Breast Milk Reduces the Risk of Illness in Children of Mothers With Cholera

Observations From an Epidemic of Cholera in Guinea-Bissau

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Background: A protective effect of breastfeeding against cholera has been demonstrated in areas endemic of cholera. To assess the protection offered by breast milk from mothers living in an area that had been free from cholera for 7 years, we investigated mothers with cholera and their children during an epidemic with *Vibrio cholerae* El Tor in the capital of Guinea-Bissau.

Methods: Eighty mothers with clinical cholera and their children were identified, and interviewed. Blood samples for vibriocidal and antitoxin antibodies were collected from mother-and-child pairs. Breast milk samples were collected from lactating mothers.

Cholera was defined as acute watery diarrhea during the epidemic and a vibriocidal reciprocal titer of 20 or above.

Results: Three (7%) of 42 breastfed children had cholera as defined above compared with 9 (24%) of 38 nonbreastfed children (RR for breastfed children, 0.19; 95% CI, 0.04–0.91, adjusted for age). The 3 breastfed children who developed cholera received milk containing lower concentrations of anticholera toxin IgA/total IgA (median, 2.0 units/mL) than 14 children who had serologic signs of colonization but did not develop the disease (median, 17.4 units/mL).

Conclusions: The protective effect of breast milk against cholera is not confined to endemic areas. Lactating mothers with cholera should receive supportive care to continue breastfeeding.

Key Words: breastfeeding, cholera, cholera epidemic, children, milk-antibodies, Guinea-Bissau

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Cholera is a threat to the health of the populations in Asia, Africa, and the Americas.[1–4] In areas where cholera is endemic, the disease primarily affects nonimmune segments of the population, including children. In addition, individuals heavily exposed to fecal matter, such as mothers of young children, can have high rates of cholera.[5] By contrast, when introduced to nonimmune populations, cholera affects persons of all ages, although the highest morbidity usually is recorded among adults.[6] Common for both cholera-endemic and -epidemic settings is the observation that infants and children of breastfeeding age usually have mild disease or low disease rates.[5,7]

Two major factors contribute to the low disease rates. First, young children may be protected against severe disease because of cross-protection from recent infections with enterotoxigenic *Escherichia coli* (ETEC) producing heat-labile toxin (LT), a toxin almost identical to the cholera toxin.[6,8] Second, protection offered by the anti-infective properties of maternal milk, both nonspecific and specific factors, may mitigate disease after exposure and colonization with *Vibrio cholerae*.[9–12]

A study from Bangladesh showed that specific breast-milk antibodies against cholera toxin protected infants from disease but not from colonization with *V. cholerae*.[9]

It is conceivable that the breast-milk antibody response is most efficient in populations where exposure to *V. cholerae* is endemic and less efficient in settings where *V. cholerae* is introduced after several years of absence. Furthermore, vaccinated lactating mothers have a higher secretory IgA response in breast milk among naturally primed mothers than among nonprimed mothers.[13,14]

The objective of the present study was to determine whether or not there is an association between specific breast milk antibodies and risk of colonization by and disease from *V. cholerae* in a population that had been free from cholera for almost 7 years.

MATERIALS AND METHODS

In October 1994, cholera was reported for the first time since 1988 in the West African country of Guinea-Bissau. During the epidemic, which was caused by *V. cholerae* O1 biotype El Tor serotype Ogawa and lasted for 6 months, 15,719 cases were reported.[15] Molecular studies of the implicated strain suggested that it was epidemiologically unrelated to the strain that caused the previous epidemic.[16] The Bandim health project, an ongoing demographic surveillance in the periurban areas of Bandim and Belem (population

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approximately 40,000), in the capital Bissau, organized several activities during the epidemic. Daily assistants from the project reviewed consultations books and discharge list from the city hospital and the 2 health centers in the study area to identify persons with cholera. This identification enabled a home-based follow-up of mothers with cholera and their children. In addition, a house-to-house-based survey in the areas of Bandim and Belem identified additional suspected cases of cholera missed by the official reporting.

Mothers who had reported cholera or acute watery diarrhea during the epidemic and with children below 3 years of age were eligible for the study. We excluded women who were visitors or were traveling during the epidemic period or from whom we could not obtain a blood sample.

The Research Committee of the Ministry of Health in Guinea-Bissau approved the study. The respondents were asked for informed consent before the interview and the collection of approximately 1 mL of capillary blood from the mother and her child. A 5-mL volume of milk was obtained by manual expression of one breast from lactating mothers before the child was breastfed. All samples were kept on ice in an insulated box. Specimens were placed in a refrigerator within 3 hours.

Laboratory Methods. Blood samples were centrifuged and serum was frozen. Breast-milk samples were centrifuged at 2500 rpm for 10 minutes to remove cells and lipids and then frozen at −20°C.

The response of specific IgA antibodies against cholera-toxin subunit B (anti-CTB IgA) in the milk samples was tested by means of GMI enzyme-linked immunosorbent assay (ELISA) method.[13]

Total IgA concentration was measured by a total IgA ELISA using a Swedish breast-milk sample with a known IgA concentration as reference. The anti-CTB IgA titer was divided by the total IgA concentration to adjust for variations that might be ascribed to dilution.

