinal tract, who visited their doctor for a general checkup or because of minor health problems'	1–12	Expired air	UBT	'The univariate associations of some of the studied risk factors with *H. pylori* positivity are shown in the table. […] The other possible risk factors did not demonstrate a significant association with *H. pylori* infection' (Breast-feeding was not included in the table)	An RR or suitable information to compute it was not provided
Okuda *et al.* (2001)[48]	Japan, Wakayama	'This study included 484 children with no gastric symptoms, […] who were examined at Wakayama Rosai Hospital'	0–12	Stool	*H. pylori* stool antigen assay	'The mean period of breast-feeding of the HpSA positive group was 5·3 ± 5·8 months, while the mean period for the HpSA negative group was 7·8 ± 7·4 months ($P = 0·02$). In the 198 children aged 1–3-years-old, the mean period of breast-feeding for the 14 HpSA positive children was 3·4 ± 3·5 and for the 184 HpSA negative children was 8·5 ± 6·9 months ($P = 0·003$, Table 2)'	It compares the mean duration of breast-feeding among *H. pylori*-infected and non-infected subjects
Rothenbacher *et al.* (2000)[54]	Germany, Ulm, Langenau and Ehingen	'In this study we included all infants and children of Turkish nationality in whom 11 participating pediatricians […] conducted a screening examination'	0–5	Stool	Enzyme immunoassay	'[…] history of breastfeeding showed no clear pattern with prevalence of current infection'	An RR or suitable information to compute it was not provided
Tindberg *et al.* (1999)[45]	Sweden, Stockholm	'children were recalled for serology of both pertussis and *H. pylori* infection and 201 of 305 identifiable children accepted.	2, 4; ND (ND)	Blood	ELISA (IgG, IgA)	'Length of breastfeeding, i.e. a mean of 3 mo for both seropositive and seronegative children, could not be correlated with later *H. pylori* infection status'	An RR or suitable information to compute it was not provided

Appendix 1 *Continued*

Authors, year of publication, reference	Country, region	Study population	Age (years): range; mean (SD)	Assessment of *Helicobacter pylori* status		Description of the main findings	Reason for exclusion
				Sample	Method		
Mahalanabis *et al.* (1996)[46]	Bangladesh, Nandipara	'The study was carried out in a periurban village named Nandipara […] settled by people of low socioeconomic status on government land. […] Infants and children over a wide age range 1–99 months were studied'	0·08–8·3; ND (ND)	Expired air	UBT	'The association between lack of breastfeeding and *H. pylori* infection could not be tested because infants and children under 3 years were nearly all breastfed. Although an association (significant at a 5 % level) was shown between breast-feeding and *H. pylori* infection in children over 5 years old, the significance of this finding is tenous and could be attributed to the effect of multiple comparison'	The exposure category was 'presently receiving some breast milk'. This exposure was assessed in groups of subjects with wide age ranges (1–3 months, 4–35 months, 36–59 months, 60–99 months)
Thomas *et al.* (1993)[21]	Gambia, ND	'We have measured the potential protective effect of specific human milk IgA by studying 12 mothers and their infants from a Gambian village in which most infants are breast-fed throughout the first 2 years of life'	3–12 months	Expired air	UBT	'We found a relation between the concentration of specific breast milk IgA and the age of acquisition of *H. pylori* infection. By ranking mothers according to the level of anti-*H-pylori* IgA they secreted […] their children could be divided into two groups according to whether or not *H pylori* infection was diagnosed by 9 months of age (Kruskal–Wallis test, $P = 0{\cdot}004$). All 5 infected children at this age came from 5 mothers with the lowest specific breast milk IgA. By 12 months of age, only 3 children were infection free, including the children of the two mothers who produced the highest specific breast milk IgA ($P = 0{\cdot}04$)'	It related the levels of IgA in the mothers and the acquisition of *H. pylori* infection in the first year of the child

ND, not defined; RR, relative risk estimate; UBT, urea breath test.
*Median age.

