Examining interactions of lead and repeated Rotavirus infection on infant cognitive development

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Master's Project

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Executive Summary

The developing world is often burdened with health issues many times over that of nations with more access to infrastructure and medical care. However, the foci of aid to these areas is frequently isolated within a single discipline, meaning that potential health issues often lack the benefit of interdisciplinary study and health professionals may not have the whole picture of interactions of environmental health threats. This project sought to cross that barrier and examine the interaction of two health risks that have been well documented to negatively affect the neurodevelopment of children.

Rotavirus is an extremely cause of severe childhood gastroenteritis globally. When children are frequently exposed to unsanitary conditions they are more likely to be repeatedly exposed to pathogens that cause diarrheal diseases and gut inflammation. This chronic inflammation can decrease cognitive function and generally stunt growth. Additionally, lead is well known to be neurodevelopmentally toxic, causing lower cognitive functioning at levels of 5ug/dL, the current CDC maximum acceptable blood lead level. Children in the developing world may be more likely to have a double burden of these growth stunting factors, disadvantaging them in global competition.

Therefore, this project evaluated if the effects of repeated early life Rotavirus infection and elevated blood lead levels increase cognitive stunting beyond what might be expected of either threat individually. To understand these potential interactions, infants from Bangladesh, India, and Pakistan were assessed for cognition at 6 months using the Bayley's Scales of Infant and Toddler Development. This score was evaluated in relation to the child's blood lead level and incidence of Rotavirus infection while controlling for covariates such as home socioeconomic status, maternal reasoning abilities, nutrition, and other pathogenic burden. No

relationship was found in this cohort, but it should be noted that the number of infants in this study with a non-zero Rota incidence was 43 of the 634 participants. Therefore, it is not able to be determined if the data is masking a potential reaction or if there is truly no interaction. It is possible that increased gut injury could be increasing lead uptake, as was hypothesized, but it may also be injuring the gut enough to decrease all absorption, thus decreasing lead uptake. Further study of this and other environmental health interactions are needed to evaluate if and how any stunting effects that may be seen are impacting these populations and potentially presenting a greater than additive risk.

Introduction

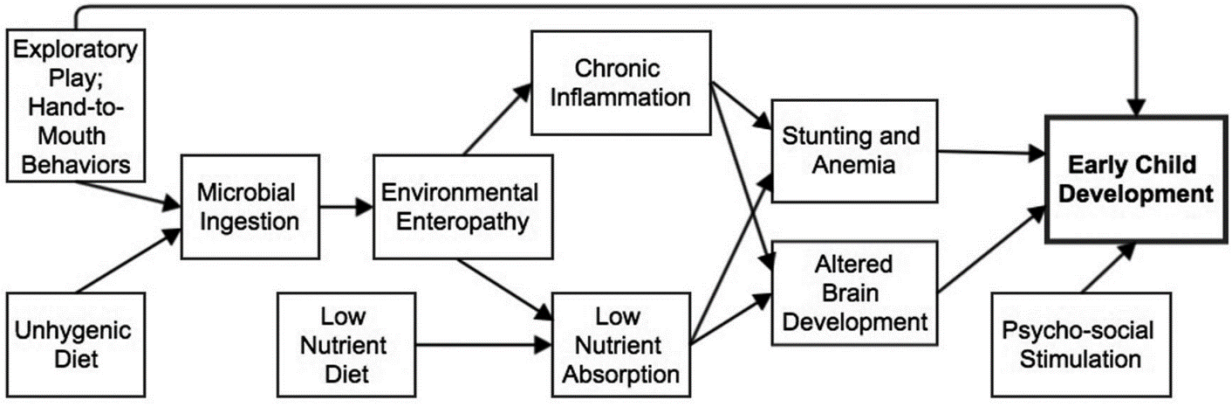
Rotavirus and gut health

Rotavirus was cited by the CDC (Payne et al., 2011) as the most common cause of infant and early childhood severe gastroenteritis worldwide. The resultant moderate to severe diarrhea can contribute to malnutrition at this stage of life (Glewwe and King, 2001), especially in developing nations (MacIntyre et al., 2014; Sudfeld et al., 2015) where children and infants may not have adequate access to medical care in order to treat the symptoms of gastroenteritis enough to prevent a significant loss of nutrient during the period of illness (Scharf et al., 2014). This malnutrition, especially in the first 2 years of life, can contribute to cognitive developmental deficits, which are likely a contributor to the global economic disparities seen in developing nations where diarrheal disease is prevalent (MacIntyre et al., 2014). This stunting stems from the impact of chronic inflammation and decreased nutrient absorption as diagrammed in the figure below (Ngure et al., 2014). Continued upset of the gastrointestinal microbiota may permanently alter immune functioning by decreasing the barrier protection of the skin and mucosal membranes (Hooper et al., 2012), leaving the child more susceptible to pathogenic attacks, and therefore more likely to be stunted cognitively.

In developing nations, diarrhea in the first 2 years of life can cause an average decrease of 10 IQ points by the age of 7 to 9 based on the associated “double burden” of diarrhea and malnutrition (Guerrant. RL. Moore SR., Scharf RJ., & Lima AA., 2013). However, Rotavirus can still impact individual health independent of malnutrition (Verkerke et al., 2016), meaning that interventions targeting only malnutrition in developing nations still may not see as great effects as if rotavirus infection were targeted as well. Conversely, protein deficiency has not been found to impair IgA rotavirus vaccine response in mice (Maier et al., 2013), indicating that a campaign to increase

vaccination rates without accompanying nutritive programs may still be an effective way to decrease the malnutrition and developmental deficits associated with diarrheal infection.

Figure 1 Ngure et al. (2014)



Lead

The neurotoxic effect of lead is a subject of increasing importance as the long term developmental effects of environmental toxins are investigated with increasing sensitivity (Earl et al., 2016). Interruption of the neuro-cellular processes can lower IQ (Kaiser et al., 2008) or cause developmental issues, such as ADHD (Chan et al., 2015; Huang et al., 2016) or contribute to neurodegenerative diseases, such as Alzheimer's (Cox et al., 2016; Lobsiger and Cleveland, 2007), depending on the timing of and extent of the environmental exposure. Children with higher blood lead levels (BLL) above those of their peers have been found to have higher rates of cognitive and behavioral disorders (Chan et al., 2015; Chen et al., 2007; Huang et al., 2016) and lower IQ (Mendelsohn et al., 1999), even when those rates are below the minimum acceptable level of 5 µg/dL (Huang et al., 2016). Additionally, toddlers with a BLL between 10 and 24.9 µg/dL had Bayley's Scale scores 6.2 points lower than those children with levels lower than 9.9 µg/dL, even when adjusted for socioeconomic factors and other confounding variables

(Mendelsohn et al., 1999). This is especially relevant to the developing world, where environmental exposure to lead can be higher than that of children in more developed countries.

