Lactoferrin reduces the risk of respiratory tract infections: A meta-analysis of randomized controlled trials

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Abstract

Background: Lactoferrin (Lf) is one of the key immunomodulatory substances found naturally in various body fluids, such as saliva, tears, and breast milk, and forms a vital part of the innate defense against invading pathogens. Various studies have demonstrated antibacterial, antifungal, and antiviral properties of Lf and its protective role against respiratory tract infections (RTIs). The present meta-analysis aims to elucidate the association of Lf administration in reducing the risk of RTIs by systematically reviewing the data from randomized controlled trials (RCTs).

Methods: We systematically searched PubMed, Cochrane Library, Medline & CINAHL, Turning Research into Practice (TRIP), ProQuest Theses & Dissertations Databases, and China National Knowledge Infrastructure (CNKI) from inception till March 15, 2021. The primary outcome measure was a reduction in respiratory illness; decrease in frequency, symptoms, and duration. Random-effects model was used to estimate the odds ratio (OR) and 95% confidence interval (CI). We used Cochrane's risk-of-bias tool version 2 (RoB-2) to appraise the risk of bias of included RCTs.

Results: A total of nine RCTs were eligible for this review, of which six were included in the meta-analysis. Overall, two studies demonstrated a high risk of bias. The meta-analysis revealed a significantly reduced odds of the development of respiratory infections with the use of Lf relative to the control (pooled odds ratio = 0.57; 95% confidence interval 0.44 to 0.74, n=1,194), with sufficient evidence against the hypothesis of 'no significant difference' at the current sample size.

Conclusions: The administration of Lf shows promising efficacy in reducing the risk of RTIs. Current evidence favours Lf fortification of infant formulas. Lf may also have a beneficial role in managing symptoms and recovery of patients suffering from RTIs, and have potential for use as an adjunct in COVID-19, however warrants further evidence from a large well-designed RCT.

Keywords: Lactoferrin; respiratory tract infection; randomized controlled trials; COVID-19; infant formula; Coronavirus

Introduction

The human respiratory tract ensures adequate assimilation of oxygen throughout the lifespan. In the life-sustaining provision of oxygen to systemic circulation and vital body tissues, the respiratory tract also offers a passageway for microorganisms and myriad of toxins, which are then diffused by the immune system to avert potential entry and damage to the lung parenchyma [1]. Respiratory tract infections (RTIs) account for a substantial tally of global morbidity and mortality. According to the Global Burden of Disease (GBD) study, lower respiratory tract infections (LRTIs) were responsible for over 2 million fatalities in 2016; it is the sixth leading cause of death among all age groups and the most common cause of mortality of children under the age of five [2].

Lactoferrin (Lf) is a 80 kDa mammalian iron-binding glycoprotein that belongs to the family of transferrins. Lf is abundantly present in saliva, nasal, bronchial, gastric, bile, seminal and vaginal secretions. Nevertheless, colostrum and milk are more copiously concentrated with Lf than any other bodily fluids. Besides, neutrophils also carry Lf in their secondary granules, and neutrophil degranulation releases Lf into plasma during systemic infection and inflammation [3, 4]. Human and bovine Lf are analogous with respect to structure and function; about 78% of the human Lf sequence is similar to the bovine Lf [5]. Owing to its antibacterial [6], antiviral [7], antifungal [8], and anticancer [9] effects, Lf is often referred to as multifunctional, nutraceutical glycoprotein [10].

Several randomized controlled trials (RCTs) have highlighted the role of Lf administration in combating RTIs [11-14]. In a RCT from Japan, Motoki *et al.* confirmed Lf as a promising treatment alternative to prevent acute respiratory tract illness in children aged <12 months [12]. Likewise, another trial found significant attenuation of RTI, with a shortening of the duration of summer colds in the interventional arm (200 mg and 600 mg of Lf tablets) compared to the placebo arm [11]. Stefanescu *et al.* reported much lower ventilator-associated pneumonia rates (VAP) in mechanically ventilated preterm newborns who were given Lf containing Biotene® gel than in those receiving sterilized water [15]. To the best of our knowledge, there has been no meta-analysis to date that has analyzed the efficacy of Lf in reducing the risk of RTIs. Given the high clinical importance of RTIs amid pandemic, we aimed to systematically examine the interventional RCTs on the efficacy of bovine Lf in preventing the occurrence of RTIs.

