RESEARCH ARTICLE

Seroprevalence of antibodies against Chlamydia trachomatis and enteropathogens and distance to the nearest water source among young children in the Amhara Region of Ethiopia

Kristen Aiemjoy[1]*, Solomon Aragie[2], Dionn0488 0 n

Author summary

Trachoma, an infection of the eye caused by the bacteria *Chlamydia trachomatis*, and many diarrhea-causing infections are associated with access to water for washing hands and faces. Measuring these different pathogens in a population is challenging and rarely are multiple infections measured at the same time. Here, we used an integrated approach to simultaneously measure antibody responses to *C. trachomatis*, *Giardia intestinalis*, *Cryptosporidium parvum*, *Entamoeba histolytica*, *Salmonella enterica*, *Campylobacter jejuni*, enterotoxigenic *Escherichia coli* (ETEC) and *Vibrio cholerae* among young children residing in rural Ethiopia. We found that the seroprevalence of all pathogens increased with age and that seropositivity to more than one pathogen was common. Children living further from a water source were more likely to be exposed to *S. enterica and G. intestinalis*. Integrated sero-surveillance is a promising avenue to explore the complexities of multi-pathogen exposure as well as to investigate associations between water, sanitation, and hygiene related exposures and disease transmission.

Introduction

Diarrhea and trachoma typically afflict the world's poorest populations and are major contributors to preventable morbidity [1,2]. Diarrhea, caused by parasitic, viral and bacterial infections, and trachoma, caused by repeated *Chlamydia trachomatis* infections of the eye, share water and hygiene related transmission pathways. Increased access to water for food preparation and washing of hands, faces, and

Health Care Administration and Control Authority, and institutional review boards at the University of California, San Francisco and Emory University. CDC staff did not have contact with study participants or access to personal identifying information and were therefore determined to be

The majority of children, 56.9% (1291/2267), lived in households whose nearest water source was unprotected. Household demographic information was available for 755 children. In this subset, 8.7% (66/755) of children lived in households with electricity, 10.1% (76/755) lived in households with a radio, 0% (0/761) lived in households with a mobile phone, 84.4% (637/755) lived in households that owned animals. For the majority of households (85.2% (643/755)), the primary occupation was agricultural work. (Table 1).

The seroprevalence among 0±9 year-olds was 43.1% (95% CI: 38, 48.4) for *C. trachomatis*, 27.5% (95% CI: 23.6, 31.6) for *S. enterica*, 70.3% (95% CI:67.7, 72.8) for *E. histolytica*, 53.9% (95% CI: 51.8, 56.0) for *G. intestinalis*, 95.6% (95% CI: 94.4, 96.5) for *C. jejuni*, 76.3% (95% CI: 74.1, 78.4) for ETEC and 94% (95% CI: 92.8, 94.9) for *C. parvum*. Seroprevalence increased with age with marked differences across pathogens. The age-dependent seroprevalence of *G. intestinalis declined after age 2*. (Fig 1). For ETEC, *E. histolytica*, *C. parvum*, *C. jejuni* and *G. intestinalis*, over 70% of children were positive at age 2 years. The age-dependent seroprevalence slopes were less steep for both *C. trachomatis* and *S. enterica*; by age 9 over 60% of children were seropositive for *C. trachomatis* and over 40% of children were seropositive for *S. enterica*. Seropositivity for more than 1 pathogen was common (Fig 2). At age 2 years, the median number of pathogens to which a child was seropositive was 4 (IQR 3±5), increasing to 5 (IQR 4±6) by age 4 years.

