Protective Immunity after Natural Rotavirus Infection: A Community Cohort Study of Newborn Children in Guinea-Bissau, West Africa

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To study the natural history of rotavirus infection and to determine the protection it confers against reinfection and diarrhea, 200 newborns in Guinea-Bissau were prospectively followed for up to 2 years. Rotavirus was detected in stool specimens collected weekly. By age 2 years, the incidence of primary rotavirus infection was 74%. In the first 3 months of life, 17% of the infections were diarrhea associated, compared with 60% at 9–11 months; after age 18 months, all infections were asymptomatic. A primary infection conferred 52% (95% confidence interval [CI], 16% to 73%) and 70% (95% CI, 29% to 87%) protection against subsequent rotavirus infection and rotavirus diarrhea, respectively. The protection was 66% (95% CI, 24% to 85%) against reinfection within the same epidemic, compared with 34% (95% CI, −29% to 67%) against reinfection in any subsequent epidemic. The high level of protection against symptomatic rotavirus infection provides an important incentive for development of a rotavirus vaccine.

Rotavirus is a leading cause of severe childhood diarrhea worldwide, accounting for about one-third of all cases of severe diarrhea that require hospitalization and causing 450,000–800,000 of the 2.2 million diarrhea-related deaths each year [1, 2]. We estimate that rotavirus causes 145,000 deaths each year in children <5 years old in sub-Saharan Africa [3]. Virtually all children are infected with rotavirus before their second birthday in both developing and industrialized countries [2]. Environmental interventions to improve hygienic conditions and provision of safe water and food are unlikely to reduce the incidence of rotavirus diarrhea [2]. Because there is no effective specific treatment, vaccination is the major strategy for disease control [4].

Immunization with live attenuated rotaviruses is intended to mimic natural rotavirus infection by providing protection against severe illness [5]. Rotavirus vaccine development experienced a major setback when the recently licensed reassor-
tant rhesus rotavirus vaccine was found to be associated with intussusception in young children [6].

The immunity provided by natural rotavirus infection must be estimated in order to obtain an evidence-based platform for evaluation of the efficacy of new rotavirus vaccines. Early epidemiologic studies of the immunity conferred by rotavirus infection have yielded diverging results, ranging from virtually no protection against rotavirus infection and/or disease [7, 8] to almost complete [9–11] protection against rotavirus diarrhea.

To describe the natural history and incidence of rotavirus infection and to estimate the protection that natural infection confers against a new rotavirus infection, we undertook a cohort study of children monitored from birth to age 2 years.

Methods

Study design. The study was undertaken as part of a prospective community-based surveillance of diarrheal diseases in the suburban districts Bandim II and Belem of Bissau, the capital of Guinea-Bissau. In total, 603 houses in the study area were randomly selected, and 200 children born in these houses between 15 January 1996 and 14 January 1997 were recruited within ~3 weeks (range, 0–24 days) after birth and monitored with weekly visits that included stool sampling, regardless of whether or not a child had diarrhea. Because of a military conflict in the spring of 1998, the follow-up of 46 children was discontinued. The study closing date was retrospectively defined as 28 April 1998.

Rotavirus detection. Stool specimens were tested for rotavirus with the IDEA ELISA kit (Dako), as described by the manufacturer.

Definitions. A child was categorized as having diarrhea or no diarrhea according to information given by the caretaker on the day of sample collection. We considered the results from each stool
Figure 1. Cumulative incidence of primary rotavirus infections in a birth cohort of 200 children monitored until age 2 years in Guinea-Bissau, 1996–1998.

specimen analysis to represent up to 7 nonoverlapping days of observation centered on the day of the specimen collection. The scheduled follow-up time was defined as the period between the inclusion day and the last day of follow-up. A child was considered to be infected with rotavirus when a stool specimen was positive in the rotavirus ELISA. A continuous infection was defined as a series of rotavirus-positive specimens collected from the same child <14 days apart and with no negative specimens. For such continuous infections, only the first rotavirus-positive sample was included in the analyses. We considered a child to be at risk for a subsequent infection after the termination of a 7-day period following an infection.