Methods

Scope of review: Eligibility criteria

The guidelines laid in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement for systematic reviews and meta-analyses of healthcare interventions were followed [16]. The review was conducted to examine the efficacy of bovine Lf in preventing the occurrence of RTIs compared to another active intervention or placebo. Only the RCTs, published in any language, investigating the occurrence of RTIs among human subjects were included in the systematic review and meta-analysis. Studies investigating infections other than RTIs or unpublished literature were deemed unfit for inclusion.

Information sources

Two investigators independently searched electronic databases including PubMed, Cochrane Library, Medline, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Turning Research into Practice (TRIP), ProQuest Theses & Dissertations Databases, and China National Knowledge Infrastructure (CNKI) from inception till March 15, 2021. References of included studies were also scanned to search for additional studies.

Search strategy

The search strategy identified original research on the development of RTIs associated with the use of bovine Lf-enhanced formula or bovine Lf capsules/tablets. We used a combination of the following keywords and Medical Subject Headings (MeSH) terms: “lactoferrin” OR “bovine lactoferrin” AND “respiratory infection” OR “respiratory tract infection”.

Outcome measures

The primary outcome measure was reduction in the occurrence of RTIs which was measured as the difference in the baseline and post-intervention frequency.

Data extraction

Data extraction was independently performed by two reviewers (SSH and ASA). A pre-designed excel sheet was used to extract required data. For continuous outcomes, the mean differences and related standard deviations were mined. For outcomes with dichotomous variable, the frequency

of participants who experienced the event were retrieved. Authors were contacted by email to request for complete datasets in case of incomplete study data.

Risk of bias assessment in included studies

Two reviewers (CSK and ASA) independently reviewed the included RCTs for risk of bias using the Cochrane Collaboration's risk-of-bias (RoB) version 2 assessment tool, with the following domains being assessed: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting. Any differences between the two reviewers were adjudicated by discussion with a third reviewer (HAM) to reach a unanimous decision.

Data analysis

We conducted the meta-analyses using random-effects model to estimate the pooled odds ratio (OR) for outcomes of interest with the use of bovine Lf-enhanced formula or bovine Lf capsule/tablets relative to the control (active intervention or placebo), at 95% confidence interval (CI). We examined the heterogeneity between studies using the I^2 statistics and the χ^2 test, with significant heterogeneity at 50% and $p<0.10$, respectively. All analyses were performed using Meta XL, version 5.3 (EpiGear International, Queensland, Australia).

Results

Identification of relevant studies

Our systematic literature search yielded 227 records, among which 199 were from English-language databases and 28 were from CNKI. The Chinese records were screened by one of the native Chinese authors (CSK). After deduplication, 223 records were included, of which 14 studies were identified for full-text review. Finally, nine [11-15, 17-20] and six [11-15, 20] RCTs were included for qualitative and quantitative synthesis, respectively *(Figure 1)*.

Characteristics of included studies

Of the nine RCTs [11-15, 17-20] the six included in the meta-analysis were published between 2007 and 2020. The included trials were from China [13, 17, 20] (n=3), the United States [15, 19] (n=2), Japan [11, 12] (n=2), Canada [14] (n=1), and Australia [18] (n=1). The number of recruited

participants in each of the included trials [11-15, 17-20] ranged from 41 to 451, with a total of 1,667 participants. Across the nine included trials [11-15, 17-20], seven [11-14, 17-19] were double-blinded trials, while the remaining two [15, 20] were open-label trials. Except for the three trials [11, 14, 18] which included adult subjects, the remaining trials [12, 13, 15, 17, 19, 20] (n=6) included infants or children. Lf was administered as fortified formula [12, 13, 17, 19] (n=4), oral dietary supplement [11, 14, 18, 20] (n=4), and oral gel [15] (n=1) across the included trials. The characteristics of the included trials are depicted in *Table 1*. All the included trials [11, 13-15, 18-20] evaluated upper or lower respiratory tract infection as an outcome of interest, except for two trials [12, 17] which evaluated non-specific respiratory-related illness.

