SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	A systematic review of probiotics and experimental necrotizing enterocolitis	
2.	Authors (names, affiliations, contributions)	Gayatri Athalye-Jape[1,2,3], Shripada Rao[1,3],Sanjay Patole[2,3] Department of Neonatal Paediatrics, Princess Margaret Hospital for Children[1], Department of Neonatal Paediatrics, KEM Hospital for Women[2], Centre for Neonatal Research and Education, University of Western Australia[3], Perth, Western Australia **Contributions:** (1)**GA-J:** GA-J will perform an independent literature search, select studies for inclusion, extract and interpret data, assess risk of bias (ROB) of included studies, handle the meta-analysis software, and write the first and final draft of the manuscript. (2) **SR:** SR will also perform an independent literature search, select studies for inclusion, contact authors for additional information, extract and interpret the data, crosscheck the data on meta-analysis software, assess the ROB of included studies, and help with the first and the final draft of the manuscript. (3) **SP:** SP will supervise the project, act as referee author in case of differences of opinion between the first 2 authors, interpret the data, and supervise the first and final versions of the manuscript.	
3.	Other contributors (names, affiliations, contributions)	None	
4.	Contact person + e-mail address	Associate Professor Shripada Rao; shripada.rao@health.wa.gov.au	
5.	Funding sources/sponsors	None	
6.	Conflicts of interest	We have no conflicts of interest to declare.	
7.	Date and location of protocol registration	7th August 2016, Perth, Western Australia	
8.	Registration number (if applicable)	-	
9.	Stage of review at time of registration	50% under progress	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	Studies in animal models are crucial for understanding the mechanisms for the benefits and adverse effects of an intervention selected for potential clinical use.	

		Investigators have evaluated the effects of probiotics in different animal models of NEC. The small sample size of individual studies makes it difficult to derive firm conclusions. Systematic review and meta-analysis of data from small but comparable individual studies is a valuable method to generate reliable evidence with higher precision and power. It is important to note that studies in animal models, though considered as the gold standard for guiding research and clinical practice, can have significant biases compromising their validity. A systematic review of studies in animal models of NEC is therefore important not only to assess their quality/validity but also to understand the pathways for benefits of probiotics in reducing the incidence and/or severity of the illness.	
	Research question		
11.	Specify the disease/health problem of interest	Necrotizing enterocolitis (**NEC**) is a devastating gastrointestinal emergency in human preterm infants (gestation <32 weeks) with significant mortality (25%) and morbidity including long-term neuro-developmental impairment (NDI). Mortality (45-100%) and morbidity is highest in infants born before 28 weeks of gestation. The economic burden associated with ≥ Stage II NEC has been estimated to be as high as one billion dollars per year in USA, not accounting for the expenses associated with ongoing care of survivors of NEC with NDI.	
12.	Specify the population/species studied	All large and small validated animal models of experimental necrotizing enterocolitis where the effect of probiotics has been studied will be included.	
13.	Specify the intervention/exposure	Studies assessing probiotic or probiotic enriched formula, killed/inactivated probiotic, probiotic DNA, or probiotic conditioned medium (PCM) vs. placebo/control/dam fed animals will be included.	
14.	Specify the control population	Controls will be dam fed (maternally fed) validated animals of the same species as the intervention group.	
15.	Specify the outcome measures	Primary outcomes will include the incidence and/or severity of NEC. Secondary outcomes will include effects of probiotics or their derivatives on pathways involved in the pathogenesis of NEC.	
16.	State your research question (based on items 11-15)	To systematically review the effects of probiotics in animal models of NEC. (Ref. Primary and secondary outcomes as abve)	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	☑MEDLINE via PubMed ☑Web of Science ☐SCOPUS ☑EMBASE ☑Other, namely: abstracts of the Pediatric Academic Societies, Animal Welfare Information Centre ☐Specific journal(s), namely:	
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	When available, please add a supplementary file containing your search strategy: [file will be updated later as a word document]	

