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Meta-analysis: efficacy of bovine lactoferrin in *Helicobacter pylori* eradication

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SUMMARY

Background
Several randomized-controlled trials (RCTs) have sought to determine the efficacy of bovine lactoferrin in *Helicobacter pylori* eradication with equivocal results.

Aim
To evaluate the effect of bovine lactoferrin supplementation in *H. pylori* eradication.

Methods
Electronic databases, reviews, bibliographies, abstracts and conference proceedings were searched. Included trials had to be randomized or quasi-randomized and controlled, using bovine lactoferrin in the intervention group, treating *Helicobacter*-infected subjects and evaluating eradication of *H. pylori* as an outcome.

Results
The search identified five eligible RCTs (of 169). Data were available for 682 subjects (bovine lactoferrin group-n = 316; control group-n = 366). The pooled odds ratio (five studies) for eradication by intention-to-treat analysis was 2.22 (95% CI 1.44–3.44; P = 0.0003) using the fixed effects model (FEM) and 2.24 (95% CI 1.15–4.35; P = 0.0003) using the random effects model (REM) (Cochran's Q = 6.83; P = 0.145). The pooled risk difference was 0.11 (95% CI 0.05–0.16; P = 0.0001) by FEM (Cochran's Q = 6.67; P = 0.154) and 0.10 (95% CI 0.04–0.17; P = 0.0023) by REM. There was no significant difference in incidence of adverse effects.

Conclusion
Bovine lactoferrin potentially improves *H. pylori* eradication rates without any impact on adverse effects, but available evidence is limited and further research is necessary to confirm the findings.

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INTRODUCTION

Helicobacter pylori infection is a global public health problem with reported prevalence rates of up to 70–90% in developing countries.[1] Standard triple therapy (proton pump inhibitor with clarithromycin with amoxicillin/nitroimidazole) achieves eradication rates ranging from 55% to 90%; 10–45% of patients fail to eradicate the bacteria and remain *H. pylori*-positive.[2] The proportion of patients achieving eradication has been progressively falling with the emergence of antibiotic resistant strains.[3] The second-line quadruple regimens are further limited by poor patient compliance because of side effects, number of tablets per day and long duration.[4] In this context, the search for alternative or complementary therapies is gaining urgency.

Many antimicrobial substances have been studied for their usefulness in eradicating *H. pylori* infection, either as a single agent or as a complementary therapy.[5, 6] One such novel therapy is bovine lactoferrin (bLF) (glycoprotein). bLF, an 80-kDa glycoprotein found in cow's milk has demonstrated antibacterial activity against *H. pylori in vitro*[7] and *in vivo*.[8] Several human, randomized-controlled trials (RCTs) in the recent past have sought to determine the efficacy of bLF in *H. pylori* eradication with or without co-interventions; however, the evidence has been limited and equivocal with some authors reporting efficacy and others lack of it. Thus, this systematic review was undertaken to assess the effectiveness of bLF alone or in combination with standard regimens to guide clinical practice and research.

METHODS

Searches

We conducted searches in MEDLINE, EXTENDED MEDLINE (1950 to 26 March 2008) using the following search words (Helicobacter or H pylori) AND (Lactoferrin OR Lactotransferrin OR bovine Lactoferrin) with limits pertaining to 'human' subjects for clinical trial, review, meta-analysis and RCT. We conducted similar searches in EMBASE (1980 to week 12 2008), CIAP Full Text Articles, AMED (1985 to 26 March 2008), Medscape (26 March 2008), KoreaMed (1997 to 26 March 2008), MEDIND (26 March 2008) and Cochrane Controlled trials register (first quarter 2008). We imposed no age or language restrictions. We also reviewed reference lists of identified articles and hand searched reviews, bibliographies of books and abstracts. Furthermore, we searched abstracts of major gastroenterological meetings, such as the Digestive Disease, Week of the American Gastroenterological Association, the World Congress of Gastroenterology, European and *H. pylori* Study Group. Authors of some identified trials were asked whether they knew of additional studies, including unpublished randomized ones. We scanned the title and abstract of the trials identified in the computerized search to exclude studies that were obviously irrelevant (Figure 1). We scrutinized the full texts of the remaining studies to identify trials that were relevant and fulfilled the inclusion criteria.

