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The epidemiology and aetiology of diarrhoeal disease in infancy in southern Vietnam: a birth cohort study

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SUMMARY

Objectives: Previous studies indicate a high burden of diarrhoeal disease in Vietnamese children, however longitudinal community-based data on burden and aetiology are limited. The findings from a large, prospective cohort study of diarrhoeal disease in infants in southern Vietnam are presented herein.
Methods: Infants were enrolled at birth in urban Ho Chi Minh City and a semi-rural district in southern Vietnam, and followed for 12 months ($n = 6706$). Diarrhoeal illness episodes were identified through clinic-based passive surveillance, hospital admissions, and self-reports.
Results: The minimum incidence of diarrhoeal illness in the first year of life was 271/1000 infant-years of observation for the whole cohort. Rotavirus was the most commonly detected pathogen (50% of positive samples), followed by norovirus (24%), Campylobacter (20%), Salmonella (18%), and Shigella (16%). Repeat infections were identified in 9% of infants infected with rotavirus, norovirus, Shigella, or Campylobacter, and 13% of those with Salmonella infections.
Conclusions: The minimum incidence of diarrhoeal disease in infants in both urban and semi-rural settings in southern Vietnam was quantified prospectively. A large proportion of laboratory-diagnosed disease was caused by rotavirus and norovirus. These data highlight the unmet need for a rotavirus vaccine in Vietnam and provide evidence of the previously unrecognized burden of norovirus in infants.

1. Introduction

Diarrhoea remains a substantial cause of morbidity and mortality amongst children globally.[1,2] In a study in rural central Vietnam, the incidence of diarrhoea in children under 5 years of age was found to exceed 115 episodes/1000 child-years.[3] Risk factors for diarrhoea in Vietnam include, as in many settings, male gender, age less than 2 years, and poor socioeconomic indicators such as household crowding and poor hygiene habits.[4,5] There are no equivalent population-based estimates of diarrhoea in southern tropical Vietnam, where approximately 40% of the country's population live.

A recent study in southern Vietnam illustrated the relative contributions of rotavirus, norovirus, and the bacterial pathogens

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Shigella spp, *Salmonella spp*, and *Campylobacter spp* as the aetiological agents of diarrhoea in hospitalized children under 5 years of age in Ho Chi Minh City (HCMC).[6] How well these data represent the community level burden of diarrhoeal disease is unclear. Further, these data suggest that the majority of hospitalized diarrhoea cases are in children <12 months of age,[6] which is the pivotal age group at which rotavirus vaccine should be targeted. Longitudinal community cohort studies provide an opportunity to evaluate the epidemiology and disease burden of diarrhoea to a fuller scale than hospital-based research. However, few studies have evaluated the incidence of diarrhoea in Vietnam,[3,7] and to date none have focused exclusively on the tropical south of the country. To address this knowledge gap, we sought to define the burden, aetiology, and risk factors for diarrhoeal disease through community cohorts of infants in two distinct settings in this densely populated, rapidly industrializing region. A better understanding of the epidemiology and aetiologies of diarrhoeal disease in southern Vietnam will inform rational public health interventions.

2. Methods

2.1. Description of the cohort

The cohort structure and methodology have been described previously.[8] Briefly, pregnant women were enrolled from 2009 to 2013 in southern Vietnam in two locations: women resident in central HCMC, the largest city in southern Vietnam, were enrolled at Hung Vuong Obstetric Hospital in HCMC; women resident in Cao Lanh District, Dong Thap Province, which is 120 km southwest of HCMC and situated in a semi-rural setting, were enrolled at Dong Thap Provincial Hospital. After delivery, infants were enrolled and followed up for the first 12 months of life with routine visits at 2, 4, 6, 9, and 12 months of age. A brief questionnaire detailing growth and illness in the preceding period since the last visit was administered, and a series of samples (blood, throat swab, nasopharyngeal swab) was collected at each routine visit.