Appendix 2 Main characteristics and results of the studies included in the meta-analysis

Authors, year of publication, reference	Country, region	Study population	Age (years): range; mean (SD)	Assessment of *Helicobacter pylori* status		Breast-feeding prevalence among the study subjects	Duration of breast-feeding/other measures	RR estimates			
				Sample	Method			Reference category used for RR estimation	Category of exposure used for RR estimation	RR (95 % CI)	Control for confounding
Jafar *et al.* (2013)[51]	Iran, Sanandaj	'This cross-sectional study was based on samples of 4-month to 15-year-old children. […] The lower age groups were selected randomly from the healthy children who referred to primary healthcare centers for vaccination and the older ones were from 12 schools with different socioeconomic status across the city'	0·3–15; 5·6 (5·4)	Stool	Enzyme immune assay	95·5 %	ND	No breast-feeding	Breast-feeding for <6 months	OR = 0·62 (0·10, 3·66)	No
								No breast-feeding	Breast-feeding for ≥6 months	OR = 0·66 (0·21, 2·06)	No
								Not breast-fed or breast-fed for <6 months	Breast-fed for ≥6 months	OR = 0·79 (0·32, 1·92)	No
O'Ryan *et al.* (2013)[53]	Chile, Colina	'Mother–infant pairs […] were enrolled during 2006 to 2007 in a 2-year cohort study. […] Only healthy 1-month-old infants were enrolled'	Baseline: birth Median time of follow-up: 60 months	Stool	ELISA	ND	ND	Not exclusively breast-fed at 6 months	Exclusive breast-feeding at 6 months	OR = 1·71 (0·66, 4·47)	No
Muhsen *et al.* (2012)[7]	Israel, Northern region	'Mothers of healthy infants aged 1 week to 2 months […] were asked to participate […] Mothers of eligible infants […] were recruited through the local family health clinics between January and August 2007'	1·5; ND (ND)	Stool	Enzyme immune assay‡	ND	ND	≤6 months	>6 months	OR = 0·92 (0·40, 2·11)	No
Ozen *et al.* (2011)[34]	Turkey, ND	'Subjects for the study were selected from the school register […]'	6–15; 9·8 (2·0)†	Blood	ELISA	ND	ND	<4 months	4–12 months	OR = 1·07 (0·66, 1·74)‖	No
								<4 months	>12 months	OR = 1·00 (0·63, 1·59)‖	
								<4 months	≥4 months	OR = 1·03 (0·69, 1·55)‖	No
Miranda *et al.* (2010)[22]	Brazil, São Paulo	'Children and adolescents were eligible for inclusion if they were registered at the outpatient service of Hospital São Paulo with a diagnosis of upper airway infection on week-days. All eligible individuals were invited to participate, without any sampling procedure'	ND; 6·82 (4·07)	Blood	ELISA (IgG)	62·2 %	ND	No breast-feeding	Exclusive breast-feeding until 4 months of age	OR = 0·83 (0·53, 1·31)	No

Appendix 2 *Continued*

Authors, year of publication, reference	Country, region	Study population	Age (years): range; mean (SD)	Assessment of *Helicobacter pylori* status		Breast-feeding prevalence among the study subjects	Duration of breast-feeding/other measures	RR estimates			
				Sample	Method			Reference category used for RR estimation	Category of exposure used for RR estimation	RR (95 % CI)	Control for confounding
Sýkora *et al.* (2009)[23]	Czech Republic, West Bohemia	'asymptomatic children, […] chosen prospectively from the general population'	0–15; ND (ND)	Stool	ELISA	80·9 %	Among HP– subjects: 13·99 (SD 19·55) weeks Among HP+ subjects: 8·37 (SD 12·73) weeks	Never have been breast-fed	Ever have been breast-fed	OR = 0·55 (0·36, 0·84)¶	No (the OR estimate remained not statistically significant after age adjustment)
Yucel *et al.* (2009)[24]	Turkey, Northern region	'children belonging to […] the outpatient clinic of a university medical center, and samples were collected from subjects who included the children of academic staff members. […] The second site was a public health center located in the city center […]. The last site was a public health center […]'	2–12; 6·8 (3·0)	Stool	Immuno-chromato-graphic assay	Prevalence of breast-feeding by duration: None: 6·0 % 0–6 months: 49·1 % 0–12 months: 15·7 % 0–24 months: 23·7 % 0–48 months: 5·5 %	A statistical correlation was found between the duration of breast-feeding and *H. pylori* positivity, but it was not significant ($P = 0·02$; 95 % CI 0·517, 7·349; $r = -0·18$)	Never have been breast-fed or breast-fed for less than 6 months	Breast-fed for more than 6 months	OR = 0·24 (0·12, 0·48)‖	No
Rodrigues *et al.* (2007)[25]	Brazil, Porto Velho	'children […] selected from a private clinic and outpatient services in surrounding neighbourhoods. […] the inclusion criteria were: […] need of venopunction for laboratory complementary exams […]'*	2–13; 7·7 (ND)	Blood	Enzyme immune assay	92·5 %	ND	Never breast-fed	Ever breast-fed	OR = 4·24 (0·93, 19·32)‖	No
Ito *et al.* (2006)[26]	Brazil, São Paulo	'The subjects of this study were volunteers with apparently good health conditions. The family units in this study were defined as husband, wife, and at least one nonadopted child aged between 0 and 19 years. The study required that both parents were Japanese or Japanese descendants whose family members all lived in the same household'	0–19; ND (ND)	Blood	Anti-*H. pylori* IgG antibody test	ND	ND	Breast-fed less frequent than 6 months	≥6 months	OR = 1·05 (0·44, 2·51)	Adjusted: for age and sex

