

1 **Title: Effect of community-initiated kangaroo mother care on fecal biomarkers of gut**  
2 **function in low birthweight infants in North India: a randomized clinical trial**

3

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31 **ABSTRACT** (Unstructured)

32 This individually randomized trial was conducted to estimate the effect of promoting community-  
33 initiated kangaroo mother care (ciKMC) in low birthweight (LBW) infants on gut inflammation  
34 and permeability. Participants included 200 stable LBW infants (weighing 1500-2250 g) in North  
35 India enrolled between May to October 2017. The ciKMC intervention included promotion and  
36 support of continuous skin-to-skin contact and exclusive breastfeeding through home visits. The  
37 mothers in the intervention arm were supported to practice ciKMC until 28 days after birth, i.e.  
38 the neonatal period, or till the baby wriggled out of KMC position, if earlier. Infant stool  
39 specimens were collected during the first week of birth, and within one week after end of the  
40 neonatal period. Concentrations of fecal neopterin (nmol/L), myeloperoxidase (ng/mL), and  
41 alpha-1-antitrypsin ( $\mu\text{g/mL}$ ) were determined using ELISA, and composite enteric enteropathy  
42 (EE) score at end of the neonatal period was calculated by principal component analysis. We  
43 did not find any substantial difference in means between the ciKMC and control arm infants in  
44 the log-transformed values of neopterin (0.03; 95% CI -0.15 to 0.21), myeloperoxidase (0.28;  
45 95% CI -0.05 to 0.61) and alpha-1-antitrypsin (0.02; 95% CI -0.30 to 0.34). The mean (SD)  
46 composite EE score was 13.6 (7.5) in the ciKMC and 12.4 (8.3) in the control arm infants, and  
47 the adjusted difference in means was negligible, 0.4 (95% CI -1.8 to 2.7). Our findings suggest  
48 that the promotion of ciKMC did not affect gut inflammation and permeability in our target  
49 population of low birthweight infants in North India.

50 **Key words:** Kangaroo mother care, Enteropathy, Gut inflammation, Gut permeability, low birth  
51 weight, infant

## 52 INTRODUCTION

53 Gut function among young children in low-income communities is postulated to be one of the  
54 important drivers of poor growth. Environmental enteric dysfunction (EED) is described as a  
55 broad syndrome with various alterations in gut function including increased gut inflammation,  
56 altered gut permeability, villous blunting, and crypt hyperplasia as a consequence of chronic  
57 exposure to enteropathogens.<sup>1</sup> EED leads to a vicious cycle of reduced intestinal absorptive  
58 capacity, which in turn causes protein-energy and micronutrient malnutrition, and thereby poor  
59 growth.<sup>1,2,3</sup> A multicentric cohort study in 8 countries showed that children with the highest  
60 enteric enteropathy (EE) score (calculated using the three fecal biomarkers neopterin,  
61 myeloperoxidase and alpha-1-antitrypsin) grew 1.08 cm less than those with the lowest EE  
62 score during the following 6-month period.<sup>4</sup> EED is of greater concern in infants born preterm or  
63 low birth weight, who are at higher risk of enteric infections and growth faltering.<sup>5, 6, 7</sup>

64 It is unclear whether EED, gut inflammation, or permeability can be prevented early in life. It is  
65 speculated that antibiotics or probiotics, improved infant feeding, zinc supplementation, or  
66 water, sanitation, and hygiene (WASH) interventions might improve gut function by reducing the  
67 enteric pathogen load, gut inflammation and/or permeability.<sup>1</sup> However, studies have not been  
68 able to demonstrate clear reproducible changes in fecal markers of gut function following these  
69 interventions.<sup>8, 9, 10, 11</sup> The World Health Organization (WHO) and the Government of India  
70 recommend Kangaroo Mother Care (KMC), an intervention encompassing skin-to-skin contact  
71 (SSC) and exclusive breastfeeding, to improve survival in low birth weight (LBW) babies.<sup>12, 13, 14</sup>

72 A large randomized controlled trial in India among 8402 LBW infants demonstrated that  
73 promotion of community-initiated KMC (ciKMC) improved post-enrolment neonatal survival by  
74 30%.<sup>15</sup> In addition, it reduced the risk of possible serious bacterial infection, diarrhea, and  
75 severe underweight during the neonatal period, i.e. the first 28 days of life. It is plausible that  
76 KMC reduces gut inflammation and permeability in LBW infants as the baby is placed in a

77 protective environment in SSC and exclusively breastfed, reducing the chance of  
78 enteropathogen exposure and clinical infection.