Risk of bias of included studies

The risk of bias of included studies is presented in *Table 1*. Majority of the included trials [11, 13, 14, 17-19] (n=6) had a low overall risk of bias. The remaining three trials either had high overall risk of bias [12, 20] or some concerns with regard to the overall risk of bias [15]: the trial by Motoki *et al.* [12] had a high risk of bias in the domain of 'missing outcome data', due to the differential missing data (7/60 in the Lf group but 1/49 in the placebo group); the trial by Chen *et al.* [20] had a high risk of bias in the domain of 'randomization' since the trial reported no information about how the allocation sequence was generated, with lack of information on the baseline characteristics, a high risk of bias in the domain of 'selection of the reported result' since the trial protocol was not available, and some concerns with the risk of bias in the domain of 'deviations from intended intervention' due to its open-label trial design; the trial by Stefanescu *et al.* [15] had some concerns with the risk of bias in the domain of 'randomization' due to baseline imbalances of the use of antenatal steroids which suggested a problem with the randomization process, and some concerns with the risk of bias in the domain 'selection of the reported result' since analysis intentions are not available for the respiratory outcomes. The aforementioned three trials had low risk of bias in the other domains as assessed via RoB v.2

Efficacy of Lf on respiratory-tract illness

The meta-analysis revealed a significantly reduced odds of the development of RTIs with the use of Lf relative to regular formula or placebo; the estimated effect indicated protective effect of Lf (*Figure 2*; pooled OR = 0.57; 95% CI 0.44 to 0.74; n = 1,194), with significant evidence against the hypothesis of 'no significant difference' at the current sample size. In addition, the subgroup

analysis after excluding two trials with high risk of bias (Motoki *et al.* [12] and Chen *et al.* [20]) remained statistically significant; the estimated effect indicated protective effect of Lf (pooled OR = 0.62; 95% CI 0.47 to 0.82; n=1005), with significant evidence against the hypothesis of 'no significant difference' at the current sample size.

Discussion

Lf has a multitude of biological functions, and is getting significant clinical attention due to its multifactorial immunomodulatory properties. Lf exerts its biological effects primarily by binding with receptors on target cells. Iron supports bacterial growth, and Lf mediated iron transfer from ceruloplasmin to Lf prevents iron utilization from pathogenic bacteria [21]. Moreover, Lf also disrupts bacterial cell membrane by binding and sequestering bacterial lipopolysaccharide (LPS), this also prevents triggering of LPS mediated proinflammatory cascade and cellular injury [22]. Lf also prevents direct entry of bacteria [23] and viruses [24, 25] by binding to cell-surface heparan sulfate proteoglycans (HSPGs) [26]. Lf also contributes to activating innate and adaptive immune responses by recruiting leukocytes and activating of dendritic cells [4]. Lf promotes innate immunity by recruiting natural killer cells, enhancing phagocytosis, and facilitating reactive oxygen species [27]. Lf also works as an adjuvant to boost vaccine efficacy by stimulating T and B lymphocytes, enhancing the cellular and humoral immune response [28, 29]. The presence of Lf receptors on a range of immune cells also confirms a diverse role of Lf in modulating both the innate and adaptive immune systems [30]. Finally, Lf has also been proposed recently to play an active role in diminishing cytokine storm [4], possibly by reducing LPS-driven tumor necrosis factor-alpha (TNF-α) as demonstrated previously in mice [31].

The meta-analysis of six RCTs demonstrated morbidity benefits with the administration of Lf where there was decreased odds of development of RTIs. Thus, we summarize a therapeutic effect of Lf in suppressing RTIs. Noteworthy to mention, the findings of the present meta-analysis are limited by undersized cohorts and substantial risk of biases in few studies. In fact, all the included RCTs were published from reputed institutions in diverse age groups. In addition, over two-thirds (72.4%) of the weight towards the pooled summary in the present meta-analysis was contributed by the RCTs from Li *et al.* [13] and Oda *et al.* [11]. We, therefore, recommend extensively well-designed RCTs to warrant the routine use of Lf to treat or as an adjunct in respiratory infections.

