# THE LANCET **Global Health**

### **Supplementary appendix**

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Khalil IA, Troeger C, Rao PC, et al. Morbidity, mortality, and long-term consequences associated with diarrhoea from *Cryptosporidium* infection in children younger than 5 years: a meta-analyses study. *Lancet Glob Health* 2018; **6:** e758–68.

### **Supplementary Material:** Morbidity, mortality, and long-term consequences associated with diarrhoea from *Cryptosporidium* infection in children younger than 5 years: a meta-analyses study

### Supplementary Tables



### Supplementary Figures



### Supplementary Material

This document provides supplementary material for the manuscript *Morbidity, mortality, and long-term consequences associated with diarrhoea from* **Cryptosporidium** *infection in children younger than 5 years: a meta-analytic study.* The information in the document is intended to provide additional detail for data extraction and modelling strategy.

### **Overview**

The burden of *Cryptosporidium* is modelled in two ways in this study. The first is the acute burden due to incident episodes and deaths due to *Cryptosporidium* diarrhoea and is the primary output of the Global Burden of Disease study. The second is quantifying the impact of *Cryptosporidium* diarrhoea on childhood physical growth.

#### Diarrhoea mortality and morbidity in GBD 2016

The Global Burden of Disease study 2016 provides comprehensive and internally consistent epidemiological estimates for over 300 causes of death and disability, 90 risk factors, for 195 locations, by year, sex, and four age groups under 5 years old. Detailed descriptions of all GBD methods have been previously published.1–4 Mortality due to diarrhoeal diseases was estimated using the Cause of Death Ensemble modeling framework (CODEm)<sup>2,5</sup> and diarrhoea incidence, prevalence, and recovery were estimated with DisMod-MR 2.1 (DisMod), a Bayesian metaregression tool.<sup>6</sup> More detail on diarrhoea modeling in GBD can be found several places including the GBD 2015 diarrhoea capstone manuscript,<sup>1</sup> the GBD 2016 cause of death publication<sup>2</sup> methods appendix (p. 58) and the GBD 2016 non-fatal publication<sup>3</sup> methods appendix (p. 54). The diarrhoea incidence, mortality, years of life lost (YLLs), years lived with disability (YLDs), and disability-adjusted life years (DALYs) estimated in the GBD 2016 framework are referred to herein as acute DALYs to differentiate them from DALYs associated with growth impairment which will be referred to as long-term sequelae DALYs. DALYs associated with growth impairment will be described in more detail below; we estimated these DALYs as part of the GBD 2016 and estimated these DALYs attributable to diarrhoea in children under-5.

### *Cryptosporidium* burden in GBD 2016

The modelling strategy for *Cryptosporidium* diarrhoea in GBD has been described elsewhere.<sup>1</sup> Etiologic attribution is estimated separately from diarrhoea mortality and morbidity. Diarrhoeal etiologies were attributed using a counter-factual approach called a population attributable fraction  $(PAF)$ .<sup>1,2</sup> Our approach accounted for pathogen codetection and detection in healthy individuals, and does not necessitate a one pathogen to one episode relationship. The population attributable fraction is defined as:<sup>7</sup>

#### $PAF = Proportion * (1-1/OR)$

The odds ratios represent the relationship between the detection of *Cryptosporidium* and moderate-to-severe diarrhoea as quantified using molecular diagnostics from the Global Enteric Multicenter Study.8,9 There are two odds ratios, one for children under-1 (3.09, 95% Confidence Interval [CI] 2.21-4.28) and one for all older age groups (1.83, 95% CI 1.3-2.44). The odds ratios were estimated in a conditional logistic mixed-effects regression model that included all other pathogens in the model with an interaction between *Cryptosporidium* and age.

The Proportion is the frequency of detection, where the molecular diagnostic is the case definition, of *Cryptosporidium* in diarrhoeal stool samples and is a modelled estimate for each age, year, sex, and geography. The number of data points by each country is shown in the map below and summarised in the table below. A data point represents the most specific estimate of the proportion of diarrhoea where *Cryptosporidium* was present by age-sexyear. Data were extracted and modeled by age-sex-location-year. For this reason, an individual study must contribute at least one data point but can contribute many to the modeling of *Cryptosporidium* proportion.

