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Astrovirus-Associated Diarrhea among Guatemalan Ambulatory Rural Children

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Fecal excretion of astroviruses was monitored in 321 children, 0 to 3 years old, living in the rural highlands of Guatemala. During the longitudinal study, from February 1987 to February 1989, we examined 5,000 stool specimens, including 1,805 collected during 1,369 episodes of diarrhea, 830 collected during the convalescent week, and 216 and 244 collected 2 weeks and 1 week, respectively, before the onset of diarrhea. Routine specimens were taken once a month from every child who had been free from diarrhea for at least three consecutive weeks. Of the children, 124 (38.6%) excreted astroviruses during the study. In total, we identified 184 infections by astroviruses. Of the samples collected 2 weeks and 1 week before the initiation of symptoms, 0.9 and 4.9%, respectively, were positive, while 7.3% of the diarrhea episodes were associated with astroviruses. Of the convalescent specimens, 3.4% were shown to be positive; 2.4% of the 1,905 specimens taken in diarrhea-free periods contained astroviruses. Infections by other potential enteropathogens were documented in 54 and 65% of the asymptomatic and symptomatic astrovirus infections, respectively. Diarrhea associated with astroviruses alone had a median duration of 5 days and was associated with vomiting in 8.6%, with fever in 17.1%, with dehydration in 5.7%, and with loss of appetite in 34.3% of the episodes. Diarrhea due to astroviruses was accompanied by negative changes in weight gain. Astrovirus diarrhea contributes to the high morbidity observed in young children living under poor conditions and has a deleterious effect on their nutritional status.

Astroviruses were first associated with human gastrointestinal disease in 1975 when Appleton and Higgins (1) and Madeley and Cosgrove (13) reported visualizing, with the aid of electron microscopy, 28-nm star-shaped particles in fecal samples obtained from young children with mild diarrhea and vomiting. Because astroviruses can not be readily isolated in cell cultures, later studies (2, 14) also used electron microscopy to detect the agents in stools from small numbers of hospitalized patients, especially infants. It soon became apparent that healthy children may shed astroviruses and, when diarrhea occurs, other potential pathogens may be excreted in feces. Kjeldsberg (11) was able to show that astroviruses were present in 4.2% of stool specimens taken from gastroenteritis patients in Norway; the age of the infected individuals was not reported, and asymptomatic subjects were not studied. A larger number, 1,751, of young children with diarrhea were studied in Canada in 1975 to 1976, and only 19 (1.1%) were shown to be excreting astroviruses (15).

The mild disease regularly associated with astroviruses, the common occurrence of asymptomatic infections (1, 2, 14), and the requirement of electron microscopy for the laboratory diagnosis made it difficult to clearly implicate astroviruses as a cause of gastroenteritis. The development of an enzyme-linked immunosorbent assay (ELISA) based on monoclonal antibodies (8) provided a more appropriate tool for the analysis of larger number of stool samples. Using the ELISA, Herrmann and colleagues (9) showed that, although 2% of healthy children excrete the agents, astroviruses are a common cause of gastroenteritis in Thai children under the age of 5 years attending outpatient clinics. Here, we report the observations regarding astrovirus infections and diarrhea among rural ambulatory children under 3 years of age, living in a rural community of Guatemala.

MATERIALS AND METHODS

The study was conducted in Santa María de Jesús, a rural village in the highlands of Guatemala, where hygienic conditions are poor (4). After informed consent was given by the parents, 321 children 0 to 30 months old, each from a different family, were enrolled in the study, which began in February 1987 and ended in February 1989. The children were kept under surveillance either until they reached their third birthday or for the duration of the study. One hundred and sixty-five (51.4%) of the children were males, and 156 (48.6%) were females. Among study families, 289 (90.6%) collected their water from public spigots and 160 (49.8%) defecated on the ground because they lacked access to latrines. Among this population, the incidence of diarrhea in children 0 to 3 years old is 7.6 episodes per child per year (3).

Field personnel visited the homes of the participants twice a week to inquire about the presence of diarrhea and other morbidity among study children; weight and height of each child were measured routinely every month. When an episode of diarrhea was detected, the initial date of the illness was recorded and anthropometric measures were obtained; sick children were visited every other day until the episode was over (72 continuous hours without symptoms); at this time, the child's weight and height were obtained again. The presence of fever, vomiting, dehydration, loss of appetite,

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TABLE 1. Detection rate of astroviruses in fecal samples from diarrhea patients and during healthy periods

Source of sample	% Detection[a]		
	By gender		Total
	Male	Female	
Child with diarrhea	7.2 (724)	7.4 (645)	7.3 (1,369)
Healthy child	2.2 (930)	2.5 (975)	2.4 (1,905)

[a] Figures in parenthesis represent the number of diarrhea episodes and the number of samples from healthy children studied.

and tiredness was also monitored and recorded during the home visits.