Lead is estimated to have cognitively stunting impacts in 34% of children in low and middle income countries (Attina and Trasande, 2013; Walker et al., 2011). It has been found to increase the odds of stunting by 1.12 per µg/dL increase in BLL when studied in a cohort in Bangladeshi children (Gleason et al., 2016). This disproportionate stunting in developing nations can contribute to economic (Gould, 2009) and social disparities (Reyes, 2007) associated with these effects. Economic costs of childhood lead exposure in low and middle- income countries are estimated to be 1.2% of the global GDP while further costs are incurred from property crimes, which have been found to decrease in association with decreases in BLL (Reyes, 2007). Developing nations are bearing the weight of the neurotoxic impacts of lead more than developed nations, further widening the global wealth gap.

Populations in developing nations that are exposed to both environmental lead and rotavirus are at increased risk of cognitive stunting. Further, based on the similarity of the endpoint effects of each of these variables I expect that those children with increased exposure to both of these variables will have significantly lower cognitive development than those exposed to either variable independently. This project will take the data from the MAL-ED cohort study (from 8 study sites worldwide) to statistically assess a relationship between lead and rotavirus exposure (both environmental and through vaccination).

Objectives

Based on this understanding, it was hypothesized that children with a blood lead level exceeding 5 µg/dL in addition to environmental exposure to rotavirus would have significantly lower Bayley's scores of cognitive development compared to those individuals exposed to each

variable individually. Additionally, it was hypothesized that those children with exposure to Rotavirus through vaccination would have a higher Bayley's score than those exposed environmentally, based on their incidence of Rotavirus- positive infection.

Methods

Data used to analyze this interaction was taken from The Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Project (MAL-ED). In this study parameters were collected from November 2009 to February 2014 across eight study sites in developing countries. These countries include Bangladesh, Brazil, India, Nepal, Peru, Pakistan, South Africa, and Tanzania.

Data collection of main variables

Lead

Up to 5mL of blood were collected from participants at the ages of 7, 15, and 24 months of age. Of that volume, 1 mL was reserved and preserved with dipotassium ethylenediaminetetracetic acid (k2 EDTA) for lead analysis through Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) with an M Series Atomic Absorption Spectro-photometer (Mohan et al., 2014).

Rota Infection

Monthly stool samples were collected from participants from the ages of 0 to 24 months. Additional stool samples were collected from participants when diarrheal incidence was reported, which was defined as 3 or greater loose samples over a 24 hour period. After collection each sample was analyzed for pathogenic burden and markers of gut inflammation (Murray-Kolb, n.d.).

Cognition

Infant cognition was evaluated using the Bayley's Scales of Infant and Toddler Development-III at the ages of 6, 15, and 24 months. Administrators were trained and had a background in child

development or psychology. Data from Tanzania and Nepal were not valid based on recordings of the test administration and therefore excluded (Murray-Kolb, n.d.).

Data collection of covariates

Additionally, this analysis sought to include the confounding variables found in the hypothesized causal pathway as demonstrated in **Error! Reference source not found.**. The quantification of t hese covariates are as follows:

Socioeconomic status

Socioeconomic status was evaluated using the WAMI index, a measure of wealth and socioeconomic status developed for use in the MAL-ED cohort to compare wealth across multiple study sites (Psaki et al., 2014).

Maternal Reasoning

The Raven's Progressive Matrices were used to measure maternal reasoning when the infant was between 6 to 8 months of age (Murray-Kolb, n.d.).

Nutrient uptake and stunting

Several variables were included in this analysis to account for and estimate nutritive status of each participant. This included vitamin B6 and B12, hemoglobin, zinc, and overall weight adjusted z-scores.

Vaccination

IgG and IgA titers were included to account for vaccination status.

Microbial ingestion

Overall microbial infection was accounted for based on the bacterial, pathogenic, and parasite scores assigned based on the stool testing which also assessed for Rotavirus infection. Pathogenic scores included an overall score as well as a non-Rota pathogen score.