Selection criteria

To be included, trials had to be randomized or quasi-randomized and controlled, using a lactoferrin

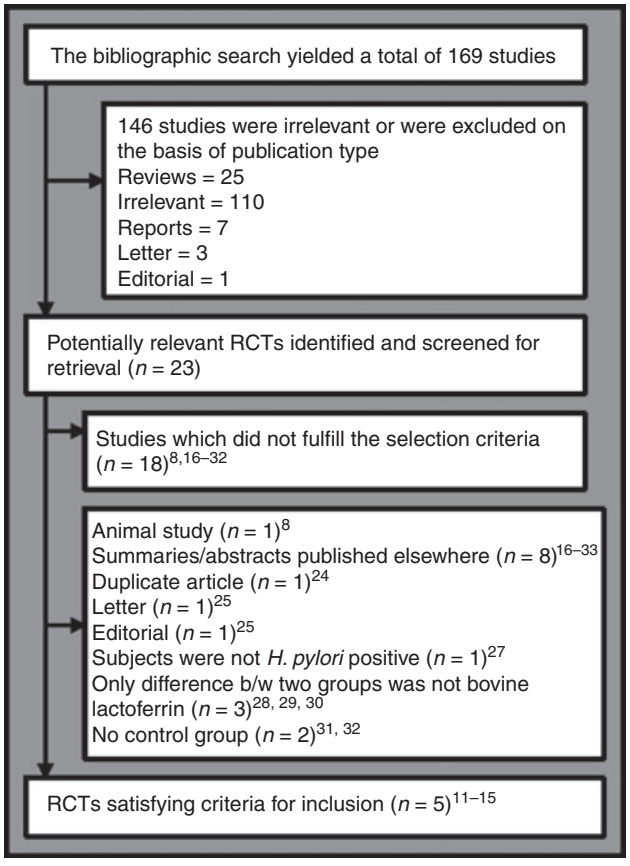

Figure 1. Trial flow for selection of randomized controlled trials to be included in meta-analysis RCT-randomized controlled trial.

in the intervention group, treating *H. pylori* infected subjects (confirmed on urea breath test or histology or stool antigen test; symptomatic or asymptomatic) and evaluating eradication of *H. pylori* as an outcome (confirmed on urea breath test or histology or stool antigen test). We considered studies in which other drugs were simultaneously administered to be eligible if the only difference between the intervention and control groups was the lactoferrin. The criteria were intended to select all studies, which would help evaluate the efficacy [odds ratio (OR)] of bLF irrespective of its use for *H. pylori* eradication as the only medication, in combination with standard therapy or in combination with rescue therapy.

Validity assessment

We assessed the quality of trials by using recommended criteria.[9, 10] We categorized randomization into randomized, quasi-randomized, not stated or unclear. We classified concealment of allocation as adequate, unclear or inadequate. To assess attrition, studies were divided by the percentage of participants lost to follow-up (<4.9%, 5–9.9%, 10–19.9% and ≥20%). For this calculation, we considered the number of patients available at the last follow-up (at which data were retrievable). We graded blinding as double blinding, single blinding, no blinding or unclear.

Data abstraction

Standardized data abstraction sheets were prepared. Data were extracted for study quality, type and duration of treatment, anti-*H. pylori* regimens, and the number and age of enrolled subjects, diagnostic methods of testing *H. pylori* infection before enrolling and after completing study and documented side effects. The key outcome data recorded included eradication rates, adverse events including occurrence of diarrhoea, nausea, taste disturbance or constipation. All articles were examined independently for eligibility by two reviewers (AS and JN). The data included in this review were derived from the published papers or published abstracts or provided by the authors. If needed and wherever possible, we contacted the authors for clarifications. When the results of a particular study were reported in more than one publication, only the most recent and complete data were included in the meta-analysis.

