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REVIEW

Probiotics for cow's milk protein allergy: a systematic review of randomized controlled trials

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Abstract

Cow's milk protein allergy (CMPA) is the commonest food allergy in infancy and is associated with significant health burden. Given their immune modulatory properties, probiotics have been proposed as a strategy for management of CMPA. We aimed to systematically review efficacy and safety of probiotics in the management of CMPA. Databases PubMed, EMBASE, CINAHL, Cochrane Central Library, and Google scholar were searched in August 2018 for randomized controlled trials (RCT) of probiotic supplementation as an adjunct in the management of infants with suspected/proven CMPA. Primary outcomes were resolution of hematochezia and acquisition of tolerance to CMP at 6, 12, 24, and 36 months. Secondary outcomes included effect on allergic symptoms (SCORAD index), growth, gut microbiota, and adverse effects. A total of 10 RCTs ($n = 845$; probiotics, 422; control, 423) with low to unclear risk of bias were included. Meta-analysis showed probiotic supplementation was not associated with earlier resolution of hematochezia ($n = 87$; RR: 1.45 (95% CI: 0.96–2.18), $p = 0.08$; level of evidence (LOE), very low), in presumed CMPA. In confirmed CMPA, probiotics were associated with higher rate of acquisition of tolerance to CMP at the end of 3 years compared with placebo ($N = 493$; RR, 1.47; 95% CI, (1.17–1.84); $p = 0.0009$; LOE, low]. Meta-analysis was not possible for other outcomes. There were no probiotic related adverse effects.

Conclusion: Limited low-quality evidence indicates that probiotic supplementation may be associated with earlier acquisition of tolerance to CMP in children with CMPA. Large well-designed trials are essential to confirm these findings.

What is Known:

- *Cow's milk protein allergy (CMPA) is one of the commonest food allergies in children. CMPA is associated with significant socioeconomic burden.*
- *Elimination diet and extensively hydrolyzed formula is the mainstay of the management of CMPA.*

What is New:

- *This first systematic review of randomized controlled trials shows that probiotics as an adjuvant can lead to earlier acquisition of tolerance to CMP in children at 36 months of age. However, the evidence is low quality and influenced by data from one large study.*
- *Probiotic supplementation was not associated with earlier resolution of hematochezia.*

Keywords Children · Hematochezia · Infants · Meta-analysis · Milk allergy · Tolerance

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Abbreviations

AAF	Amino acid-based formula
CMPA	Cow's milk protein allergy
DBPCFC	Double-blind placebo-controlled food challenge
IgE	Immunoglobulin E
LGG	*Lactobacillus rhamnosus* GG
RCT	Randomized controlled trial
SCORAD	Symptomatic improvement as per severity scoring of atopic dermatitis
PRISMA	Preferred reporting items for systematic reviews and meta-analyses

Introduction

Cow's milk protein allergy (CMPA) is most common food allergy in infancy. CMPA is defined as a reproducible adverse reaction to one or more cow's milk proteins (CMP) (usually caseins or whey β-lactoglobulin) mediated by one or more immune mechanisms. The incidence of CMPA during first year of life is estimated to be around 5% [1]. In Australia and New Zealand, around 2% (1 in 50), infants are allergic to cow's milk and other dairy products [2]. CMPA is associated with significant impact on the families and financial burden on the health services all over world [3, 4]. CMPA can be either immunoglobulin E (IgE) or non-IgE-mediated. IgE-mediated reactions typically occur immediately after ingestion, whereas non-IgE-mediated are delayed and take up to 48 h to develop [5]. IgE-mediated reactions can vary in severity and may present as a life-threatening anaphylaxis. They may also manifest with skin, respiratory, cardiac, and gastrointestinal signs and symptoms, whereas the non-IgE-mediated reactions can present as allergic food protein induced proctocolitis and enteropathy.

A double-blind placebo-controlled food challenge (DBPCFC) is the gold standard for diagnosis of CMPA where both the doctor and parents are blinded of introduction to CMP [6]. However, cost, extensive preparations, and time-consuming nature of the test makes it hard to perform routinely [7]. The diagnosis of CMPA is therefore largely clinical. A thorough history and examination, family history of atopy is important. A skin prick test (SPT) can be carried out in children with high suspicion of IgE-mediated reactions. However, the sensitivity and specificity of SPT is low [8]. Patch testing can be used for diagnosis of non-IgE-mediated reactions [6]. Endoscopic evaluation (flexible sigmoidoscopy or colonoscopy) is generally reserved for patients of CMPA with atypical symptoms, such as diarrhea, constipation, and severe rectal bleed despite of the cow's milk elimination diet [6]. The endoscopic findings are generally limited to the distal colon and include patchy erythema and edematous mucosa with loss of vascularity, with the biopsy typically showing high eosinophils in lamina propria and muscularis mucosa [9].

CMPA usually resolves in the first few years of life with 80 to 90% of children developing tolerance to CMP by 5 years [10]. Non-IgE-mediated CMPA usually resolves earlier than IgE-mediated CMPA (2.5 vs. 5 years) [11, 12].

