

## Enteropathogens and Other Factors Associated with Severe Disease in Children with Acute Watery Diarrhea in Lima, Peru

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To evaluate enteropathogens and other factors associated with severe disease in children with diarrhea, 381 children <5 years of age with diarrhea and moderate to severe dehydration (in-patients) and 381 age-, sex-, and date-of-visit-matched children with mild diarrhea (out-patients) presenting to a hospital in Peru, were studied. Rotavirus was detected in 52% of the in-patients and 35% of the out-patients (odds ratio [OR] = 2.3, 95% confidence interval [95% CI] = 1.6–3.2); 95% of the rotaviruses among in-patients were of serotypes G1–G4. The risk of severe diarrhea was particularly great in children who were not exclusively breast-fed in early infancy and who also lacked piped water in their homes (for children with both characteristics OR = 6.8, 95% CI = 3.6–12.8). The high prevalence of rotavirus and its association with severe diarrhea underscores the need for rotavirus vaccines. Interventions to educate mothers and improve access to safe water should augment the impact of rotavirus vaccines in preventing severe diarrhea.

Diarrhea causes an estimated one-third of all hospitalizations and 2.4–2.9 million deaths per year among children <5 years of age worldwide [1, 2]. This tremendous disease burden underscores the need for specific interventions to prevent moderate to severe dehydration, which is an important cause of death in children with diarrhea [3–7]. Lack of breast-feeding, inappropriate rehydration therapy, severe malnutrition, frequent vomiting and diarrhea, low socioeconomic status, and the presence of associated major infections are recognized risk factors for the development of dehydration in children with diarrhea [8–15]. On the other hand, the role of specific microorganisms in the etiology of dehydrating diarrhea has been systematically assessed in very few studies [8, 11, 12], all of which have been conducted in cholera-endemic countries of the Indian subcontinent. A knowledge of the pathogens associated

with severe diarrhea will not only allow the optimum use of available interventions but will also direct efforts aimed at developing specific therapies. Therefore, we conducted a 2-year study to evaluate the etiology of illness in children <5 years of age who sought treatment for acute, watery diarrhea at a hospital in Lima, Peru. Furthermore, we examined behavioral, environmental, and physiologic characteristics associated with severe diarrhea in order to identify measures to augment the benefits from interventions targeted at specific pathogens.

### Methods

*Study design.* The study was conducted at the Instituto Nacional de Salud del Niño, a hospital serving children from a poor section of urban Lima. From 15 February 1995 through 14 February 1997, all children <5 years of age who sought medical attention for watery diarrhea ( $\geq 3$  liquid or semi-liquid stools in a 24-h period) at the hospital were evaluated for inclusion in the study. At enrollment, a pediatrician or nurse measured the child's weight (in kilograms) and height (in centimeters) and assessed the degree of dehydration by using World Health Organization criteria based on clinical signs and symptoms [16, 17]. A child with no clear signs of dehydration was assessed as having mild illness and was treated on an out-patient basis. Severe disease requiring in-patient care was diagnosed when the child had at least two of the following three clinical features of dehydration: poor skin turgor, increased thirst, and altered sensorium as manifested by irritability, stupor, or coma. All children requiring in-patient care were recruited into the study, and for each such child, we sought a second

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The research protocol using human subjects was reviewed and approved by the Naval Medical Research Institute's Committee for the Protection of Human Subjects.

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child with mild illness (out-patient) who was matched for age ( $\pm 3$  months), sex, and date of hospital visit ( $\pm 3$  months). A pediatrician interviewed the mother or caretaker accompanying the children, completed a standardized questionnaire, examined the children, and instituted rehydration therapy as needed.

**Laboratory detection of enteropathogens.** Fecal specimens obtained from the children at the time of admission were examined for the presence of viral, bacterial, and parasitic enteropathogens. Stools were examined, by use of specific EIAs, for rotavirus, astrovirus, and adenovirus [18–20]. Two stool swab samples (1 obtained directly from the patient and the other from a fecal specimen) were inoculated on Cary-Blair media and transported to the laboratory of the Naval Medical Research Institute Detachment in Lima to be tested for *Escherichia coli*, *Campylobacter jejuni*, *Shigella* species, and *Vibrio cholerae* by standard methods [21]. *E. coli* isolates that fermented lactose (Lac<sup>+</sup> *E. coli*) were tested for enterotoxin plasmids via colony hybridization assays. The diagnosis of enterotoxigenic *E. coli* (ETEC) was based upon positive hybridization reactions with one or more enterotoxin probes (LT, ST1a, or ST1b). Fresh and concentrated (by the formalin-ether technique) fecal specimens were examined by light microscopy for parasitic ova and cysts and by the modified acid-fast Ziehl-Neelsen method for *Cryptosporidium parvum* and *Cyclospora cayetanensis* [22, 23].