19.	Identify other sources for study identification	☑Reference lists of included studies ☐Books ☑Reference lists of relevant reviews ☐Conference proceedings, namely: British Maternal and Foetal Society Meetings, European Academy of Paediatric Societies, Perinatal Society of Australia and New-Zealand ☑Contacting authors/ organisations, namely: ☑Other, namely: Grey literature (open grey, trove, ntis, google scholar)	
20.	Define search strategy for these other sources	Not applicable	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	All the above databases and e abstracts from relevant conference proceedings as well as grey literature sources will be searched from inception till current date for the words highlighted in the search terminology.	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	(a) Two authors (GA-J and SR) will review each title/abstract in the screening phase and (b) any discrepancy will be resolved by discussion among all authors (GA-J, SR and SP)	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	**Inclusion criteria:** Only randomised controlled trials (RCTs) will be eligible for inclusion. **Exclusion criteria:** Narrative reviews, systematic reviews, case reports, letters, editorials and commentaries will be excluded, but will be read to identify potential additional studies. Studies in hamsters, nematodes, and invertebrates will be excluded as their relevance to NEC as it occurs in human preterm infants is uncertain.	
24.	Type of animals/population (*e.g.* age, gender, disease model)	**Inclusion criteria:** Validated animal models (rats, mice, piglets, rabbit, quail) **Exclusion criteria:** Hamsters, nematodes, and invertebrates	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	**Inclusion criteria:** Enteral probiotic supplementation in any dose, duration, frequency, type and combination vs. placebo/control. Studies assessing probiotic, probitic enriched formula, killed/inactivated probiotic, probiotic DNA, or probiotic conditioned medium (PCM) vs. placebo/control/dam fed animals will also be included. **Exclusion criteria:** Not fitting the inclusion criteria	
26.	Outcome measures	Primary outcomes will include the incidence and/or severity of NEC. Secondary outcomes will include effects of probiotics or their derivatives on pathways involved in the pathogenesis of NEC	
27.	Language restrictions	No language restrictions will be applied	
28.	Publication date restrictions	**Inclusion criteria:** No restrictions	
29.	Other	Not applicable	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase: 1. Non-RCTs, and narrative reviews, systematic reviews, case reports, letters, editorials 2. Studies not done on in validated models of NEC 3. Studies not assessing probiotic, probitic enriched formula, killed/inactivated probiotic, probiotic DNA, or	

		PCM vs. placebo/control/dam fed animals	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	Authors, year	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	RCT vs. non-RCT, sample size, type of experimental groups	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	Characteristics of the animal model, including species, gender (where available) and disease induction will be included.	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	Probiotic protocol (Dose, duration, type, frequency)	
35.	Outcome measures	Primary outcome will be Incidence and/ or severity of experimental NEC	
36.	Other (*e.g.* drop-outs)	Pre-euthanasia mortality	
	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	(a) GA-J and SR will assess the study quality as per SYRCLE guidelines and (b) any discrepancy will be resolved by discussion among all authors (GA-J, SR and SP)	
38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	☑By use of SYRCLE's Risk of Bias tool[4] □By use of SYRCLE's Risk of Bias tool, adapted as follows: □By use of CAMARADES' study quality checklist, e.g [22] □By use of CAMARADES' study quality checklist, adapted as follows: □Other criteria, namely:	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	Incidence of any NEC: Binary outcome Severe NEC: Binary outcome Secondary outcomes (e.g. laboratory markers, injury scores): N (%), and summary statistics (e.g. mean/std dev.)	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	Data will be extracted either from the results section or the graphs in the manuscript otherwise authors would be contacted for additional data/clarifications on methodology.	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	(a) GA-J and SR will extract the data from each study and (b) any discrepancy will be resolved by discussion among all authors (GA-J, SR and SP)	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Primary outcomes : Pooled when data available in requisite format (Ref : 39) Secondary outcomes: Descriptive summary	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	Meta-analysis will be performed when requisite data is available in a particular format	
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean	Mean difference and risk ratio will be used to measure the effects of binary outcomes.	

	difference, risk ratio, odds ratio)		
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effects model will be used for meta-analysis and results will also be confirmed by fixed effects model (to confirm the effect size of individual studies).	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I^2 will be used to assess heterogeneity	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	A subgroup analysis is planned for each of the animal species in the included studies as the effects of probiotics could be species specific.	
48.	Any sensitivity analyses you propose to perform	None pre-planned.	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)		
50.	The method for assessment of publication bias	Funnel plot will be used for assessment of publication bias.	
Final approval by (names, affiliations):		Prof. Sanjay Patole, A/Prof. Shripada Rao and Dr Gayatri Jape	Date: 7/8/2016