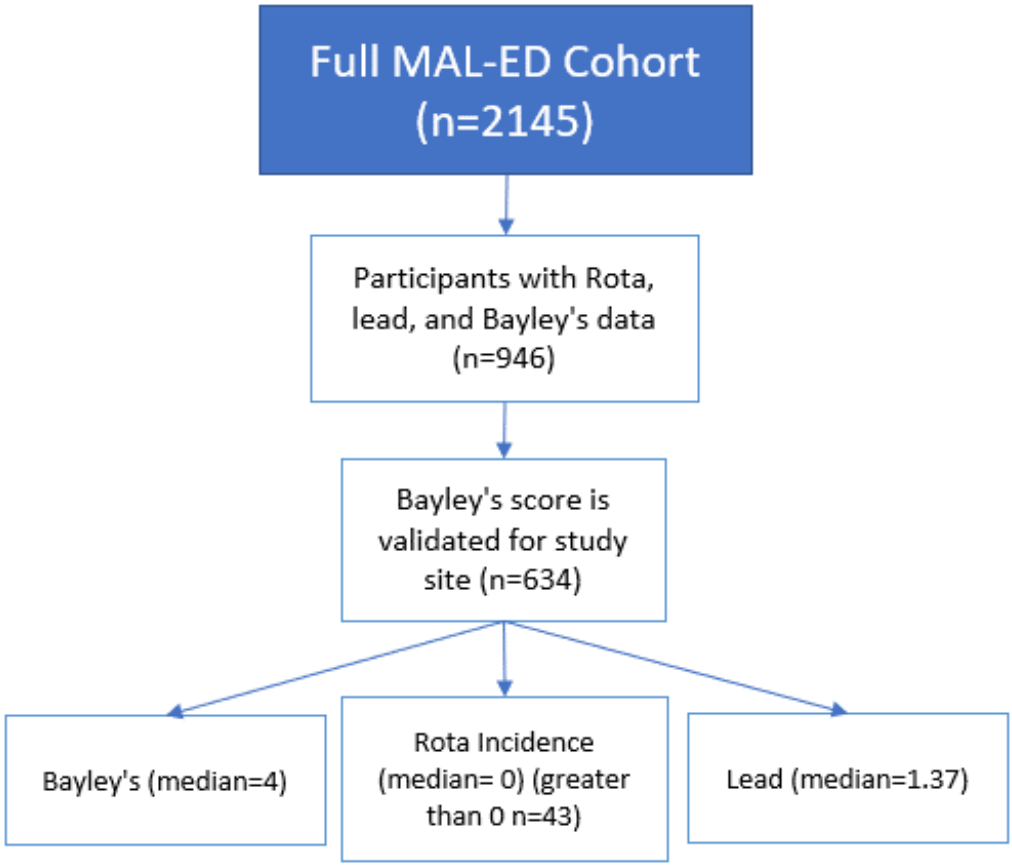


Figure 2: Demonstration of the filtered data used for analysis.

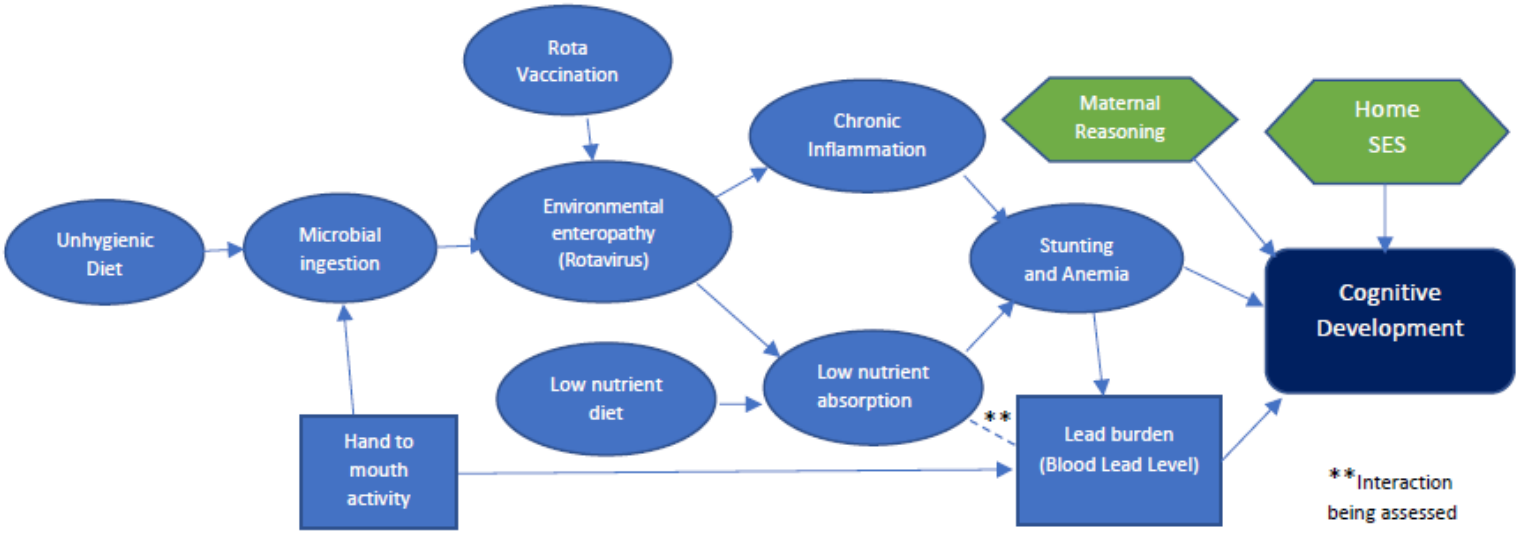


Figure 3: Hypothesized causal pathway of environmental influences on cognitive development.

Results

The original data from the MAL-ED cohort (n=2048) was filtered for this analysis by participants that had values for the three main variables of interest: lead, cumulative Rotavirus, and Bayley's Scales of Infant and Toddler Development-III (n=946). This was further filtered by the validation of the Bayley's Scale by study site. Since Nepal was not validated for this parameter, the Nepalese participants were excluded from analysis (n=643) (Figure 2). All values used in analysis were collected under 200 days of age, to approximate the exposures at between 6 to 7 months of age, which would influence the main variables of interest.

Based on the distribution of the lead and Rotavirus data these analyses were non-parametric. To test the correlations between variables the Spearman Rank Correlation was used. For further analysis the parameters were regressed using a median regression and sample differences were evaluated using the Wilcoxon Signed Rank test.

Correlations

The main variables of this analysis were not normally distributed, as demonstrated in . Lead had a median of 1.37 µg/dL with a maximum of 19.9 µg/dL, though this average was skewed by the lead values found in Bangladesh, the multivariate Spearman Rank Correlation of lead and Rotavirus incidence on participant Bayley's score was -0.018. This correlation is broken down further to a 0.264 correlation of lead and Rotavirus incidence and -0.286 between lead and Bayley's score. Rotavirus and Bayley's score correlated at a value of -0.017 (Table 1). Correlations separated by study site can be found in the appendices 8-10.

Table 1: Overall Spearman Rank correlations of the main effects variables.

Variable	Rota	Lead	Bayley's
Rota (n=634)	1		-0.018
Lead (n=634)	0.264	1	
Bayley's (n=591)	-0.017	-0.286	1