There was no indication for trend in community-level seroprevalence by community-level median distance to the nearest water source; however, there was considerable variability on community-level seroprevalence for some pathogens (*C. trachomatis*, *G. intestinalis*, *E. histolytica and S. enterica* (Fig 3)). The between-community variance in seroprevalence was highest for *C. trachomatis* (SD .20) and *S. enterica* (SD 0.13). More community-level heterogeneity was apparent among young children (under 3) compared with older children, the exceptions being *C. parvum* and *C. jejuni* which both had very high seroprevalence even among young

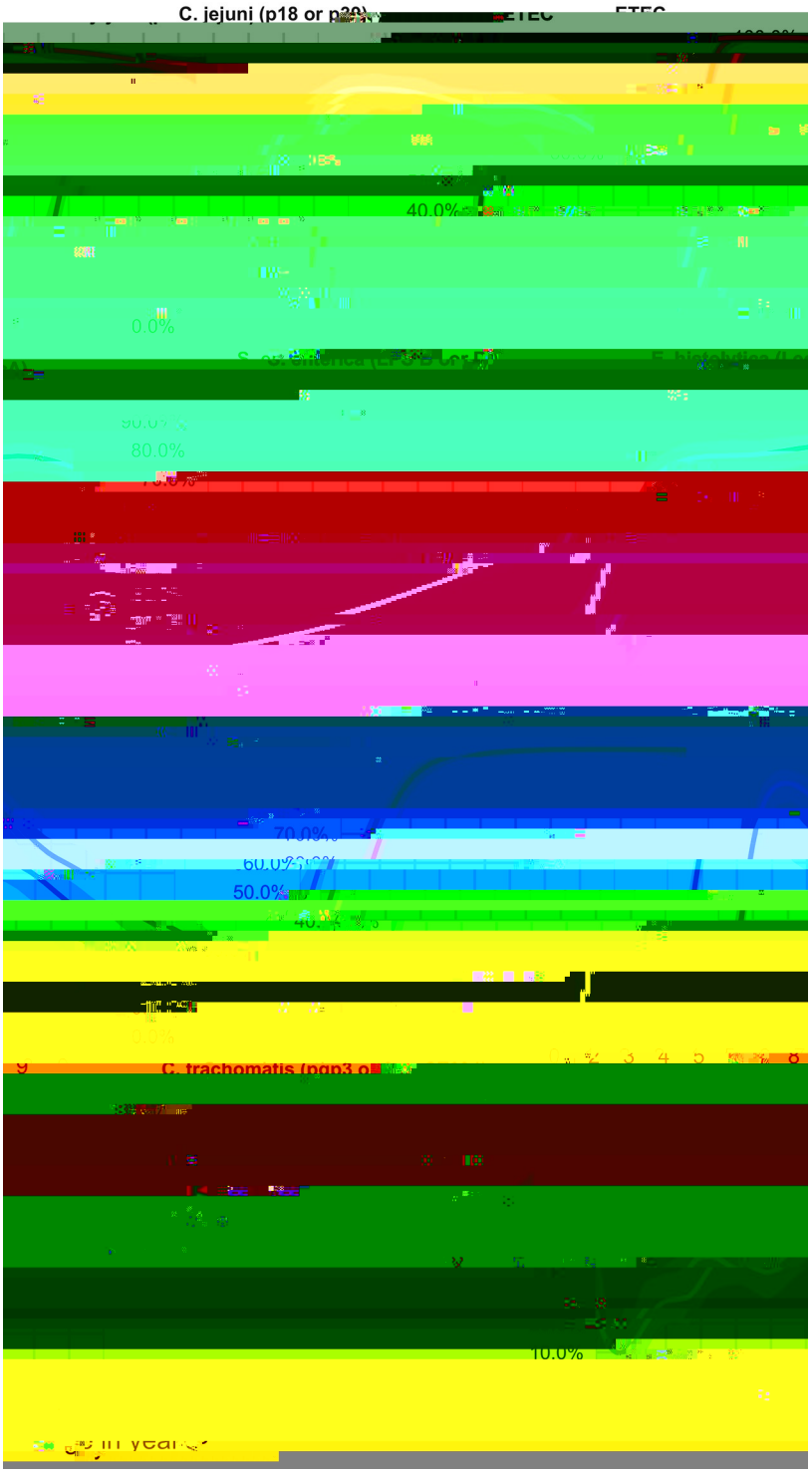

Fig 1. Age-dependent seroprevalence of trachoma and enteropathogens in the Amhara region of Ethiopia. Age-dependent seroprevalence curves were fitted using generalized additive models (GAM) with a cubic spline for age. Seropositivity cutoffs were derived using ROC curves, if available, or by fitting finite mixture models (S1 Fig). Seropositivity cutoffs could not be estimated for V. cholerae in this study, so seroprevalence curves are not shown. For pathogens with more than one antigen, positivity to either antigen was considered positive. IgG response measured in multiplex using median fluorescence units minus background (MFI-bg) on the Luminex platform on 2267 blood samples from 2267 children Td (minus)Tj 2.7284 0 Td (background)Tj 5.0032 0 Td ((MFI-b)Tj 2.9339 0 T085 -1.1835 Td (Sero1 0

Children in the quartile living farthest from any water source had a 12% (95% CI: 2.6, 21.4) higher seroprevalence of *S. enterica* and a 12.7% (95% CI: 2.9, 22.6) higher seroprevalence of *G. intestinalis* compared to children living in the nearest quartile (Table 2). Quantitative antibody levels demonstrated the same pattern for *S. enterica*, with antibody levels for *S. enterica* LPS group D 0.32 (95% CI: 0.13, 0.52) log

age 9 years, over 60% of children were seropositive for *C. trachomatis* and over 40% of children were seropositive for *S. enterica*.

Unlike for other pathogens in the study, G. intestinalis seroprevalence declined after age two years. Giardia has been shown to exhibit increasing infection prevalence with age in other cohorts in low-resource settings with a high proportion of asymptomatic infections [48], suggesting that the IgG response is weaker at older ages despite infection. The precise immunological mechanism for lower mean IgG levels among older ages is not currently known, but the phenomena