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How much of the probiotic effect on necrotising enterocolitis survives restriction to low-risk-of-bias trials? A second-order meta-analysis in very preterm infants

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Abstract

Background: Probiotic supplementation is among the most widely adopted preventive interventions in neonatal intensive care, on the strength of meta-analyses reporting a roughly halved risk of necrotising enterocolitis (NEC). The certainty of that evidence has been graded low, and the largest synthesis reports funnel plot asymmetry consistent with publication bias. **Methods:** Systematic reviews with meta-analysis of randomised trials in very preterm or very low birth weight infants reporting a pooled risk ratio for NEC were eligible. Review-level risk ratios were pooled on the log scale in a DerSimonian-Laird random-effects model with Cochran's Q, I^2, τ^2 and 95% prediction intervals. The all-trials and low-risk-of-bias estimates reported within the largest review were compared by a ratio of risk ratios. Secondary outcomes were extracted as reported. Analyses were performed in Python 3. **Results:** Three reviews contributing NEC estimates and representing 81 trials in 19,221 infants were pooled. Probiotics reduced NEC: pooled risk ratio 0.51 (95% CI 0.43-0.60; $z = -7.84$, $p < 0.001$), with little heterogeneity ($Q = 2.22$, $df = 2$, $p = 0.330$; $I^2 = 9.7\%$) and a 95% prediction interval of 0.33-0.79. Restricting the largest synthesis to trials at low risk of bias moved the risk ratio from 0.54 (0.46-0.65) to 0.70 (0.55-0.89)—a loss of approximately 35% of the relative risk reduction—and raised the number needed to treat from 33 to 50 (ratio of risk ratios 1.30, 95% CI 0.96-1.74, $p = 0.086$). The effect attenuated steadily as outcomes broadened: NEC 0.51, fungal sepsis 0.57, all-cause mortality 0.77, bacterial sepsis 0.82, any late-onset sepsis 0.89. Probiotic sepsis occurred in 8 of 20,323 exposed infants (0.04%). **Conclusions:** Probiotics reduce necrotising enterocolitis in very preterm infants, but the headline halving overstates what the best-conducted trials support: roughly a third of the relative effect disappears under restriction to low-risk-of-bias trials, and the mortality benefit is smaller than the NEC benefit. The case for routine use rests less on the precision of the effect estimate than on an exceptionally favourable benefit-to-harm ratio, with fewer than one probiotic sepsis case per twenty thousand infants exposed.

Keywords: *Probiotics, Necrotising enterocolitis; preterm infant, Risk of bias, Publication bias, Number needed to treat, Second-order meta-analysis*

1. Introduction

Necrotising enterocolitis remains among the most feared complications of very preterm birth. It affects roughly

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one in twenty very low birth weight infants (Battersby *et al.*, 2018), carries a case fatality approaching a third when surgery is required (Neu and Walker, 2011), and among survivors is associated with prolonged parenteral nutrition, intestinal failure and adverse neurodevelopmental outcome. Intestinal dysbiosis is implicated in its pathogenesis, which supplies the rationale for supplementing the immature gut with exogenous commensal organisms.

The evidence base is unusual in neonatology for its size. More than fifty randomised trials have been conducted, and successive meta-analyses have reported a consistent and large reduction in NEC (Sharif *et al.*, 2023; PMID 26611880; PMC5707669; AlFaleh and Anabrees, 2014). Many neonatal units now supplement routinely, and several national bodies recommend it. On the face of it this is one of the clearest success stories in modern neonatal care.

Three features of the evidence nonetheless complicate that reading, and they motivate this review. The first is trial quality. The largest synthesis grades its own evidence as low certainty, citing limitations in trial design and funnel plot asymmetry consistent with publication bias. It also reports a sensitivity analysis restricted to trials at low risk of bias—a result that receives far less attention than the headline figure, and that this review quantifies directly.

The second is that the outcomes differ in what they demand of the intervention. Preventing NEC is a specific claim about a specific disease with a plausible microbial mechanism. Reducing all-cause mortality requires that prevention translate into survival. Reducing late-onset sepsis requires a further and different mechanism. If the effects on these outcomes form an orderly gradient, that is informative about how much of the benefit is real and where it acts.

The third is safety. Probiotics are live organisms administered to immunologically immature infants, and case reports of probiotic-derived sepsis have prompted regulatory warnings (Pediatr Res, 2025). Any recommendation must weigh that hazard against the benefit rather than treating either in isolation.