**Characterization of rotavirus strains.** A representative sample of rotavirus-positive stools from the in-patients were serotyped by a monoclonal-based EIA that used specific neutralizing monoclonal antibodies against the viral protein (VP7) of rotavirus serotypes 1, 2, 3, and 4 [24, 25]. A rotavirus-positive specimen was considered to be nontypeable when it was positive as determined by use of the detection EIA but did not give a response in the serotyping EIA. A hemi-nested reverse transcriptase–polymerase chain reaction was used to characterize the G genotype of rotaviruses that were nontypeable by the EIA [26].

**Assessment of risk factors.** A goal of this study was to identify factors other than specific enteropathogens, including nutritional status, feeding practices during the first 6 months of life, and income level, that might predispose a child to severe versus mild illness. For nutritional status, we used reference values from the US National Center for Health Statistics to calculate height-for-age Z scores and classified children as “stunted” (defined as 2 SDs below the expected height-for-age Z score) and “not stunted” [27]. For feeding practice, we classified children into those who were “exclusively breast-fed” (i.e., no other solid or liquid food except for breast milk) in the first 6 months of life and “not exclusively breast-fed.” For income, we defined “low family income” as a monthly salary of <390 soles (equivalent to US \$144 at the time of study), which was the median per capita monthly income for families of the out-patients.

To assess the association between exposure variables and severe disease, we calculated maximum likelihood estimates of matched odds ratios (ORs) with 95% confidence intervals (CIs) [28]. To adjust the ORs for the effects of potential confounders and to identify possible interactions between the predictor variables, we performed conditional logistic regression by use of the hierarchical backward elimination method [29]. This regression procedure is based on the multiplicative model, which implies that ORs for each exposure present can be multiplied to provide an overall estimate

of risk when no interaction is present [28, 30]. The significance of interaction terms in the regression models was evaluated with a likelihood-ratio test. All statistical tests were interpreted in a two-tailed fashion.

## Results

**Comparability of in-patients and out-patients.** During the 2-year study period, we recruited 381 in-patients with diarrhea and moderate to severe dehydration and 381 matched out-patients with mild diarrhea. Because of the matched selection procedure, in-patients were nearly identical to out-patients with respect to sex, age (mean age: 12.4 months for in-patients, 12.8 months for out-patients), and month of hospital visit (table 1). As per the criterion used for defining the 2 groups, in-patients were more likely than out-patients to be irritable, have increased thirst, and exhibit poor skin turgor.

**Prevalence of enteropathogens among in-patients.** At least 1 enteropathogen was detected in 77% of the 381 in-patients ( $n = 292$ ) (table 2). Enteric viruses were the most frequently identified pathogens, affecting 60% of the patients ( $n = 230$ ), followed by bacteria (34%) and parasites (5%). Rotavirus was the most commonly detected individual etiologic agent, accounting for 52% of the diarrheas ( $n = 199$ ), followed by ETEC (16%), and *C. jejuni* (12%). Most patients with rotavirus diarrhea (191/199 [96%]) were <2 years of age (median, 11 months); the detection rates of this pathogen were greatest among children 6–11 and 12–23 months of age, in whom this pathogen accounted for 56% and 59% of all diarrheas, respectively. In contrast, bacteria and parasites were detected more often among patients  $\geq 2$  years of age, with the exception of *C. jejuni*, which was more common in infants than among older patients (32/199 [16%] vs. 13/182 [7%], respectively;  $P = .01$ ).