79 Our primary objective was to estimate the effect of ciKMC promotion in LBW infants on gut  
80 inflammation and permeability at the end of the neonatal period. In addition, we estimated the  
81 effect of ciKMC promotion on enteric enteropathy. We hypothesized that the promotion of  
82 ciKMC reduces gut inflammation and permeability as reflected in a lower concentration of fecal  
83 neopterin, myeloperoxidase, and alpha-1-antitrypsin, and enteric enteropathy as reflected in a  
84 lower composite EE score and EE index.

85

## 86 **METHODOLOGY**

### 87 *Ethics Statement*

88 Ethics approval was obtained from the Society for Applied Studies' ethics committee  
89 (SAS/ERC/KMCS/2017) and the Regional Committee for Medical and Health Research Ethics  
90 (REK) in Western Norway. The study was registered with Clinical trials registry-India  
91 (CTRI/2017/04/008430). Written informed consent including permission for storage of  
92 specimens for future research was obtained from the mothers of the eligible infants before  
93 enrollment.

### 94 *Study design and participants*

95 This individually randomized clinical trial was developed as a sub-study within a larger primary  
96 trial (ClinicalTrials.gov NCT02653534).<sup>15, 16</sup> The trial was conducted in rural and semi-urban low-  
97 income populations of Faridabad and Palwal districts in Haryana, India. As part of the primary  
98 trial<sup>15, 17</sup>, pregnant women were followed up by a surveillance team periodically till delivery.  
99 Newborns weighing 1500 to 2250 g and their mothers were eligible for inclusion if they were

100 screened within 72 hours of birth. Infants were excluded if KMC had already been initiated in a  
101 birth facility, or infants were unable to feed, had breathing problems, gross congenital  
102 malformations, less than normal movements, or mothers were not living with their babies or  
103 intending to move away over the next six months. Additionally, in this sub-study, we excluded  
104 twins and triplets. In the primary trial enrolments were done between July 2015 to October 2018.  
105 For evaluation of fecal biomarkers, we enrolled consecutive 200 infants from May 2017 onwards  
106 who provided consent; only one eligible child was enrolled from each household (Figure 1).

107

### 108 *Intervention and Usual Care*

109 The ciKMC intervention comprised of promotion and support of continuous and prolonged SSC  
110 and exclusive breastfeeding. The intervention delivery team visited homes of the infant-mother  
111 dyads allocated to the ciKMC trial arm to initiate and support KMC. The team visited on days 1,  
112 2, 3, 5, 7, 10, 14, 21, and 28 after birth to observe and solve any problems related to KMC  
113 Mothers and family members were taught to daily record the duration of SSC. Mothers were  
114 counselled to practice SSC for as long as possible during day and night, with the assistance of  
115 other family members. Visits continued till 28 days of age or if the baby wriggled out of KMC  
116 position and no longer accepted SSC, whichever was earlier. Referral of ill infants in both trial  
117 arms was facilitated through government Accredited Social Health Activists (ASHAs).<sup>18</sup> All  
118 infants in the intervention and control arms received usual care, i.e. home-based postnatal care  
119 visits by ASHAs as implemented through the health system.<sup>19</sup>

120

### 121 *Study Outcomes*

122 The primary outcomes were the concentration of the individual fecal biomarkers viz. neopterin  
123 (nmol/L), myeloperoxidase (ng/mL), and alpha-1-antitrypsin ( $\mu\text{g/mL}$ ) at the end of the neonatal  
124 period (day-28 of birth up to plus 7 days). Other outcomes were EE score and EE index which is

125 a composite score calculated using three fecal biomarkers neopterin, myeloperoxidase and,  
126 alpha-1-antitrypsin. Neopterin is an indicator of T-helper cell 1 activity. Myeloperoxidase reflects  
127 neutrophil activity in the intestinal mucosa while alpha-1-antitrypsin indicates intestinal  
128 permeability and protein loss. These fecal biomarkers have been previously used in multiple  
129 studies as proxy measures of gut inflammation and permeability.<sup>3, 20, 21, 22</sup>