### <span id="page-3-0"></span>**Supplementary Figure 1. Number of unique data sources for** *Cryptosporidium* **proportion modelling by country.**



Data sources were identified from the scientific literature.

#### <span id="page-4-0"></span>**Supplementary Table 1. Data points used for** *Cryptosporidium* **modelling.**

Data points represent the number of unique values for the proportion of diarrhoea episodes where *Cryptosporidium*  was detected. The number of data points used in *Cryptosporidium* proportion modelling by geography is shown. The number from an inpatient population and the number of data points that tested for *Cryptosporidium*, excluding other diarrhoeal etiologies, are shown in the second and third columns.





Many studies investigating the etiology of diarrhoea tested for the presence of *Cryptosporidium* using ELISA or microscopy. In order to make these estimates comparable with our case definition from quantitative polymerase chain reaction (qPCR), we had to make several data adjustments. The first was to determine a cut point in the continuous qPCR test result that differentiates between negative and positive results. To do this, we found the cycle threshold (Ct) that maximised the diagnostic accuracy in discriminating between cases and controls in GEMS. Functionally this represents the point that is most likely to dichotomise test results between *Cryptosporidium*  infection likely to cause diarrhoea and *Cryptosporidium* infection unlikely to cause diarrhoea or no

*Cryptosporidium.* A Ct value of 35 indicates that no *Cryptosporidium* gene target was identified in a sample. This is equivalent to determining the Youden's index- maximizing sensitivity and specificity. The relationship between diagnostic accuracy and Ct is shown below.

### <span id="page-6-0"></span>**Supplementary Figure 2. Relationship between qPCR cycle threshold and diagnostic accuracy**

This figure shows the relationship between the qPCR cycle threshold and the accuracy in discriminating between cases and controls. The blue curve indicates a loess curve fitted to the data while the vertical blue line indicates the cut-point determined in this analysis. Any Ct value below the blue line indicates a positive test result for *Cryptosporidium.* 



Cryptosporidium

### **Growth faltering due to** *Cryptosporidium*

Our primary long-term outcomes of interest were changes in Z-scores of height or length for age (HAZ), weight for age (WAZ), and weight for height (WHZ) subsequent to diarrhoea. We defined mild, moderate, and severe stunting, underweight, and wasting as -1 to -2, -2 to -3, and <-3 Z-scores of HAZ, WAZ, and WHZ, respectively. When possible, HAZ, WAZ, and WHZ were defined according to the World Health Organization (WHO) 2006 growth charts<sup>10</sup>, rather than the CDC 2000<sup>11</sup> or the 1977 National Center for Health Statistics (NCHS) growth charts. We selected these outcomes because they are health related, quantifiable, have sufficient literature or available microdata for meta-analyses, and are available in the Global Burden of Disease Study (GBD).<sup>1,4</sup>

We performed a systematic review of published scientific literature for the impact of *Cryptosporidium* on physical growth in children under five years old. The PubMed search was performed in July 2015 and updated in July 2017. The search string is provided below and returned 598 results.

*cryptospor\* AND (stunting[Title/Abstract] OR wasting[Title/Abstract] OR growth[Title/Abstract] OR underweight[Title/Abstract] OR development[Title/Abstract] OR malnutrition[Title/Abstract]) AND Humans[Mesh] NOT (rats or mice)*

From these 598 initial results, 49 were included for full-text screening and 7 provided data. We specifically looked for data describing the change in height or weight either measured in metric units or z-scores. Metric units were converted to height-for-age and weight-for-age z-scores based on the WHO sex-specific growth curves.

We supplemented the systematic literature review with individual-level data from several case control and cohort studies. We performed panel-based linear regression models with these data accounting for an interaction term between *Cryptosporidium* and diarrhoea, and adjusting for age in days, days between anthropometric measurements, and the previous anthropometric measurement.

The input data can be found in Table S2.