Appendix 2 *Continued*

Authors, year of publication, reference	Country, region	Study population	Age (years): range; mean (SD)	Assessment of *Helicobacter pylori* status		Breast-feeding prevalence among the study subjects	Duration of breast-feeding/other measures	RR estimates			
				Sample	Method			Reference category used for RR estimation	Category of exposure used for RR estimation	RR (95 % CI)	Control for confounding
Nguyen *et al.* (2006)[27]	Vietnam, Northern region	'Every consecutive outpatient aged more than 6 months and less than 15 years presenting every Wednesday at the pediatric department of a university hospital was included. However, children with acute diarrhea, ulcer disease, and repeated abdominal pain or immunocompromised status were excluded'	0·5–15; ND (ND)	Blood	ELISA (IgG)	ND	ND	Breast-feeding for ≤6 months Breast-feeding for ≤6 months	Breast-feeding for >6 months Breast-feeding for >6 months	OR = 0·6 (0·4, 0·8) OR = 0·5 (0·3, 0·9)	No Adjusted for: age (3–6 years, >6 years) and offspring number >1
Rodrigues *et al.* (2006)[28]	Brazil, Ceará, Fortaleza	'[…] involving a community in Parque Universitário […] All houses in the community had been previously numbered and households to be surveyed were chosen by means of a table of random numbers. Children […] as well as their mothers were invited to participate in the study'	0·5–14; ND (ND)	Expired air	UBT	93·2 %	ND	Not breast-fed Breast-fed ≤6 months Breast-fed Breast-fed ≤6 months	Breast-fed Breast-fed >6 months Not breast-fed Breast-fed >6 months	OR = 1·12 (0·45, 2·82) OR = 0·86 (0·56, 1·35) OR = 0·89 (0·31, 2·56)‖,¶ OR = 0·64 (0·35, 1·18)	No** No** Adjusted for: *H. pylori* status of the mother, age, nutritional status, education of the mother, history of antibiotic use, smoking of the mother, number of persons per room and number of children per household
Rowland *et al.* (2006)[29]	Ireland, Dublin, Mallow and Kingscourt	'Nineteen family doctors were approached to provide patients for the study […] Parents of eligible children were invited by letter from their family doctor to participate in this study'	Baseline: 2–4; 2·75 (0·6)	Expired air	UBT	35 %	There was no difference in the rate or duration of breast-feeding between infected and non-infected index children	Not breast-fed	Breast-fed	OR = 0·54 (0·26, 1·09)	No
Ndip *et al.* (2004)[30]	Cameroon, Buea and Limbe	'The study population consisted of 176 apparently healthy children […]. Eighty eight children were sampled from each of the two study sites'	0–10; 4·29 (ND)	Stool	ELISA	ND	ND	Breast-fed for ≤2 months	Breast-fed for ≥6 months	OR = 0·20 (0·08, 0·49)‖	No

Appendix 2 *Continued*

Authors, year of publication, reference	Country, region	Study population	Age (years): range; mean (SD)	Assessment of *Helicobacter pylori* status		Breast-feeding prevalence among the study subjects	Duration of breast-feeding/other measures	RR estimates			
				Sample	Method			Reference category used for RR estimation	Category of exposure used for RR estimation	RR (95 % CI)	Control for confounding
Moraes and da Silva (2003)[32]	Brazil, Pernambuco	'A cross-sectional study was done […] then, we did a comparative study between the seropositive children and the seronegative children of HP. The sample was non probabilistic, of convenience, meaning all children who presented during the morning shift in the outpatient clinic of Pediatrics since the day of start of the study […]'*	2–10; ND (ND)	Blood	ELISA (IgG)	81·6 %	ND	Never breast-fed	≤4 months ≥5 months	OR = 0·78 (0·41, 1·49)	No
Ertem *et al.* (2003)[33]	Turkey, ND	'[…] in a population of pre-school and school-aged healthy children […]'	3–12; 8·2 (2·1)	Expired air	UBT	93·0 %	ND	Not breast-fed	≤1 month 2–3 months 4–5 months 6–24 months	OR = 1·09 (0·43, 2·76)‖ OR = 0·59 (0·23, 1·53)‖ OR = 0·65 (0·26, 1·64)‖ OR = 1·55 (0·60, 4·02)‖	No
								Not breast-fed	Breast-fed	OR = 0·22 (0·05, 0·96)¶	Adjusted for: socio-economic class (high, middle, low), number of siblings (none, 1, ≥2), heating system (central heating, coal-stove), age, percentile values for weight and height, household density, education of parents
Dore *et al.* (2002)[35]	Italy, urban area: Sardinia, Porto Torres	'A cross-sectional study of *H. pylori* prevalence was conducted among elementary and middle school children who lived in the same region but in different settings (rural *v.* urban)'	5–16; ND (ND)	Blood	ELISA (IgG)	70–75 %	ND	Not breast-fed	Breast-fed	OR = 1·25 (0·92, 1·69)¶	Adjusted for: age, sex, occupational category for the head of household, ownership of animals, day care attendance