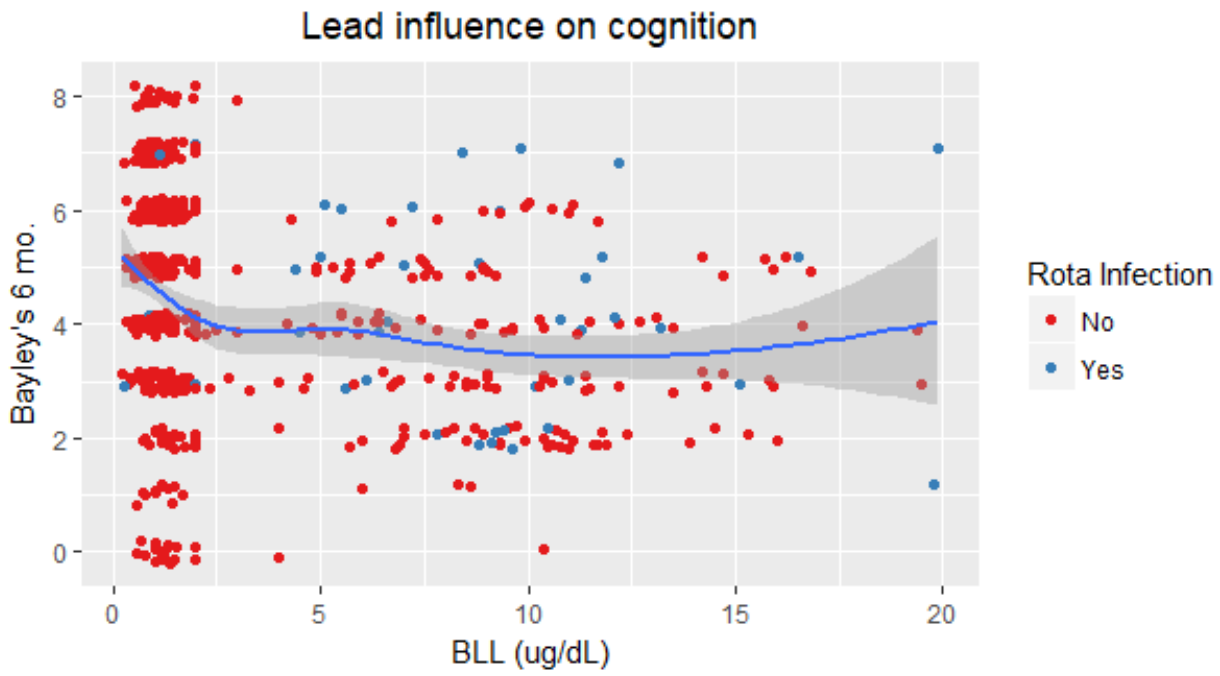


Figure 4:Non-zero Rota infection differences reflected in the regression of mean blood lead level and Bayley's cognitive score.

Regressions

To evaluate this relationship further in and out of the context of the confounding variables mapped in Figure 3, a median regression was used to model the relationship based on the nonparametric distribution of lead and Rotavirus. The model with the lowest AIC had an equation as follows:

Equation 1: BLL= Mean blood lead (ug/dL), RTA = Cumulative Rota infection, ZN = Low Zinc mml, WAMI = WAMI index score, RVN = Maternal Raven score, ZWEI = Weight adjusted z-score, SEX = Participant sex

$$\text{Bayley's Score} = 4.46 - 0.04\text{BLL} - 1.32\text{RTA} + 0.48\text{ZN} + 0.15\text{WAMI} + 0.02\text{RVN} + 0.27\text{ZWAZ} - 0.31\text{SEX} + 0.18\text{BLL} * \text{RTA}$$

This formula was then regressed with the 25th and 75th percentiles to evaluate potential differences but again, the median regression had the lowest AIC (Table 2).

Table 2: Model of best fit varied by percentile. Median estimates yield the best fitting model.

Variable	**Median**	**25th Percentile**	**75th Percentile**
B_0	4.460174	2.569788	5.639346
BLL	-0.06577	-0.07169	-0.09475
RTA	-1.31785	-0.35075	-0.46428
ZN	0.478303	0.209595	0.633525
WAMI	0.145334	0.12114	0.110823
RVN	0.015042	0.017834	0.019575
WAZ	0.268068	0.104996	0.10828
SEX	-0.31286	0.173508	-0.2879
BLL:RTA	0.175464	0.088385	0.10055
AIC	2167.99	2257.517	2196.424

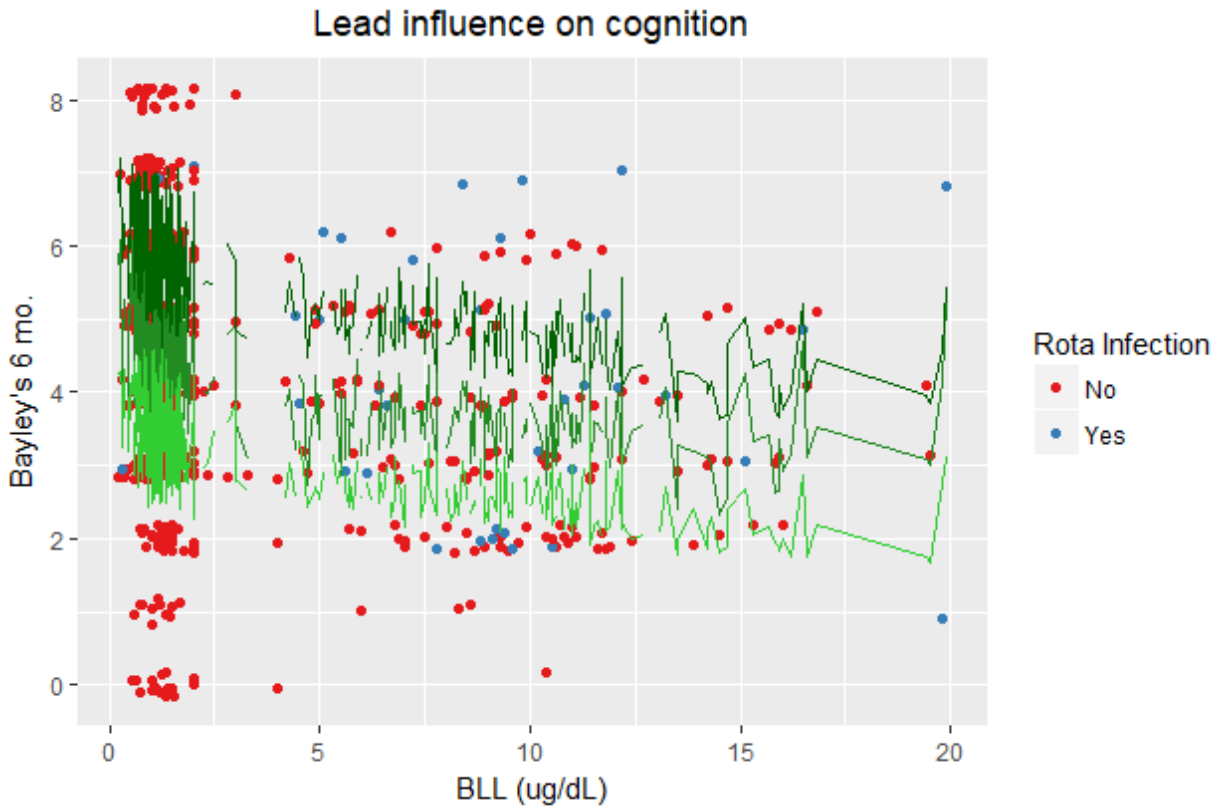


Figure 5:Expected Bayley's score based on the 25th Percentile, Median, and 75th Percentile Regressions.