The IgA and IgG antibody responses against CTB in serum were measured by GMI-ELISA.[17] The vibriocidal antibody responses in serum were measured by a microtiter technique, as described. Titers were determined against El Tor serotype Ogawa, as well as El Tor serotype Inaba *V. cholera* O1.[17,18]

Case Definitions and Statistical Methods. A case of clinical cholera was defined as a person who had acute watery diarrhea during the epidemic and had a vibriocidal reciprocal titer against *V. cholerae* O1 serotype Ogawa or Inaba of 20 or above.

Comparing proportions in age groups of children was by χ^2 for trend and adjusting for age in estimation of the relative risk of cholera was done by Mantel-Haenszel technique. We used nonparametric methods (Kruskal-Wallis test) to compare antibody titers among age groups. Linear regression of log-transformed values was used to correlate amounts of anti-CT IgA/total IgA against maternal vibriocidal and antitoxin antibody titers.

RESULTS

The study included 80 mother-child pairs, of whom 74 were officially notified cases of cholera-patients and 6 were identified in the house-to-house survey. The median age of the mothers was 27 years (range, 16 to 39 years), 66 (82.5%) had sought health care at a hospital, and 41 (53.2%) were admitted as inpatients. The median serum vibriocidal reciprocal antibody titer against serotype Ogawa was 1280 (interquartile range, 320 to 5120), median IgG reciprocal titer against CT was 410 (interquartile range, 205 to 878), and median IgA reciprocal titer against CT was 35 (interquartile range, 16 to 69).

Forty-two (51%) of the children of these mothers had vibriocidal titers of ≥20. Fifteen (19%) had had a diarrhea episode during the epidemic, and 12 of these had vibriocidal titers of antibodies ≥20 and were thus considered cholera cases (Table 1). With increasing child age, there were increased proportions of children who had samples positive for vibriocidal antibodies ($P = 0.003$, χ^2 for trend), and the titers of antibodies against cholera toxin were higher by increasing child age ($P = 0.001$ for IgG and $P = 0.014$ for IgA). By contrast, there was no significant increased disease rate by age. Among 38 weaned children, 9 (24%) had cholera, compared with 3 cases among 42 (7.1%) breastfed children

TABLE 1. Results From Examination of 80 Children Whose Mothers Had Cholera During the 1994/1995 Epidemic in Guinea-Bissau

Variable	Child Age		
	2–11 mo (n = 28)	12–23 mo (n = 22)	24–36 mo (n = 30)
Blood samples			
No. (%) with vibriocidal antibodies ≥20	11 (39)	8 (36)	22 (73)
No. (%) with cholera*	4 (14)	2 (9)	6 (20)
Geometric mean (25%–75%)			
Vibriocidal antibodies†	12.5 (5–560)	5 (5–640)	640 (5–1280)
Anti-CTB IgG	132 (43–503)	324 (140–615)	585 (278–2162)
Anti-CTB IgA	10 (5–25)	30 (15–43)	33 (15–100)
Milk samples			
No. (%) breastfed	25 (89)	15 (68)	2 (7)
Geometric mean (25%–75%) of breast milk anti-CTB IgA/total IgA	16.7 (2.5–25)	7.7 (1.7–14.3)	0.5 (0.5–0.5)

*Children who experienced a diarrheal disease plus vibriocidal antibodies ≥20.
†Against serotype Ogawa or Inaba.

TABLE 2. Attack Rate of Cholera in the 80 Children, by Age and Breastfeeding Status, Guinea-Bissau, 1994–1995

Age Group	Cholera (%)	
	Breastfed	Not Breastfed
2–11 months	2/25 (8%)	2/3 (67%)
12–23 months	1/15 (7%)	1/7 (14%)
24–36 months	0/2 (0%)	6/28 (21%)
Total	3/42 (7%)	9/38 (24%)

(RR, 0.30; 95% CI, 0.09–1.03) (Table 2). After adjusting for age, the relative risk of cholera among breastfed children was 0.19 (95% CI, 0.04–0.91). Among breastfed children, 17 of 42 (40%) had vibriocidal titers ≥20, compared with 24 (63%) of the nonbreastfed children.

Breast-milk samples were available from all 42 lactating mothers. With increasing child age, the titers of anti-CT IgA/total IgA in breast milk decreased ($P = 0.0001$, Kruskal-Wallis test, Table 1). This was due to larger amounts of total IgA/mL with increasing age of the children (geometric mean, 0.85 mg/mL among infants compared with 1.08 mg/mL among children 12–36 months of age [$P < 0.01$]), whereas anti-CT IgA titers were independent of child age. Three breastfed children who had cholera received milk with a median anti-CT IgA/total IgA of 2.0 units/mL (range, 1.3 to 2.4) compared with an anti-CT IgA/total IgA of 17.4 units/mL (range, 2.5 to 28.6) in milk consumed by 14 children who were seropositive for vibriocidal antibodies but had no diarrhea. Among the total of 39 children who had vibriocidal antibodies titers <20 or were vibriocidal-positive but had no diarrhea, the geometric mean milk values were 14.0 units/mL (range, 2 to 20).

