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REVIEW

# The relationship between *Helicobacter pylori* infection and recurrent aphthous stomatitis: a systematic review and meta-analysis

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## Abstract

**Objective** This meta-analysis was designed to provide new insights into the relationship between *Helicobacter pylori* (*H. pylori*) infection and recurrent aphthous stomatitis (RAS).

**Materials and methods** We included and evaluated studies on *H. pylori* infection and RAS from PubMed, EMBASE, Cochrane Library, and Web of Science databases published up to January 31, 2023. The characteristics of these studies were collected, and the quality was evaluated by Newcastle-Ottawa Scale (NOS). The random effects model was used to calculate the pooled odds ratio (OR) and 95% confidence interval (CI). To further explore the sources of heterogeneity, meta-regression analysis and subgroup analyses were performed. Funnel plot, Egger's test, and Begg's test were used to assess publication bias.

**Results** In total, fifteen case-control studies with 1137 individuals (601 cases and 536 controls) were included. The *H. pylori* was found to be significantly associated with RAS (OR: 1.83 95% CI: 1.41–2.37, $P = 0.001$). In the subgroup analyses, studies that used PCR (OR: 2.03 95% CI: 1.31–3.15) or UBT (OR: 1.83 95% CI: 1.13–2.96) yielded a significant positive association, while a non-significant association (OR: 1.12 95% CI: 0.61–2.08) was found from studies that used ELISA method. Sensitivity analyses showed that the results were robust. No significant publication bias was found.

**Conclusions** The current evidence does not rule out an association between *H. pylori* and RAS. The effect of *H. pylori* on RAS varies in detection methods and sources of sample. Large samples, multiple clinical studies, and improved methods are still needed to determine the exact effect of *H. pylori* on RAS.

**Clinical significance** *H. pylori* infection may be a risk factor for the pathogenesis of RAS.

**Keywords** *Helicobacter pylori* · Recurrent aphthous stomatitis · Meta-analysis

## Introduction

Recurrent aphthous stomatitis, also known as aphthous ulcers (AU), is one of the most common oral mucosal lesions. It is characterized by recurrent round or oval superficial ulcer on the mucous membrane of the mouth, with periodic, progressive, and self-limiting features. Nearly 2 to 66% of the population suffers persistent and repeated pain causes by RAS in the world, which can interfere with important functions such as eating, drinking, swallowing, speaking, and negatively affect the quality of life [1, 2]. The etiology and pathogenesis of RAS remain unclear, but several factors have been proposed as possible etiopathogenesis, including nutritional deficiency (vitamins and minerals), microbial infections, local trauma, food allergy, psychological factors, and genetic factors [3].

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*Helicobacter pylori* (*H. pylori*) is a gram-negative, microaerophilic, rod-shaped bacteria. It was first isolated from gastritis mucosal biopsies of patients suffering from chronic gastritis in 1983 and recognized as a pathogenic factor for many upper gastrointestinal diseases, such as chronic gastritis and peptic ulcer [4, 5]. More than half of the global population is infected with *H. pylori*, and the prevalence rates vary across regions, ranging from approximately one-third in North Europe and North America, to higher than 50% in South, East Europe, South America, and Asia [6, 7]. *H. pylori* has been detected in oral cavity and has been reported to be associated with oral diseases, such as periodontitis, erosive oral lichen planus (OLP), and oral squamous cell carcinoma (OSCC) [8–10].

In recent years, there has been growing scientific interest in the association between *H. pylori* infection and RAS. Numerous studies have shown that anti-pyloric therapy can promote RAS healing, such as shortening the improvement time and reducing the recurrence rate [11]. To date, there have been three meta-analyses explored the relationship between *H. pylori* and RAS, but the results were conflicting [12–14]. Li L et al. conducted a meta-analysis in 2014 included articles limited to September 2013, which showed a positive association [12]. After the previous meta-analyses, many relevant original studies were published. It is necessary to update the pooled OR of *H. pylori* for RAS with the latest evidences. Besides, there is still lack evidence regarding the effects of test methods, sample sources on the results. Therefore, we conducted this meta-analysis included recently published studies and addressed the aforementioned shortcomings to explore the correlation between *H. pylori* infection and RAS.

## Methods

### Protocol and registration

This systematic review was performed according to Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines [15]. It was registered at PROSPERO under CRD42023404955.

### Search strategy

We searched for a detailed systematic literature published up to January 31st, 2023 from PubMed, EMBASE, Cochrane Library, and Web of Science for all relevant articles.