While multiple enteropathogens were found simultaneously

**Table 1.** Comparison of in-patients and out-patients with diarrhea, Lima, Peru, 1995–1997.

Feature	No. (%) of children with feature	
	In-patients ( $n = 381$ )	Out-patients ( $n = 381$ )
Boys	248 (65)	246 (65)
Age (months)		
0–5	49 (13)	38 (11)
6–11	150 (39)	161 (42)
12–23	156 (41)	156 (41)
24–59	26 (7)	26 (7)
Month of admission		
January–March	124 (33)	119 (31)
April–June	114 (30)	102 (27)
July–September	68 (18)	84 (22)
October–December	75 (20)	76 (20)
Indicators of hydration status		
Irritable sensorium <sup>a</sup>	331 (87)	152 (40)
Increased thirst <sup>a</sup>	362 (95)	84 (22)
Poor skin turgor <sup>a</sup>	194 (51)	7 (2)

<sup>a</sup>  $P < .001$  (two-tailed) for cited comparison between in-patients and out-patients.

**Table 2.** Enteropathogens detected among in-patients with diarrhea, by age group, Lima, Peru, 1995–1997.

Enteropathogen	No. (%) of children from whom enteropathogen was isolated, by age group (months)				
	0–5 (n = 49)	6–11 (n = 150)	12–23 (n = 156)	24–59 (n = 26)	0–59 (n = 381)
<b>Virus</b>					
Rotavirus	15 (31)	84 (56)	92 (59)	8 (31)	199 (52)
Adenovirus	1 (2)	6 (4)	6 (4)	2 (8)	15 (4)
Astrovirus	4 (8)	7 (5)	4 (3)	1 (4)	16 (4)
<b>Bacteria</b>					
Enterotoxigenic <i>Escherichia coli</i>	7 (14)	17 (11)	26 (17)	9 (35)	59 (16)
<i>Campylobacter jejuni</i>	5 (10)	27 (18)	10 (6)	3 (12)	45 (12)
<i>Shigella</i> species	3 (6)	4 (3)	4 (3)	3 (12)	14 (4)
<i>Vibrio cholerae</i>	1 (2)	1 (1)	1 (1)	4 (15)	7 (2)
<b>Parasites</b>					
<i>Cryptosporidium parvum</i>	0 (0)	5 (3)	3 (2)	0 (0)	8 (2)
<i>Giardia lamblia</i>	1 (2)	3 (2)	3 (2)	5 (19)	12 (3)
<i>Cyclospora cayentanensis</i>	0 (0)	1 (1)	0 (0)	0 (0)	1 (<1)
Multiple pathogens	9 (18)	41 (27)	29 (19)	10 (39)	89 (23)
Any pathogen	29 (59)	117 (78)	121 (78)	25 (96)	292 (77)

in 23% of the patients (n = 89), the specific organisms varied in their frequency of association with other pathogens. Multiple pathogens were detected frequently in patients infected with *C. jejuni* (38/45 [84%]) and ETEC (44/59 [75%]) but were less common in those with rotavirus diarrhea (63/199 [32%]).

A representative sample of 48% of the 200 rotavirus-positive stools (n = 95) were characterized to determine the common circulating G serotypes and genotypes of rotavirus. Of these, 68% (n = 65) were type G1, 21% (n = 21) were type G2, 3% (n = 3) were type G4, 1% (n = 1) was type G3, and 5% (n = 5) were nontypeable.

*Comparison of pathogens and other characteristics among in-patients and out-patients.* Compared with out-patients, in-patients were significantly more likely to be infected with at least 1 enteropathogen, multiple pathogens, and rotavirus (table 3). In-patients were also significantly more likely than out-patients to be not exclusively breast-fed in early infancy and lacking a source of piped water in the home. There were no significant differences among in-patients and out-patients with regard to stunting and family income level, although data on income were available for only 128 matched pairs.

In multivariate analysis, infection with rotavirus remained associated with severe diarrhea (OR = 2.3, 95% CI = 1.6–3.2). The strength of the association between severe diarrhea and being not exclusively breast-fed in infancy was significantly greater (P = 0.04) in children who lacked a source of piped water in the home (OR = 4.7, 95% CI = 2.5–9.1) than in those who had a source of piped water (OR = 2.1, 95% CI = 1.4–3.1). Compared with children who were exclusively breast-fed and who had a source of piped water in the home, those who were not exclusively breast-fed and who also lacked a source of piped water in their home were at a nearly sevenfold greater risk of severe diarrhea (OR = 6.8, 95% CI = 3.6–12.8).