Appendix 2 *Continued*

Authors, year of publication, reference	Country, region	Study population	Age (years): range; mean (SD)	Assessment of *Helicobacter pylori* status		Breast-feeding prevalence among the study subjects	Duration of breast-feeding/other measures	RR estimates			
				Sample	Method			Reference category used for RR estimation	Category of exposure used for RR estimation	RR (95 % CI)	Control for confounding
Dore *et al.* (2002)[35]	Italy, rural area: Sardinia, Alà dei Sardi, Bono, Padria, Buddusò, Sedini, Laerru, and Nughedu	'A cross-sectional study of *H. pylori* prevalence was conducted among elementary and middle school children who lived in the same region but in different settings (rural *v.* urban)'	5–16; ND (ND)	Blood	ELISA (IgG)	70–75 %	ND	Not breast-fed	Breast-fed	OR = 1·67 (1·01, 2·76)¶	Adjusted for: age, sex, occupational category for the head of household, ownership of animals, day care attendance
Rothenbacher *et al.* (2002)[36]	Germany, Ulm	'[…] 1201 children who were to attend first grade in the school year 1997/98 and who lived within the city limits of Ulm'	5–8; 5·9 (0·46)	Expired air	UBT	82·5 % 33·1 % of the children were exclusively breast-fed until 3 months 17 % were exclusively breast-fed for ≥6 months 36·7 % were breast-fed for ≥6 months	ND	Never breast-fed	Ever breast-fed	OR = 1·22 (0·68, 2·22)**	No
								Never breast-fed	Ever breast-fed	OR = 1·67 (0·89, 3·14)**	Adjusted for: *H. pylori* status of the mother
								Never breast-fed	Ever breast-fed	OR = 1·56 (0·79, 3·11)	Adjusted for: *H. pylori* status of the mother, nationality, age, sex, place of birth, birth weight, education of the father, education of the mother, history of antibiotic use, housing density, number of siblings, household smoking of mother, household smoking of father
								Never breast-fed	<3 months	OR = 1·07 (0·47, 2·46)	
									3–6 months	OR = 1·19 (0·52, 2·75)	
									≥6 months	OR = 2·57 (1·19, 5·55)	
Belkind-Gerson *et al.* (2001)[37]	Mexico, Morelos, Cuernavaca	'A birth cohort study followed up during the first years of life. Blood from 100 healthy children who were presented to in the Hospital del Ninõ Morelense to routine immunization […]'*	Baseline: 2 months Followed up to 24 months	Blood	ELISA§	ND	ND	Breast-fed for less than 6 months	Breast-fed for at least 6 months	RR = 2·43 (0·30, 20·1)	No