The following MeSH terms and combinations of words were used for the literature search: ('*Helicobacter pylori*' [MeSH] OR 'Helicobacter nemestrinae' OR 'Campylobacter pylori' OR 'Campylobacter pylori subsp. pylori' OR 'Campylobacter pyloridis') AND ('Stomatitis, Aphthous' [MeSH] OR 'Aphthous Stomatitides' OR 'Aphthous Stomatitis' OR 'Stomatitides, Aphthous' OR 'Ulcer, Aphthous' OR 'Aphthous Ulcer' OR 'Aphthous Ulcers' OR 'Ulcers, Aphthous' OR 'Aphthae' OR 'Canker Sore' OR 'Canker Sores' OR 'Sore, Canker' OR 'Sores, Canker'). Furthermore, a manual retrieval was performed to obtain additional studies (Table S1).

### Inclusion criteria and exclusion criteria

The inclusion criteria were as follows: (1) cohort studies or case-control studies; (2) the clinical diagnosis of RAS was based on the description of its clinical presentation and classification: one or several round, shallow, painful oral ulcers, recurrent from months to days apart [16]; and (3) the outcome index of interest was the number of *H. pylori* positive patients in the RAS group and the control group (non-RAS group), respectively.

The exclusion criteria were as follows: (1) cross-sectional studies, animal experiments, conference abstracts, reviews, duplicate publications, and case reports; (2) incomplete or unavailable original data; (3) studies in specific population; and (4) the low-quality studies (a score of less than 5 points).

### Data extraction

The following data items were extracted by two trained reviewers (JS & ZY) independently; any discrepancy between the two reviewers was solved by a third reviewer (YW):

We retrieved relevant information from the final selected studies:

A. Demographic and clinical characteristics: (1) the first author, (2) publication year, (3) country of the study, (4) region, (5) mean age, (6) sex ratio, (7) the number of case group (RAS patients)/control group, and (8) the number of *H. pylori* positive patient.
B. Methodological characteristics: (1) study design, (2) sample sources of the study, (3) detection methods, and (4) the effect sizes and confidence intervals reported in the studies.

### Quality assessment

The quality of the included studies was independently assessed by two researchers (JS & ZY). The Newcastle-Ottawa Scale (NOS) consisting of a nine-point scale

was used as a standard international tool to evaluate the quality of the studies [17, 18]. Studies with scores of 5 or higher were categorized as medium to high-quality studies.

### Statistical analysis

A meta-analysis was performed to assess the association between *H. pylori* infection and RAS using STATA/SE 17.0 statistical software. ORs and CIs were calculated. Since the included studies had a small total number of events, ORs were obtained using Peto's assumption-free method, which is considered the least biased and most powerful approach [19, 20]. Heterogeneity was assessed by the Cochrane $Q$ test and $I^2$ statistics. Given the substantial level of heterogeneity observed ($I^2 > 60\%$, $P > 0.05$), we employed a random effects model for the statistical analysis [21]. To explore the sources of heterogeneity, we conducted subgroup analyses based on factors such as geographic region, detection method, source of sample, study quality score, and publication year. The Funnel plots, Begg's test, and Egger's test were used to explore publication bias in the included studies [22, 23]. Sensitivity analyses were conducted to investigate the effect of each individual study on overall analysis.

## Results

### Systematic search

A systematic search identified 169 articles, including 38 articles from PubMed, 93 articles from EMBASE, 5 articles from Cochrane Library, 33 articles from Web of Science. No additional articles were identified through manual searches of reference lists. After excluding duplicate publications ($n = 38$), 131 articles remained for full-text screening. Based on the predetermined eligibility criteria, a total of 13 articles with 15 case-control studies were included in the review (Fig. 1). In instances where articles encompassed multiple studies featuring diverse clinical groups, we regarded each study as a distinct analysis.

### Study characteristics

The main characteristics of the included studies are presented in Table 1. All articles represented a range of geographical areas in Asia ($n = 6$), Europe ($n = 5$), South America ($n = 1$), Africa ($n = 1$), and Turkey, which straddles both Asia and Europe ($n = 2$). Regarding the detection methods, 7 studies used PCR (PCR and nest-PCR); 3 studies used $^{13/14}$C-UBT, 1 study used RUT; 3 studies used ELISA, and