Statistical analysis

Data were entered into the MIX software v 1.7 (Kitasato Clinical Research Center, Evidence Synthesis division-MIX, Sagamihara, Kanagawa, Japan). The primary outcome measure evaluated was the ORs of *H. pylori* eradication rates of the two groups (bLF vs. without bLF). A similar evaluation of individual adverse effects and the number of subjects with any adverse event was conducted as a secondary outcome. Eradication rates were used as directly specified (four studies)[11–14] or imputed using pre- and post-urease breath test (UBT) values (from scatter graphs in the study by Okuda *et al.*) and an assumed UBT eradication value of 5‰.[15] For one study,[11] both groups using standard triple therapy (for 7 or 10 days) were pooled as the control group as these were compared to a third group using bLF + triple therapy for 7 days. Heterogeneity between the studies was assessed by Cochran's *Q*-test and by visual inspection (non-overlapping) of the confidence intervals on the forest plot. Statistical significance for the test of heterogeneity was set at 0.10. Eradication rates were calculated by an intention-to-treat and by a per-protocol analysis. To assess the stability of the results, we performed an exclusion sensitivity analysis. Egger's regression test (weighted least squares) was used to detect any publication bias.

Subanalyses for the meta-analysis were planned depending on symptoms before enrolment and age of subjects if the total number of studies exceeded 10.

We chose to present the results by the fixed and random effects models as the number of studies was small and the Cochrans test of heterogeneity was significant on per protocol analysis.

RESULTS

The bibliographic search yielded a total of 169 publications from all specified sources after excluding duplicates. These articles were screened for relevance and fulfilment of selection criteria. Of the 23 potentially relevant publications, 12 publications represented abstracts of studies published later elsewhere,[16–23] duplicate publications,[24] animal studies,[8] letters to editor[25] or editorials[26] and were hence found irrelevant on detailed full text evaluation. Thus, of the 11 human trials on the subject, in one of the trials, all recruited subjects were not positive for *H. Pylori*;[27] in three of the studies, the only difference between the two groups was not bLF (the two groups had more than one differ-

Table 1. Baseline characteristics of included studies

Study	Location, income group*	Age group	Methods of randomization†, allocation concealment‡, lost to follow-up§, blinding¶	Inclusion criteria	Exclusion criteria	Test used for confirmation of *Helicobacter pylori* at initiation, cut off value, test used for confirmation at conclusion	bLF dose (mg), no. times/day, no. weeks	Co-intervention**
Di Mario *et al.*[11]	Italy, Europe, A	Adult	RCT, D, A, C	*Helicobacter pylori*-positive patients suffering from dyspeptic symptoms, gastritis and peptic ulcer disease	Previous attempt at *H. pylori* eradication, h/o of definitive acid lowering surgery, reflux oesophagitis >A previous oesophageal surgery, treatment with PPI within last 2 weeks or any antibiotics within the last 4 weeks before study enrolment, proved allergy from clarithromycin or benzimidazole, pregnant or lactating females, having chronic renal or hepatic disease and subjects with any neoplasm	Histology plus UBT or histology plus *H. pylori* stool antigen test, ≥5‰, UBT or *Helicobacter pylori* stool antigen (HPSA) test	200 mg, 2, 1	Triple therapy (PPI, C, T)
Di Mario *et al.*[12]	Italy, Europe, A	Adult	RCT, D, A, C	*Helicobacter pylori*-positive patients suffering from dyspeptic symptoms, gastritis and peptic ulcer disease	Previous *H. pylori* eradication therapy, h/o of definitive acid lowering surgery, reflux oesophagitis >A, previous oesophageal surgery, treatment with PPI within last 2 weeks or any antibiotics within the last 4 weeks before study enrolment, proved allergy to clarithromycin or benzimidazole, pregnant or lactating females, having chronic renal or hepatic disease and subjects with any neoplasm	Histology plus UBT or histology plus *H. pylori* stool antigen test, ≥5‰, UBT or HPSA test	200 mg, 2, 1	Triple therapy (PPI, C, T)

