



Fecal biomarkers for Environmental Enteric Dysfunction (EED) and child growth: A longitudinal study in rural areas of Cambodia



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Introduction

Malnutrition in early childhood is an intractable public health problem associated with increased mortality, cognitive dysfunction, and loss of productivity. It is responsible for approximately 45% of all death among children under 5 years, globally.

The aims of this study:

- (1) to investigate on potential factors of each EED biomarker (MPO and NEO) and combined scores of the two biomarkers, and
- (2) to investigate on the association of each EED biomarker and the combined scores with child growth among children aged under 5 years in rural areas of Cambodia.

Methods

Longitudinal data of the Cambodia Health and Nutrition Monitoring Study (MyHealth) was used for linear regression models to estimate potential factors of each EED variables while generalized linear-mixed effect models were used to estimate associations between each EED variables and child growth.

Table 1: General characteristics of the participants in the study.

Characteristics	Total	% or mean (SD)
Provinces	133	
Katie		121 (91.0)
Ratanakiri		12 (9.0)
Sex (boys)	133	69 (51.9)
Age in months at FU1	133	19.9 (±8.4)
Age in months at FU2	120	24.03 (±8.5)
Age in months at FU3	102	37.1 (±8.8)
Maternal education levels	132	
No education		15 (11.4)
Primary		67 (50.8)
Secondary		36 (27.3)
Highschool or higher		14 (10.6)
Hygiene practices:		
Unimproved main households drinking water sources	133	47 (35.3)
Untreated child's drinking water	133	49 (36.8)
Unimproved type of toilet	133	71 (53.4)
Had animal feces around houses	127	119 (93.7)
Had chicken around houses	119	104 (87.4)
Had cow around houses	119	72 (60.5)
Had pig around houses	119	39 (32.8)
Morbidity last in the past two weeks		
Number of days being sick at FU1	104	3.8 (±3.9)
Children had diarrhea	104	17 (16.4)
Fecal biomarkers at FU1:		
NEO concentration in nmol/L, median (IQR)*	131	860 (245.5 ; 2088.0)
MPO concentration in ng/mL, median (IQR)*	143	4013 (1695.5 ; 11517.4)
Combined MPO and NEO scores	130	4.5 (±1.9)

HAZ= heigh for age z-score; WHZ= weight for heigh z-score; NEO= neopterin; MPO=Myeloperoxidase; Combined MPO and NEO scores= NEO quartiles + MPO quartiles ranging scores from 2-8 then categorized into 3 categories: 2-3, 4, 5 and 6 to 8. *NEO and MPO concentration were presented as median and interquartile range (IQR).