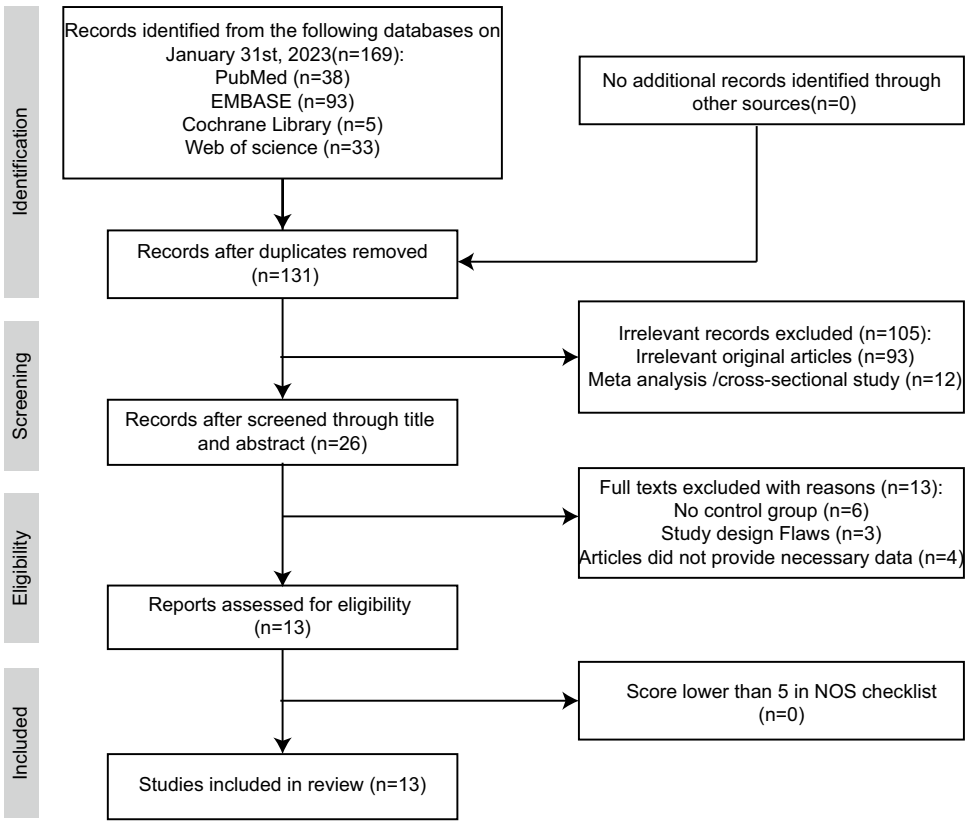


**Fig. 1** Flowchart diagram of the study selection according to Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines [24]

**Table 1** Main characteristics of included studies

| Author | Country | Region | RAS group | | | | Control group | | | | Source of controls | Diagnostic method | Sample source | Infection site | *H. pylori* infection was associated with RAS (Y/N) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Age (mean) | Sex (F/M) | Number of RAS patients | *H. pylori* positive | Age (mean) | Sex (F/M) | Number of controls | *H. pylori* positive | | | | | |
| Long et al. (2007) [25] | China | Asia | 38.7 | 50/32 | 82 | 36 | 41.3 | 38/36 | 74 | 12 | Non-RAS patients | PCR | Saliva | Intraoral | Y |
| S.H. Al-Amad et al. (2020) [26] | The United Arab Emirates | Asia | NA[a] | NA[a] | 52 | 30 | NA[a] | NA[a] | 52 | 29 | Non-RAS patients | $^{14}$C-UBT | Expired air | Gastric | N |
| Gülseren et al. (2016) [27] | Turkey | Asia/ Europe | 35.11 | 19/19 | 38 | 34 | 34.93 | 22/21 | 43 | 24 | Non-RAS patients | RUT | Dental plaque | Intraoral | Y |
| Rajendra et al. (2017) [28] | India | Asia | NA | NA | 15 | 3 | NA | NA | 15 | 9 | Non-RAS patients | Nest-PCR | Intraoral soft tissue | Intraoral | N |
| Maleki et al. (2009) [29] | Iran | Asia | 25 | 28/15 | 43 | 16 | 23.7 | 29/15 | 44 | 14 | Healthy controls | $^{13}$C-UBT | Expired air | Gastric | N |
| Iamaroon et al. (2003)a [30] | Thailand | Asia | NA | NA | 22 | 2 | NA | NA | 15 | 3 | Healthy controls | Nest-PCR | Intraoral soft tissue | Intraoral | N |
| Victória et al. (2003) [31] | Brazil | South America | NA[a] | NA[a] | 36 | 14 | NA[a] | NA[a] | 48 | 16 | Healthy controls | Nest-PCR | Intraoral soft tissue | Intraoral | N |
| Riggio et al. (2000) [32] | UK | Europe | NA | NA | 28 | 3 | NA | NA | 13 | 0 | Non-RAS patients | Nest-PCR | Intraoral soft tissue/ saliva | Intraoral | N |
| Porter et al. (1997) [33] | England | Europe | 34 | 51/24 | 75 | 23 | 26 | 19/6 | 25 | 6 | Non-RAS patients | ELISA-IgG | Venous blood | Intraoral or gastric | N |