130

### 131 *Assessment of Outcomes*

132 Stool specimens were collected at baseline within the first week of birth and within 1 week after  
133 the end of the neonatal period. A stool kit consisting of a cold box with 4 ice-packs, a labelled  
134 sterile (gamma-irradiated) stool container with a spatula, a plastic nappy, and tissue paper was  
135 provided to the participant's mother. The process of specimen collection was demonstrated to  
136 the mother and family members by the field worker. The mother was instructed to store ~5g  
137 stool in the cold box only between 6 AM and 3 PM to enable prompt transportation to the  
138 laboratory. The mother called the study team upon specimen collection, after which a  
139 fieldworker transported the cold box to reach the Clinical and Research Laboratories, Society for  
140 Applied Studies (CRL SAS), New Delhi within 6 to 8 hours. The specimens were stored without  
141 fixatives<sup>4</sup> in a -80°C freezer until analysis.

142 Laboratory analysis was initiated in CRL SAS, New Delhi, in October 2018. Fecal biomarkers of  
143 gut function were analyzed by the ELISA method using the automated EVOLIS™ Twin Plus  
144 system (BioRad, California, USA). We used IBL International Kit (Hamburg, Germany) for  
145 neopterin assessment; K6630 IDK MPO ELISA kit (Immundiagnostik AG, Bensheim, Germany)  
146 for myeloperoxidase; and the Human A1AT kit (Immuchrom GmbH, Heppenheim, Germany) for  
147 alpha-1-antitrypsin. All three kits were verified to identify the acceptable range of values for  
148 accuracy using the manufacturer standards, inter and intra-assay precision, and linearity before  
149 conducting the experiments with the study stool specimens. Standard kit instructions were

150 followed; specimens with values out of range were diluted as required and the dilution factor  
151 was accounted for when calculating the final values.

152

### 153 *Sample Size*

154 With 95% confidence and 90% power, a total sample size of 168 infants (84 in each arm) was  
155 deemed sufficient to detect at least a 0.5 SD difference in the mean concentration of fecal  
156 biomarkers between the trial arms. Assuming a 15% attrition due to loss to follow-up or failed  
157 stool specimen collection or processing, we enrolled 200 infants (100 in each of the ciKMC and  
158 control arms) into our trial.

159

### 160 *Statistical Analysis*

161 Analyses were conducted on an intent-to-treat basis using STATA version 16 (Stata  
162 Corporation, College Station, TX). Given the right-skewed distribution of the fecal biomarkers,  
163 we reported medians with interquartile ranges (IQRs), presented violin plots for both study arms,  
164 and log-transformed (natural logarithm) the data prior to statistical analyses. Pearson correlation  
165 coefficient in pairwise comparisons was estimated for the three fecal biomarkers.

166

167 Using the approach of MAL-ED investigators<sup>4</sup>, a composite score based on the percentile  
168 category of the myeloperoxidase, neopterin, alpha-1-antitrypsin concentrations was developed  
169 using the weightage factor as per principal component analysis. The principal component  
170 analysis indicated a 6-fold higher weight for myeloperoxidase and neopterin compared to alpha-  
171 1-antitrypsin. The EE score calculation is shown in the equation below, where myeloperoxidase,  
172 neopterin, alpha-1-antitrypsin categories are defined as 0 ( $\leq$  25<sup>th</sup> percentile), 1, (25–75<sup>th</sup>  
173 percentile), or 2 ( $\geq$  75<sup>th</sup> percentile). The score ranged from 0 to 26.



174  
175  $EE\ score = 6*(neopterin\ category) + 6*(myeloperoxidase\ category) + 1*(alpha-1-antitrypsin$   
176  $category)$

177  
178 To calculate the EE index we used Stata's "factor" command including the three log-transformed  
179 fecal biomarker variables and thereafter generated the index using the "predict" command. The  
180 value of this index ranged from -4.3 to 1.73.

181  
182 We applied the Student's t-test to estimate if there were any substantial differences in the  
183 unadjusted mean of the log-transformed concentrations of the fecal biomarkers at 1 month of  
184 age between the ciKMC arm and the control arm. We used multivariable generalized linear  
185 models (GLM) of the Gaussian family with an identity link to estimate the difference in means of  
186 the log-transformed fecal biomarkers between the trial arms adjusted for its baseline  
187 concentration and unequally distributed potential confounding factors at baseline. Unequal  
188 distribution of a potential confounding factor was *a priori* defined as a relative difference of more  
189 than 10% across the study arms.<sup>23</sup> Wealth quintiles, WASH factors (toilet facility and source of  
190 drinking water), birth order, baby sex, weight at enrolment, and gestational age were the  
191 potential confounders associated with the primary outcome at  $P < 0.10$  in univariable analysis.