has been observed in multiple other cohorts. For example, Arnold et al. demonstrated declining mean IgG with age for Giardia (VSP-3, VSP-5), ETEC (LTB) and Campylobacter (p18, p39) in cohorts from Haiti and Kenya [13]. Age-dependent antibody kinetics in that study suggest that much of the decline of mean IgG with age for these pathogens is likely due to acquired immunity, which results in either lower rates of infection, or more likely, if children are infected they experience less severe disease and potentially a less robust IgG boost.

Use of a multiplexed immunoassay allowed us to expediently identify that seropositivity to more than one pathogen was common in the Amhara region of Ethiopia and that, by age three, most children were seropositive for five of the seven pathogens under investigation. Similarly, we were able to identify notable correlation in seroprevalence between some pathogens (for example, *C. parvum* and *E. histolytica)* at the Td Tj 6.1511 0 Td (between)Tj 3.5263ere

in studies using microscopy in the region. In one recent study of protozoan prevalence in the Amhara region, the single-stool prevalence of *Entamoeba spp. (histolytica and dispar)* by microscopy among three year old children was 7.1% [49]. However, differences between seroprevalence and prevalence by microscopy are expected given that IgG response integrates information over time and microscopy measures active presence and shedding. The seroprevalence of C. trachomatis identified in this study is consistent with the high burden of trachoma documented in the Amhara region [50].

Children living farther from a water source had higher seroprevalence of *S. enterica* and *G. intestinalis*. The absence of heterogeneity in seroprevalence in this high transmission setting may have masked other potential relationships between exposure to enteric pathogens and distance to water. For example, among children 0 to 3 years old, the seroprevalence of *C. parvum* and *C. jejuni* were both very high (77% and 91% respectively). In a sensitivity analysis restricted to children younger than 12 months, there was an indication that the quantitative antibody levels for children living in the farthest quartile of distance compared to the nearest quartile of distance were higher for *V. cholerae* toxin beta subunit, *C. parvum* cp17 and cp23. However, the differences among this age sub-group were not statistically significant; the statistical power was likely limited by the lower number of children in this subset.