### <span id="page-8-0"></span>**Supplementary Table 2. Sources included in the** *Cryptosporidium* **effect size meta-analyses.**

Gray sources are ones for which individual-level microdata were used.



To standardise the change in growth associated with *Cryptosporidium* diarrhoea, we use height-for-age z-scores and weight-for-age z-scores. The HAZ and WAZ values are intended to be universally usable and are produced by the World Health Organization. Some studies report changes in growth as change in HAZ or WAZ. For studies that don't, we convert the change in weight (kilograms) and in height (centimeters) to z-scores using the WHO Growth Charts [\(http://www.who.int/childgrowth/standards/en/\)](http://www.who.int/childgrowth/standards/en/). At a given age, the difference in weight or height per zscore is used to convert to z-scores for analysis. When possible, we use sex specific z-scores but if a study makes no distinction between sexes, we use the z-score for girls. The age for the z-score lookup is the median age in the time period in months.

The WAZ distribution is assumed to be right-tailed, meaning that the standard deviations below the median get progressively smaller and those above the median get progressively larger. For this analysis, the kilograms/z-score change are the first standard deviation below the median.

A number of studies report p-values on associations but fail to report the confidence intervals. To approximate the confidence intervals, we use the equation  $23$ :

$$
Z = -0.862 + \sqrt{(0.743 - 2.404 * \ln(p))}
$$
  
Standard Error = 
$$
\frac{Estimate}{Z}
$$
  

$$
CI = Estimate - SE * 1.96; Estimate + SE * 1.96
$$

We performed a series of random effects meta-analyses to summarise the relationship between childhood growth indicators, HAZ, WAZ, and WHZ, and *Cryptosporidium* diarrhoea. We quantified the effect in z-scores on each of these indicators per episode of *Cryptosporidium* diarrhoea. The results of these analyses are shown in Figures S3-S5. We also performed a random effects meta-analysis on the impact of *Cryptosporidium* infection in the absence of diarrhoea on each indicators. This relationship was statistically significant only for change in HAZ, shown in Figure S6.

<span id="page-10-0"></span>**Supplementary Figure 3. Forest plot of the effect per** *Cryptosporidium* **diarrhoea episode on height-for-age zscores based on a random effects meta-analysis**



### Cryptosporidium diarrhea and HAZ

<span id="page-11-0"></span>**Supplementary Figure 4. Forest plot of the effect per** *Cryptosporidium* **diarrhoea episode on weight-for-age zscores based on a random effects meta-analysis**



### Cryptosporidium diarrhea and WAZ

<span id="page-12-0"></span>**Supplementary Figure 5. Forest plot of the effect per** *Cryptosporidium* **diarrhoea episode on weight-for-height z-scores based on a random effects meta-analysis**



### Cryptosporidium diarrhea and WHZ

#### <span id="page-13-0"></span>**Supplementary Figure 6. The effect of non-diarrhoea** *Cryptosporidium* **infection on height-for-age z-scores from a random effects meta-analysis**

The point estimates in the forest plot indicate the summary estimate of the change in HAZ scores wherein *Cryptosporidium* infection in the absence of diarrhoea was identified between anthropometric measurements.

# Cryptosporidium w/o diarrhea and HAZ



### <span id="page-14-0"></span>**Supplementary Figure 7. Sensitivity analyses of the effect of Cryptosporidim diarrhoea on height-for-age and weight-for-age z-scores.**

The analysis is stratified by age of exposure (A and B) and by data source type (C and D). There were insufficient data to perform these analyses for weight-for-height. Note that the results in these forest plots are not exactly the same as the primary analyses because the input data were stratified for these sensitivity analyses.