192  
193 Subgroup analyses, decided *a priori*, were conducted to estimate whether the effect of ciKMC  
194 on gut inflammatory biomarkers at the end of 1 month after birth was different in preterm infants  
195 (<37 weeks gestation) compared to term infants ( $\geq 37$  weeks gestation). Gestational age was  
196 estimated from the ultrasonography reports, when available, or based on the last menstrual  
197 period as documented in hospital records or as per maternal recall, in the given order of  
198 preference.

199

200 **RESULTS**

201 Stool specimens were collected from all 200 infants enrolled in the trial at baseline and the end  
202 of the neonatal period. Fecal biomarker assessments were completed in 99% to 100% of the  
203 infants in both study arms for all three biomarkers (Figure 1).

204 The mean (SD) age when the baseline stool specimens were collected was 4.2 (1.6) days in the  
205 ciKMC arm and 5.1 (1.2) days in the control arm. The mean (SD) age of stool specimen  
206 collection at the end of the neonatal period was 31.6 (3.4) days in the ciKMC arm and 32.0 (5.5)  
207 days in the control arm. Baseline characteristics were similar in the two study arms other than  
208 for wealth quintiles (lower 3 quintiles), availability of toilet facility in the household, source of  
209 drinking water (public tap), birth order ( $\geq 5$ ), and sex of the baby, where the relative differences  
210 between study arms exceeded 10% (Table 1).

211 All mothers in the intervention arm and 4% in the control arm reported practice of SSC during  
212 the neonatal period. In the intervention arm, the median (IQR) age of the infant at ciKMC  
213 initiation was 27.5 (12.5 to 38.5) hours. The mothers in the intervention arm practiced SSC for a  
214 median of 28 days with a mean (SD) of 12.2 (3.1) hours per day. Exclusive breastfeeding  
215 prevalence (24-hour recall) at day-28 was 84% in the ciKMC arm and 60% in the control arm  
216 (Online supplemental table 1). Diarrhea or dysentery during the neonatal period was reported in  
217 3% in the ciKMC arm and 9% in the control arm infants. The chi-square test showed that there  
218 were no significant differences in diarrhea or dysentery between the trial arms ( $p=0.075$ )

219 Pearson's correlation coefficient ( $r$ ) suggested a low correlation between the baseline  
220 concentrations of neopterin or myeloperoxidase with alpha-1-antitrypsin ( $r = <0.1$ ). At baseline,  
221 the median (IQR) concentrations of fecal neopterin were 1497 (993 to 2397) nmol/L and 1268  
222 (879 to 1893) nmol/L, myeloperoxidase were 201 (94 to 388) ng/mL and 208 (98 to 340) ng/mL,

223 and alpha-1-antitrypsin were 282 (148 to 544)  $\mu\text{g/mL}$  and 302 (187 to 549)  $\mu\text{g/mL}$  in the ciKMC  
224 arm and control arm infants, respectively (Figure 2).

225 At the end of the neonatal period, the median (IQR) concentration of fecal neopterin were 1866  
226 (1022 to 2371)  $\text{nmol/L}$  and 1689 (1055 to 2355)  $\text{nmol/L}$ ; myeloperoxidase were 324 (137 to 498)  
227  $\text{ng/mL}$  and 262 (93 to 392)  $\text{ng/mL}$ ; alpha-1-antitrypsin were 310 (164 to 649)  $\mu\text{g/mL}$  and 298  
228 (178 to 605)  $\mu\text{g/mL}$  in the ciKMC and control arm infants, respectively (Figure 3). The mean  
229 (SD) composite EE Score was 13.6 (7.5) in the ciKMC arm and 12.4 (8.3) in the control arm  
230 infants.