We were likely underpowered to determine differences in seroprevalence adjusted for socio-economic status. In the random 33% subset of children with available household asset information, children living in the furthest quartile of distance still had a higher seroprevalence of *S. enterica* and *G. intestinalis*, however the differences were not statistically significant.

There were several limitations of this study with respect to how the nearest water source was measured. First, we measured absolute Euclidean distance rather than walking distance or time it takes to collect water. The study site region has tremendous gradation in altitude, with

many high plateaus and steep valleys. In some cases, the distance to the nearest water source may not reflect the time it would take to ascend, descend or otherwise traverse the terrain. Future studies may consider alternative methods for calculating distance that accommodate land type and elevation changes. Second, we did not ask household which water source they were using. Households may use water sources that are further away via linear distance because of taste preference, ease of access, water source type or other reasons, namely terrain [51]. Third, the study site region is arid and there is variation in water availability by season. We simply measured the distance to the nearest water source at the time of the census and this may have not reflected a water source that was flowing and available at different times of the year. Third, we assumed that distance to the nearest water source was associated with the

based on external negative controls (solid) and finite Gaussian mixture models (dash). For Chlamydia trachomatis pgp3 & CT694 cutoffs were derived using receiver operating characteristic (ROC) curves, for Cryptosporidium parvum Cp17 & Cp23 cutoffs were derived using a standard curve and for Giardia intestinalis VSP-3 & VSP-5 and Entamoeba histolytica LecA cutoffs were derived using the mean plus 3 standard deviations above a negative control panel.
(TIFF)

S2 Fig. Community-level correlation in seroprevalence. Correlation between the mean community seroprevalence depicted with circles, greater circle area represents higher correlation. For pathogens with more than one antigen, positivity to either antigen was considered positive. IgG response measured in multiplex using median fluorescence units minus background (MFI-bg) on the Luminex platform on 2267 blood samples from 2267 children aged 0 to 9 years.
(TIFF)

S1 Table. Quantitative antibody levels by distance quartile and differences comparing Quartile 4 to Quartile 1.
(DOCX)

Acknowledgments

We gratefully acknowledge the study participants for their valuable time. Purified Cp17, Cp23, VSP-3, VSP-5, p18, and p39 antigens were kindly provided by Jeffrey Priest (US CDC), and LecA antigen was kindly provided by William Petri (University of Virginia) and Joel Herbein (TechLab).

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

Author Contributions

Conceptualization: Kristen Aiemjoy, Diana Martin, Jeremy D. Keenan, Benjamin F. Arnold.

Data curation: Kristen Aiemjoy, Benjamin F. Arnold.

Formal analysis: Kristen Aiemjoy, Sarah Gwyn, Diana Martin, Benjamin F. Arnold.

Funding acquisition: Jeremy D. Keenan.

Investigation: Kristen Aiemjoy, Jeremy D. Keenan, Benjamin F. Arnold.

Methodology: Kristen Aiemjoy, Solomon Aragie, Dionna M. Wittberg, Sarah Gwyn, Diana Martin, Jeremy D. Keenan, Benjamin F. Arnold.

Project administration: Solomon Aragie, Dionna M. Wittberg, Zerihun Tadesse, E. Kelly Callahan.

Resources: Diana Martin.

Software: Benjamin F. Arnold.

Supervision: Solomon Aragie, Dionna M. Wittberg, Diana Martin, Jeremy D. Keenan, Benjamin F. Arnold.

Validation: Sarah Gwyn.

Visualization: Kristen Aiemjoy, Benjamin F. Arnold.

Writing ± original draft: Kristen Aiemjoy, Benjamin F. Arnold.

Writing ± review & editing: Solomon Aragie, Dionna M. Wittberg, Zerihun Tadesse, E. Kelly Callahan, Sarah Gwyn, Diana Martin, Jeremy D. Keenan.

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