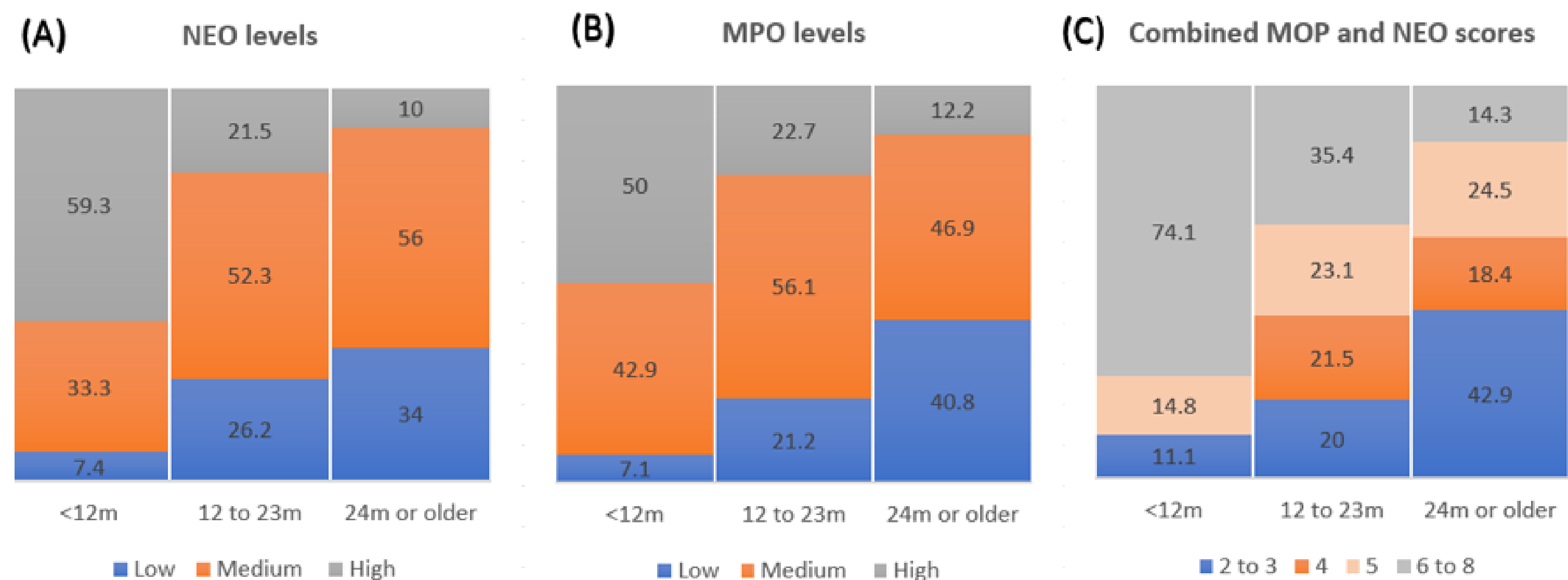
Results

More than 90% of these children were living in households contaminated with animal feces (Table 1).

Children with high levels of all MPO, NEO and combined scores were found to be high prevalent among younger children aged under 12 months (Figure 1).



Figure 1: Distribution of EED biomarkers and a combined score over child's age in months.



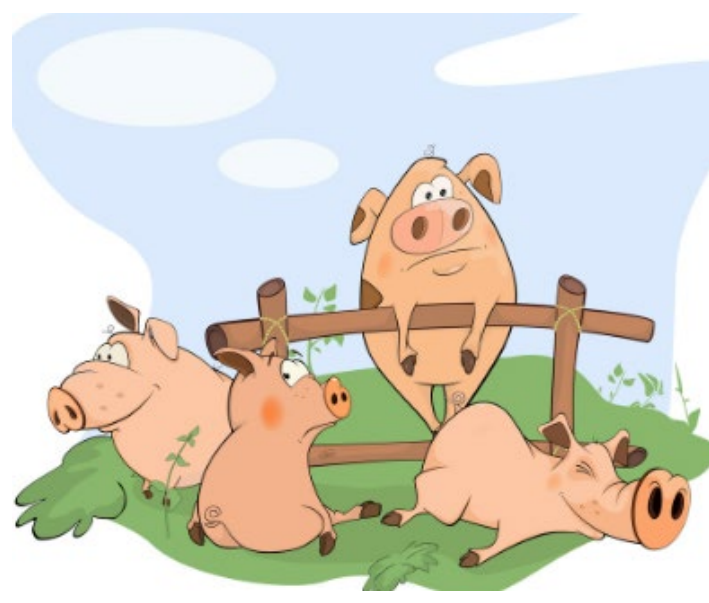
(A)Distribution of NEO levels over child's age in months at FU1.
(B)Distribution of MPO levels over child's age in months at FU1.
(C)Distribution of combined MPO NEO scores over child's age in months at FU1.
NEO levels= low (concentrations in first quartile), medium (concentrations in the interquartile range), or high (concentrations in fourth quartile).
MPO levels= low (concentrations in first quartile), medium (concentrations in the interquartile range), or high (concentrations in fourth quartile).
A combined MPO and NEO scores= NEO quartiles + MPO quartiles ranging scores from 2-8 then categorized into 3 categories: 2-3, 4, 5 and 6 to 8.

Table 2: Unadjusted and adjusted linear regression of the association between each EED biomarker concentration and other determinants.