231 The adjusted difference in means between the ciKMC arm and control arm in the log-  
232 transformed concentration of neopterin was 0.03 (95% CI -0.15 to 0.21), myeloperoxidase was  
233 0.28 (95% CI -0.05 to 0.61), and alpha-1-antitrypsin was 0.02 (95% CI -0.30 to 0.34). The  
234 adjusted difference in means in the EE score was 0.44 (95% CI -1.81 to 2.69), that for the EE  
235 index 0.17 (95% CI -0.11 to 0.45). Unadjusted analysis showed similar results (Table 2).

236 In term infants, there was virtually no difference between the study arms in the mean log-  
237 transformed concentrations of fecal biomarkers, nor in the EE score or the EE index (Table 3).  
238 Among the preterm infants, the adjusted difference in means between study arms in the log-  
239 transformed concentration of myeloperoxidase was 0.41 (95% CI 0.02 to 0.82), and that for EE  
240 index was 0.38 (95%CI 0.01 to 0.75).

241

## 242 **DISCUSSION**

243 We aimed to estimate the effect of ciKMC promotion among LBW infants on fecal biomarkers of  
244 gut inflammation and permeability, enteric enteropathy score and index at the end of the  
245 neonatal period. In our trial of 200 North Indian LBW infants, we did not find evidence of any

246 substantial effect of ciKMC promotion on the concentrations of fecal neopterin,  
247 myeloperoxidase, or alpha-1-antitrypsin, nor on the enteric enteropathy score or index.

248 Our effectively implemented randomization, no loss to follow-up and adjustment for potential  
249 confounders makes it unlikely that selection bias compromised the validity of our findings. Errors  
250 in the measurement of fecal biomarkers are unlikely, given the use of an automated ELISA  
251 system and pre-study kit validation exercises. Similar to the protocol followed in the MAL-ED  
252 study sites<sup>4</sup>, we did not use any fixatives or protease inhibitors while storing fecal specimens.  
253 Therefore, we cannot rule out the possibility of some degree of natural degradation of the  
254 biomarker proteins. Nonetheless, given that the fecal specimens were stored within 6-8 hours of  
255 stool passage at -80°C, with no freeze-thaw events, we believe this not to be a major concern.

256 There is a possibility that stool specimens collected during a diarrheal episode may lead to  
257 inaccurate measurement of fecal biomarker concentrations. We included all eligible LBW infants  
258 in the primary analysis, as history-based ascertainment of diarrhea in neonates is not always  
259 reliable. Although we did not find a statistically significant difference in this substudy, the  
260 proportion of children with diarrhea or dysentery in the trial arms was comparable to that in the  
261 primary trial.<sup>15</sup> A sensitivity analysis excluding the infants with diarrhea during the neonatal  
262 period showed similar estimates as described in the results section (data not shown). With the  
263 low likelihood of bias, the study findings seem internally valid and suggest that the promotion of  
264 ciKMC is unlikely to affect gut inflammation and permeability in the target population of stable  
265 LBW infants in our study setting.

266 We did not find previous studies that examined the effect of KMC on infant gut function. Some  
267 trials evaluated the effect of different interventions like WASH or improved infant feeding  
268 practices on gut function but failed to demonstrate clear effects on reducing gut inflammation or  
269 permeability.<sup>8, 22</sup> The SHINE I cluster-randomized trial, estimated the effect of improved WASH  
270 and infant and young child feeding (IYCF) practices on environmental enteric dysfunction in

271 children aged 1 to 18 months.<sup>8</sup> The trial found no effect of improved WASH or IYCF  
272 interventions on fecal myeloperoxidase, neopterin, alpha-1-antitrypsin levels in the first 6  
273 months of life. Another trial in Bangladesh showed that age-appropriate nutrition counselling  
274 plus a lipid-based nutrient supplement substantially reduced neopterin concentration at 3 and 14  
275 months of age, but there was no effect on myeloperoxidase, alpha-1-antitrypsin levels, or  
276 lactulose-mannitol ratio, another marker of gut permeability. At 28 months of age, however,  
277 myeloperoxidase and the lactulose-mannitol ratio were higher among children in the intervention  
278 arm than in the control arm.<sup>22</sup> Our trial did not show evidence of any effect of KMC promotion on  
279 fecal biomarker concentration at the end of the neonatal period. Better resource availability  
280 could have enabled a longer follow-up period, which would have been useful to study the effect  
281 over time.