Rank Tests

Wilcoxon Signed Rank tests were used to further evaluate group differences. Sex differences of both Rotavirus incidence and lead level were evaluated. Based on this data, females had significantly higher Rotavirus infection than males (W= 394110, p= 2.2×10^{-16}), where the estimated difference of location was 1.000026. Conversely, males were estimated to have a greater lead burden (W=185630, p=0.01572), with an estimated difference in location of -4.9228×10^{-5}.

Additionally, using Kruskal-Wallis tests the three sites were determined to be from non-identical populations with respect to blood lead levels (chi-squared= 457.77, df=2, p=2.2×10^{-16}), Rota incidence (chi-squared=62.528, df= 2, p=2.644×10^{-14}), and Bayley's score (chi-squared= 66.344, df=2, p=3.922×10^{-15}).

Discussion

Of the initial 2,084 participants in the study from the 8 study sites, only 946 were used in this analysis based on availability of data across the main variables of interest: cumulative Rotavirus infection, blood lead level, and 6 month Bayley's Scale score of cognitive development. The cohort was then further limited based on validation of the Bayley's Scale. Based on these requirements of inclusion, data from Brazil, Nepal, Peru, South Africa, and Tanzania were excluded. These exclusions left participants from Bangladesh (n= 192), India (n= 227), and Pakistan (n= 215), yielding a total of 634 participants.

This inclusion criteria significantly limited the scope of the analysis. What could have been analysis of a potential problem impacting the developing world became an analysis of 3 countries in the same global corridor, sharing borders.

Of the 634 participants included in the analysis only 43 participants had a non-zero incidence of Rotavirus infection in the temporal scope of this analysis. Of these 43 participants only 4 experienced multiple infections, further limiting the ability to address any sort of lead-Rota interaction. Further, all repeated infections occurred within the Bangladeshi cohort and only 4 of the Indian participants and 3 of the Pakistani participants had a non-zero incidence. This is likely a significant factor contributing to the lack of significant site differences in the regressions. While the cohorts were found to be significantly different the regression fit increased when site was not included in the model.

The lack of significance found in this study may be due to a variety of causes. Firstly, the low incidence of Rotavirus infection in this population may be masking any potential interactions with lead exposure or any of the other covariates analyzed. Secondly, the early life data used may have been too early in development to see differences in effect. Third, infants at this age

may not be exposed to the same level of environmental contaminants and toxins as older, more mobile children in these areas. Lastly, there may not be an interaction between lead and Rotavirus.

While recognizing these limitations of this study and the inherent challenges of analysis with this data set, future research in the intersection of environmental health and other studies in enteric disease may be essential in protecting populations which may have greater burdens of both diseases that increase susceptibility as well as exposures to environmental toxins.

Conclusion

Based on these findings it is inconclusive whether or not there is an interaction of these factors. It may be beneficial to reexamine this relationship at a later age to determine long term effects of chronic exposure as well as consider if there are behavioral factors that are limiting exposure and therefore decreasing hazard of the interaction occurring. While this interaction may be present, this data does not support it, though there are significant limitations of the data and its ability to evaluate this interaction.