For microbiological studies, fecal specimens were collected the day the episode was detected; if the episode lasted for more than 6 days, additional samples were taken weekly and during convalescence (7 days after the episode was over). Routine stools were obtained from each child once a month, if he or she had been free of gastroenteric symptomatology for at least three consecutive weeks.

Suspensions of fecal material were prepared at the community in buffered Formalin, polyvinyl alcohol, selenite broth, phosphate-buffered saline, Cary Blair medium, and veal infusion broth with 0.5% albumin and were handled as described previously (5) to identify *Salmonella* spp.; *Shigella* spp.; *Campylobacter jejuni*; enteropathogenic, enterotoxigenic, and HEp2-adherent *Escherichia coli*; *Plesiomonas shigelloides*; *Yersinia enterocolitica*; *Cryptosporidium* spp.; *Giardia* spp.; rotaviruses; and adenovirus types 40 and 41. The identification of astroviruses was done by means of the ELISA in the material in veal infusion broth (8). All astrovirus-positive specimens were retested for confirmation.

Nutritional status of the children was expressed as a Z score; this score represents the difference in standard deviations from the mean of the U.S. National Center for Health Statistics reference curve (10). Comparisons of rates and proportions were done according to Fleiss (7).

RESULTS

We studied 1,805 samples collected during 1,369 episodes of diarrhea among the 321 children; 830 additional samples were collected during the convalescent period, and 1,905 specimens were obtained during diarrhea-free times. Because of the routine microbiological surveillance, 216 and 244 stools were collected within 2 weeks and 1 week, respectively, before the onset of diarrhea. In total, the presence of astroviruses was determined in 5,000 fecal samples. Of the 321 children, 124 (38.6%) excreted astrovirus during the study period. In total, we detected 184 infections by astroviruses. Seventy-seven children had one infection, 34 had two infections, and 13 had three infections during their follow-up. The fecal excretion of astroviruses was more common, 7.3%, during diarrhea episodes than during symptom-free times, 2.4% ($\chi^2 = 24.85$, $P < 0.001$), with no differences in relation to gender (Table 1). Of the 45 asymptomatic infections, 15 were detected in children who had previously had an episode of diarrhea associated with astroviruses. The peak detection rate of astroviruses was observed in children 6 to 11 months old, among both sick and healthy individuals (Fig. 1). The highest monthly detection rate in sick children was observed in May, when the rainy season starts (Fig. 2); in general, the monthly detection rates of astroviruses among sick children paralleled the monthly incidence rates of diarrhea among the study population (Fig. 2). Astrovirus infections were more common and the illness-to-infection ratio tended to be higher during the peak months of diarrhea than in the remainder of the year (Fig. 2).

The detection rates of astroviruses in relation to the phase of the diarrhea episode are presented in Table 2. Astroviruses were more commonly shed during the days of illness than immediately before and after that period ($\chi^2 = 18.45$, $P = 0.01$).

Astrovirus shedding was accompanied by the excretion of other potential enteropathogens in 20 (54.4%) and 65 (65.0%) of asymptomatic and symptomatic infections, respectively (Table 3); the detection rates of astroviruses alone were 2.5% for sick children and 1.3% for healthy ones ($\chi^2 = 6.86$, $P = 0.009$). In every age group studied, astroviruses alone were detected more often among the ill (Fig. 1). In the instances where only astroviruses were detected during the diarrhea episodes, the duration of illness was shorter (mean = 6.5 days, median = 5 days) than when astroviruses and other potential pathogens were found (mean = 15.5 days, median = 9 days); furthermore, when only pathogens other than astroviruses were isolated, the duration of the illnesses