	Log NEO concentration*				Log MPO concentration*			
	Unadjusted Coefficient (95%CI*)	p-Value	Adjusted Coefficient (95%CI*)	p-Value	Unadjusted Coefficient (95%CI*)	p-Value	Adjusted Coefficient (95%CI*)	p-Value
Provinces (Kratie)	Reference				Reference			
Ratanakiri	0.6 (-0.3 ; 1.4)	0.202			0.4 (-0.5 ; 1.4)	0.360		
Sex,(girls)	Reference		Reference		Reference			
Boys	0.3 (-0.1 ; 0.8)	0.168			0.01 (-0.5 ; 0.5)	0.972		
Age in months	-0.1 (-0.1 ; -0.04)	0.000	-0.1 (-0.1 ; -0.03)	0.000	-0.1 (-0.1 ; -0.05)	0.000	-0.1(-0.1 ; -0.04)	0.000
Maternal education level	0.02 (-0.04 ; 0.1)	0.392			-0.1 (-0.1 ; 0.01)	0.074	-0.04(-0.1 ; 0.03)	0.214
Wealth index	-0.03 (-0.2 ; 0.1)	0.728			-0.1 (-0.4 ; 0.1)	0.160		
Households water sources (no)	Reference				Reference			
Improved	-0.1 (-0.6 ; 0.4)	0.720			0.04 (-0.5 ; 0.6)	0.879		
Treated drinking water (no)	Reference				Reference			
Yes	0.2 (-0.3 ; 0.6)	0.438			0.1 (-0.4 ; 0.6)	0.693		
Type of toilet (no)	Reference				Reference			
Improved	0.1 (-0.3 ; 0.6)	0.610			-0.2 (-0.8 ; 0.3)	0.395		
Pig around houses (No)	Reference		Reference		Reference			
Yes	0.4 (-0.05 ; 0.9)	0.077	0.4 (-0.1 ; 0.8)	0.083	0.1 (-0.5 ; 0.6)	0.849		
Diarrhea past 2 weeks (No)	Reference				Reference			
Yes	-0.1 (-0.8 ; 0.6)	0.724			-0.2 (-1.1 ; 0.6)	0.530		

*Neopterin (NEO) and Myeloperoxidase (MPO) concentrations were not normally distributed so log 10 was applied for the two concentration. *CI= confident interval.

Having pigs around child households showed an increase in NEO concentration at p-value 0.083 (AOR=0.4; 95%CI=-0.1;0.8) (Table 2).



None of the EED biomarkers were associated with either HAZ nor WHZ (Table 3).

Table 3: Adjusted generalized linear mixed effect model of the association between individual EED biomarker and child growth (HAZ or WHZ).

	HAZ ¹		WHZ ²	
	Adjusted coefficient (95%CI)	p-value	Adjusted coefficient (95%CI)	p-value
MPO levels ^a (Low)	Reference		Reference	
Medium	0.09(-0.26 ; 0.45)	0.598	0.11(-0.21 ; 0.43)	0.496
High	-0.10(-0.52 ; 0.32)	0.638	0.22(-0.15 ; 0.60)	0.239
NEO levels ^b (Low)	Reference		Reference	
Medium	0.08(-0.28 ; 0.43)	0.673	-0.28(-0.59 ; 0.03)	0.073
High	0.08(-0.33 ; 0.49)	0.711	0.17(-0.19 ; 0.53)	0.346
Combined MPO and NEO scores ^c (2 to 3)	Reference		Reference	
4	-0.19(-0.64 ; 0.27)	0.427	0.26(-0.15 ; 0.67)	0.212
5	0.14(-0.29 ; 0.57)	0.531	0.14(-0.23 ; 0.51)	0.455
6 to 8	-0.4(-0.42 ; 0.34)	0.839	0.21(-0.13 ; 0.55)	0.223

¹Generalized linear mixed effect of association between HAZ and each EED biomarker indication adjusted for child's age, maternal education, wealth index, treatment of drinking water and type of toilet.

²Generalized linear mixed effect of association between WHZ and each EED biomarker indication adjusted for region and child's age.

^aNEO levels= low (concentrations in first quartile), medium (concentrations in the interquartile range), or high (concentrations in fourth quartile).

^bMPO levels= low (concentrations in first quartile), medium (concentrations in the interquartile range), or high (concentrations in fourth quartile).

^cA combined MPO and NEO scores= NEO quartiles + MPO quartiles ranging scores from 2-8 then categorized into 3 categories: 2-3, 4, 5 and 6 to 8.

Conclusion

Children at younger age, especially in their first year of life, had the highest prevalence of high levels of the EED biomarkers than older children suggesting a critical time for prevent children from this infection.

None of the three traditional WASH indicators (improved main water sources, improved type of toilet, and treated children's drinking water) showed any statistically significant association with the EED biomarkers or child growth but having pigs around children's houses showed an increase in NEO concentration.

This cohort study in rural areas in Cambodia suggests children in the first year of life are vulnerable to EED and the potential source of the infection was from pigs which may suggest that WASH program should therefore consider a wider intervention.