Table 1. Continued

Study	Location, income group*	Age group	Methods of randomization†, allocation concealment‡, lost to follow-up§, blinding¶	Inclusion criteria	Exclusion criteria	Test used for confirmation of *Helicobacter pylori* at initiation, cut off value, test used for confirmation at conclusion	bLF dose (mg), no. times/day, no. weeks	Co-intervention**
Zullo *etal.*[13]	Italy, Europe, A	Adult	RCT, D, A, C	Patients with dyspepsia defined as pain or discomfort centred in upper abdomen and referred for upper endoscopy by primary care physician	Patients taking PPI, H2 receptor antagonist, antibiotics in the 4 weeks preceding the study, patients with known antibiotic allergy and those with hepatic impairment or kidney failure, patients with peptic ulcer	Endoscopy for histology and rapid urease test, ≥5‰, UBT	200 mg, 2, 1	Triple therapy (PPI, C, A)
Tursi *etal.*[14]	Italy, Europe, A	Adult	CCT, C, A, C	Patients with persistent *H. pylori* infection after failure of a 1st std treatment schedule. Patients with h/o of peptic ulcer or with gastritis with severe histological abnormalities (atrophy, diffuse intestinal metaplasia, dysplasia) or with a family h/o of gastric cancer	Patients who had antibiotics, bismuth compounds, PPI or H2 blockers in the month preceding the study, pregnancy, previous gastroduodenal surgery, allergy to penicillin or to nitroimidazoles or concomitant serious illnesses (renal or hepatic insufficiency)	Confirmed by endoscopy and biopsy, NA, Confirmed by endoscopy and biopsy in subjects where clinically relevant (peptic ulcer, duodenal ulcer, severe histological abnormality) or by UBT	200 mg, 2, 1	Quadruple therapy (PPI, RBC, T, A)

Table 1. Continued

Study	Location, income group*	Age group	Methods of randomization†, allocation concealment‡, lost to follow-up§, blinding¶	Inclusion criteria	Exclusion criteria	Test used for confirmation of *Helicobacter pylori* at initiation, cut off value, test used for confirmation at conclusion	bLF dose (mg), no. times/day, no. weeks	Co-intervention**
Okuda *etal.*[15]	Japan, Asia, A	Adult & Child	CCT, B, A, A	*Helicobacter pylori*-infected healthy volunteers without GI symptoms or minimal UGIT symptoms who were not being treated	H/o of milk intolerance and use of antimicrobial and/or antiacid drugs within 4 weeks before or during the study	UBT and serum based or urine based ELISA, >2.5% (children) and >3.5 (adults) UBT	200 mg, 2, 12	None

NA, not available, bLF, bovine lactoferrin.
* Income group (according to World Bank list of economies):[32] A, high income; B, upper middle income; C, not specified.
† Randomization: randomized – RCT, quasi randomized – CCT, not stated or unclear – CCT, Using a predetermined or biases method – X, not sure – NS.
‡ Allocation concealment: A, adequate; B, unclear; C, inadequate; D, not used.
§ Loss to follow-up based on percentage of excluded participants: A, <4.9%; B, 5–9.9%; C, 10–19.9%; D, ≥20%; E, not mentioned.
¶ Blinding: A, double blinding; B, single blinding; C, no blinding; D, unclear.
** PPI, proton pump inhibitor; A, amoxycillin; C, clarithromycin; T, tinidazole; RBC, ranitidine bismuth citrate.

ence; therefore, the effect could not be attributed to lactoferrin[28–30]), while in two trials, there was no control group (they were not RCTs.[31, 32] We therefore included five trials in this systematic review.[11–15]