A plausible explanation of the morbidity benefits of Lf seen in patients with RTIs could be due to its immunomodulatory [32], antibacterial [10], and antiviral activity [33]. Lf has demonstrated strong anti-inflammatory effects by reducing leukocyte infiltration in bronchoalveolar lavage fluid (BALF) and ameliorate lung inflammation in mice infected with influenza. [34]. Moreover, *in vitro* studies have confirmed the significant antiviral potential of Lf against influenza A virus by suppressing influenza virus-driven apoptosis [35] and viral hemagglutination and subsequent infection [36].

Most importantly, the findings of Li *et al.* [13] may have been limited by the non-inclusion of the breastfed reference group. Indeed, it would have helped to evaluate the efficacy of Lf fortified formula-fed infants to that of infants receiving natural Lf through breastfeeding in achieving the desired respiratory outcomes. Moreover, the separate effects of milk fat globule membrane (MFGM) and Lf were not distinguished [13]. In the trial led by Muscedere *et al.* [14], the use of antibiotics in the intervention cohort may have confounded the outcomes. However, the study did not observe statistically significant antibiotic-free days with use of Lf. Finally, only three RCTs precisely mentioned the identification of virus or bacteria associated RTI either by established diagnosis or through microbial culture [12, 14, 15].

With regards to the safety profile of Lf administration, the trial by Chen *et al.* [20] noted significantly higher rate of vomiting events with LF compared to placebo ($n = 5$ vs $n = 13$; $P<0.05$), to which the authors attributed the findings to the coadministration of iron supplementation. The trial by Vitetta *et al.* [18] noted adverse events included mild to moderate asthma, gastrointestinal upsets, headaches and sinusitis, and some environmental allergies. Still, none of these adverse events were deemed to be associated with Lf. The trials by Motoki *et al.* [12], Oda *et al.* [11] and Stefanescu *et al.* [15] noted statistically insignificant differences in adverse effects with the use of Lf; only the trial by Motoki *et al.* [12] described the nature of the adverse events which included eye discharge, asthma, and eczema. The trials by Chen *et al.* [17], Muscedere *et al.* [14], Li *et al.* [13] and King *et al.* [19] did not report any serious adverse events associated with Lf. The adverse effects described may have been triggered from co-interventions (as in the trial by Chen *et al.* [20]) or co-ingredients besides Lf itself, as only three [14, 18, 20] studies used pure Lf supplementation versus placebo.

To the best of our knowledge, this is the first meta-analysis on Lf efficacy in reducing RTIs. In the context of the present meta-analysis findings, Lf supplementation has a promising role in preventing RTIs in the vulnerable population. Lf may also have a beneficial role in managing symptoms and recovery in patients suffering from RTIs. Lf has shown a remarkable efficacy in randomized clinical trials for the prevention of upper and lower respiratory infection, nosocomial infections and ventilator associated pneumonia across the age spectrum, including pre-term infants, infants, children, adults and elderly patients *(Table 1)*. The scientific and clinical evidence for Lf has evolved significantly over decades and supports its multifaceted antiviral, anti-inflammatory, immunomodulatory properties. Lf has shown efficacy against range of viruses including naked or enveloped viruses, and also DNA or RNA viruses; for e.g., cytomegalovirus, herpes simplex virus, human immunodeficiency virus (HIV), rotavirus, poliovirus, respiratory syncytial virus, hepatitis B and C (HCV) viruses, parainfluenza virus, alphavirus, hantavirus, human papillomavirus, adenovirus, enterovirus 71, echovirus 6, influenza A virus, and Japanese encephalitis virus, and various coronavirues (murine CoV, hCOV-NL63, and SARS-CoV) [26].
Lf can be promising candidate in the prevention and treatment of COVID-19 as an adjunct therapy. Lf can not only inhibit the viral entry via binding to HSPGs (heparan sulfate proteoglycans), but can also modulate an overactive immune and inflammatory response to a viral infection [4, 26] . The immunomodulatory properties of Lf can prevent the tissue injury by moderating cytokines, chemokines and inflammatory cascade. The evidence support that Lf can reduce IL-6, TNF-α and downregulate ferritin – the mediators of the 'cytokine storm', a hyper-inflammatory state attributed to the significant COVID-associated morbidity and mortality [4, 26]. The current evidence, therefore, also supports the potential of Lf as an adjunct for the prevention and management of COVID-19, however, this warrants further clinical evidence from a well-designed large RCT.