**Table 1** (continued)

| Author | Country | Region | RAS group | | | | Control group | | | | Source of controls | Diagnostic method | Sample source | Infection site | *H. pylori* infection was associated with RAS (Y/N) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Age (mean) | Sex (F/M) | Number of RAS patients | *H. pylori* positive | Age (mean) | Sex (F/M) | Number of controls | *H. pylori* positive | | | | | |
| Saeed et al. (2020) [34] | India | Asia | 36 | 30/40 | 50 | 36 | NA | NA | 35 | 11 | Healthy controls | $^{13}$C-UBT | Expired air | Gastric | Y |
| Uyar et al. (2014)a [35] | Italy | Europe | 34.3 | 26/10 | 36 | 9 | 27.4 | 14/8 | 22 | 0 | Healthy controls | PCR | Intraoral soft tissue/ saliva | Intraoral | Y |
| Uyar et al. (2014)b [35] | Italy | Europe | 34.3 | 26/10 | 36 | 24 | 27.4 | 14/8 | 22 | 18 | Healthy controls | ELISA-IgA | Venous blood | Intraoral or gastric | N |
| Uyar et al. (2014)c [35] | Italy | Europe | 34.3 | 26/10 | 36 | 20 | 27.4 | 14/8 | 22 | 9 | Healthy controls | ELISA-IgG | Venous blood | Intraoral or gastric | Y |
| Elsheikh et al. (2005) [36] | Egypt | Africa | 24 | 47/41 | 88 | 9 | NA[a] | NA[a] | 20 | 0 | Non-RAS patients | PCR | Intraoral soft tissue | Intraoral | Y |
| Yakar T et al. (2015) [37] | Turkey* | Asia/ Europe | 40 | 21/15 | 36 | 27 | 50 | 71/59 | 130 | 87 | Non-RAS patients | Gastroscope | Intragastric tissue | Gastric | Y |

*RUT* rapid urease test, *PCR* polymerase chain reaction, *Nest-PCR* nested polymerase chain reaction, *$^{13}$C-UBT* $^{13}$C-urea breath test, *$^{14}$C-UBT* $^{14}$C-urea breath test, *ELISA* enzyme-linked immunosorbent assay

Turkey*: Turkey located at the border was included in the both Asian and Europe groups in subgroup analysis

NA[a]: In this project, the original data of the experimental group and the control group were not provided in the original article, but were matched

Y: *H. pylori* infection was associated with RAS

N: *H. pylori* infection was not associated with RAS

1 study used gastroscopy. In terms of sample sources, there were 8 studies from intraoral tissues, 4 studies from gastric tissue, and 3 studies from venous blood. The results for assessment of quality according to the NOS are presented in Table 2 and Fig. S4.

## Main results of the included studies

In the RAS patient group, 601 subjects were included, out of which 242 cases were *H. pylori* positive. In the control group, 536 cases were included, with 211 positive for *H. pylori*. The estimated results indicated a statistically significant association between *H. pylori* infection and RAS, with OR value was 1.83 (95% CI: 1.41–2.37, $P = 0.001$, as shown in Fig. 2). These findings suggested that *H. pylori* infection may be linked to RAS. Fig. 2 shows forest plot of the relationship between *H. pylori* infection and RAS.

## Subgroup analyses and meta regression

Subgroup analysis (Table 3) revealed significant positive associations between *H. pylori* infection and the risk of RAS in studies conducted in Europe (OR: 1.90, 95% CI: 1.26–2.86) and Asia (OR: 1.97, 95% CI: 1.44–2.71) (Fig. S1). Stratification by different *H. pylori* detection methods showed a significant positive association in studies using PCR (OR: 2.03, 95% CI: 1.31–3.15) and UBT (OR: 1.83, 95% CI: 1.13–2.96), rather than in studies using ELISA (OR: 1.12, 95% CI: 0.61–2.08)