Strategies for management of CMPA include elimination of CMP from mother's diet by going dairy free in an exclusively breast-feed infant. In case of non-resolution of symptoms in breast-fed or exclusively formula fed infants, use of extensively hydrolyzed formulas is recommended. Amino acid-based formula (AAF) is generally used for management of complex CMPA, multiple food allergies, or when extensively hydrolyzed formula is not tolerated. After resolution of acute symptoms, CMP is gradually introduced in stepwise fashion at 6–12 months, for promoting tolerance.

Recent studies have shown that gut microbiota plays an important role in the development of immune response [13]. Altered gut microbiota in early life is associated with food allergy, and may predict persistence of disease or acquisition of tolerance [14]. Gut dysbiosis is linked with increased risk of allergic disorders including CMPA in childhood [15]. Fecal metagenomic studies in infants with CMPA have showed high counts of total bacteria and anaerobes [16]. Presence of *Clostridia* and *Firmicutes* species in fecal samples is associated with resolution of milk allergy in children with CMPA [17]. Animal models have suggested pathways by which specific bacterial taxa within gut microbiota may promote oral tolerance [18].

Considering the significance of dysbiosis in the pathogenesis of the condition, probiotics have been proposed as a strategy for management of CMPA [19]. Probiotics are live organisms which when administered in an adequate dose confer health benefits to the host [20]. Probiotics could potentially restore intestinal homeostasis and prevent allergy through interaction with the intestinal immune cells especially in early life. The pathways for benefits of probiotics could include enhancement of gut mucosal barrier function, competitive inhibition of pathogenic bacteria, modulation of the immune response towards non-allergy, and degradation of protein antigen [21].

Recent studies have indicated beneficial role of probiotics in management of children with CMPA [22, 23]. However, there are no systematic reviews in this field. Given the health burden associated with the condition, and the mechanisms suggesting their benefits, we aimed to systematically assess the efficacy and safety of probiotic supplementation in management of CMPA in children.

Materials and methods

The Cochrane methodology and preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines were used for conducting and reporting this systematic review respectively [24, 25]. Ethics approval was not required.

Eligibility criteria

Types of studies Randomized controlled trials (RCTs) and quasi-RCTs assessing role of probiotics as (1) treatment for suspected (suggestive symptoms) CMPA and (2) in achieving acquisition of tolerance to CMP in cases with confirmed CMPA (based on food challenge) were eligible for inclusion. Non-RCTs, reviews, and commentaries were excluded, but read to identify other potential studies.

Types of participants Children under 5 years of age ware suspected or confirmed diagnosis of CMPA. Infants with rectal bleeding due to bacterial or viral infections, necrotizing enterocolitis or coagulopathy, and those with prior or current exposure to probiotic or symbiotic supplementation were excluded.

Types of interventions Oral probiotic (any strain, dose, or duration) with/without prebiotic oligosaccharide (symbiotic) as an adjuvant to standard treatment including dietary restriction for CMP compared with control as placebo or standard treatment alone.

Primary outcomes (1) Resolution of hematochezia in infants with presumed CMPA defined as absence of visible speckles or streaks of blood mixed with mucous or occult blood in the stool of otherwise healthy infant. (2) Acquisition of tolerance to CMP in infants with confirmed CMPA based on the DBPCFC at 6, 12, 24, and 36 months of age.

Secondary outcomes (1) Symptomatic improvement in severity scoring of atopic dermatitis (SCORAD) index; (2) resolution of other clinical symptoms (gastrointestinal, respiratory, dermatological symptoms); (3) effects on growth parameters such as weight, length, and head circumference; (4) improvement in endoscopic and histological parameters of rectum and sigmoid colon—the endoscopic parameters included focal rectal erythema or erosions or lymphoid nodular hyperplasia are seen in CMPA. The histological parameters included inflammation, or eosinophilic infiltration of the colonic epithelium, lamina propria, or muscularis; eosinophils 6–20 per high-powered field; (5) duration of rectal bleeding; (6) stool calprotectin levels; (7) intestinal microflora analysis, to determine the effect of probiotics on intestinal microbiota; (8) adverse effects secondary to intervention.

Search strategy Reviewer (SQ and MD) conducted the literature search independently. We searched Pub Med, EMBASE, the Cochrane central register (CENTRAL) databases, and Google Scholar for studies reported from the earliest available online year of indexing until August 2018 using the following search terms in various combinations: (a) population—neonate(s), infant*, pediatric; (b) intervention—probiotic, probiotics, *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*; (c) outcome—cow's milk protein allergy, milk allergy, allergic proctocolitis; and (d) publication type— "Randomized controlled Trial," "Controlled Trial," or "Clinical Trial." Online abstracts of Pediatric Academic Society (PAS) meetings were reviewed from 2002.