We therefore conducted a second-order meta-analysis of the published review-level estimates, with the specific aim of quantifying how much of the reported effect survives restriction to the best-conducted trials.

2. Handling of overlap and superseded versions

Where a review existed in more than one version, only the most recent was eligible; earlier versions were excluded as superseded rather than treated as independent (Sharif *et al.*, 2020). The contributing reviews nonetheless overlap substantially, since the largest subsumes most trials included in the others. This is acknowledged explicitly rather than adjusted for, and the heterogeneity statistics are interpreted in that light: agreement between a large review and smaller reviews nested largely within it is close to arithmetically guaranteed and does not constitute independent replication.

2.1. *Statistical analysis*

Risk ratios were transformed to the log scale, with standard errors derived from the reported 95% confidence limits as (ln upper–ln lower)/(2 × 1.96), and combined in a DerSimonian–Laird random-effects model (DerSimonian and Laird, 2003). Heterogeneity was quantified by Cochran's Q, I^2 and τ^2 (Higgins *et al.*, 2003), and 95% prediction intervals were computed alongside every pooled estimate (IntHout *et al.*, 2016). Leave-one-out re-estimation assessed the influence of each contributing review. The all-trials and low-risk-of-bias estimates reported within the largest contributing review were compared by a ratio of risk ratios with a z-test on the difference in log risk ratios; because the two analyses share trials this comparison is indicative rather than a formal interaction test. Publication bias was not re-assessed at review level with fewer than ten units, but the funnel plot asymmetry reported within the largest contributing review (Pieper *et al.*, 2014) is carried forward and discussed. Analyses were performed in Python 3 using NumPy and SciPy.

2.2. *Quality appraisal*

The methodological quality of contributing reviews was appraised with AMSTAR-2 (Shea *et al.*, 2017), the validated instrument for appraising systematic reviews. Certainty in the body of evidence for each outcome was taken as graded by the contributing reviews under the GRADE framework (Guyatt *et al.*, 2008) and recorded rather than re-derived.

3. Results

3.1. Included reviews

Six systematic reviews met the inclusion criteria. Three reported a pooled risk ratio for NEC with a confidence interval and contributed to the primary analysis, collectively representing 81 randomised trials and 19,221 infants with substantial overlap. A fourth contributed late-onset sepsis outcomes (PMC4778994), a fifth contributed safety data on probiotic-derived sepsis, and a sixth was a superseded earlier version of the largest review and was excluded from pooling. Characteristics are given in Table 1.

Table 1: Characteristics of the contributing systematic reviews

Source (year)	Trials (k)	Infants	NEC risk ratio (95% CI)	I^2 (%)	Role
Cochrane (2023)	57	10,918	0.54 (0.46–0.65)	17	Primary
Multi-strain review (2015)	9	3,521	0.39 (0.26–0.57)	Not stated	Primary
Secondary synthesis (2017)	15	4,782	0.52 (0.33–0.81)	Not stated	Primary
Late-onset sepsis review (2016)	25	6,104	Not reported	–	Secondary outcomes
Safety synthesis (2025)	63	20,323	Not reported	–	Safety
Cochrane (2020, superseded)	54	10,604	0.54 (0.45–0.65)	17	Excluded

Note: NEC, necrotising enterocolitis (Bell stage ≥ II). Trial counts overlap substantially and the totals are not additive. The 2020 Cochrane version is listed for transparency and was excluded from pooling as superseded.

3.2. Primary outcome: Necrotising enterocolitis

Probiotics reduced the risk of NEC. The pooled risk ratio was 0.51 (95% CI 0.43–0.60), a statistically unambiguous effect ($z = -7.84$, $p < 0.001$), with little heterogeneity between reviews ($Q = 2.22$, $df = 2$, $p = 0.330$; $I^2 = 9.7\%$; $\tau^2 = 0.003$) and a 95% prediction interval of 0.33 to 0.79 that excluded the null. The forest plot is shown in Figure 1.