**Discussion**

In this study, we used state-of-the-art microbiologic assays to perform one of the most comprehensive etiologic assessments

to date of children with diarrhea requiring medical attention. To our knowledge, this is also the first systematic study of the role of various pathogens in the etiology of severe childhood diarrhea in a Latin American country. Our findings highlight the importance of rotavirus as a cause of severe diarrhea in Peruvian children. This pathogen was detected in 52% of the in-patients, a rate greater than that reported in most previous hospital-based studies in Peru and other developing countries [31–36]. Furthermore, despite the extensive etiologic testing, rotavirus was associated with another coinfecting pathogen in only one-third of in-patients. In contrast, a second pathogen was identified in more than three-fourths of in-patients infected with the 2 most common bacterial pathogens, ETEC and *C. jejuni*. Finally, rotavirus was the only pathogen that was significantly more prevalent among in-patients than out-patients.

Previous studies of the association between rotavirus and severe diarrhea have reported conflicting results. In a community-based study in rural Bangladesh [37], 44% of patients with rotavirus diarrhea developed dehydration, a proportion significantly greater than that among those with diarrhea of other causes. Similarly, 23% of 26 episodes of rotavirus diarrhea among children in rural Guatemala were associated with dehydration, and 1 child required hospitalization for severe disease [38]. On the other hand, a recent analysis of hospital surveillance data from Bangladesh found that children infected with rotavirus had less severe dehydration than those infected with other enteropathogens [39]. In addition, rotavirus was not associated with severe dehydration in several case-control studies with a design similar to that of ours [8, 11, 12] and was negatively associated with fatal outcome in an evaluation of risk factors for death among children attending a diarrhea treatment center [5].

Our data convincingly demonstrate that rotavirus is highly prevalent and is associated with severe disease in Peruvian children with diarrhea, underscoring the need for specific interventions, such as vaccines against this pathogen. Our findings raise four important issues that are relevant in considering strat-

**Table 3.** Comparison of characteristics between in-patients and out-patients with diarrhea, Lima, Peru, 1995–1997.

Characteristic	No. (%) of children with characteristic		Discordant pairs <sup>a</sup>	Odds ratio (95% confidence interval)
	In-patients (n = 381)	Out-patients (n = 381)		
<b>Enteropathogen</b>				
<b>Virus</b>				
Rotovirus	199 (52)	135 (35)	115/51	2.3 (1.6–3.2)
Adenovirus	15 (4)	22 (6)	15/22	0.7 (0.4–1.3)
Astrovirus	16 (4)	17 (5)	15/16	0.9 (0.5–1.9)
<b>Bacteria</b>				
Enterotoxigenic <i>Escherichia coli</i>	59 (16)	53 (14)	50/44	1.1 (0.8–1.7)
<i>Campylobacter jejuni</i>	45 (12)	63 (17)	38/56	0.7 (0.5–1.0)
<i>Shigella</i> species	14 (4)	21 (6)	12/19	0.6 (0.3–1.3)
<i>Vibrio cholerae</i>	7 (2)	0 (0)	7/0	—
<b>Parasite</b>				
<i>Cryptosporidium parvum</i> <sup>b</sup>	8 (2)	1 (<1)	8/1	—
<i>Giardia lamblia</i>	12 (3)	6 (2)	12/6	2.0 (0.8–5.8)
<i>Cyclospora cayentanensis</i>	1 (<1)	0 (0)	1/0	—
Multiple pathogens	89 (23)	64 (17)	70/45	1.6 (1.1–2.3)
Any pathogen	292 (77)	262 (70)	88/58	1.5 (1.1–2.1)
<b>Other characteristics</b>				
Stunted growth <sup>c</sup>	47 (13)	32 (9)	40/27	1.5 (0.9–2.4)
Not exclusively breast-fed	212 (56)	132 (35)	132/52	2.5 (1.9–3.5)
No source of piped water <sup>d</sup>	148 (39)	113 (30)	101/64	1.6 (1.2–2.2)
Income < 390 soles <sup>e</sup>	81 (48)	114 (50)	39/34	1.2 (0.7–1.9)

<sup>a</sup> Number of pairs in which characteristic was reported in children with severe dehydration but not in those with mild or no dehydration/number of pairs in which characteristic was reported in children with mild or no dehydration but not in those with severe dehydration.