Statistical analysis. The incidences were calculated as the number of episodes per 100 child-months at risk. To estimate the median age for children at the end of breast-feeding, we used the Kaplan-Meier method. To determine the cumulative incidence of primary rotavirus infections, we used a modification of the Kaplan-Meier estimator [12] that takes into account the gaps in the observation periods. We used logistic regression to estimate age-adjusted odds ratios (ORs) for the association between rotavirus infection and diarrhea and to estimate the protective immunity induced by rotavirus infections. To account for repeated entries of the same child, we used generalized estimating equations with a compound symmetry (exchangeable) variance-covariance matrix [13]. Stool specimens were collected weekly on a routine basis, reflected in the average at-risk time associated with each visit being the same (6.5 days), regardless of whether a child was or had been infected with rotavirus. Hence, the obtained ORs were direct measures rather than biased estimates of the corresponding incidence ratios [14]. The percentage protection against subsequent infection or disease was calculated as \((1 - OR) \times 100\%\). A given exposure was considered to be a confounder for the pathogenicity estimate if the OR changed >10% after adding a variable indicating the status of the exposure to the model.

In Guinea-Bissau, rotavirus infections appear in annual epidemics from January through March [3]. Infections in the same child >180 days apart were defined as occurring in different epidemics, whereas infections occurring within 180 days were regarded as occurring in the same epidemic. We used nested indicator coded variables [15] to compare the protection against rotavirus reinfection and diarrhea in the same versus in any subsequent epidemic. The fold-difference in protection was calculated as the OR for infection or diarrhea in any subsequent epidemic divided by the OR for infection or diarrhea in the same epidemic. Statistical analyses were performed with the SAS System (version 8.02; SAS Institute).

Results

Cohort monitoring. Of the 200 study children, 104 (52%) were boys. The study area is inhabited by most of the major ethnic groups of Guinea-Bissau [16]. Prolonged breast-feeding is common in Guinea-Bissau [16], and 93.5% of the children in our study were breast-fed at the time of sample collection. At age 1 year, 99% of the children were partially breast-fed, 83% at age 1.5 years, and 30% at age 2 years. The observed median age for ending breast-feeding was 21.5 months (interquartile range [IQR], 18.5–24.0 months). During the observation period, 18 children died, 10 within their first year of life.
Table 1. Age-specific incidences of rotavirus infections in a birth cohort of 200 children monitored for up to 2 years in Bissau, Guinea-Bissau, 1996–1998.

<table>
<thead>
<tr>
<th>Age, months</th>
<th>Person-time, days</th>
<th>No. of rotavirus infections</th>
<th>Incidencea</th>
<th>Disease: infection ratiob</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>With diarrhea</td>
<td>Total</td>
<td>With diarrhea</td>
</tr>
<tr>
<td>0–2</td>
<td>12,873</td>
<td>17</td>
<td>3</td>
<td>4.0</td>
</tr>
<tr>
<td>3–5</td>
<td>11,632</td>
<td>27</td>
<td>12</td>
<td>7.1</td>
</tr>
<tr>
<td>6–8</td>
<td>10,939</td>
<td>14</td>
<td>6</td>
<td>3.9</td>
</tr>
<tr>
<td>9–11</td>
<td>10,886</td>
<td>15</td>
<td>9</td>
<td>4.2</td>
</tr>
<tr>
<td>12–14</td>
<td>10,135</td>
<td>20</td>
<td>11</td>
<td>6.0</td>
</tr>
<tr>
<td>15–17</td>
<td>8617</td>
<td>10</td>
<td>5</td>
<td>3.5</td>
</tr>
<tr>
<td>18–20</td>
<td>6062</td>
<td>6</td>
<td>0</td>
<td>3.0</td>
</tr>
<tr>
<td>21–23</td>
<td>3647</td>
<td>7</td>
<td>0</td>
<td>5.8</td>
</tr>
</tbody>
</table>

a Infections/100 child-months at risk.
b Ratio between symptomatic and all rotavirus infections.