Study selection and data extraction Reviewers SQ and MD identified potentially eligible studies, read the abstracts of the citations obtained from the initial broad search independently. Full-text articles of these studies were obtained and assessed independently for eligibility using the predefined eligibility criteria. Multiple publications of the same study were excluded. Data was extracted using a pre-specified data extraction form. For dichotomous outcomes, the number of patients with the event and the number of patients analyzed in each treatment group of each study were entered into the form. For continuous outcomes, the mean and standard deviations (SD) were entered. Disagreements were resolved by group discussion until consensus was reached.

Assessment of risk of bias We used the Cochrane "Risk of Bias Assessment Tool" to assess the methodological quality of the included trials [26]. For each trial, information was sought regarding the method of randomization, allocation concealment, blinding of participants and outcome assessors, completeness of follow-up, selective reporting, and other biases. The studies were assigned as of high, low, or unclear ROB risk of bias. Reviewer SQ, MD assessed each study independently. Disagreements were resolved by discussion.

Data synthesis Meta-analysis was planned using Review Manager 5.3 [Cochrane Collaboration, Nordic Cochrane Centre] if pooling of data was possible and justified according to the "intention to treat" principle. We used a random-effect model for meta-analysis assuming heterogeneity. Categorical measure of effect size was expressed as risk difference (RR) (Mantel Haenszel method) and mean difference (MD) (inverse variance method) was used for continuous measures. A narrative synthesis was planned if meta-analysis was not possible due to significant heterogeneity in included studies and/or non-availability of the outcome measures in the desired form.

Subgroup analyses We aimed to conduct subgroup analyses based on pathogenesis of CMPA (IgE vs. non-IgE) and strain-specific effects of probiotics. The risk of publication bias was to be assessed by a funnel plot [27].

Grading the evidence and summary of findings We used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) for assessment and grade pro guidelines development tool to create the summary of finding table to report the quality of evidence [28, 29]. Reviewer SQ under supervision of reviewer SP independently assessed the quality of evidence.

Results

Our search retrieved 623 potentially relevant citations (Fig. 1). After removing 153 duplicates, 570 records screened for eligibility. A total of 542 citations were excluded as they were not relevant to the review. Finally, 28 studies were read in detail. After careful scrutiny, we identified 10 RCTs ($N = 845$; probiotics, 422; control, 423) that assessed effects of probiotics ($n = 7$) and synbiotic ($n = 3$) on CMPA. Total seven RCTs reported the primary outcome of interest in our review (i.e., resolution of hematochezia as a marker of suspected CMPA; $n = 3$ [21, 22, 30] and acquisition of tolerance in proven CMPA; $n = 4$) [23, 31–33]. The baseline characteristics of these studies are shown in Tables 1 and 2. The type of probiotics and the primary outcomes varied among these trials. Except for Hol (*Lactobacillus casei CRL431* and *Bifidobacterium lactis Bb-12*), all studies used *Lactobacillus* GG (LGG) [21–23, 31, 32, 34]. Canani 2012, 2017, Baldassarre, Szajewska, Burk, Hol, Candy et al. carried low ROB risk of bias in most of the domains, whereas Canani 2013, Ahanchian and Kirjavainen et al. were deemed to carry high to unclear ROB risk of bias (Supplementary Fig. 1).

Primary outcome

(1) Resolution of hematochezia in presumed CMPA

Three studies ($n = 87$; probiotics, 42; control, 45) reported this outcome [21, 22, 30]. Definition of hematochezia was based on presence of blood-streaked stools as per parental report in two studies by Szajewska and Ahanchian and physician observation along with Guiauc card test by Baldassarre [21, 22, 30]. Only Baldassarre ($n = 26$) reported significant reduction in hematochezia in probiotic vs. placebo group (12/12 vs. 9/14, $p = 0.02$) [21]. Szajewska ($n = 29$) and Ahanchian ($n = 32$) reported no significant difference between the groups in resolution of hematochezia. Despite contacting the author, the data on this outcome was not available from Ahanchian. Meta-analysis of data from Baldassarre and Szajewska ($n = 55$) showed no significant reduction in rectal bleeding between probiotic and placebo group infants (RR, 1.45; (95% CI), 0.96–2.18), heterogeneity: $\text{chi}^2 = 1.01$, $I^2 = 1\%$, $p = 0.08$) [21, 22] (Supplementary Fig. 2).

(2) Acquisition of tolerance to CMP in confirmed CMPA

Data on this outcome was available from four studies ($n = 493$; probiotics, 255; control, 238) at different time points (6, 12, 24, and 36 months) [23, 31–33]. Overall, significantly more children achieved tolerance to CMP in probiotic vs. placebo group (RR, 1.47; 95% CI, (1.17–1.84); heterogeneity: $\text{chi}^2 = 28.76$; $I^2 = 76\%$; $p = 0.0009$) after 36 months (Fig. 2). There was no significant difference in acquisition of tolerance at 6 and 12 months as reported by Canani (2012, 2013, and