Study characteristics

Table 1 summarizes the characteristics of the included trials. Five RCTs involving 682 participants (316 in the experimental group and 366 in the control group) met our predefined inclusion criteria. As depicted, most studies ($n = 4$) were conducted on symptomatic *H. pylori* positive adults except for the one conducted on symptomatic or asymptomatic adults and children.[15] The initial screening test used for confirmation of *H. pylori*, the cutoff for eradication and the test used for confirmation of eradication were variable as specified in Table 1. One study was conducted in patients with treatment failure on standard triple therapy regimen.[14] Four of the studies co-administered various standard [triple ($n = 3$),[11–13] quadruple ($n = 1$)[14]] therapy regimens. All the five studies were conducted in high income countries[11–15] (World Bank List of economies).[33] The treatment was administered for 1 week in all except one study[15] (12 weeks).

Methodological quality

The methodological quality of the studies is reported in Table 1. As depicted, most studies were not of good quality. Three of the studies did not conceal allocation[11–13] and in two studies it was inadequate[14] or unclear.[15] Four studies conducted intention-to-treat analysis.[11–14] Only one study was double-blinded and placebo-controlled.[15]

Eradication rate

Eradication OR was available for 682 subjects (five studies; 316 in the experimental group and 366 in the control group). The pooled OR by intention-to-treat analysis in the bLF vs. non-bLF group was 2.22 (1.44–3.44; $P = 0.0003$) and 2.24 (1.15–4.35; $P = 0.0003$) using the fixed effects model (Figure 2a) and random effects model (Figure 2b) respectively [Cochran's $Q = 6.83$; $P = 0.145$; $I^2 = 41.42\%$ (95% CI 0–78.42)]. On exclusion sensitivity analysis, it was noted that the results were rendered insignificant on exclusion of any one of two studies[11, 12] by random effects model (Figure 3a), but this was not the case with fixed effect model (Figure 3b). There was no evidence of publication bias (Egger's weighted least squares method; P for bias = 0.88).

Risk difference data were available for all the five studies. The pooled risk difference was 0.11 (95% CI 0.05–0.16; $P = 0.0001$) by the fixed effects model (Cochran's $Q = 6.67$; $P = 0.154$) and 0.10 (95% CI 0.04–0.17; $P = 0.0023$) by random effects model.

Similar results with per protocol analysis were available for 606 subjects (four studies; treatment group = 276; control group = 330[11–14]).The pooled OR of eradication was 2.63 (95% CI 1.61–4.31) by the fixed effects model and 2.69 (95% CI 1.02–7.10; $P = 0.0459$) by the random effects model (Cochran's $Q = 9.83$; $P = 0.0201$). Exclusion of any one of two studies were noted to impact the results[11, 12] and there was no evidence of publication bias (Eggers' weighted least squares: P-value = 0.774) using either the fixed or random effects models.

Change in UBT values

Only one study by Okuda *et al.*[15] has reported on the actual UBT values before and after treatment with bLF. The author noted that 10 of 31 bLF treated subjects and 1 of 28 control subjects ($P = 0.01$) had a positive response (positive response as more than 50% decrease in UBT at end of administration). Although the authors have not reported any comparison of change in UBT between the two groups, when the UBT values were imputed from the graphs provided (least count 1%) and the difference in change calculated, we noted that the difference was statistically insignificant (mean difference 7.17 $\pm$ 5.16; $P = 0.169$). Also, the UBT values rose back to baseline levels by 4 weeks after end of administration.