In total, 38 children moved from the study area, and follow-up of another 46 children was interrupted by the outbreak of a military conflict in June 1998. In all, 102 children were lost to follow-up before age 2 years. We monitored the children for a median of 18.4 months (IQR, 13.1–22.3 months). The 200 children were monitored for 74,791 child-days, which represented 88% (IQR, 74%–95%) of the total scheduled follow-up time.

In total, we collected 11,987 specimens, representing 88% (IQR, 73%–95%) of the scheduled specimens, and analyzed 11,406 of these for rotavirus. Rotavirus was identified in 132 stool specimens. Sixteen isolates were regarded as being part of continuous infections, leaving 116 distinct rotavirus infections, of which 94 were considered to be primary infections, 18 were second infections, and 4 were third infections.

Incidence and association with diarrhea. The overall incidence of rotavirus infections, including 22 reinfections, was 0.6 infections per child-year. According to the Kaplan-Meier estimates, 26% of the children would experience a primary infection by age 6 months, 46% by age 1 year, and 74% by age 2 years (figure 1). Of the 116 rotavirus infections, 46 (40%) were associated with diarrhea, compared with 10% (1135 of 11,272) of the rotavirus-negative specimens. The age-adjusted OR for the association between rotavirus and diarrhea was 5.9 (95% confidence interval [CI], 3.9–8.8). Breast-feeding and sex were not confounders for this pathogenicity estimate. Symptomatic infections were infrequent in early infancy but became more common with increasing age and peaked at ages 9–11 months (table 1). Rotavirus infection was not associated with diarrhea in children 18–24 months old.

Seasonality of rotavirus infections. With a few exceptions, rotavirus infections were limited to the relatively cooler and drier period from January to March (figure 2).

Protection conferred by natural rotavirus infection. Of the primary rotavirus infections, 44% were symptomatic. During second and third infections, only 20% of the children experienced diarrhea. The incidence of primary rotavirus infections was 5.37/100 child-months, compared with 3.08/100 child-
months for subsequent infections. The corresponding crude OR was 0.57, whereas the OR adjusted for age and repeated entries of the same child was 0.48 (95% CI, 0.27–0.84; table 2). Similarly, the crude OR for rotavirus diarrhea in primary versus subsequent infections was identical to that of the adjusted OR (0.50 [95% CI, 0.13–0.71]). In other words, natural rotavirus infection conferred 52% and 70% protection against subsequent rotavirus infection and diarrhea, respectively.

The OR for a symptomatic postprimary infection was 0.30 (95% CI, 0.13–0.71), corresponding to 70% protection against rotavirus diarrhea. The protection estimates against reinfection within the same epidemic and in any subsequent epidemic were 66% (95% CI, 24%–85%) and 34% (95% CI, −29% to 67%), respectively (table 3). Thus, the protection was 1.9-fold higher (95% CI, 0.8–4.6) against a new rotavirus infection in the same epidemic than against a rotavirus infection in a birth cohort of 200 children monitored for up to 2 years in Guinea-Bissau.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No. of diarrhea episodes</th>
<th>ORb</th>
<th>Protection % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any infection</td>
<td>116</td>
<td>4.71</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>94</td>
<td>5.37</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Subsequent</td>
<td>22</td>
<td>3.08</td>
<td>0.48 (0.27–0.84)</td>
</tr>
<tr>
<td>Any diarrhea</td>
<td>46</td>
<td>1.87</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>41</td>
<td>2.34</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Subsequent</td>
<td>5</td>
<td>0.70</td>
<td>0.30 (0.13–0.71)</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval.