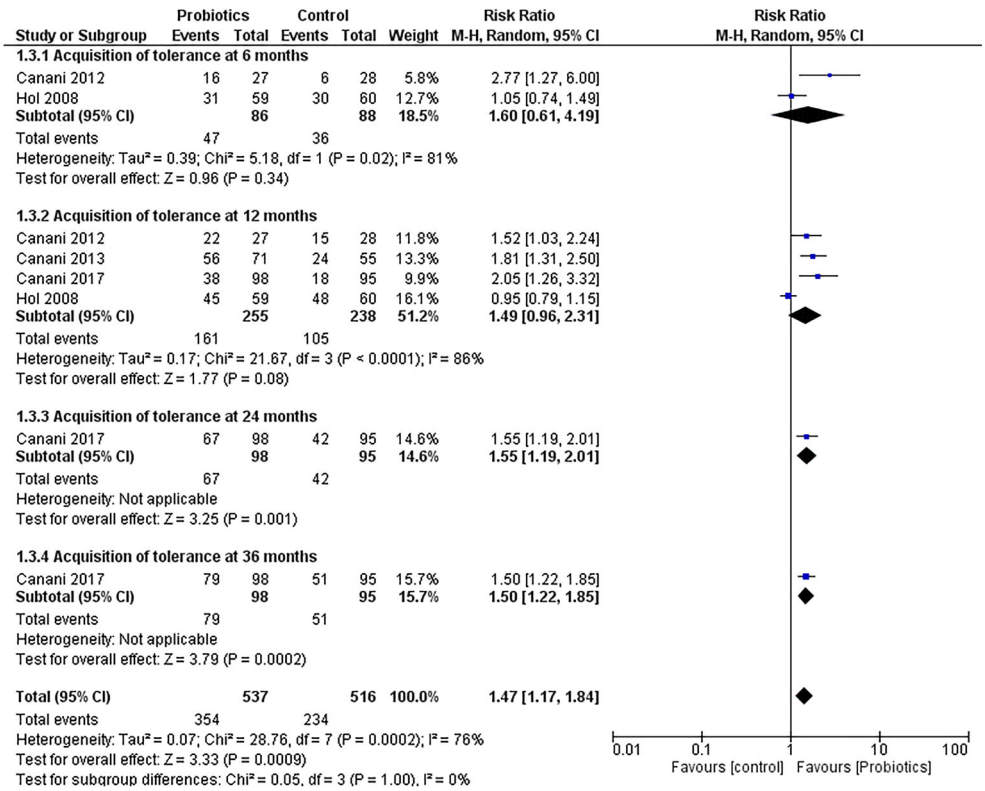

Fig. 1 Flow chart of study selection process after screening of electronic search

Table 1 Characteristics of RCTs evaluating effects of probiotics on resolution of hematochezia

Author/year	Study design	Age (months)	Sample size	Intervention/dose/duration	Primary outcomes	Results
Szajewska 2007	RCT	< 6	$N = 26$ $I = 11$ $C = 15$	I = mothers milk on cow's milk restriction + *L. rhamnosus* GG (3×10^9 CFU) C = mothers milk on cow's milk restriction + placebo; twice daily for 4 weeks	Duration of rectal bleeding based on parenteral report (days)	No difference in mean duration of rectal bleeding (MD, − 1.9; 95% CI, − 4 to 7; $p = 0.54$), clinical resolution of rectal bleeding within 72 h and no relapse afterward (RR, 0.9; 95% CI, 0.2–3.9, $p = > 0.99$) and clinical resolution of rectal bleeding within 72 h followed by relapse of symptoms (RR 1.4; 95% CI, 0.5–3.5, $p = 0.69$) in probiotics vs placebo
Baldassarre 2010	RCT	0–10	$N = 26$ $I = 12$ $C = 14$	I = EHCF + *L. rhamnosus* GG (1.46×10^7 CFU/100 ml) or BF C = EHCF or BF; for 4 weeks	Fecal calprotectin levels and hematochezia based on clinician observation and Guiac test	1) Higher fecal calprotectin levels ($P < 0.0001$) in hematochezia group 2) Higher fecal calprotectin decrease, mean ± SD (− 214.5 ± 107.93 vs 112.7 ± 105.27 μg/g, $p = 0.02$) and resolution of hematochezia (12/12 vs. 9/14; $p = 0.002$) in probiotics vs. placebo group.
Ahanchian 2014	RCT	1–12	$N = 32$ $I = 16$ $C = 16$	I = synbiotic, 1 billion CFU of Protexin Restore* C = placebo; for 1 month	Clinical gastrointestinal symptoms including hematochezia (based on parental report). Growth (weight, length and HC) at beginning, end of 1st and 3rd month	1) No differences in rectal bleeding, intestinal colic after 72 h and 2 weeks and symptoms at 1, 2, 3, and 6 months 2) Higher increment in head circumference (at 1 month, $p = 0.048$, at 3 months, $p = 0.03$) and weight (at 1 month, $p = 0.008$, at 3 months, $p = 0.02$) in synbiotic group vs. placebo but no difference in height increment (at 1 month, $p = 0.7$, at 3 months, $p = 0.9$)