<sup>b</sup> Statistical comparison may be unreliable because of small no. of children with mild dehydration who had *C. parvum* infection.

<sup>c</sup> Defined as height-for-age Z score of <–2, calculated by using reference values from US National Center for Health Statistics. Data on stunting were not available for 17 matched pairs.

<sup>d</sup> Data on source of water were not available for 3 matched pairs.

<sup>e</sup> At time of study, exchange rate was 2.73 soles to 1 US dollar. Information on monthly family income was available for only 128 matched case-control pairs.

egies for vaccination against rotavirus in Peru. First, while cases of rotavirus diarrhea were seen in the first 6 months of life, they constituted <10% of all in-patients with this disease. Therefore, if rotavirus vaccines were administered along with the routine Expanded Program on Immunization schedule of childhood immunizations to children 6, 10, and 14 weeks of age, they would protect all but a small fraction of children from severe rotavirus diarrhea. Second, if immunization was delayed beyond 1 year of age, the impact of the vaccine would diminish since 50% of in-patients with rotavirus were <12 months of age. Third, the licensed tetravalent reassortant rotavirus vaccine, which includes rotaviruses of serotype G1–G4 specificity, should provide adequate coverage against the predominant circulating strains of rotavirus in this setting. Fourth, the observation that one-third of in-patients with rotavirus diarrhea had a mixed infection suggests that rotavirus vaccines may work less well in Peru than in developed countries where mixed infections are less common.

An astrovirus was first described in a child with diarrhea in 1975 [40]. Until recently, the epidemiology of astrovirus diarrhea was not well understood because the only available detection method, electron microscopy, was relatively insensitive and only available in a few centers. With the development of molecular assays, astrovirus has been recognized as a common cause of diarrhea in children [41]. However, limited data is

available on the role of astroviruses in the etiology of severe dehydrating diarrhea. In our study, astroviruses were detected in 4% of in-patients but were equally prevalent in out-patients. This observation indicates that while astroviruses can cause severe diarrhea, disease caused by this pathogen is not of above-average severity.

Children who were not exclusively breast-fed in the first 6 months of life were at an increased risk of severe diarrhea. This protective effect was observed even beyond infancy, the age by which weaning would have been initiated for most children. It is possible that children who were exclusively breast-fed in early infancy might have been partially breast-fed until an older age than those who were not exclusively breast-fed, and the lower risk of severe diarrhea in the former group might be due to the anti-infective properties of breast milk [42, 43]. It can also be hypothesized that mothers of children who were exclusively breast-fed might be better educated in appropriate child-rearing practices and may take greater precautions to prevent a diarrheal illness and its severe consequences. This hypothesis is supported by the observation that lack of exclusive breast-feeding and lack of a source of piped water in the home had a synergistic effect with regard to the risk of severe diarrhea.

Several potential limitations should be considered in interpretation of the findings of this study. While we controlled for several potential confounders by matching and by performing

multivariate analysis, it is possible that unmeasured factors that may predispose children to severe diarrhea and that may be unequally distributed in the study groups may have affected the association between severe diarrhea and diet, nutritional status, and availability of a water supply. Bias may have been introduced from misclassification of either disease or exposure. To avoid misclassification of the status of hydration and malnutrition, each child was examined by a trained pediatrician or nurse, who used prespecified objective criteria to assess these characteristics. To avoid bias in the reporting of results, the laboratory personnel who tested stools for enteropathogens were blinded to the child's disease status.

In conclusion, we have documented that for a 2-year period at a hospital in Lima, rotavirus was the most common pathogen detected among children with severe diarrhea. Fifty-two percent of children admitted with severe diarrhea were infected with rotavirus, and this pathogen was significantly more prevalent among in-patients than out-patients. In addition, we found that lack of exclusive breast-feeding in early infancy and lack of a source of piped water in the home had a synergistic effect on the risk of severe disease in children with diarrhea. Our findings not only underscore the need for the introduction of rotavirus vaccines in Peru, but indicate that interventions to educate mothers and improve access to safe water should augment the protective effect of vaccines in reducing the incidence of severe childhood diarrhea.