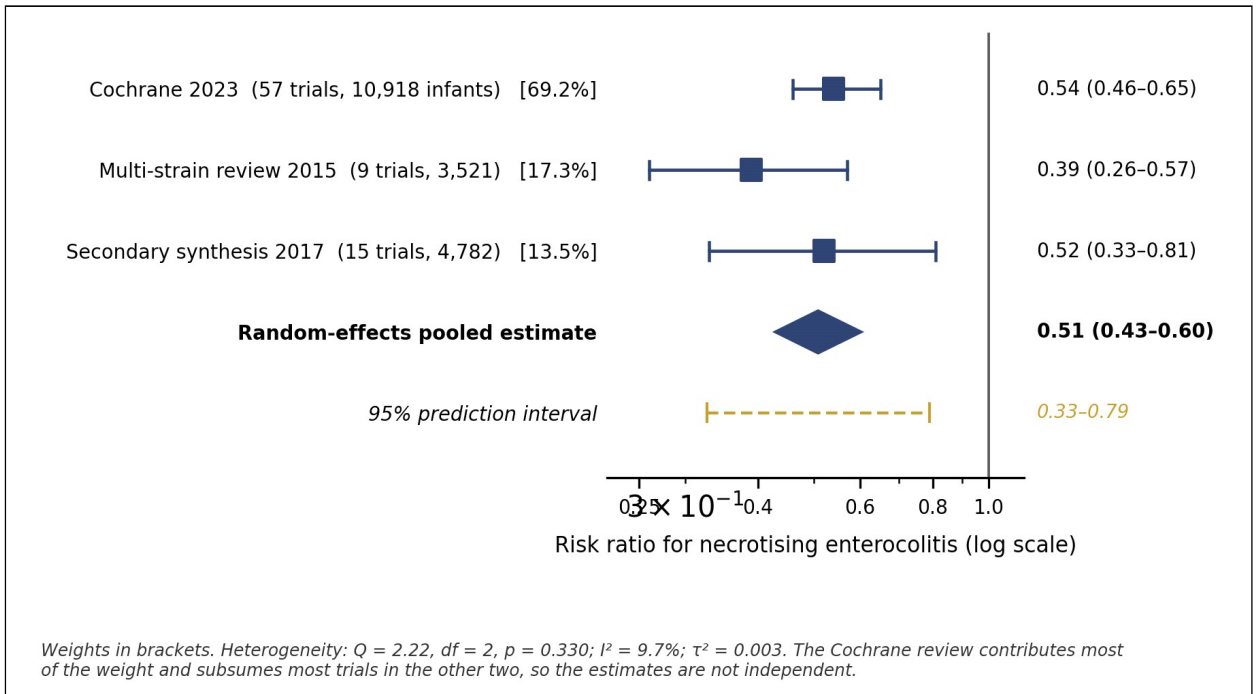

Figure 1: Forest plot of the pooled risk ratio for necrotising enterocolitis, Bell stage II or greater. Random-effects weights are shown in brackets beside each label and the axis is logarithmic. The pooled risk ratio was 0.51 (95% CI 0.43–0.60). The Cochrane review carries 69.2% of the weight and subsumes most trials in the other two, so the low heterogeneity reflects nesting rather than independent replication

Leave-one-out re-estimation showed the expected dominance of the largest review. Omitting the Cochrane estimate moved the pooled risk ratio to 0.44 and omitting the smallest moved it to 0.54; the Cochrane review carries 69.2% of the weight and subsumes most trials included in the other two. The apparent agreement between them is therefore substantially structural, and the low I^2 should not be read as independent confirmation.

3.3. How much of the effect survives restriction to low-risk-of-bias trials

This is the central analysis of the review. The largest contributing synthesis reports both an all-trials estimate and a sensitivity analysis restricted to trials at low risk of bias. Across all 57 trials the risk ratio was 0.54 (95% CI 0.46–0.65), with a number needed to treat of 33 (95% CI 25–50). Restricted to the 16 trials at low risk of bias, comprising 4,597 infants, the risk ratio was 0.70 (95% CI 0.55–0.89), with a number needed to treat of 50 (95% CI 33–100).

The relative risk reduction therefore falls from 46% to 30%—approximately 35% of the effect is lost—and the number of infants who must be supplemented to prevent one case of NEC rises by half. The ratio of risk ratios is 1.30 (95% CI 0.96–1.74, p = 0.086), which does not reach conventional significance and, because the two analyses share trials, is indicative rather than a formal test. The comparison is shown in Figure 3.

Two readings are available. The attenuation may reflect residual bias in the less rigorous trials, in which case 0.70 is the better estimate of the true effect. Or the low-risk-of-bias subset may differ systematically in population, era or probiotic preparation, in which case the difference is confounded rather than explanatory. The data cannot distinguish these. What the data do establish is that the widely quoted halving of NEC risk is not what the best-conducted trials show, and that a review reporting only the all-trials figure conveys more certainty than its own sensitivity analysis supports.