RCT, randomized controlled trial; *N,* total number of subjects; *I,* intervention; *C,* control; *EHCF,* extensively hydrolyzed casein formula; *L, Lactobacillus*; *LGG*, *Lactobacillus rhamnosus GG*; *CFU,* colony forming unit; *BF,* breast-feeding; *SD,* standard deviation; *OR,* odd's ratio; *CI,* confidence interval; *MD,* mean difference; *RR,* relative risk; *HC,* head circumference

*Protexin Restore: a mixture of *Lactobacillus casei*, *Lactobacillus rhamnosus, Streptococcus thermophilus*, *Bifidobacterium breve*, *Lactobacillus acidophilus*, *Bifidobacterium infantis*, *Lactobacillus bulgaricus*, and *Fructooligosaccharide*

Table 2 Characteristics of RCTs evaluating effects of probiotics on acquisition of tolerance to CMP, growth, and gut microbiota

Author/ year	Study design	Age (months)	Sample size	Intervention/dose/duration	Primary outcomes	Results
Kirjavainen 2003	RCT	3.5–6.8	*N* = 35 *I* = 14 (viable) +13 (heat-inactivated LGG); *C* = 8	*I* = EHCF+ viable or heat-inactivated *L. rhamnosus* GG (1×10^9 CFU/g); *C* = placebo, EHWF; for mean duration of 7.5 weeks	Efficacy of viable and heat-inactivated LGG for atopic eczema. Effects on gut bacterial genera	1) Significant decrease in the SCORAD scores median (IQR) within the viable LGG from 19 (4–47) to 5 (0–18) units than in placebo from 13 (4–29) to 8 (0–29) units and in the heat-inactivated LGG group from 15 (0–29) to 7 (0–26) units. ($p = 0.02$) 2) No changes in the bacterial numbers with any treatment but treatment with heat-inactivated LGG caused more adverse gastrointestinal symptoms and diarrhea (5/13, $p = 0.05$).
Hol 2008	RCT	< 6	*N* = 119 *I* = 59 *C* = 60	*I* = *L. casei CRL 431* and *B. lactis Bb-12*/10^7 CFU/g *C* = placebo For 12 months	Clinical tolerance at 6 and 12 months by DBPCFC	1) No difference in tolerance at 6 months (56% vs. 54%, $p = 0.92$) or cumulative tolerance at 12 months (77% vs. 81%, $p = 0.95$) in probiotics vs. placebo 2) Strong predictor of persisting CMPA at 6 ($p = 0.009$) and 12 months ($p = < 0.001$) was positive SPT response at randomization.
Canani 2012	RCT	1–12	*N* = 80 *I* = 40 *C* = 40	*I* = EHCF + *L. rhamnosus* GG (1.46×10^7 CFU/100 ml; *C* = EHCF; for 12 months	Tolerance acquisition based on clinical evaluation, SPT + APT and DBPCFC	1) Higher clinical tolerance acquisition in probiotic vs. placebo group at 6 and 12 months both in IgE ($p = 0.171$, $p = 0.046$) and non-IgE-mediated group ($p = 0.017$, $p = 0.006$) respectively 3) No difference in adverse events, decrease in SPT responses and APT in non-IgE-mediated CMPA after 6 and 12 months
Canani 2013	Quasi RC-T	1–12	*N* = 126 *I* = 71 *C* = 55	*I* = EHCF + *L. rhamnosus* GG (1.46×10^7 CFU/100 ml); *C* = EHCF; for 12 months	Tolerance acquisition after 12 months based on DBPCFC	1) Higher oral tolerance rate after 12 months in EHCF + LGG vs. EHCF; OR (95%CI) (4.82 (2.21–10.52); $p < 0.001$)
Burks 2015	RCT	0–8	*N* = 110 *I* = 54 *C* = 56	*I* = Neocate with synbiotics (prebiotic 8 g/l + *B breve M-16V*, 1.47×10^9 CFU/100 ml formula) *C* = Neocate without synbiotics For 16 weeks	Growth (weight, length and HC)	No differences in increase in weight, mean difference (90% CI) in *Z*-scores (test–control) 0.147 (− 0.10; 0.39, $p = 0.32$), increase in length − 0.299 (− 0.69; 0.09, $p = 0.21$) and head circumference 0.152 (− 0.15; 0.45, $p = 0.40$)
Candy 2017	RCT	< 13 only non-IgE-mediated CMPA	*N* = 71 *I* = 35 *C* = 36 HBR = 51	*I* = AAF + synbiotic (probiotics-*B breve M-16V*/1.47×10^9 CFU/100 ml and prebiotic); *C* = AAF without synbiotics; for 8 weeks	Effect of test formula on gut microbiota in Non-IgE-mediated CMPA	1) Significant higher fecal *Bifidobacteria* in test vs. control (35.4% vs. 9.7%; $p < 0.001$), and lower *E. rectale/C. coccoides* (ER/CC) (9.5% vs. 24.2%; $p < 0.001$)
Canani 2017	RCT	1–12 Only IgE-mediated CMPA	*N* = 220 *I* = 110 *C* = 110	*I* = EHCF+ *L. rhamnosus* GG *C* = EHCF; for 36 months	Occurrence of AM (eczema, urticaria, asthma, or rhino-conjunctivitis) or other food allergy. Tolerance acquisition based on DBPCFC	1) Absolute risk difference (95% CI) for occurrence of at least one AM over 36 months for EHCF + LGG vs. EHCF was − 0.23 (− 0.36 to − 0.10; $p < 0.001$) 2) Lesser other FA + AM in EHCF + LGG group (49%) vs. control (64.2%)