2.2. Diarrhoeal episode detection

During the 12 months of follow-up, passive detection of diarrhoeal illness was performed, in which families were asked to take their child to a designated study clinic if the infant was unwell. At presentation, a brief clinical report was collected, as well as a stool sample. If the child was admitted, a detailed clinical evaluation was recorded. Blood samples were collected at the discretion of the treating physician. A new episode of diarrhoea was defined by ≥7 days between the onset dates of symptoms. Diarrhoea was defined as three watery loose stools or at least one bloody/mucoid diarrhoeal stool within 24 h,[9] or an increase in stool frequency as determined by the parent's judgement.

A secondary source of data on diarrhoeal episodes were self-reports by the mother of diarrhoeal illness in their infant for the period prior to each study visit.

2.3. Laboratory analysis

Stool samples collected from diarrhoeal episodes were stored at 4 °C until transport within 24 h and were then stored at −80 °C until further testing. One-step reverse transcriptase (RT) PCRs for rotavirus and norovirus genogroups I and II (GI and GII) were performed using RNA Master Hydrolysis Probes (Roche Applied Sciences, UK) on a LightCycler 480 (Roche Applied Sciences, UK) with the primers and probe sequences and PCR cycling conditions described previously.[10] Real-time PCR cycling conditions for Shigella (target *ipa*H) and Campylobacter (*Campylobacter jejuni* target: *hip*O; *Campylobacter coli* target: *gly*A) were as follows: 95 °C for 15 min, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s, 72 °C for 30 s, as described previously.[11,12] Salmonella was detected using an in-house assay targeting the *inv*A gene, which is conserved across the eight *Salmonella* subspecies, with cycling conditions as follows: 95 °C for 15 min, followed by 45 cycles of 95 °C for 5 s, 60 °C for 60 s. The sequences of the primers and probe for the *inv*A gene were as follows: forward 5′-TCATCGCACCGT-CAAARGA-3′, reverse 5′-CGATTTGAARGCCGGTATTATT-3′, probe: 5′-FAM-ACGCTTCGCCGTTCRCGYGC-BHQ1-3′. The limit of detection was 5 copies/reaction. Stool samples were not available from self-reported diarrhoea episodes.

2.4. Statistical analyses

Two separate incidence measurements were calculated: one evaluating diarrhoeal presentations at a study clinic and/or admitted to hospital, and the other based solely on self-reported diarrhoeal illness derived from information collected at the routine follow-up visits. These data were not merged. Infant-years of observation (IYO) for each infant were derived from the date of birth and date of exit from the study due to either completion of follow-up, documented early withdrawal, or loss to follow-up, defined by the last routine visit or illness presentation, whichever was later, if the full 12-month follow-up period was not completed. Pathogen-specific incidence estimates were not calculated due to low counts, but the incidence of aetiological groups (bacterial, viral, or mixed infection) was evaluated. Comparisons between groups were made using the Kruskal–Wallis test for continuous variables with non-normal distributions and the Chi-square test for categorical variables.

Multivariable negative binomial regression was used to identify risk factors associated with severe diarrhoea presenting to a study clinic and/or admitted to hospital. Regression was performed independently for each study site due to the heterogeneity in risk profiles between HCMC and Dong Thap. Factors were included in the multivariable model according to hypothesized associations determined a priori (maternal characteristics, socioeconomic indicators, household elevation), as well as those found to be significantly associated in the univariable analysis ($p < 0.05$). All analyses were performed in Stata v. 13 (StataCorp, College Station, TX, USA).

2.5. Spatial clustering analyses

To investigate the presence of spatial clustering of diarrhoeal illness, we used a Bernoulli model with all diagnosed episodes of diarrhoea as cases, and children without any reported history of diarrhoeal episodes as the background population using SaTScn v. 9.1.1 (http://www.satscan.org/). Each pathogen in turn was also considered as a case, with the control group remaining all children in the cohort with no reported episode. For the analyses, the upper limit for cluster detection was specified as 50% of the study population. The significance of the detected clusters was assessed by a likelihood ratio test, with a *p*-value obtained by 999 Monte Carlo simulations generated under the null hypothesis of a random spatiotemporal distribution.

2.6. Ethics

Four hospitals in HCMC (Hospital for Tropical Diseases, Hung Vuong Obstetric Hospital, District 8 Hospital, Children's Hospital 1) and Dong Thap Provincial Hospital participated in the study. The protocol was approved by the institutional review boards of all these hospitals, as well as the Oxford Tropical Research Ethics