**Table 2** Quality assessment of included studies using the Newcastle-Ottawa Scale (case-control studies)

| Author | Selection | | | | Comparability | Exposure | | Non-response rate | Total scores |
|---|---|---|---|---|---|---|---|---|---|
| | Is the case definition adequate | Representativeness of the cases | Selection of controls | Definition of controls | Comparability of cases and controls on the basis of the design or analysis | Ascertainment of exposure | Same method of ascertainment for cases and controls | | |
| Long et al. (2007) [25] | ★ | ★ | ☆ | ★ | ★ | ☆ | ★ | ★ | 6 |
| S.H. Al-Amad et al. (2020) [26] | ★ | ★ | ☆ | ★ | ★★ | ☆ | ★ | ★ | 7 |
| Gülseren et al. (2016) [27] | ★ | ★ | ☆ | ★ | ★★ | ☆ | ★ | ★ | 6 |
| Rajendra et al. (2017) [28] | ★ | ★ | ☆ | ★ | ★ | ☆ | ★ | ★ | 6 |
| Maleki et al. (2009) [29] | ★ | ★ | ☆ | ★ | ★★ | ☆ | ★ | ★ | 7 |
| Iamaroon et al. ( 2003) [30] | ★ | ★ | ☆ | ★ | ★ | ☆ | ★ | ★ | 6 |
| Victória et al. ( 2003) [31] | ★ | ★ | ☆ | ★ | ★★ | ☆ | ★ | ★ | 7 |
| Riggio et al. (2000) [32] | ★ | ★ | ☆ | ★ | ☆ | ☆ | ★ | ★ | 5 |
| Porter et al. (1997) [33] | ★ | ★ | ☆ | ★ | ★★ | ☆ | ★ | ★ | 7 |
| Saeed et al. (2020) [34] | ★ | ★ | ☆ | ★ | ☆ | ☆ | ★ | ★ | 5 |
| Uyar et al. (2014) [35] | ★ | ★ | ☆ | ★ | ★ | ☆ | ☆ | ★ | 5 |
| Elsheikh et al. (2005) [36] | ★ | ★ | ☆ | ★ | ★★ | ☆ | ★ | ★ | 7 |
| Yakar et al. (2015) [37] | ★ | ☆ | ☆ | ★ | ★ | ☆ | ★ | ★ | 5 |

★Indicates that the study met this entry

☆Indicates that the study did not meet the entry

★★A maximum of two stars can be obtained in comparability items, indicating that both the most important factor and other important confounders have been controlled in the study

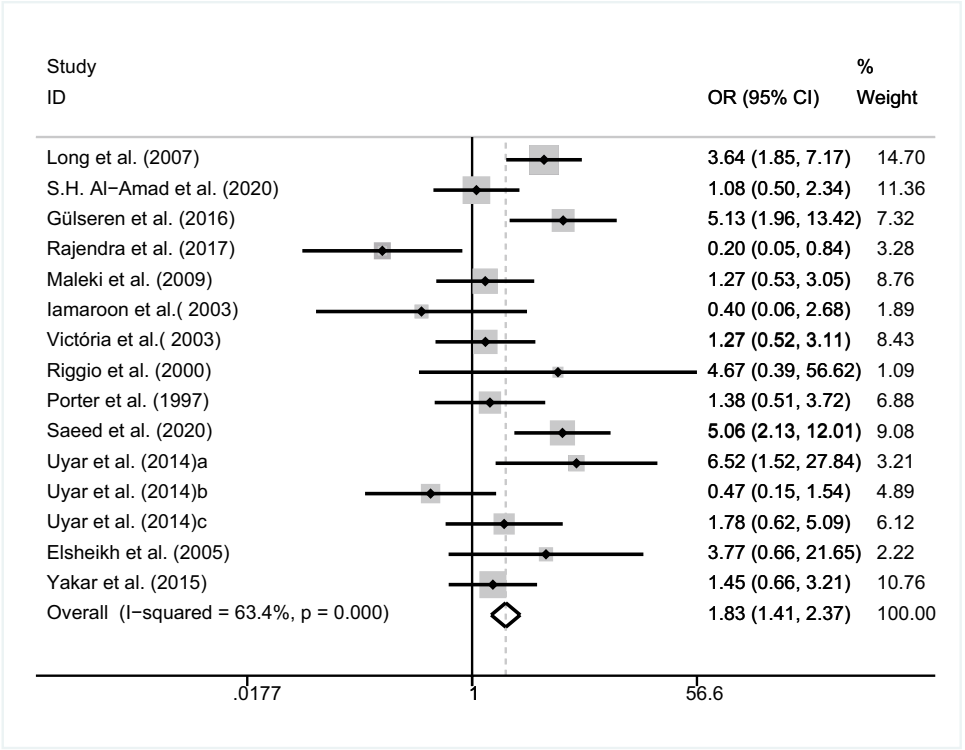


**Fig. 2** A forest plot of the relationship between *H. pylori* infection and oral ulceration

(Fig. S2). Furthermore, an association between *H. pylori* and RAS was found in samples from oral (OR: 2.38, 95% CI: 1.60–3.56) and gastric (OR: 1.72, 95% CI: 1.14–2.60) samples but not venous blood (Fig. S3).