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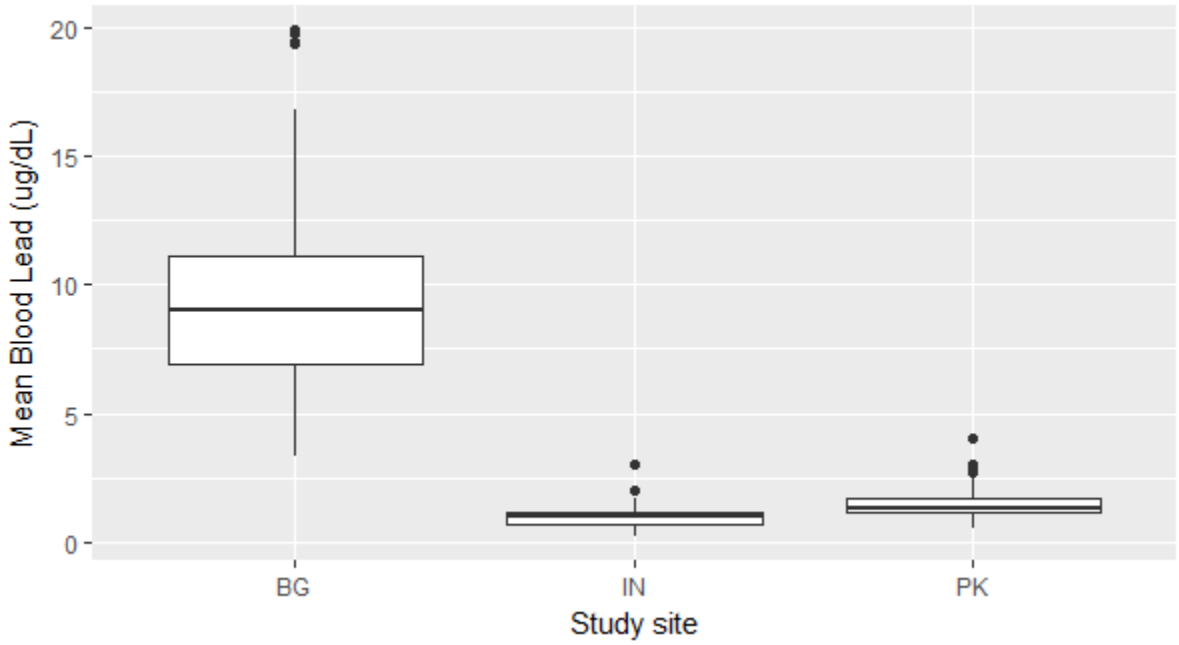
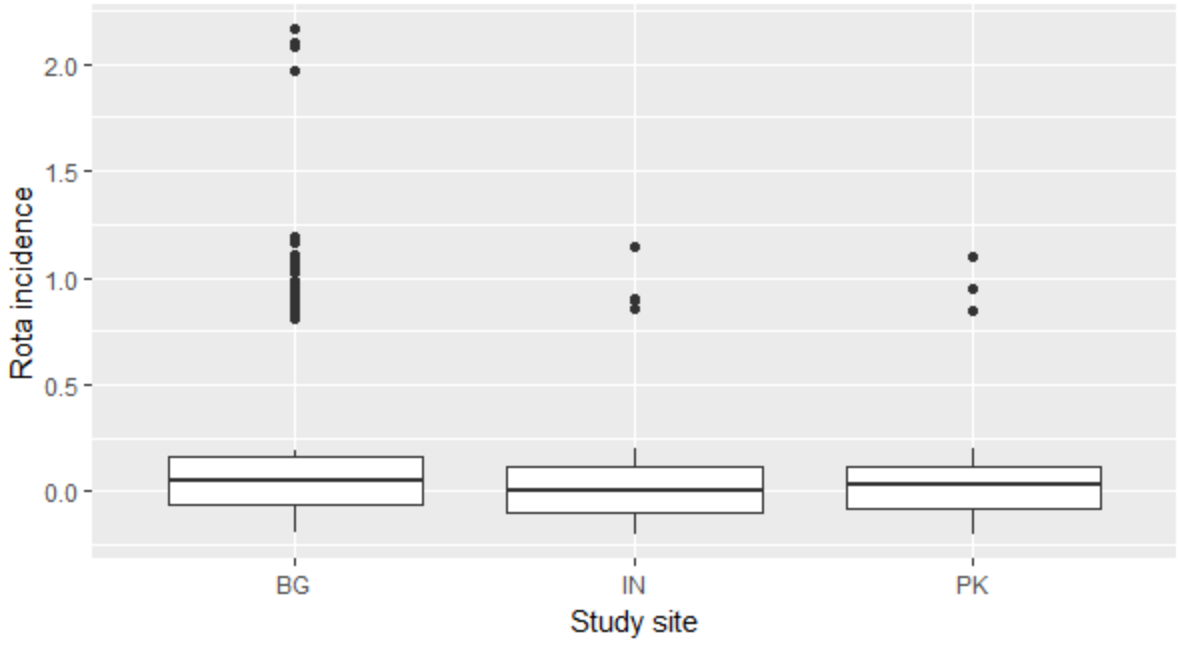
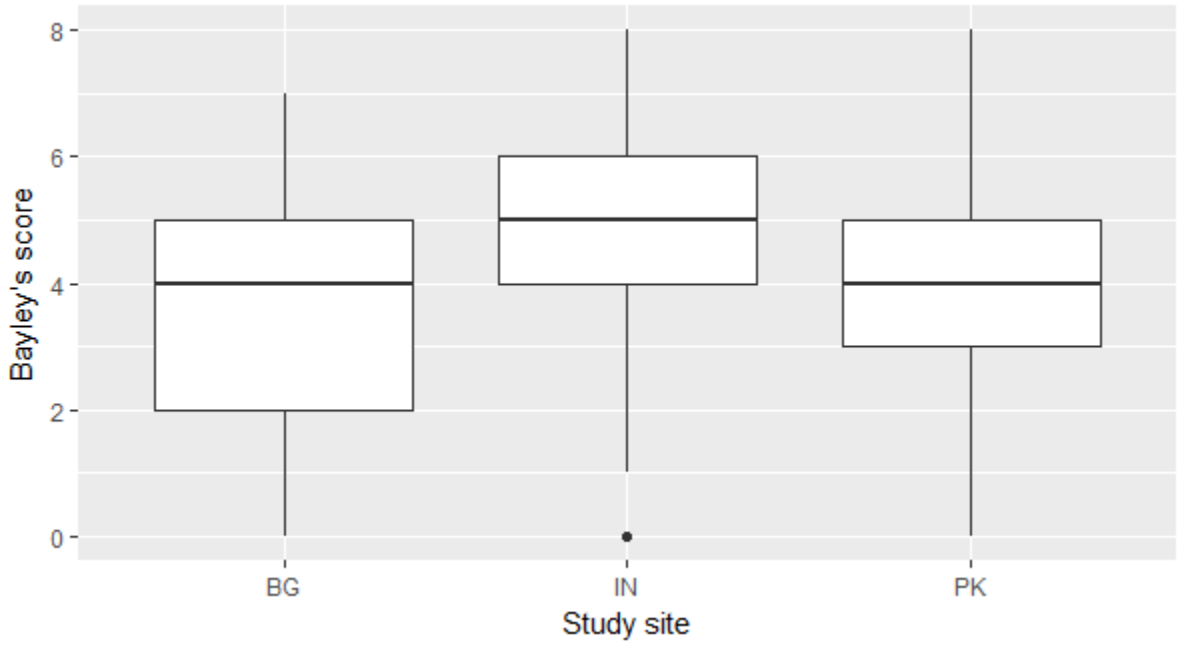
Appendices



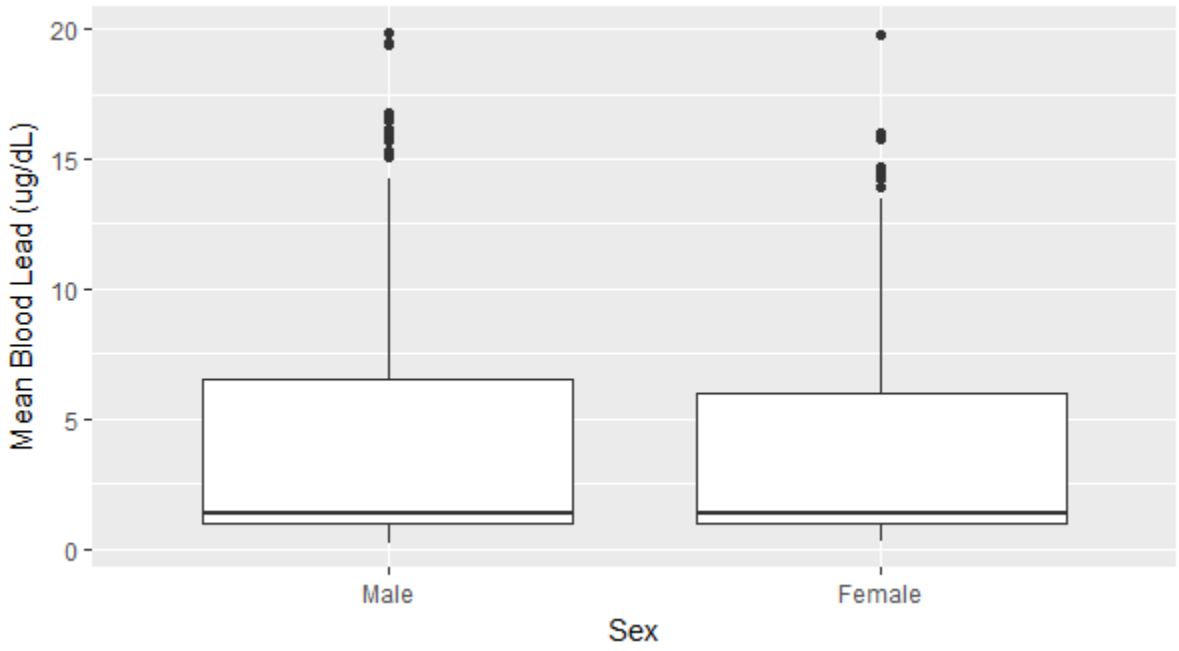
Appendix 1: Distribution of blood lead levels across study sites.