RCT, randomized controlled trial; *N,* total number of subjects; *I,* intervention; *C,* control; *EHCF,* extensively hydrolyzed casein formula; *LGG, Lactobacillus rhamnosus GG; CFU,* colony forming unit; *BF,* breast-feeding; *SD,* standard deviation; *IgE,* immunoglobulin *E; OR,* odd's ratio; *CI,* confidence interval; *L, Lactobacillus*; *S, Streptococcus*; *B, Bifidobacterium*; *SPT,* skin prick test; *APT,* atopic patch test; *DBPCFC,* double-blind placebo-controlled food challenge; *CMPA,* cow's milk protein allergy; *IQR,* interquartile range; *MD,* mean difference; *RR,* relative risk; *EHWF,* extensively hydrolyzed whey formula; *HBR,* healthy breast-fed reference; *AAF,* amino acid formula; *AM,* allergic manifestation; *HC,* head circumference

2017) and Hol. Data on acquisition of tolerance at 24 and 36 months was available from only Canani et al. (2017) [23]. Significantly, more infants in the probiotic vs. placebo group achieved acquisition of tolerance at 24 and 36 months ($p = 0.001$, $p = 0.0002$) respectively (Fig. 2).

Subgroup analysis

Based on pathogenesis of CMPA, we conducted a subgroup analysis using data from Canani (2012 and 2013) [31, 32]. Both studies reported at 6 and 12 months of age a greater number of infants in non-IgE group achieved tolerance to CMP- vs. IgE-mediated group. However, there was no significant effect of probiotic on this outcome. Subgroup analysis of LGG specific data showed significant increase in acquisition of tolerance at 6, 12, 24, and 36 months (not shown).

Secondary outcomes (Supplementary Table 1)

(1) Symptomatic improvement as per SCORAD index

Three trials reported this outcome ($n = 251$; probiotics, 127; placebo, 124) [33–35]. Only Kirjavainen reported significant reduction in SCORAD index at the end of probiotic supplementation for 8 weeks [34]. Burk reported no difference in the SCORAD index between the probiotic and placebo group at the end of 4 month [35]. Hol reported overall improvement in the SCORAD index within the groups at 6 and 12 months. However, this improvement was not significant on comparison between the groups at 6 months and 12 months [33]. Meta-analysis was not possible as outcome was reported in different timeframe in the included studies.