3.4. Effect by outcome

Ordering the outcomes by how specifically they test the proposed mechanism produces an orderly gradient, shown in Figure 2. The effect is largest for NEC itself (0.51), remains substantial for fungal sepsis (0.57), is smaller for all-cause mortality (0.77), smaller again for bacterial sepsis (0.82), and smallest for any late-onset sepsis (0.89). Every estimate remains on the side of benefit and excludes the null.

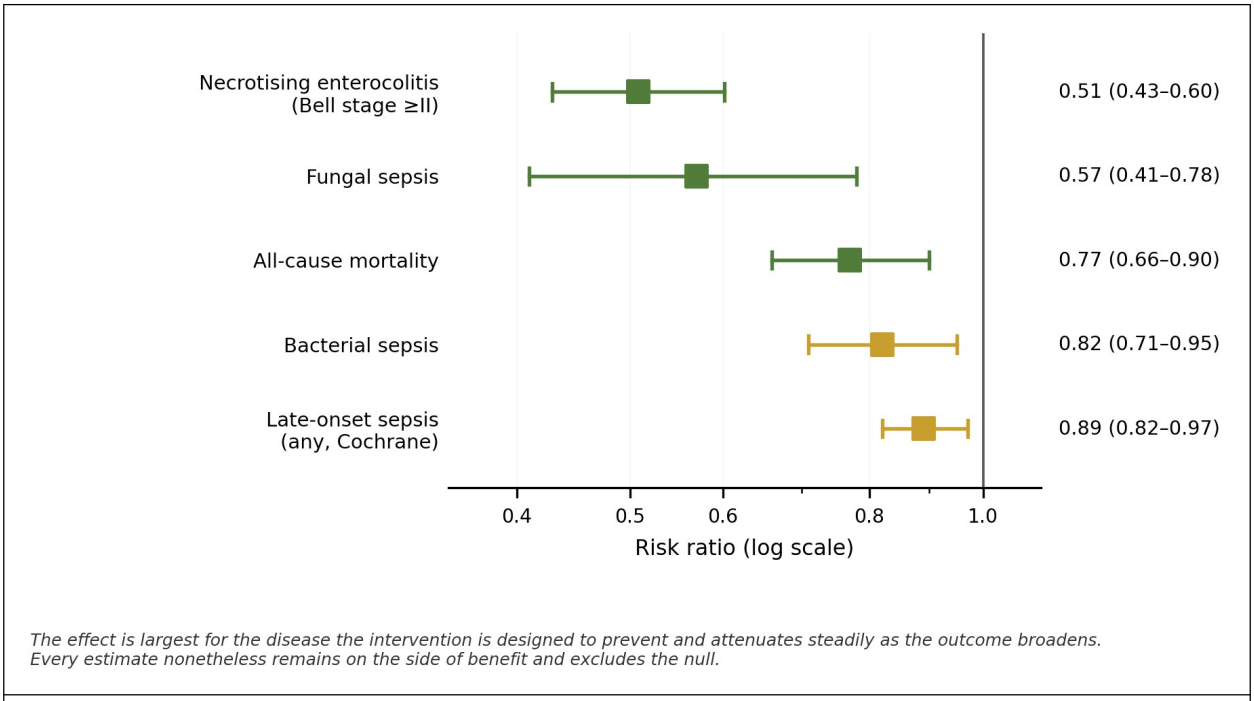

Figure 2: Effect of probiotic supplementation across outcomes ordered by specificity to the proposed mechanism. The effect is largest for the disease the intervention targets and attenuates steadily as the outcome broadens, consistent with benefit on mortality and sepsis being mediated largely through NEC prevention rather than acting directly