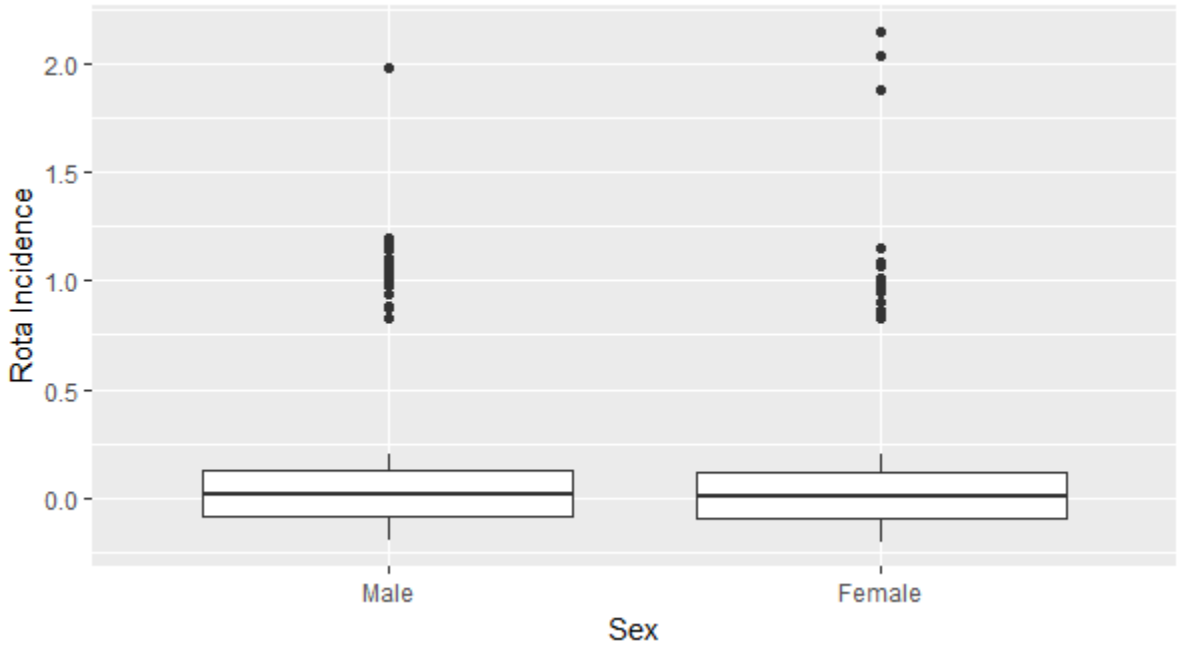
Appendix 2: Distribution of Rotavirus incidence across study sites.



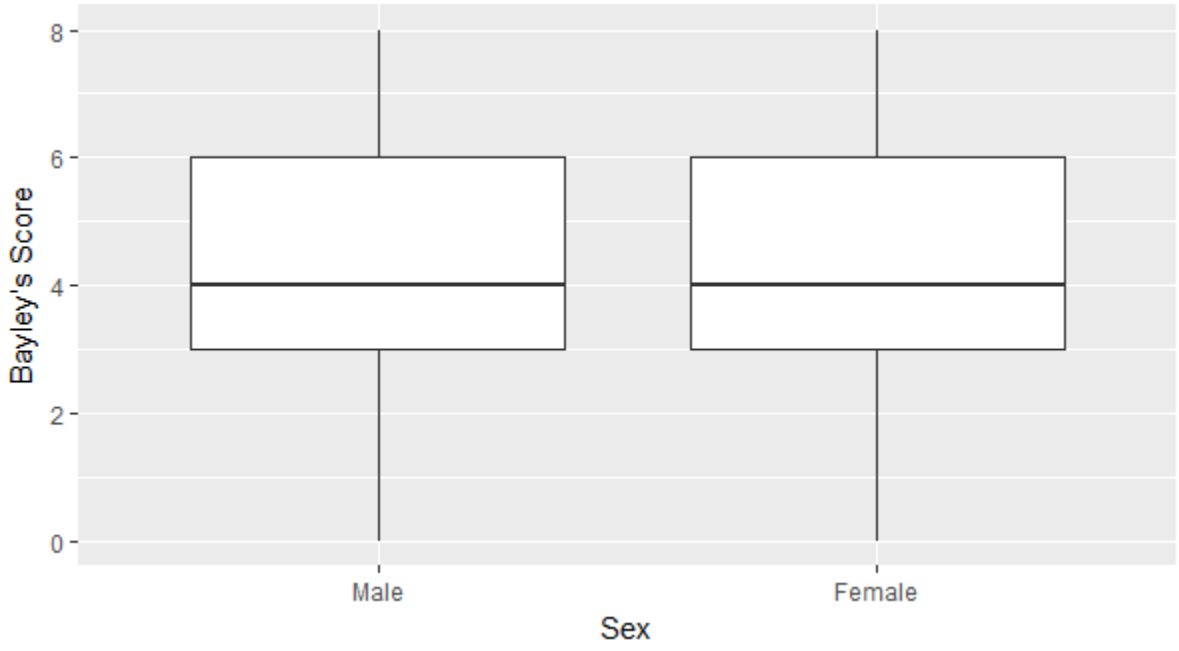
Appendix 3: Distribution of Bayley's scores across study sites.