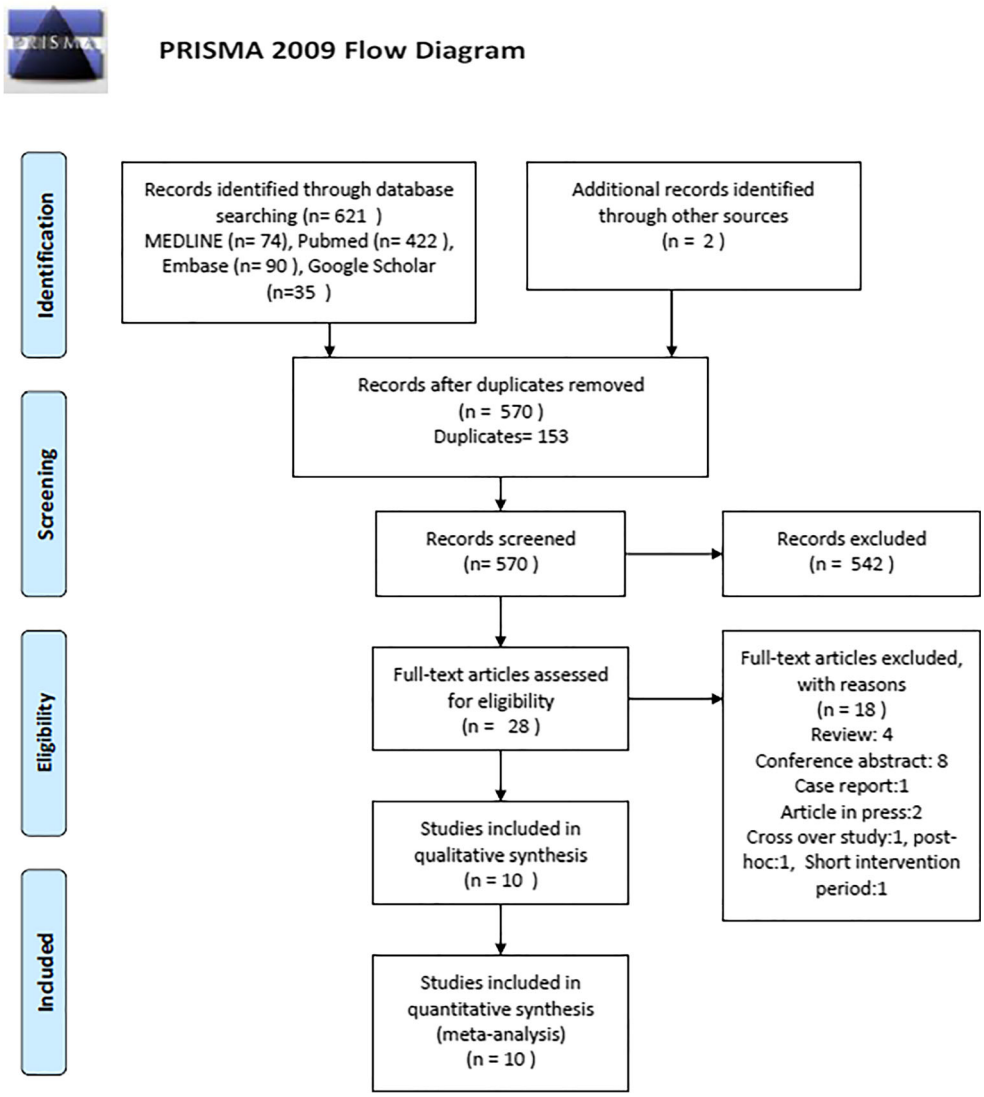

Fig. 2 Effect of probiotics on acquisition of tolerance to CMP

(2) Resolution of other clinical symptoms

Four studies ($n = 324$; probiotics, 160; control, 164) which reported on symptoms other than rectal bleeding and SCORAD index showed no difference between probiotic vs. control group [30, 33, 35, 36]. Only Candy reported lower stool frequency scores in probiotics group [36]. Three studies reported on use of antibiotic for adverse events classified as infection [33, 35, 36]. Hol reported comparable antibiotic usage in probiotic vs. placebo groups [33]. Burks reported lower use of antibiotics in probiotics group, especially of amoxicillin [35].Candy noted lower use of antibiotics in probiotics group [36].

(3) Effects on growth

Three studies reported this outcome ($n = 261$; probiotics, 130; control, 131) [30, 35, 37]. Burk and Dupont (cases from Hol 2008) reported no significant effect of probiotics on growth parameters [35, 37]. Ahanchian reported significantly more increase in weight and head circumference in synbiotic group. Increase in height was not significantly different [30]. Meta-analysis was not possible as the outcome was reported in different units.

(4) Improvement in endoscopic and histological parameters of rectum and sigmoid colon

Only Szajewska reported this outcome ($n = 26$; probiotics, 11; control, 15). Due to lack of consent only 5 infants had endoscopic and histological examination before and after completing the study. Author reported no difference in endoscopic and histological remission between probiotic and placebo group [22].

(5) Duration of rectal bleeding

Only Szajewska ($n = 26$; probiotics, 11; control, 15) reported that there was no difference in this outcome after 1 month in LGG vs. placebo group [22].

(6) Stool calprotectin levels

Only Baldassarre reported higher fecal calprotectin levels in infants with hematochezia ($n = 26$; probiotics, 12; control, 14). At the end of 4 weeks, these levels reduced significantly from the baseline in both probiotic and placebo group. However, fecal calprotectin levels showed significantly greater reduction in probiotic vs. placebo group ($p = 0.02$) [21].

(7) Intestinal microflora analysis

Burks, Kirjavainen, Hol, and Candy reported this outcome ($n = 322$; probiotics, 162; control, 160) [33–36]. Burk noted significantly higher proportion of *Bifidobacteria* in synbiotic group [35]. Kirjavainen reported no significant change in percentage of *Bifidobacteria* and *Bacteroides* before vs. after intervention in both groups [34]. Hol noted significantly higher percentage of *B. animalis* and *L. casei*, *L. paracasei* in probiotics arm [33]. Candy reported higher percentage of *Bifidobacterium* and lower percentage of *Eubacterium rectale/Clostridium coccoides* group bacteria (ER/CC) in synbiotic group similar to 51 healthy breast-feed infants [36].

(8) Adverse effects secondary to intervention

Only Kirjavainen reported that 5/13 children in heat-inactivated probiotic group (LGG) experienced diarrhea from several days to weeks after the intervention [34]. No probiotic related adverse effects were reported by other authors.

Grading of evidence and summary of findings (Table 3)

The evidence was considered low for acquisition of tolerance in view of the small sample size, heterogeneity, and high risk of bias in some of the included studies. For resolution of hematochezia evidence was graded as very low due to small sample size, high risk of bias in some of the included studies and wide CI. Given the small number of studies, we did not assess for publication bias [38].

Discussion

The results of our systematic review showed that in presumed CMPA, probiotic supplementation was not associated with earlier resolution of hematochezia compared to placebo. However, in confirmed CMPA, probiotic supplementation showed higher rate of acquisition of tolerance to CMP at the end of 3 years compared with placebo. Overall, the evidence is low quality and the findings regarding acquisition of tolerance to CMP were significantly influenced by Canani et al. 2017 [23]. The data was inadequate to assess effect of probiotics on symptoms of allergy, and growth.