282 The median concentrations of myeloperoxidase, neopterin, and alpha-1antitrypsin in our study  
283 were lower than that observed in many of the previous studies. This may be because of the  
284 specific population of LBW infants included in our trial, the timing of fecal specimen collection  
285 and/or differences in the ELISA kits used. We analysed the concentration of fecal specimens  
286 which were collected within 7 days of birth and at the end of the neonatal period, whereas in  
287 earlier studies fecal specimens were studied mostly in children 3 months and older.<sup>4, 21, 24, 25</sup> A  
288 study documenting trends in fecal biomarker concentrations over time suggests that they are  
289 probably higher when babies are 3 to 6 months of age than younger infants.<sup>24</sup>

290  
291 The exploratory subgroup analyses in preterm infants did not suggest any meaningful  
292 differences between the study arms in the measured fecal biomarkers at the end of the neonatal  
293 period. The somewhat higher myeloperoxidase levels among preterm infants in the ciKMC arm  
294 could be an incidental finding.<sup>26</sup> Alternatively, it may be explained by the higher rates of  
295 exclusive breastfeeding in the ciKMC arm which are believed to be associated with increased

296 fecal myeloperoxidase levels.<sup>24</sup> More research on the effect of interventions on gut function in  
297 preterm infants and association of breastfeeding with fecal biomarkers of inflammation would be  
298 helpful.

299

300 The study did not show evidence to support our hypothesis that KMC promotion can reduce  
301 fecal biomarkers of gut inflammation and permeability in stable LBW neonates in rural and peri-  
302 urban settings in North India. Our study had some limitations. The findings may not be  
303 generalizable to different settings, or among unstable or very low birth weight infants. Additional  
304 information on maternal nutrition parameters that might influence infant gut function could be  
305 useful to document adequate randomization and contextualize our findings. Further research is  
306 needed to substantiate our findings and to study if the intervention has an impact on EED, which  
307 is a broader entity encompassing several aspects of gut function and systemic inflammation.<sup>27</sup>  
308 Biomarkers of EED are seen to be associated with the presence of multiple viral and bacterial  
309 pathogens (enteroviruses, adenoviruses, *Campylobacter* spp., and diarrheagenic *Escherichia*  
310 *coli*) in the gut.<sup>25</sup> Future assessment of intervention effects on gut function may consider  
311 detection and quantitation of fecal enteropathogens in addition to the biomarkers. The fact that  
312 promotion of ciKMC reduced the risk of severe neonatal stunting and wasting<sup>15</sup>, yet in the  
313 current study we did not find a measurable effect on fecal biomarker concentrations,  
314 underscores the need to look for additional mechanisms that can explain growth faltering.  
315 Because EED may be influenced by multiple factors it may be worthwhile to explore the role of  
316 integrated health interventions on gut function in LBW infants.

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331 in the study and take responsibility for the integrity of the data and the accuracy of the data  
332 analysis.

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**Table 1. Baseline characteristics of the study participants\***

<b>Variables</b>	<b>Control arm (n=100)</b>	<b>ciKMC arm (n=100)</b>
	<b>%</b>	<b>%</b>
<b>Household characteristics</b>		
Wealth Quintiles		
Least poor	19	16
Less poor	25	27
Poor	17	26
Very poor	25	13
Most poor	14	18
Religion		
Hindu	86	85
Muslim	10	14
Others	3	1
Type of family		
Nuclear	33	35
Joint	67	65
Toilet not available inside household	17	13
Source of drinking water		
Piped water	20	19
Tube well	31	33
Public tap	28	22
Bottled water	19	25
Other	2	1
Mean number of family members (SD)	7.0 (2.8)	7.6 (3.7)
<b>Maternal and paternal characteristics</b>		
Mean maternal age: years (SD)	24.1 (3.7)	23.1 (3.2)
Mean paternal age: years (SD)	27.2 (4.8)	26.4 (4.2)
Maternal education: years of schooling (SD)	7.1 (5.3)	5.7 (5.2)
<b>Birth related characteristics</b>		
Place of delivery: Home	14	16
Birth order		
1	35	37
2-4	56	56
≥5	9	7
<b>Infant characteristics</b>		
Sex of the baby: Female	52	59
Mean weight at enrolment in gm (SD)	2094.5 (162.1)	2086.2 (139.1)
Mean gestational age in weeks (SD)**	35.9 (1.9)	35.9 (1.6)
Preterm <37 weeks	62	63