A meta-regression was conducted to explore possible sources of heterogeneity. There were significant differences in the heterogeneity between strata by detection method (*P* in meta-regression = 0.032).

### Sensitivity analysis

A sensitivity analysis was performed to evaluate the stability and reliability of the meta-analysis. Similar results were obtained when each study was sequentially excluded, indicating that no individual study had a significant impact on the overall risk estimates (Fig. 3). To assess the potential publication bias, we examined the symmetry of the funnel plot (Fig. S6) with Begg's (Fig. S7) and Egger's tests (Fig. S8). The results indicated a low risk of publication bias. There was no substantial publication bias detected in any of the analyses.

## Discussion

The etiology of RAS still remains unclear. Most attempts to culture *H. pylori* from oral samples have failed [38–40]. While *H. pylori* has been detected on RAS lesions, a definitive connection between microbial infection and RAS has not yet to be established [41]. In this study, our meta-analysis provides evidence to the correlation between *H. pylori* infection and RAS.

Our results showed that *H. pylori* was found to be significantly associated with RAS. To the best of our knowledge, *H. pylori* infection can effect gastric mucosal inflammation and may also act on systemic autoimmune responses [42, 43]. The exact mechanism of tissue damage induced by *H. pylori* is not clear. Many researchers suggest the immune-mediated mechanisms induced by *H. pylori* in the development of RAS. *H. pylori* has the ability to stimulate the production of immune cytokine, particularly IL-8 and lymphocyte chemotactic factors which plays a role inthe formation of particular T lymphocyte subpopulations. Infiltrating neutrophils can also be activated by *H. pylori* or its extracts, resulting in the production of reactive oxygen metabolites (hydrogen peroxide and hypochlorous acid) [44]. Furthermore, the involvement of cytokine production, over expression of lymphocyte adhesion molecules, and the recruitment of specific subsets of T-lymphocytes have been demonstrated to play a role in both RAS and *H. pylori*–associated gastritis [11].

The subgroup analyses conducted by regions revealed a positive association between the risk factor *H. pylori* and RAS in Europe and Asia. Although heredity is widely recognized as one of the well-established underlying causes of RAS [45], its geographical variations have not been reported.

**Table 3** Pooled odds ratios (ORs) and estimates (95% confidence interval (CI)) in subgroup analyses based on region, detection method, source of sample, study quality score, and publication year