Differences in effects of probiotics on hematochezia reported by Baldassarre et al. (reduced), Szajewska et al., and Ahachian et al. (no difference) may relate to insufficient adherence to dairy free diet, inadequate dosing or no effect of the probiotic in short duration [21, 22, 30]. As for fecal calprotectin, it is important to note its variability and questionable correlation with intestinal inflammation in infants with CMPA [39, 40]. Large studies are required to explore this outcome further.

Extensively hydrolyzed formula is the first-line therapy for management of CMPA given that it is associated with quicker acquisition of tolerance compared to other formulas [32]. However, its mechanisms of benefits are not yet clear. Extensively, hydrolyzed formulas have immunomodulatory

Table 3 Summary of finding for pooled data as per GRADE guidelines

Outcome	Absolute risk		Relative effect RR (95% CI)	Number of participants	Quality of evidence GRADE
	Estimated risk in control group	Corresponding risk in probiotics group			
Resolution of hematochezia	414 per 1000	571 per 1000 (368 to 886)	RR 1.38 (0.89 to 2.14)	52 (2 RCTs)	⊕○○○ Very low#
Acquisition of tolerance for CMP - Overall	453 per 1000	667 per 1000 (531 to 834)	RR 1.47 (1.17 to 1.84)	1053 (4 RCTs)	⊕⊕○○ Low*
Acquisition of tolerance for CMP - at 6 months	409 per 1000	655 per 1000 (250 to 1000)	RR 1.60 (0.61 to 4.19)	174 (2 RCTs)	⊕⊕○○ Low*
Acquisition of tolerance for CMP at 12 months	441 per 1000	657 per 1000 (424 to 1000)	RR 1.49 (0.96 to 2.31)	493 (4 RCTs)	⊕⊕○○Low*
Acquisition of tolerance for CMP at 24 months	442 per 1000	685 per 1000 (526 to 889)	RR 1.55 (1.19 to 2.01)	193 (1 RCT)	⊕⊕○○ Low*
Acquisition of tolerance for CMP at 36 months	537 per 1000	805 per 1000 (655 to 993)	RR 1.50 (1.22 to 1.85)	193 (1 RCT)	⊕⊕○○ Low*

CMP, cow's milk protein; *RR,* relative risk; *CI,* confidence interval; *RCT,* randomized controlled trial

*High-risk bias in included RCTs, small sample size, and heterogeneity

#High-risk of bias, small sample size, wide CI

properties as shown in animal models of type 1 diabetes [41]. Evidence from mice models show that the benefits of extensively hydrolyzed formula such as reduced production of IL-4, IL-5, IL-13, and increased expression of IFN-c and IL-10 are enhanced by probiotics [42]. Our systematic review also indicates the potential of probiotics as an adjuvant to extensively hydrolyzed formula for earlier acquisition of tolerance compared to extensively hydrolyzed formula alone.

One of the concerns of extensively hydrolyzed formula is its bitter taste that can lead to inadequate intake resulting in suboptimal growth. Rzehak et al. reported that except for the slower weight gain in infancy, there is no effect of extensively hydrolyzed formula on subsequent weight in children [43]. Meta-analysis of growth outcomes with probiotics as adjuvant was not possible in our review as the data was provided in different units.

Safety of probiotics supplementation needs to be discussed. Probiotic sepsis, long-term altered immune responses, and development of antibiotic resistance are important concerns with use of probiotics. There are many reports of fungaemia and bacteremia associated with probiotics [44]. A systematic review on safety of probiotic supplementation in children < 18 years which included 74 studies, has concluded that probiotics/synbiotic supplementation was safe, well-tolerated, and without adverse events. The studies included in this systematic review comprised of healthy as well as immune compromised, and obese children, and those with intestinal disorders, infections, and inflammatory disorders [45].

To our knowledge, this is the first comprehensive systematic review on the effects of probiotics in management of CMPA in children. The limitations of our review include small sample size, high statistical heterogeneity, the differences in the probiotics (type, dose, and duration) used, variation in follow-up period and the high risk of bias in the included trials. Our results should be interpreted with caution considering that except for Canani (2017) none of the included studies have reported outcomes beyond 12 months. Our conclusions are therefore influenced by the results of Canani et al. [23]. Considering these data, the routine use of probiotics for management of CMPA cannot be recommended and their use should be limited only to clinical research.

In summary, current evidence on the effects of probiotics in management of CMPA is limited and of low quality. Adequately, powered RCTs with long-term follow-up are needed to assess the potential of probiotics as an intervention for children with CMPA.

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Dr Deshmukh (MD): Independent literature search, coordinated and supervised data collection, handling meta-analysis software, carried out the initial analyses and help in writing the first and final draft of the manuscript. Addressing reviewers' comments.

Dr Patole (SP): Conceptualized and designed the study, independent literature search, interpretation of the data, critically reviewed the manuscript for important intellectual content. Addressing reviewers' comments.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent The paper is systematic review of randomized controlled trial. There was no involvement of patient so consent is not required.

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