The concentrations of anti-CT IgA/total IgA in milk correlated with maternal vibriocidal antibodies titer (correlation coefficient $r = 0.40$, $P = 0.009$, linear regression, Fig. 1), as well as with maternal blood IgA titers against cholera toxin in serum ($r = 0.51$, $P < 0.001$, linear regression).

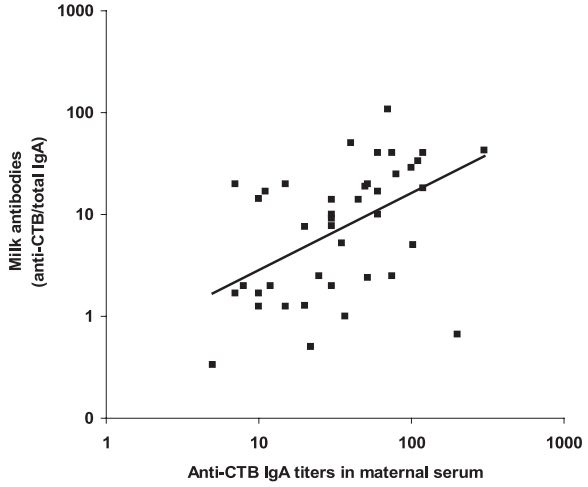

FIGURE 1. The correlation between maternal anti-CTB IgA milk antibodies vs. maternal anti-CTB IgA serum antibodies.

DISCUSSION

During the 7-year interepidemic period of this study there were no notified cases of cholera, and microbiologic studies carried out in that period did not isolate *V. cholerae* from patients with gastroenteritis (A. Dalsgaard, unpublished data).

A study from Chile showed low preimmune titers (GMT approximately 10 a.u.) in infants and toddlers before vaccination.[19] It is known that a small percentage of persons from nonendemic areas have naturally occurring low titers of vibriocidal antibodies.[20]

During an epidemic of cholera, it is reasonable to assume that the presence of vibriocidal antibodies ≥20 a.u. by and large is due to clinical or subclinical infection with *V. cholerae*. We therefore assume that the presence of vibriocidal antibodies was due to exposure from *V. cholerae* in the present epidemic, and our working definition of cholera may thus be valid for epidemiologic purposes. According to these assumptions, we show that approximately half of the children of mothers with cholera may have been colonized with *V. cholerae* and that colonization frequency increased with increasing child age. The study also suggests that breast milk, even in an epidemic situation, may protect against cholera. There was a higher risk of disease from cholera among nonbreastfed children, and there was also an indication that children who developed cholera in spite of breastfeeding were fed with breast milk with a low content of specific cholera-toxin antibodies. Breastfeeding did not seem to protect against colonization with *V. cholerae*, only against developing the disease. Our data from a cholera epidemic corroborate previous data from a highly endemic setting in Bangladesh.[9]

Previous studies with focus on the protective effects of breastfeeding against cholera have mainly been made in endemic areas or after vaccine induction. Vaccination may induce an IgA antitoxin-response in milk in only 27% of women versus 80%–90% of women with naturally induced cholera.[13,14] The present study is one of the first studies measuring specific anticholera IgA antibodies after natural cholera disease in an epidemic setting.

There were lower titers of anti-CT IgA/total IgA in breast milk of mothers of older than younger children. This should not be regarded as an "inferior immunologic quality" of breast milk from mothers who feed their children after 1 year of age. Rather, this finding was due to increased amounts of total IgA with increasing age, perhaps because of less milk production in mothers of partially weaned children. This observation is corroborated by a Guatemalan study that reported a higher concentration of IgA in the milk samples from mothers with more than 1 year of lactation.[21]

Large amounts of anticholera toxin antibodies secreted in breast milk were related to maternal antitoxin antibody concentrations of IgA but also of IgG in serum and, to a certain degree, also to maternal vibriocidal antibody titers. These findings suggest that heavily exposed mothers can have powerful breast milk antibody responses, which in turn may protect their children from cholera. Similar observations have been made in Bangladesh.[9]

It is a limitation of the study that specimens were collected at a variable interval of 2 weeks to 3 months after illness. Another limitation is that it is not possible to measure the amount of milk consumed by the children.

Immunologic memory from the 1987/1988 epidemic in Guinea-Bissau may have played a role in the maternal serologic response observed in the present study. Because anti-CT is strongly cross-reactive with the heat-labile enterotoxin (LT) of ETEC and ETEC are highly endemic in Guinea-Bissau,[8,22] it is possible that previous exposures to LT producing ETEC enhanced maternal antibody responses to cholera toxin during the recent epidemic.

Even gravely undernourished women are capable of producing high titers of anti–*V. cholerae* enterotoxin IgA in their milk.[14] All mothers should be encouraged to continue breastfeeding even if they become ill. We believe that guidelines for the control of cholera should emphasize the positive value of breastfeeding, and lactating mothers with cholera should receive the necessary supportive care to continue or restart breastfeeding as soon as possible.

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