| Subgroup | No. of studies | Cases with *HP* (+) | Controls with *HP* (+) | OR (95 % CI) | *Z* | *P* value | Tests of heterogeneity | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | *Q* | df | *P* value for heterogeneity | $I^2$ (%) |
| Region | | | | | | | | | | |
| Asia [25–30, 34, 37] | 8 | 184/338 | 189/408 | 1.97 (1.44–2.71) | 4.19 | 0.00 | 27.84 | 7 | 0.000 | 74.90 |
| Europe [27, 32, 33, 35, 37] | 7 | 14/22 | 16/32 | 1.90 (1.26–2.86) | 3.06 | 0.02 | 11.96 | 6 | 0.035 | 55.80 |
| South America [31] | 1 | 140/285 | 144/277 | 1.27 (0.52–3.11) | 0.52 | 0.60 | 0.00 | 0 | NA | NA |
| Africa [36] | 1 | 9/79 | 0/20 | 3.77 (0.66–21.65) | 1.49 | 0.13 | 0.00 | 0 | NA | NA |
| Detection method | | | | | | | | | | |
| PCR [25, 28, 30–32, 35, 36] | 7 | 76/307 | 40/207 | 2.03 (1.31–3.15) | 3.14 | 0.002 | 20.09 | 6 | 0.003 | 70.10 |
| UBT [26, 29, 34] | 3 | 82/145 | 54/131 | 1.83 (1.13–2.96) | 2.46 | 0.014 | 7.80 | 2 | 0.020 | 74.30 |
| ELISA [33, 35] | 3 | 67/147 | 33/69 | 1.12 (0.61–2.08) | 0.37 | 0.711 | 2.96 | 2 | 0.228 | 32.40 |
| RUT [27] | 1 | 34/38 | 24/43 | 5.13 (1.96–13.42) | 3.33 | 0.001 | 0.00 | 0 | NA | NA |
| Gastroscope [37] | 1 | 27/36 | 87/130 | 1.45 (0.66–3.21) | 0.92 | 0.357 | 0.00 | 0 | NA | NA |
| Source of sample | | | | | | | | | | |
| Oral [25, 27, 28, 30–32, 35, 36] | 8 | 110/345 | 64/250 | 2.38 (1.60–3.56) | 4.25 | 0.000 | 23.04 | 7 | 0.002 | 69.6 |
| Gastric [26, 29, 34, 37] | 4 | 109/181 | 141/261 | 1.72 (1.14–2.60) | 2.58 | 0.010 | 8.04 | 3 | 0.045 | 62.7 |
| Blood [33, 35] | 3 | 67/147 | 33/69 | 1.12 (0.61–2.08) | 0.37 | 0.711 | 2.96 | 2 | 0.228 | 32.4 |
| Study quality score | | | | | | | | | | |
| > 5 [25–31, 33, 36] | 9 | 167/451 | 113/336 | 1.69 (1.22–2.34) | 3.19 | 0.001 | 23.78 | 8 | 0.002 | 66.40 |
| = 5 [32, 34, 35, 37] | 6 | 119/222 | 125/244 | 2.11 (1.36–3.28) | 3.34 | 0.001 | 13.78 | 5 | 0.017 | 63.70 |
| Publication year | | | | | | | | | | |
| 1990–2000 [32, 33] | 2 | 26/103 | 6/38 | 1.63 (0.65–4.09) | 1.04 | 0.300 | 0.79 | 1 | 0.373 | 0.0 |
| 2001–2010 [26, 29–31, 36] | 5 | 77/271 | 45/201 | 1.96 (1.27–3.03) | 3.05 | 0.002 | 8.25 | 4 | 0.083 | 51.5 |
| 2011– [25, 27, 28, 34, 35, 37] | 8 | 183/299 | 187/341 | 1.78 (1.25–2.52) | 3.24 | 0.001 | 28.97 | 7 | 0.000 | 75.8 |
| All studies | 15 | 242/601 | 211/536 | 1.83 (1.41–2.37) | 2.08 | 0.040 | 35.69 | 14 | 0.634 | 60.80 |

*PCR* polymerase chain reaction, *UBT* $^{13}$C-urea breath test or $^{14}$C-urea breath test, *RUT* rapid urease test, HP *H. pylori*

*Turkey is included in the Asian and Europe subgroups due to its geographical location between the two

Gastroscope: enzyme-linked immunosorbent assay

Oral: The objective was to investigate the correlation between RAS and oral *H. pylori* and exclude the influence of gastric *H. pylori* infection on the test results

Gastric: The objective was to investigate the correlation between RAS and *H. pylori* in stomach and to exclude the influence of oral *H. pylori* infection on the test results

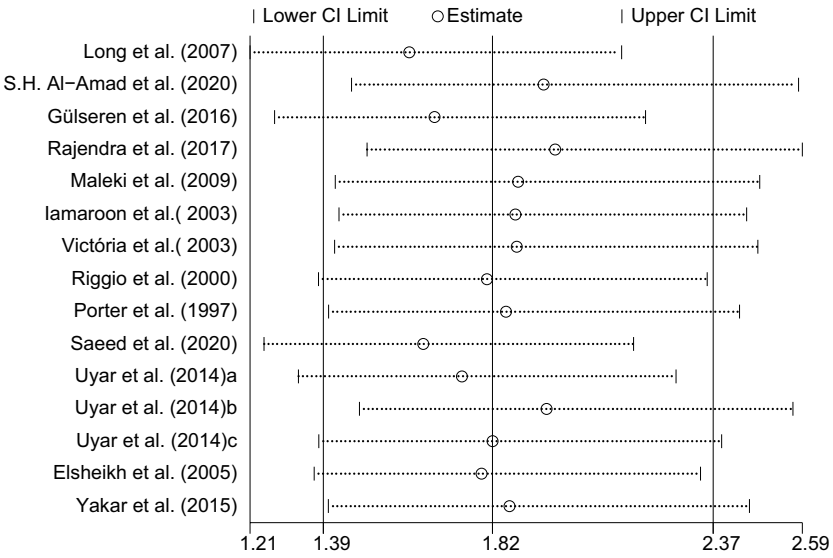


**Fig. 3** Sensitivity analysis of the relationship between *H. pylori* and RAS

The results in South America and Africa subgroups were affected by the limitation of study number, since there was only one study included in each subgroup.