Adverse effects

Data on number of subjects with any one or more adverse effects was available for 682 subjects (five studies; treatment group = 316; control group = 366).[11–15] The pooled OR by fixed effects model was 0.73 (95% CI 0.44–1.20; $P = 0.22$; Figure 4). There was no evidence of publication bias for this measure (Eggers weighted least squares: P-value = 0.918). Exclusion of any one of the trials did significantly change the outcome. Details of the adverse effects were provided in two studies. Tursi

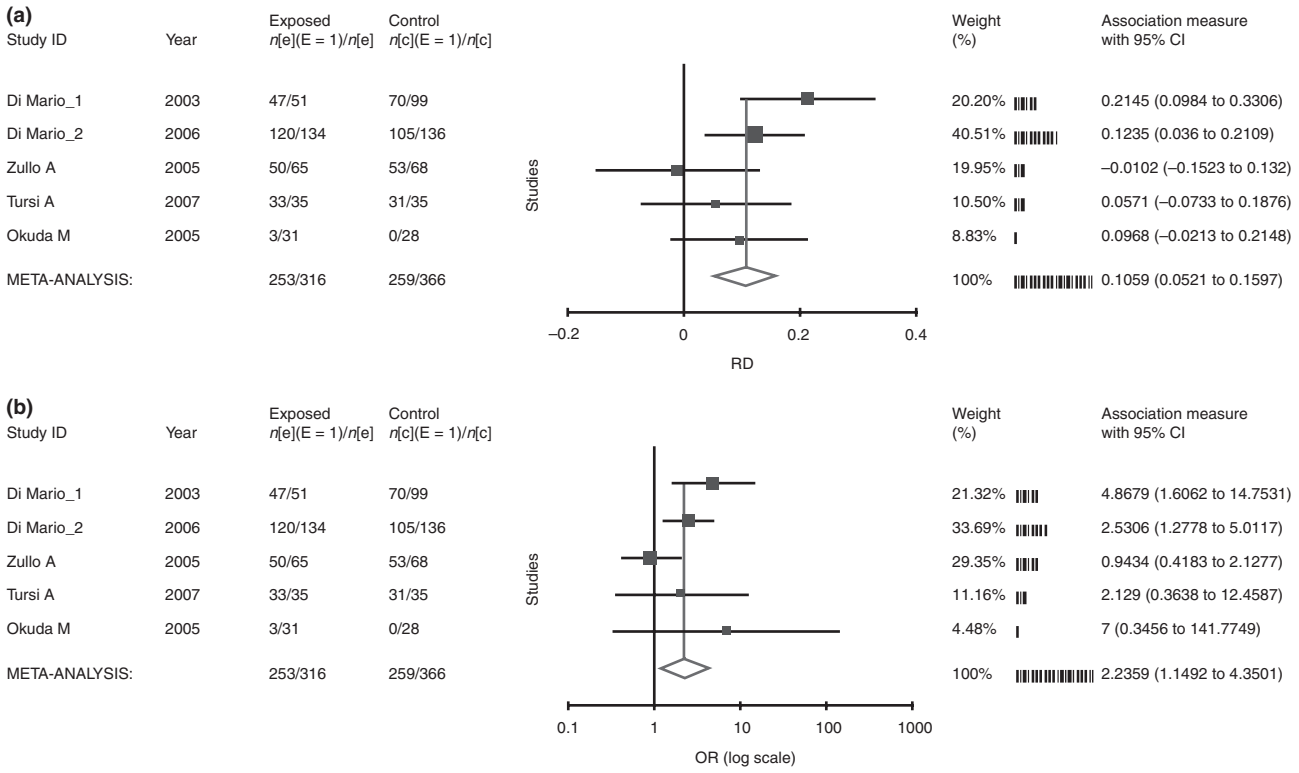

Figure 2. (a) Annotated forest plot of odds ratio of *Helicobacter pylori* eradication in supplemented (bLf) vs. control groups by fixed effects model. (b) Annotated forest plot of odds ratio of *H. pylori* eradication in supplemented (bLf) vs. control groups by random effects model.