Conclusions

This meta-analysis demonstrated promising efficacy of lactoferrin (Lf) to reduce the risk of respiratory tract infections (RTIs). The evidence also favours Lf fortification of infant formulas. Lf may also have a beneficial role in managing symptoms and recovery in patients suffering from RTIs. The evidence also supports potential use of Lf as an adjunct for the prevention and management of COVID-19, however, this warrants further clinical evidence from a well-designed large RCT.

Authors contributions

HAM conceptualized and designed the study, SSH, CSK ASA, HAM extracted and analysed the data and drafted the manuscript. All authors read and approved the final version of the manuscript.

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The authors did not receive any funding or financial assistance for this work.

Conflict of Interest

HAM is on a scientific advisory board for a lactoferrin supplement product but does not have any financial or other affiliation with the company and has neither received any funding or financial reimbursements. HAM is committed to promote the science behind Lf and its immunomodulatory role in the prevention and management of infections.

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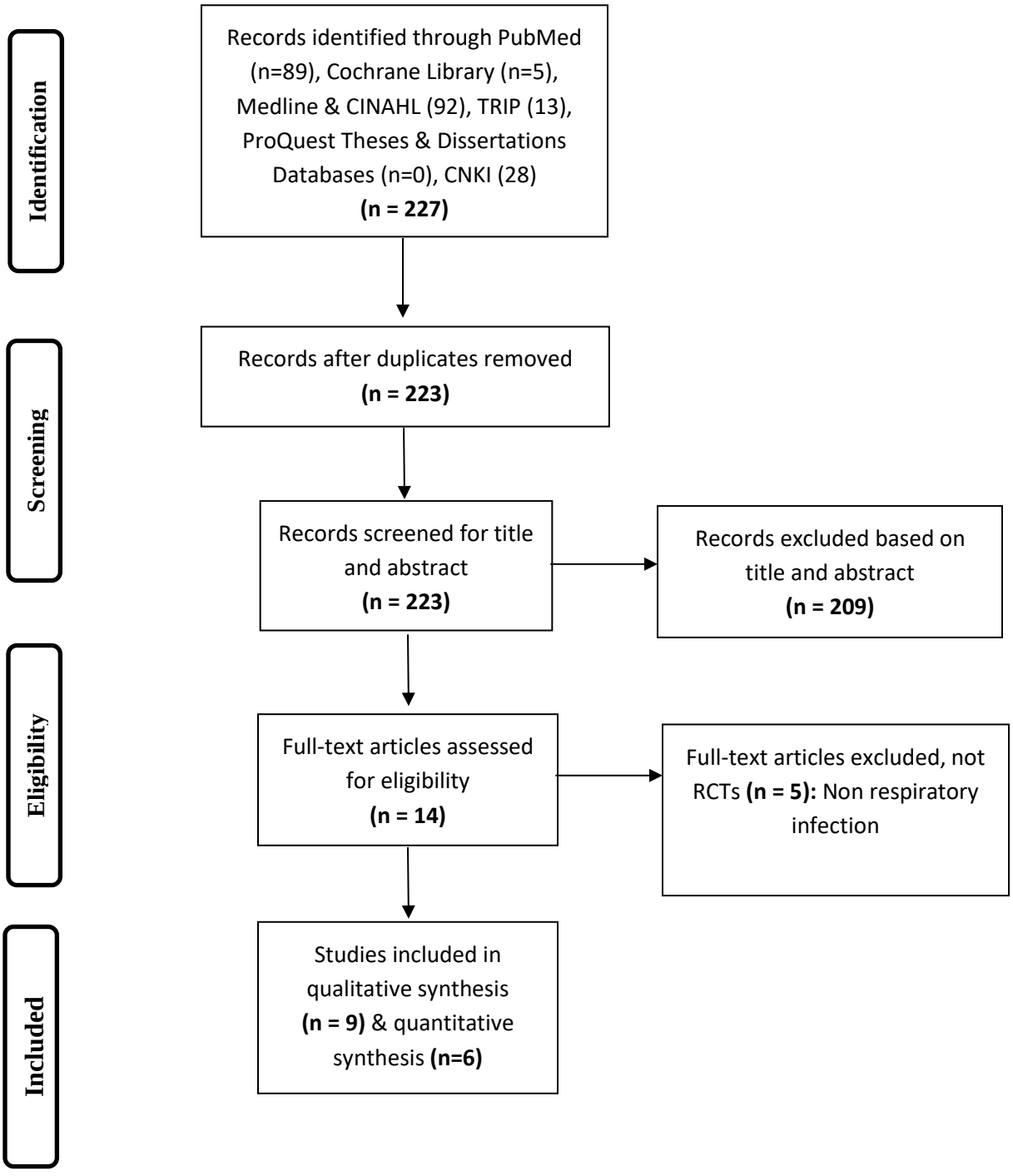