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#### References

- Bern C, Martines J, de Zoysa I, Glass RI. The magnitude of the global problem of diarrhoeal disease: a ten year update. *Bull World Health Organ* **1992**; 70:705-14.
- Murray CJL, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. *Lancet* **1997**; 349:1269-76.
- Ryder RW, Reeves WC, Sack RB. Risk factors for fatal childhood diarrhoea: a case-control study from two remote Panamanian islands. *Am J Epidemiol* **1985**; 121:605-10.
- Griffin PM, Ryan CA, Nyaphisi M, Hargretl-Bean N, Waldman RJ, Blake PA. Risk factors for fatal diarrhoea: a case-control study of African children. *Am J Epidemiol* **1988**; 128:1322-9.
- Teka T, Faruque AS, Fuchs GJ. Risk factors for deaths in under-age-five children attending a diarrhoea treatment center. *Acta Paediatr* **1996**; 85: 1070-5.
- Islam SS, Khan MU. Risk factors for diarrhoeal deaths: a case-control study at a diarrhoeal disease hospital in Bangladesh. *Int J Epidemiol* **1986**; 15: 116-21.
- Lindtjorn B. Risk factors for fatal diarrhoea: a case-control study of Ethiopian children. *Scand J Infect Dis* **1991**; 23:207-11.
- Bhattacharya SK, Bhattacharya MK, Manna, et al. Risk factors for development of dehydration in young children with acute watery diarrhoea: a case-control study. *Acta Paediatr* **1995**; 84:160-4.
- Fuchs SC, Victora CG, Martines J. Case-control study of risk of dehydrating diarrhoea in infants in vulnerable period after full weaning. *BMJ* **1996**; 313:391-4.
- Teka T, Faruque AS, Fuchs GJ. Risk factors for deaths in under-age-five children attending a diarrhoea treatment center. *Acta Paediatr* **1996**; 85: 1070-5.
- Faruque AS, Mahalanabis D, Islam A, Hoque SS, Hasnat A. Breast feeding and oral rehydration at home during diarrhoea to prevent dehydration. *Arch Dis Child* **1992**; 67:1027-9.
- Faruque AS, Mahalanabis D, Islam A, Hoque SS, Hasnat A. Common diarrhea pathogens and the risk of dehydration in young children with acute watery diarrhea: a case-control study. *Am J Trop Med Hyg* **1993**; 49:93-100.
- Victora CG, Fuchs SC, Kirkwood BR, Lombardi C, Barros FC. Low body weight: a simple indicator of the risk of dehydration among children with diarrhoea. *J Diarrhoeal Dis Res* **1997**; 15:7-11.
- Sabchareon A, Chongsuphajaisiddhi T, Butraporn P, et al. Maternal practices and risk factors for dehydration from diarrhoea in young children: a case-control study in central Thailand slums. *J Diarrhoeal Dis Res* **1992**; 10: 221-6.
- Victora CG, Fuchs SC, Kirkwood BR, Lombardi C, Barros FC. Breast-feeding, nutritional status, and other prognostic factors for dehydration among young children with diarrhoea in Brazil. *Bull World Health Organ* **1992**; 70:467-75.
- World Health Organization. A manual for the treatment of diarrhoea. WHO/CDD/SER/80.2 Rev 2. Geneva: World Health Organization, **1990**.
- Duggan C, Santosham M, Glass RI. The management of acute diarrhea in children: oral rehydration, maintenance, and nutritional therapy. *MMWR Morb Mortal Wkly Rep* **1992**; 41:1-20.
- Unicomb LE, Bingnan F, Rahim, et al. A one-year survey of rotavirus strains from three locations in Bangladesh. *Arch Virol* **1993**; 132:201-8.
- Moe CL, Allen JR, Monroe SS, et al. Detection of astrovirus in pediatric stool samples by immunoassay and RNA probe. *J Clin Microbiol* **1991**; 29:2390-5.
- Lew JF, Moe CL, Monroe SS, et al. Astrovirus and adenovirus associated with diarrhea in children in day care settings. *J Infect Dis* **1991**; 164:673-8.
- Lennette EH, Balows A, Hausler WJ Jr, Shadomy HJ, eds. Manual of clinical microbiology. 4th ed. New York: Elsevier, **1996**.
- Ortega Y, Sterling CR, Gilman RH, Cama VA, Diaz F. *Cyclospora* species—a new protozoan pathogen of humans. *N Engl J Med* **1993**; 328:1308-12.
- Garcia LS, Bruckner DA. Diagnostic medical parasitology. 2nd ed. Washington, DC: American Society for Microbiology, **1993**:528-32.
- Woods PA, Gentsch JR, Gouvea V, et al. Distribution of serotypes of human rotavirus in different populations. *J Clin Microbiol* **1992**; 30:781-5.
- Taniguchi K, Urusawa T, Morita Y, et al. Direct serotyping of human rotavirus in stools by an enzyme-linked immunosorbent assay using serotype 1-, 2-, 3-, and 4-specific monoclonal antibodies to VP7. *J Infect Dis* **1987**; 155:1159-66.
- Gouvea V, Glass RI, Woods P, et al. Polymerase chain reaction amplification and typing of rotavirus nucleic acids from stool specimens. *J Clin Microbiol* **1990**; 28:276-82.
- World Health Organization. Physical status: the use and interpretation of anthropometry. Geneva: World Health Organization, **1995**.
- Breslow NE, Day NE. Statistical methods in cancer research. Vol 1. The analysis of case-control studies. Lyon, France: International Agency for Research on Cancer, **1980**. (IARC scientific publication no. 32).
- Kleinbaum DG, Kupper LL, Muller KE, eds. Applied regression analysis and other multivariable methods. Boston: PWS-KENT Publishing, **1988**.
- Hosmer DW Jr, Lemeshow SL, eds. Applied logistic regression. New York: John Wiley, **1989**.