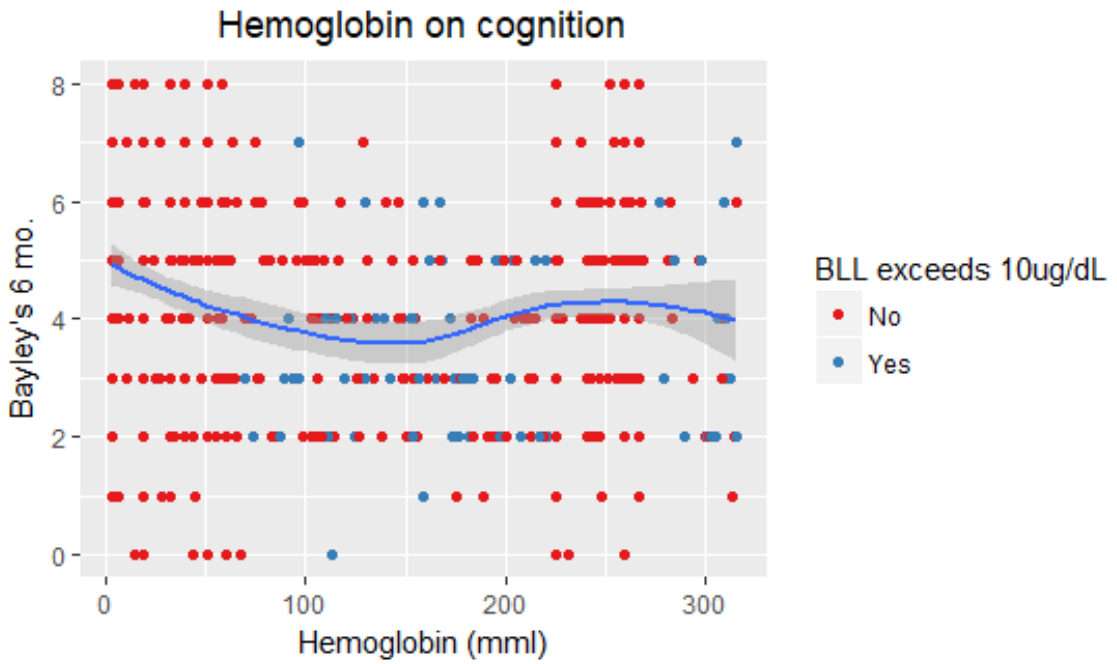
Appendix 2: Sex differences of distribution of mean blood lead.



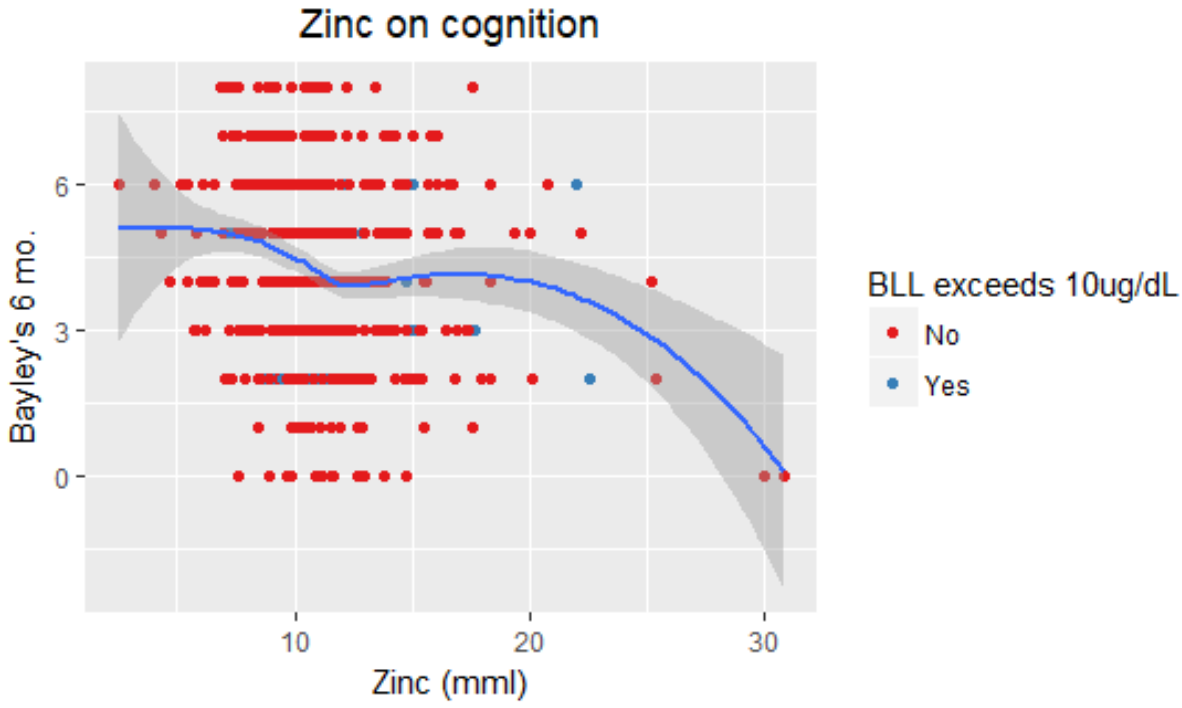
Appendix 3: Sex differences of Rotavirus incidence distribution.



Appendix 4: Sex differences of Bayley's score distributions.



Appendix 5: The effect of hemoglobin levels and blood lead on cognition.



Appendix 6: The effect of zinc and blood lead on cognition.

Appendix 7: The effect of weight adjusted z-score and Rota infection on cognition.

Variable	Rota	Lead	Bayley's
Rota (n=192)	1		0.136
Lead (n=192)	0.010	1	
Bayley's (n=173)	0.143	-0.1385	1

Appendix 8:Bangladesh Spearman Rank correlations

India: Variable	Rota	Lead	Bayley's
Rota (n=192)	1		-0.031
Lead (n=192)	-0.0008	1	
Bayley's (n=173)	-0.032	-0.002	1

Appendix 9: India Spearman Rank Correlations

Pakistan: Variable	Rota	Lead	Bayley’s
Rota (n=192)	1		0.031
Lead (n=192)	0.028	1	
Bayley's (n=173)	-0.062	0.032	1

Appendix 10:Pakistan Spearman Rank Correlations