**Discussion**

In this longitudinal cohort study of newborn children in West Africa, we estimated that 74% of the children would experience a rotavirus infection before their second birthday. This is likely a conservative estimate, because our weekly stool sampling probably failed to identify infections with rotavirus shedding shorter than 7 days and because we did not include serologic testing [11]. Moreover, children may have been lost to follow-up during the rotavirus epidemics because some accompanied their mothers outside the study area during the annual cashew harvest toward the end of the epidemics.

The high rotavirus pathogenicity (OR, 5.9) corroborates the previous observation that rotavirus is an important cause of diarrheal disease among children in Guinea-Bissau [3]. The low proportion of symptomatic infections during the first 3–6 months of life, when almost 98% of the children in our study were breast-fed, confirms the findings of earlier longitudinal studies [17, 18] and supports suggestions of a protective role for maternal immunity and breast-feeding [7, 18, 19]. The peak of rotavirus diarrhea incidence at age 12–14 months occurred at about the same age as that observed in other studies in Africa [17, 18].

The incidence of postprimary (i.e., subsequent) rotavirus infections was substantially lower than of primary rotavirus infections. Natural rotavirus infection conferred a 52% and 70% protection against subsequent infection and rotavirus diarrhea, respectively. The risk of introducing a bias by the selective loss to follow-up of older children prompted us to undertake an additional analysis in which we restricted the dataset to include only children with ≥85% of the scheduled follow-up time. The protection conferred by natural rotavirus infection against reinfection was then found to be 64% (95% CI, 17%–84%) (data not shown). Therefore, the inclusion of children with <85% of the scheduled follow-up time is unlikely to have artificially inflated the protection estimates.

A cohort study in Mexico reported a higher incidence of...
rotavirus than we observed, possibly in part because serologic testing was included to identify the infections [11]. However, our study corroborates the main conclusion from the Mexican study, in which the protection conferred by rotavirus infection against subsequent rotavirus infection and rotavirus diarrhea was 38% and 77%, respectively. The low pathogenicity in the children 18–24 months old in the present study corroborates findings from other cohort studies [17, 18, 20] and likely reflects the high degree of protection conferred by natural rotavirus infections early in life.

In the present study, protection against rotavirus infection was 1.9-fold lower in any subsequent epidemic than in the same epidemic. Because our estimates are based on incidence, there should be no bias related to the time of the postprimary event (i.e., whether it occurred early or late in the epidemic season). To explore whether the higher level of protection during the same epidemic could be attributed to bias introduced by the criteria we used to classify whether a postprimary infection occurred in the same or in a subsequent epidemic, we restricted the data to include only the rotavirus epidemics. The fold difference was then 3.0, indicating that 1.9 may be a conservative estimate.

Although our estimates are imprecise and should be interpreted with caution, others have also reported a loss of protection against reinfection with time from infection [21, 22]. A possible reduction in protection over time may be due to waning immunity to rotavirus, of year-to-year shifts in predominating rotavirus serotypes [23] with insufficient cross-protection, or be related to the transmission dynamics in the child’s immediate environment. Irrespective of the underlying mechanisms, our findings underscore the need to include long-term follow-up (with a minimum duration spanning 2 rotavirus seasons), including genotype profiling of the isolated rotaviruses in future cohort studies of rotavirus immunity and in rotavirus vaccine trials.

We conclude that natural rotavirus infection induces a high level of protection against rotavirus infection and rotavirus diarrhea. This is an important finding for the strategy of immunizing young infants and thereby protecting them against rotavirus diarrhea during their first critical years of life.

Acknowledgments

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References


11. Velazquez FR, Matson DO, Calva JJ, et al. Rotavirus infections in infants as related to the transmission dynamics in the child’s immediate environment. Irrespective of the underlying mechanisms, our findings underscore the need to include long-term follow-up (with a minimum duration spanning 2 rotavirus seasons), including genotype profiling of the isolated rotaviruses in future cohort studies of rotavirus immunity and in rotavirus vaccine trials.