\*Data presented are number/ percentages unless indicated otherwise

\*\*67.5% (135/200) had an ultrasound for gestational age assessment

**Table 2. Fecal biomarker concentration, enteric enteropathy (EE) score and EE index among study participants at the end of the neonatal period**

<b>Fecal biomarkers</b>	<b>Control arm</b>	<b>ciKMC arm</b>	<b>Unadjusted Difference in means (95% CI)</b>	<b>Adjusted<sup>†</sup> Difference in means (95% CI)</b>
Log Neopterin nmol/L				
Mean (SD)	7.30 (0.68)	7.37 (0.61)	0.07 (-0.11 to 0.25)	0.03 (-0.15 to 0.21)
Log Myeloperoxidase ng/mL				
Mean (SD)	5.12 (1.29)	5.49 (1.01)	0.38 (0.05 to 0.70)	0.28 (-0.05 to 0.61)
Log Alpha1antitrypsin µg/mL				
Mean (SD)	5.57 (1.09)	5.61 (1.16)	0.04 (-0.27 to 0.35)	0.02 (-0.30 to 0.34)
EE score				
Mean (SD)	12.36 (8.32)	13.57 (7.51)	1.21 (-1.01 to 3.43)	0.44 (-1.81 to 2.69)
EE index				
Mean (SD)	-0.12 (1.12)	0.12 (0.86)	0.25 (-0.03 to 0.53)	0.17 (-0.11 to 0.45)

<sup>†</sup> Adjusted for potentially confounding baseline factors when the relative difference at baseline between trial arms were >10%, i.e., wealth quintiles, toilet facility, source of drinking water, birth order, sex of the baby, and baseline concentration of the respective gut inflammatory markers.



**Table 3. Effect of ciKMC on infant fecal biomarkers at the end of the neonatal period in subgroups of preterm and term infants**

Gut Biomarkers at 1 month	Infant subgroup	Control arm	ciKMC arm	Unadjusted	Adjusted <sup>†</sup>
		Mean (SD)	Mean (SD)	Difference in means (95% CI)	Difference in means (95% CI)
Log Neopterin nmol/L	Preterm (n=125)	7.27 (0.75)	7.43 (0.61)	0.17 (-0.07 to 0.41)	0.16 (-0.09 to 0.40)
	Term (n=75)	7.37 (0.57)	7.27 (0.60)	-0.10 (-0.37 to 0.17)	-0.26 (-0.55 to 0.04)
Log Myeloperoxidase ng/mL	Preterm (n=125)	5.13 (1.13)	5.51 (0.98)	0.39 (0.01 to 0.76)	0.41 (0.02 to 0.82)
	Term (n=75)	5.11 (1.54)	5.47 (1.09)	0.36 (-0.25 to 0.98)	0.01 (-0.58 to 0.60)
Log Alpha1 antitrypsin µg/mL	Preterm (n=125)	5.39 (1.24)	5.55 (1.23)	0.16 (-0.28 to 0.60)	0.24 (-0.21 to 0.69)
	Term (n=75)	5.86 (0.74)	5.70 (1.02)	-0.16 (-0.57 to 0.25)	-0.21 (-0.66 to 0.24)
EE Score at 1 month	Preterm (n=125)	11.7 (7.9)	14.2 (7.6)	2.5 (-0.27 to 5.26)	2.5 (-0.40 to 5.36)
	Term (n=75)	13.5 (8.9)	12.5 (7.2)	-0.99 (-4.77 to 2.80)	-3.84 (-7.76 to 0.08)
EE index at 1 month	Preterm (n=125)	-0.18 (1.1)	0.19 (0.8)	0.37 (0.02 to 0.72)	0.38 (0.01 to 0.75)
	Term (n=75)	-0.04 (1.1)	0.01 (0.8)	0.04 (-0.43 to 0.51)	-0.29 (-0.76 to 0.18)

<sup>†</sup> Adjusted for potentially confounding baseline factors i.e., wealth quintiles, toilet facility, source of drinking water, birth order, sex of the baby, and baseline concentration of the respective gut inflammatory markers.

**Figure 1. Participant flow in the trial**

**Figure 2. Fecal biomarkers of inflammation and permeability across study arms at baseline**

**Figure 3. Fecal biomarkers of inflammation and permeability across study arms at the end of the neonatal period**