Considering the effect of detection methods and sample sources on the results, we performed subgroup analyses based on these two factors. In the studies using PCR and UBT, we found a positive association between *H. pylori* and RAS. However, no statistically significant difference was found when ELISA was used as the detection method. Currently, the combination of culture and histologic examination is generally regarded as the "gold standard" for the diagnosis of *H. pylori* [44–46]. The detection of *H. pylori* in oral cavity mostly relied on traditional methods, such as RUT, immunological method, PCR, and UBT. UBT is one of the commonly used detection methods for gastric *H. pylori* in clinic, which requires $^{13}C$ and $^{14}C$ to react with *H. pylori* [47]. Whether UBT is suitable for detecting *H. pylori* in the oral cavity is still worth exploring, given the differences in oral and gastric micro-environment. PCR detection has the advantages of high sensitivity, strong specificity, and low requirements for specimens compared to other diagnostic methods [47, 48]. But the possible transmission of *H. pylori* by the gastro-oral route and specificity of primers may lead to false positive results [38, 49]. In RUT detection, the urease produced by *H. pylori* breaks down urea to form ammonia, which increases the *PH* value of mucosal tissue and changes the color of the detection indicator [47]. However, the oral cavity has many bacteria with a weak alkaline *PH* value, which may easily lead to false positives [50]. ELISA is a non-invasive, simple, and inexpensive method for the diagnosis of *H. pylori* infection. While, the serology ELISA detection was unable to distinguish infection status and site [48, 51]. Given the comparative analysis of the above detection mechanism, PCR and UBT methods are relatively accurate, which is also the source of heterogeneity in this meta-analysis.

Serology detection of *H. pylori* in studies often used ELISA, which could not distinguish the infection site, and the serum antibodies could persist for a period of time after the eradication of the pathogen [52]. This may lead to the inaccurate results, and the limited number of studies may also lead to false negative results. Therefore, the result of ELISA detection methods was consistent with that of serum source in subgroup analyses.

Our meta-analysis included the latest research in recent years compared to previous meta-analyses. We strictly screened the study design and RAS diagnostic basis of the included studies, which increased the comparability of study data and the authenticity of the results. We also performed subgroup analyses according to region, detection method, and source of sample. This study aims to provide new insights into the relationship between *H. pylori* and RAS. To better interpret the results, some limitations of this meta-analysis should be noted, such as the small sample size of each included study, the absence of a cohort study with high reliability, and the poor quality of the included articles. Furthermore, this study did not analyze the source of *H. pylori*. There is still controversy on whether *H. pylori* is resident or transient in oral cavity. Many literatures suggest a correlation and homology between oral *H. pylori* and gastric *H. pylori* [24, 53–55]. In oral sample collection, many researches did not consider reflux and hiccup, which may bring *H. pylori* from the stomach to the oral [49]. Therefore, for exploring the relationship between different sources of *H. pylori* and RAS, careful sampling techniques can help reduce the likelihood of contamination, for instance, taking oral samples at least 2 h after reflux [49]. For many detection methods, the positive results may not mean the *H. pylori* infection since the potential cross-reactions with other

bacteria. The whole-genome sequencing of the isolates and subsequent deposition of the organisms in a culture collection are needed to confirm the *H. pylori* colonization of the oral cavity [49]. If possible, comparing the genetic characteristics of the oral *H. pylori* strains to strains isolated from the stomach can provide insights into whether they are similar or distinct. This may help us clarify the association between *H. pylori* and oral diseases.

## Conclusion

In conclusion, the current evidence does not rule out an association between RAS and *H. pylori*. The effect of *H. pylori* on RAS varies in detection methods and the sources of sample. However, due to the limitations of oral *H. pylori* detection methods, we cannot determine the source of *H. pylori* in detection samples definitely. Large samples, multiple clinical studies, and improved detection methods are still needed to determine the exact effect of *H. pylori* on RAS.

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**Data availability** The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author.

## Declarations

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Consent to participate** For this type of study, formal consent is not required.

**Competing interests** The authors declare no competing interests.

**Registration** The review was registered in PROSPERO (ref: CRD42023404955).

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