### **A)**



### Cryptosporidium diarrhea and HAZ



# Cryptosporidium diarrhea and HAZ



# Cryptosporidium diarrhea and WAZ



### Cryptosporidium diarrhea and WAZ

Undernutrition is a risk factor for diarrhoea, measles, and lower respiratory infections (LRI) in GBD 2016, based on the statistically significant relative risks from a systematic review.24,25 Each of these outcomes has a relative risk given undernutrition status (stunted, underweight, wasted) and the relative risks are adjusted for covariance between undernutrition indicators.24 We performed a log-linear interpolation of the reported relative risks at each level of undernutrition reported in the original study (severe, moderate, and mild, stunting, wasting, and underweight) for each outcome. This created a continuous relative risk curve for the increased risk of diarrhoea, LRIs, and measles for each undernutrition indicator.26 Undernutrition was then attributed to a fraction of the DALYs from each of the outcomes based on a counter-factual proportion called the population attributable fraction (PAF).

To determine the number of undernutrition-associated DALYs attributable to Cryptosporidium, we calculated the change, at the population level, in mean HAZ, WAZ, and WHZ due to the pathogen for each age group, year, sex, and geography from GBD. This PAF is defined as:

$$
PAF = 1 - \frac{1}{Diarchea Episodes * Proportion_{crypto} * \left(\frac{\Delta Z score}{Crypto diarchea episode}\right) * RR}
$$

Where the Diarrhoea Episodes is the modelled number of diarrhoea episodes (GBD 2016),27 Proportion Crypto is the frequency of detection of Cryptosporidium in diarrhoea cases (GBD 2016)28, ∆Zscore is the change in z-score per Cryptosporidium diarrhoea episode from the meta-analysis, and RR is the relative risk of a given outcome (e.g. measles) per z-score change in malnutrition category (i.e. per HAZ, WHZ, or WAZ unit change).24 We did not calculate an undernutrition PAF for children under one month old as neonatal weight is predominantly related to birthweight.

To get a final PAF for undernutrition due to Cryptosporidium diarrhoea, we accounted for covariance in WHZ, WAZ, and HAZ. We used the same approach as the risk factor analysis for undernutrition in GBD 2016 which is defined by:25

$$
PAF_{\text{}}}(1 - PAF_{\text{}}}) * (1 - PAF_{\text{}}}) * (1 - PAF_{\text{}}}) * (1 - PAF_{\text{}}})
$$

The last step was to multiply our PAF by the LRI, measles, and diarrhoea DALYs estimates to determine the total number of DALYs from those outcomes attributable to Cryptosporidium diarrhoea.

Cryptosporidium affects weight gain. Protein-energy malnutrition (PEM) is a burden of disease that is due to low weight. To estimate the amount of PEM that was due to Cryptosporidium, we estimated the shift in the weight-forage and weight-for-height distribution due to Cryptosporidium diarrhoea. This was done by evaluating the percent difference in the observed WAZ and WHZ distribution. The shift in the mean WAZ and WHZ at the population level was represented by:

$$
Shift = \text{Diarrhea Episodes} * \text{Proportion}_{\text{Crypto}} * \left( \frac{\Delta Z score}{\text{Crypto diarrhea episode}} \right)
$$

And the counterfactual prevalence of wasting and underweight was:

$$
Prevalence (Counterfactual) = Prevalence (GBD 2016 Estimate) + Shift
$$

The prevalence of underweight and wasting was converted to a z-score, and we estimated the percent change in the cumulative density from a normal distribution compared to the observed prevalence.

$$
PAF = \frac{Prevalence(GBD\ 2016\ Estimate) - Prevalence(Counterfactual)}{Prevalence(GBD\ 2016\ Estimate)}
$$

PAFs were calculated independently for mild, moderate, and severe undernutrition, age group, geography, sex, and year. Final DALYs due to Cryptosporidium diarrhoea are the PAF multiplied by the number of DALYs due to protein-energy malnutrition.

### 1 **Supplementary Table 3. Cryptosporidium deaths, incidence, cases, DALYs among children under 5 in 2016 by country**

2 The burden of *Cryptosporidium* by country is shown. The number of deaths, episodes, acute, undernutrition, and total DALYs due to *Cryptosporidium* are

3 accompanied by the incidence per 1,000 child-years and the percent increase in the total number of DALYs after accounting for the long-term outcomes

4 associated with *Cryptosporidium* diarrhoea. Numbers in parentheses are the 95% Uncertainty Intervals

<span id="page-19-0"></span>























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