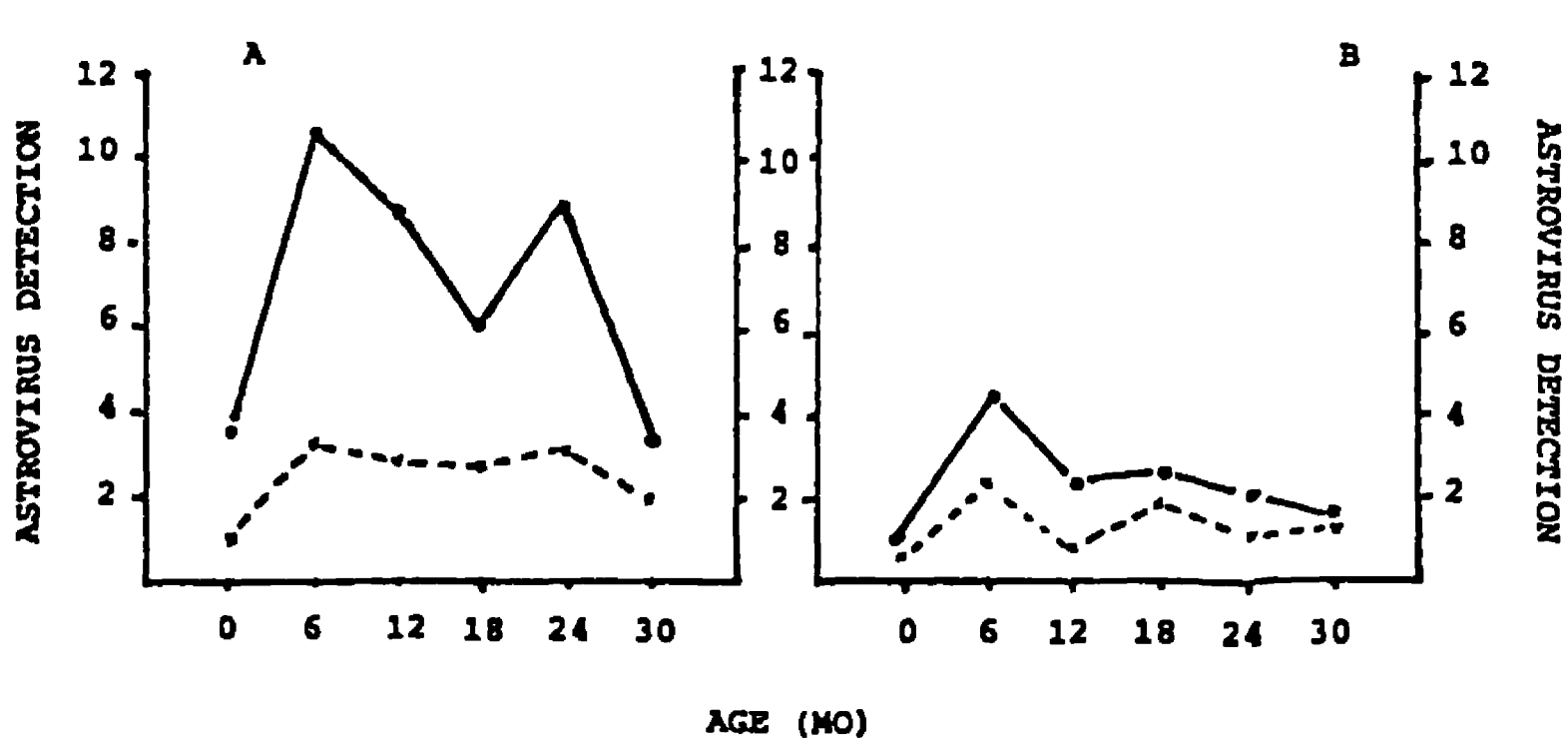

FIG. 1. Detection rate of astroviruses from diarrhea cases (——) and from healthy individuals (– – –), according to age (months). (A) All positive episodes; (B) astroviruses alone.