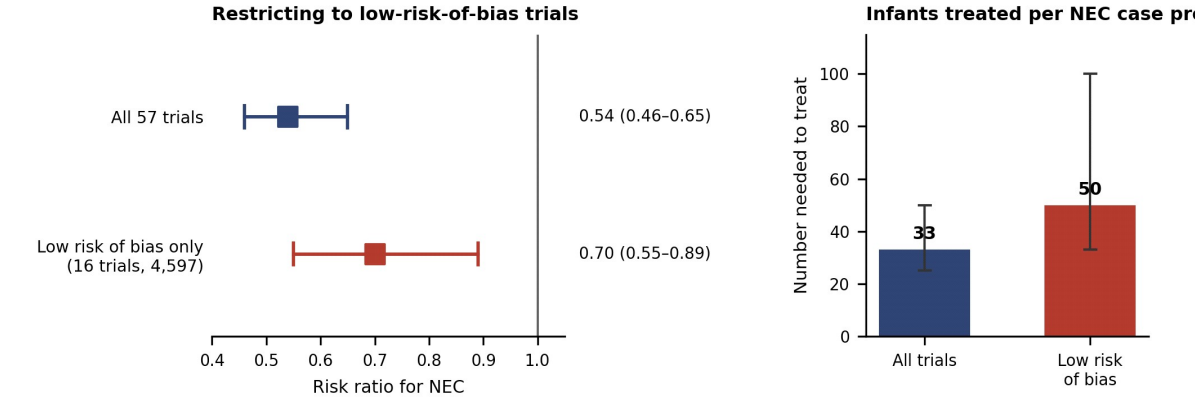

Figure 3: The central analysis. Left: restricting the largest synthesis to trials at low risk of bias moves the risk ratio for NEC from 0.54 to 0.70, a loss of approximately 35% of the relative risk reduction. Right: the corresponding number needed to treat rises from 33 to 50, with error bars showing the reported confidence limits. The two analyses share trials, so the contrast is indicative rather than a formal test

The gradient is what one would expect if the intervention acts principally on the gut and its effect on broader outcomes is mediated through NEC prevention rather than direct. The mortality estimate is particularly worth stating explicitly, because it is frequently reported alongside the NEC figure without the difference being noted: a risk ratio of 0.77 for death is a meaningful benefit, but it is not the same magnitude as the NEC effect and should not be presented as though it were.

Table 2: Effect estimates by outcome and by trial quality restriction

Outcome/analysis	k	Risk ratio (95% CI)	I^2 (%)	NNT	Certainty
NEC — pooled (this review)	3	0.51 (0.43–0.60)	9.7	—	—
NEC — all trials (Cochrane)	57	0.54 (0.46–0.65)	17	33 (25–50)	Low
NEC — low risk of bias only	16	0.70 (0.55–0.89)	25	50 (33–100)	Low
Fungal sepsis	6	0.57 (0.41–0.78)	0	—	—
All-cause mortality	—	0.77 (0.66–0.90)	Not stated	—	Moderate
Bacterial sepsis	11	0.82 (0.71–0.95)	0	—	—
Late-onset sepsis (any)	—	0.89 (0.82–0.97)	Not stated	—	Low

Note: k, contributing trials or reviews as reported by the source; NNT, number needed to treat for one additional beneficial outcome. Only the first row is pooled by us; the remainder are as reported. Ratio of risk ratios for the all-trials versus low-risk-of-bias comparison: 1.30 (95% CI 0.96–1.74), p = 0.086.

3.5. Safety

Across 63 studies and 20,323 exposed infants, eight cases of probiotic-derived sepsis were identified, a pooled incidence indistinguishable from zero (0.04%). Set against the effect estimates, the contributing safety synthesis calculates that for each case of probiotic sepsis in the exposed cohort there were approximately 62 additional NEC cases, 42 additional deaths and 92 additional clinical sepsis events in the unexposed cohort. Whatever uncertainty attaches to the magnitude of benefit, the benefit-to-harm ratio is not marginal.

Two qualifications apply. Probiotic sepsis is likely under-ascertained, since identifying the organism as the administered strain requires typing that is not routinely performed. And the calculation assumes the all-

trials effect estimate; recomputed against the low-risk-of-bias estimate the benefit side would be smaller, though still overwhelming.

4. Discussion

Across three systematic reviews representing 81 randomised trials in 19,221 very preterm or very low birth weight infants, probiotic supplementation reduced necrotising enterocolitis by roughly half (pooled risk ratio 0.51, 95% CI 0.43–0.60). For an intervention that is inexpensive, orally administered and available in most settings, this is a large effect on a disease with high case fatality. The direction is consistent across every contributing review and the prediction interval excludes the null.