Figure 1: Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram for the study selection.

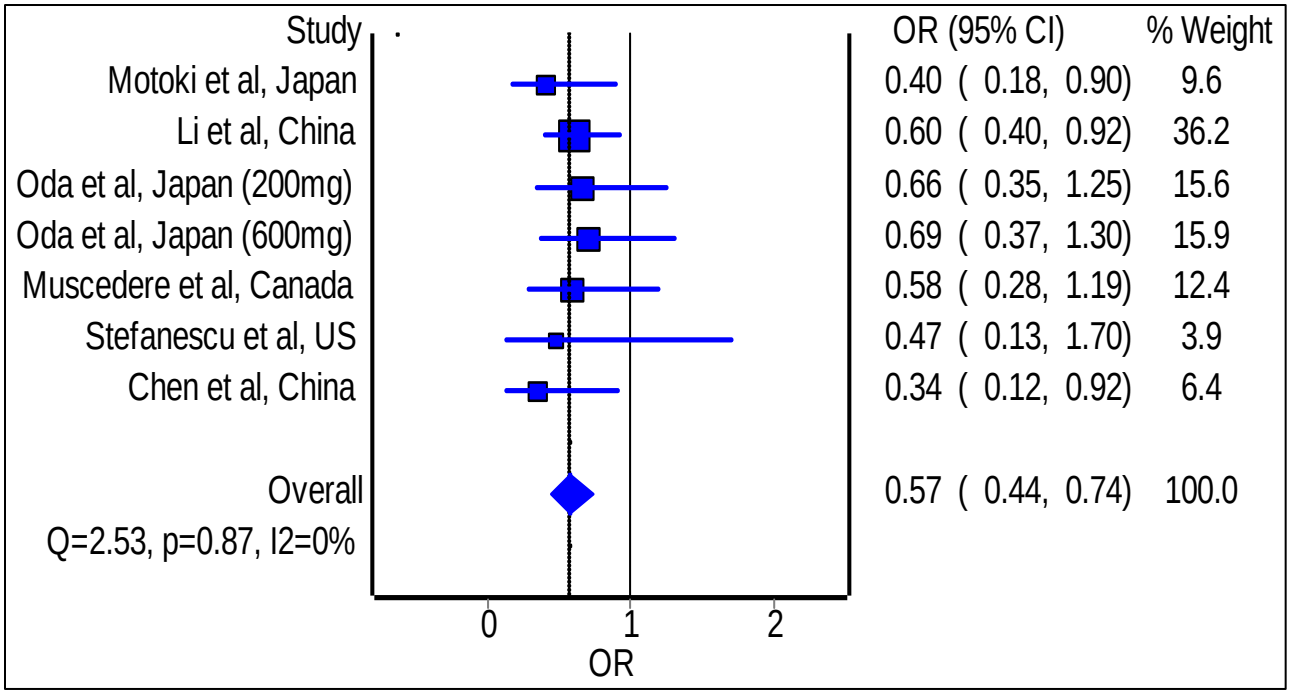

Figure 2: Pooled odds ratio of developing respiratory infections with the use of lactoferrin relative to the control.