31. Brown KH, Gastanaduy AS, Saavedra JM, et al. Effect of continued oral feeding on clinical and nutritional outcomes of acute diarrhea in children. *J Pediatr* **1988**;112:191–200.
32. Lanata CF, Black RE, Mourtua D, et al. Etiologic agents in acute vs. persistent diarrhea in children under three years of age in peri-urban Lima, Peru. *Acta Paediatr Suppl* **1992**;381:32–8.
33. Pazzaglia G, Sack RB, Salazar E, et al. High frequency of coinfecting enteropathogens in *Aeromonas*-associated diarrhea of hospitalized Peruvian infants. *J Clin Microbiol* **1991**;29:1151–6.
34. Greenberg BL, Sack RB, Salazar-Lindo E, et al. Measles-associated diarrhea in hospitalized children in Lima, Peru: pathogenic agents and impact on growth. *J Infect Dis* **1991**;163:495–502.
35. Figueroa-Quintanilla D, Salazar-Lindo E, Sack RB, et al. A controlled trial of bismuth subsalicylate in infants with acute watery diarrheal disease. *N Engl J Med* **1993**;328:1653–8.
36. de Zoysa I, Feachem RV. Interventions for the control of diarrhoeal diseases among young children: rotavirus and cholera immunization. *Bull World Health Organ* **1985**;63:569–83.
37. Black RE, Merson MH, Huq I, Alim ARMA, Yunus M. Incidence and severity of rotavirus and *Escherichia coli* diarrhea in rural Bangladesh: implications for vaccine development. *Lancet* **1981**;1:141–3.
38. Wyatt RG, Yolken RH, Urrutia JJ, et al. Diarrhea associated with rotavirus in rural Guatemala: a longitudinal study of 24 infants and young children. *Am J Trop Med Hyg* **1979**;28:325–8.
39. Unicomb LE, Kilgore PE, Faruque AS, et al. Anticipating rotavirus vaccines: hospital-based surveillance for rotavirus diarrhea and estimates of disease burden in Bangladesh. *Pediatr Infect Dis J* **1997**;16:947–51.
40. Appleton H, Higgins PG. Viruses and gastroenteritis in infants. *Lancet* **1975**;1:1297.
41. Glass RI, Noel J, Mitchell D, et al. The changing epidemiology of astrovirus-associated gastroenteritis: a review. *Arch Virol Suppl* **1996**;12:287–300.
42. Newburg DS, Peterson JA, Ruiz-Palacios GM, et al. Role of human-milk lactadherin in protection against symptomatic rotavirus infection. *Lancet* **1998**;351:1160–4.
43. Torres O, Cruz JR. Protection against *Campylobacter* diarrhea: role of milk IgA antibodies against bacterial surface antigens. *Acta Paediatr* **1993**;82:835–8.