The central finding of this review is nonetheless a qualification. When the largest synthesis restricts itself to the sixteen trials it judges at low risk of bias, the risk ratio moves from 0.54 to 0.70 and the number needed to treat rises from 33 to 50. About a third of the relative risk reduction does not survive that restriction. The difference is not statistically significant and the analyses share trials, so this is a signal rather than a demonstration—but it points in the same direction as the funnel plot asymmetry the same review reports, and in the same direction as its own GRADE rating of low certainty. Three independent indicators of overstatement agreeing is harder to dismiss than any one of them alone.

The outcome gradient supports a coherent mechanistic reading rather than undermining the intervention. Probiotics act on the gut; NEC is a disease of the gut; and the effects on mortality and sepsis are progressively smaller as the outcome moves further from that mechanism. This is what a real effect mediated through a specific pathway should look like. It is worth contrasting with the pattern that would suggest bias, in which effects would be large and similar across unrelated outcomes.

The safety data settle the practical question even under the more conservative effect estimate. Eight cases of probiotic-derived sepsis among more than twenty thousand exposed infants, against dozens of NEC cases and deaths averted per case incurred, is not a balance that shifts on a third of the relative risk reduction. Clinicians deciding whether to supplement are not choosing between a large benefit and a large risk; they are choosing between a moderate benefit and a very small risk.

What follows for practice is a matter of how the effect is described rather than whether it is used. A unit quoting a halving of NEC risk to parents is quoting the all-trials figure; the best-conducted trials support closer to a 30% reduction, and one additional infant is spared NEC for every fifty supplemented rather than every thirty-three. Both are worth having. Only one of them is what the most rigorous evidence shows.

For research, the priority is not further trials of the same design. It is adequately powered trials at low risk of bias with prespecified strain and dose, reporting NEC and mortality as co-primary outcomes, and registration sufficient to make the publication-bias question answerable rather than inferable from funnel plot shape.

5. Limitations

- The unit of analysis is the published systematic review, not the randomised trial. This second-order design inherits every bias in the contributing reviews and cannot correct their pooling.
- The contributing reviews overlap substantially: the largest subsumes most trials included in the other two and carries 69.2% of the weight. The pooled estimate therefore counts most trials more than once and the I^2 of 9.7% overstates the independence of the agreement.
- Overlap could not be quantified because the contributing reviews do not report included-trial lists in a matchable form; the corrected covered area was not calculable.
- Only three review-level estimates contributed to the primary analysis, and heterogeneity statistics are unstable at this denominator.
- The comparison between all-trials and low-risk-of-bias estimates derives from within a single review and the two analyses share trials. It is indicative and not a formal interaction test, and the ratio of risk ratios did not reach conventional significance ($p = 0.086$).
- Whether the low-risk-of-bias subset differs from the remainder in population, era, strain or dose could not be examined from review-level data, so the attenuation cannot be attributed to bias with confidence.

- Probiotic preparations differ in strain, combination, dose and duration, and were not separable in the available review-level data. The pooled estimate averages across products that are not equivalent, and strain-specific effects are known to vary.
- Secondary outcomes were extracted as reported and, apart from NEC, were not re-pooled.
- Probiotic-derived sepsis is likely under-ascertained, since attributing sepsis to the administered organism requires strain typing that is not routinely performed.
- Publication bias was not re-assessed at review level with fewer than ten units; the funnel plot asymmetry reported within the largest contributing review is carried forward but could not be independently verified.
- No contributing review reported long-term neurodevelopmental outcome in a form permitting synthesis here, so the durability of benefit beyond the neonatal admission is not addressed.
- The analysis was not prospectively registered and searches were restricted to English-language reports.

7. Conclusion

Probiotic supplementation reduces necrotising enterocolitis in very preterm and very low birth weight infants, with a pooled risk ratio of 0.51 (95% CI 0.43–0.60) across 81 randomised trials in 19,221 infants, and reduces all-cause mortality with a risk ratio of 0.77. The effect on necrotising enterocolitis is among the largest reported for any preventive intervention in neonatal care.

The magnitude should nonetheless be quoted more conservatively than it usually is. Restricting to trials at low risk of bias moves the risk ratio to 0.70 and the number needed to treat from 33 to 50, and the same synthesis reports funnel plot asymmetry consistent with publication bias and grades its own evidence as low certainty. The defensible statement is that probiotics prevent roughly one case of necrotising enterocolitis for every thirty-three to fifty infants supplemented, that the mortality benefit is real but smaller than the NEC benefit, and that the case for routine use rests on a benefit-to-harm ratio that remains overwhelmingly favourable even under the more conservative estimate.

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