Table 1: Study characteristics of included trials

Study	Study design	Country	Age (median/mean)	Respiratory infection/ illness outcome	Regimen of LF	Acute respiratory infection		Risk of bias[1]
						LF (n/N; %)	Non-LF (n/N; %)	
King et al [19], 2007	Randomized, double-blind, placebo-controlled trial	USA	LF=39.1 weeks Placebo=38.3 weeks	Prevention of upper and lower respiratory tract infection	850 mg/L bovine LF as LF-fortified formula for 1 year	Incidence of URTI: 3.92 episodes/infant-year Incidence of LRTI: 0.15 episodes/infant-year	Incidence of URTI: 4.00 episodes/infant-year Incidence of LRTI: 0.50 episodes/infant-year	Low risk
Vitetta et al [18], 2013	Randomized, double-blind, placebo-controlled trial	Australia	LF=32.9 years Placebo=33.9 years	Prevention of upper respiratory tract infection/ common cold	LF (200 mg)/IgF (100 mg) [2 × 300 mg/cap daily] for 90 days	Incidence: 0.93 events/90 patient-days	Incidence: 2.26 events/90 patient-days	Low risk
Stefanescu et al [15], 2013 [§]	Randomized, open-label, controlled, pilot trial at level IV neonatal intensive care unit	USA	Pre-term infants LF Plus=24 weeks Placebo=25weeks	Prevention of lower respiratory tract infection (Ventilator-associated pneumonia)	Biotene gel (lactoperoxidase, lysozyme, lactoferrin) – oral care with 2 ml single use vials	Frequency: 6/20; 30 9/1000 ventilator days	Frequency: 10/21; 48 17/1000 ventilator days	Some concerns
Chen et al [17], 2016	Randomized, double-blind, controlled trial	China	LF=38.4 weeks LF-free=38.7 weeks Breastfed=38.6 weeks	Prevention of respiratory-related illness	38 mg/100 g bovine LF as LF-fortified formula for 3 months	Incidence: 2.01 events/100 child-days	Incidence (LF-free): 2.94 events/100 child-days Incidence (Breastfed): 2.18 events/100 child-days	Low risk

Chen et al [20], 2016 §	Randomized, open-label, controlled trial	China	Preterm infants born between 34-36 weeks	Prevention of recurrent respiratory tract infection	LF 25 mg once or twice daily	Frequency: 8/40; 20.0	Frequency: 17/40 (42.5)	High risk
Muscedere et al [14], 2018 §	Multicenter, randomized, double-blind, placebo, controlled study	Canada	LF=66.3 years Placebo=62.5years	Prevention of nosocomial infections (including lower respiratory tract infection)	2 g of LF administered in four divided doses per day up to 28 days	Frequency: 15/107; 14.0	Frequency: 23/105; 21.9	Low risk
Li et al [13], 2019 §	Randomized, double-blind, controlled trial	China	37-42 weeks of gestation	Prevention of upper respiratory tract infection	600 mg/L bovine LF as LF-fortified formula for 1 year	Frequency of URTI: 151/223; 67.7	Frequency of URTI: 177/228; 77.6	Low risk
Oda et al [11], 2020 §	Randomized, double-blind, placebo-controlled, parallel-group comparative trial	Japan	LF 200 mg=23.7 years LF 600 mg=24.0 years Placebo=23.3 years	Prevention of upper respiratory tract infection/ common cold	LF 200 mg or 600 mg daily for 12 weeks	Frequency (200 mg): 20/95; 21.0 Frequency (600 mg): 21/96; 21.9	Frequency: 25/99; 25.3	Low risk
Motoki et al [12], 2020 §	Randomized, double-blind, placebo-controlled trial	Japan	LF=25.9 months Placebo=25.7 months	Prevention of r espiratory-related illness	48 mg/day as LF-fortified formula for 13 weeks	Frequency: 16/53; 30.2	Frequency: 25/48; 52.1	High risk

§ *included in quantitative synthesis*