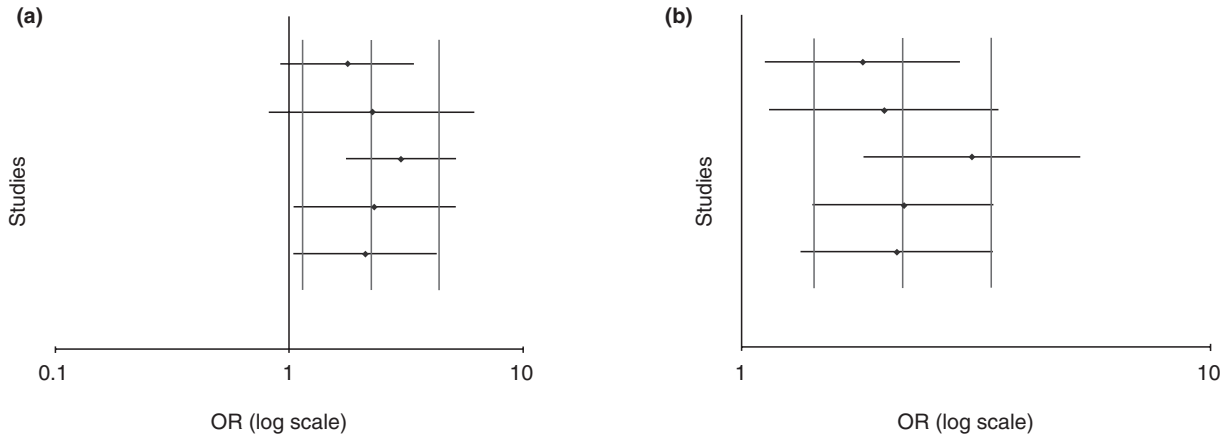

Figure 3. (a) Exclusion sensitivity plot (random effects model). (b) Exclusion sensitivity plot (fixed effect model).

et al.[14] documented a lower incidence of diarrhoea (one of 35), abdominal pain (one of 35) and black faeces (one of 35) in the bLF group. Zullo *et al.*[13] have reported almost equal incidence of diarrhoea and abdominal pain in the bLF group and non-bLF groups. Also, one case each of taste disturbance and vomiting was reported in the supplemented group in this study.

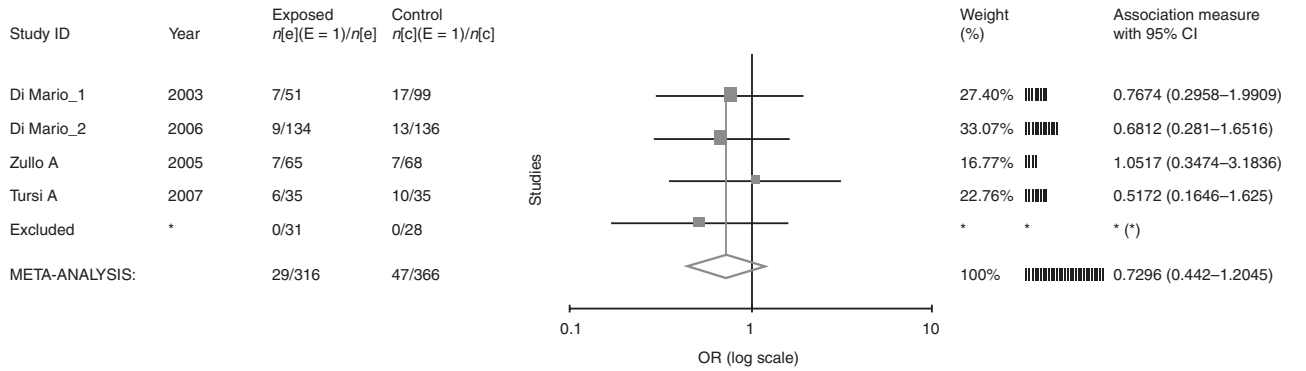

Figure 4. Forest plot of number of subjects with one or more adverse events in supplemented vs. control groups.

Sensitivity analysis

As per predefined criteria, the number of studies was inadequate to perform sensitivity analysis or metaregression.