Appendix 2 *Continued*

Authors, year of publication, reference	Country, region	Study population	Age (years): range; mean (SD)	Assessment of *Helicobacter pylori* status		Breast-feeding prevalence among the study subjects	Duration of breast-feeding/other measures	RR estimates			
				Sample	Method			Reference category used for RR estimation	Category of exposure used for RR estimation	RR (95 % CI)	Control for confounding
Malaty *et al.* (2001)[11]	USA, Houston	'The study involved 13 licensed day care centers from different locations in Houston. […] Children 15 years old attended day care centers in the late afternoon, after regular school hours, and in the morning before school. […] The sampling of the study was not random but depended on invitation and eligibility as determined by the entry criteria'	2–16; ND (ND)	Expired air	UBT	62·9 %	ND	Not breast-fed	Breast-fed	OR = 0·33 (0·17, 0·66)¶	Adjusted for: age
Omar *et al.* (2001)[38]	Egypt, Cairo	'Children attending the pediatric outpatient clinic of Damanhour Teaching Hospital for minor illnesses during a six months period'	1·5–16; 6·8 (3·7)	Blood	ELISA (IgG)	ND	ND	Not breast-fed Breast-fed for ≥12 months	Breast-fed Breast-fed for <12 months	OR = 0·48 (0·18, 1·29)¶ OR = 4·3 (1·5, 125·6)	No Adjusted for: age and bed sharing
Naficy *et al.* (2000)[39]	Egypt, Abu Homos	'[…] a house-to-house census of the study population was performed […] Following the census, all children under 24 months and new births into the censused housed were eligible for enrolment into the cohort'	0·5–2·9; ND (ND)	Blood	ELISA (IgG)	ND	ND	Never breast-fed	Ever breast-fed	OR = 1·4 (0·37, 5·8)	Adjusted for: age
Lindkvist *et al.* (1999)[40]	Ethiopia, 9 highland and lowland villages and the town Butajira situated in the Rift Valley	'The children were selected randomly by computer from the BRHP database, and only one sibling from each family was selected. (…)'	Baseline: 1·8–4; 2·9 (ND) End of 30-month follow-up: 4·3–6·5; 5·5 (ND)	Blood	Immunoblot assay	ND	ND	Exclusive breast-feeding <6 months	Exclusive breast-feeding ≥6 months	RR = 0·91 (0·61, 1·34)	No

Appendix 2 *Continued*

Authors, year of publication, reference	Country, region	Study population	Age (years): range; mean (SD)	Assessment of *Helicobacter pylori* status		Breast-feeding prevalence among the study subjects	Duration of breast-feeding/other measures	RR estimates			
				Sample	Method			Reference category used for RR estimation	Category of exposure used for RR estimation	RR (95 % CI)	Control for confounding
Gold *et al.* (1997)[49]	Republic of China, Taiwan, Taipei	'Study subjects have been enrolled voluntarily from a group of Taiwanese women and infants entered in a hepatitis B vaccine efficacy study at the National Taiwan University, Taipei, Taiwan'	Baseline: birth date End of follow-up: 14 months of age	Blood	ELISA (IgG)	28 %	'Forty-eight of the 80 infants were *H. pylori*-positive at birth (*i.e.* blood drawn at 3 d of life); all of those had seropositive mothers. Ninety four percent of the infants with evidence of passive transfer of maternal IgG antibodies showed no detectable antibodies by 3 mo of age and 98 % of these infants by 6 mo of age'	Not breast-fed	Breast-fed	OR = 13·2 (1·2, 347)	No (this association remained when analysis was limited to those infants whose mothers were the primary care givers)
McCallion *et al.* (1996)[12]	Ireland, Belfast	'children […] attending the Royal Belfast Hospital for Sick Children for routine non-gastrointestinal day surgery'	3–15; ND (ND)	Blood	ELISA (IgG)	20·2 %	There was a significant negative association between infection and breast feeding	Never breast-fed or breast fed for less than 2 weeks	Breast fed for 2 weeks or longer	OR = 0·52 (0·29, 0·96)‖,** OR = 0·54 (0·28, 1·06)	No Adjusted for: age, social class and housing density

Appendix 2 *Continued*

Authors, year of publication, reference	Country, region	Study population	Age (years): range; mean (SD)	Assessment of *Helicobacter pylori* status		Breast-feeding prevalence among the study subjects	Duration of breast-feeding/other measures	RR estimates			
				Sample	Method			Reference category used for RR estimation	Category of exposure used for RR estimation	RR (95 % CI)	Control for confounding
Klein *et al.* (1991)[52]	Peru, San Juan de Miraflores	'266 children from families of low socioeconomic status (recruited at local health posts) and 141 children from families of high economic status (recruited from private schools, churches, and social organisations) were studied. Children from families of low socioeconomic status were regarded as representative of the community population on the basis of family demographic comparisons with randomly selected age-matched children from the same region'	0·16–12; ND (ND)	Expired air	UBT	65·1 %	ND	Not breast-fed	Breast-fed	OR = 1·25 (0·62, 2·52)	No

RR, relative risk; ND, not defined; HP, *Helicobacter pylori*; UBT, urea breath test.
*Translated from original language to English by the authors of the present review.
†Weighted mean of the mean age of *H. pylori*-positive and -negative subjects.
‡*H. pylori* infection was defined as having at least two positive tests at examinations obtained at age 6 months or later.
§Only considered *H. pylori* infection when the test was positive after the age of 6 months.
‖Estimates computed from the information presented in the paper.
¶Study providing OR estimates for the comparison of the subjects who were never breast-fed with those who were ever breast-fed. To estimate OR for the comparison of those who were ever breast-fed with those who were never, we computed the inverse of the OR and the respective 95 % CI.
**OR estimate not considered for meta-analysis.