DISCUSSION

With the limited evidence available, the review documents that bLF based therapy could potentially improve *H. pylori* eradication rates by ~10% without any significant impact on the treatment-associated adverse effects.

This is the first systematic review undertaken to evaluate the efficacy of bLF for eradication of *H. pylori* infection in humans. The main conclusion about improvement in eradication rates was drawn on the basis of a comprehensive systematic review of literature including non-English journals and appears to be consistent across intention to treat/per protocol analysis and with fixed/random effect models. The review includes studies using bLF as the sole intervention, as an adjunct to standard therapy or as an adjunct to rescue. This is not expected to bias the results, but could lead to underestimation of the efficacy because in the case of rescue therapy, the selected subjects may be expected to have a higher proportion of subjects with multi-drug resistant organisms or chronic anatomical changes, which may compromise the efficacy of bLF (most drugs in this condition are poorly effective), while in the case of use as sole intervention,[15] bLF alone eradicated *H. pylori* in very few subjects compromising the power of the study (three of 62; if a 5‰ definition was used). Nonetheless, several limitations merit consideration. The conclusions are based primarily on eradication data using author defined cutoffs, which were variable. Most of the trials (except one) did not specifically evaluate change in UBT values allowing only dichotomous evaluation of eradication. The interpretation of the results is also limited by the small number of trials available and the poor methodological quality of the trials (only one double-blind RCT).

Bovine lactoferrin is a glycoprotein found primarily in milk and other secretions like saliva.[34] It has documented antimicrobial activity against *H. pylori* species both *in vitro*[7] and *in vivo*.[8] Antibiotic actions of bLf have been attributed to its ability to bind to iron with great affinity and prevent iron utilization by bacteria.[35] It has also been reported to act as an antioxidant[34] and to exert a significant inhibitory effect on the attachment of the *H. pylori* colonizing the stomach *in vivo*.[8] A number of other diverse potential mechanisms by which lactoferrin inhibits the growth of several microorganisms have been suggested, including structural changes in the microbial cell wall, complete loss of membrane potential and integrity, indirect effects on enzyme activation, an increased generation of metabolic by-products of aerobic metabolism, iron deprivation and a combination of these factors.[36–40]

To understand the results better as an exploratory exercise, we subanalysed the studies included in a published meta-analysis[41] to differentiate the impact of fermented milk- or yogurt-based probiotic preparations [FMP; OR 2.51 (1.43–3.51)] with capsule-based probiotics [CBOP; OR 1.57 (1.06–2.32)]. Furthermore, the subanalysis on CBOP preparations (eight studies[42–49]) fails on exclusion sensitivity analysis with the exclusion of one study by Canducci *et al.*[49] rendering the results statistically insignificant (OR 1.36). These findings

allow speculation of equivalent or better efficacy of fermented milk based probiotic preparations. It may be hypothesized that bLF could be one of the active biological principals, which could potentially explain the potentially better efficacy of FMPs.

The results have important clinical implications and provide direction for future research. The poor eradication and return to baseline after end of sole administration in the Okuda *et al.* trial possibly suggest that bLF alone suppresses *H. pylori*, but may be more effective in eradication in combination with standard regimens. Although the results indicate potential benefits, the available data are inadequate to define the exact clinical setting (symptomatic or asymptomatic subjects; standard treatment, treatment failure or prevention; optimal dose) for benefit. Further confirmation is also necessary with larger studies in a double-blind RCT design in diverse populations including low income and developing countries where interference with other bacteria might be an important confounder and preferably with simultaneous estimation of costs incurred. Future studies also need to document difference in changes in UBT between the study and control groups to allow stronger conclusions.

Bovine lactoferrin potentially improves *H. pylori* eradication by ~4–17% without any significant impact on adverse effects, but the poor quality of studies available and the limited evidence available prevent a robust conclusion on the issue and necessitate further research.

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