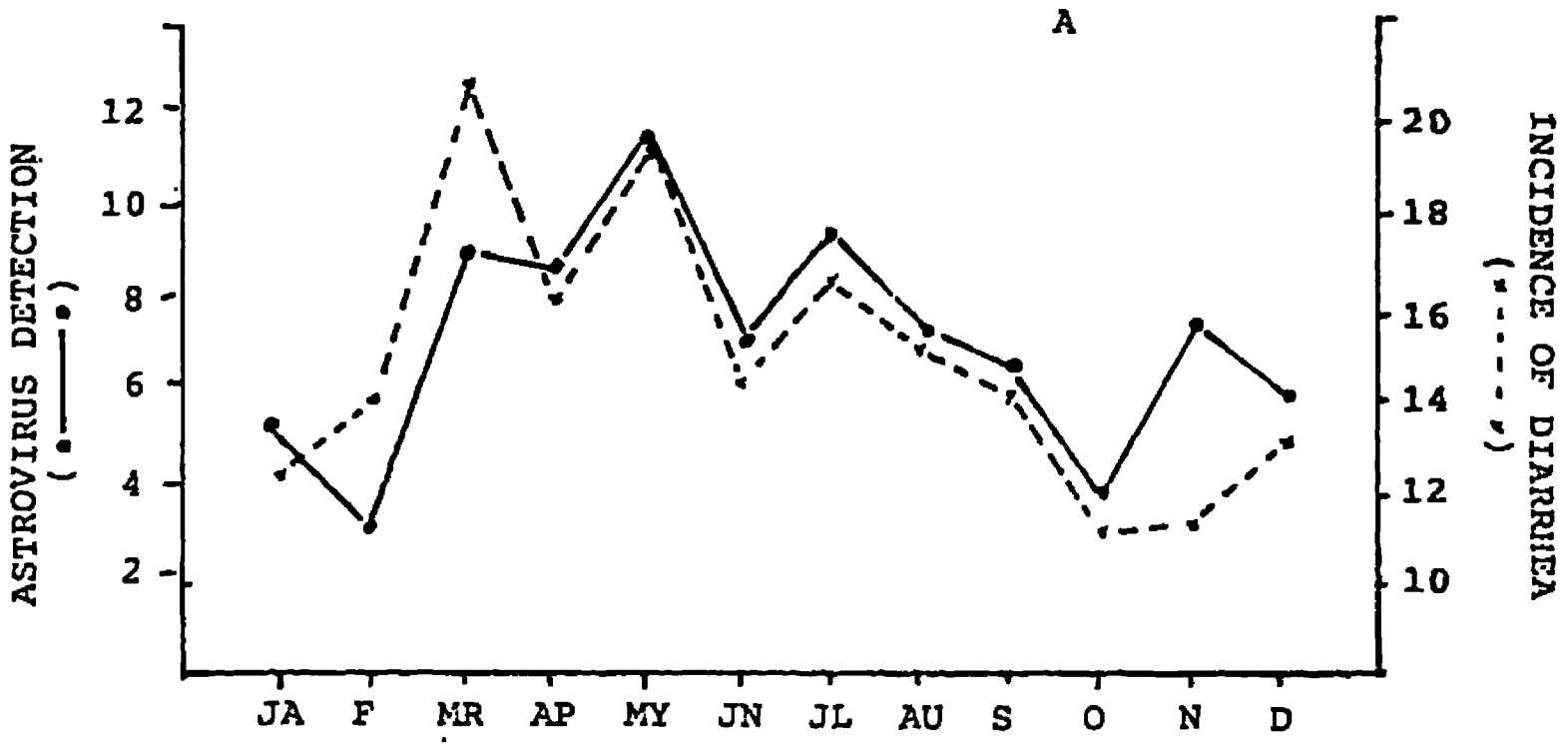

FIG. 2. Astrovirus detection according to month of the year. (A) Astrovirus-associated diarrhea and monthly incidence of diarrhea per 100 child weeks. (B) Number of symptomatic (■) and asymptomatic (□) infections.

was intermediate (mean = 11.0 days, median = 8 days; Table 4). The presence of fever, vomiting, dehydration, loss of appetite, and tiredness during the episode followed the same pattern (Table 4). Of the diarrheal episodes associated with astroviruses alone, 17.1% were accompanied by fever, 8.6% by vomiting, and 5.7% by dehydration; loss of appetite was reported in 34.3% and tiredness was reported in 20.0% of the episodes (Table 4).

TABLE 2. Detection of astroviruses in relation to phase of the diarrhea episode[a]

Time sample was obtained	No. of samples tested	No. (%) of positive samples
2 wk before onset	216	2 (0.9)
1 wk before onset	244	12 (4.9)
Phase of episode (days)		
1–3	976	50 (5.1)
4–7	391	28 (7.2)
8–13	250	14 (5.6)
14–21	131	6 (4.6)
≥22	57	5 (8.8)
Convalescence	830	28 (3.4)

[a] $\chi^2_{DF} = 18.45$; $P = 0.01$ for all samples tested.

The detection rates of astroviruses were not affected by nutritional status, be it expressed as adequacy of weight for age, height for age, or weight for height (Table 5). Diarrheal diseases of known etiology had a negative effect on nutritional status, as shown by the changes in weight and in adequacy of weight for age and of weight for height during the disease (Table 4). Astroviruses in combination with other potential enteric pathogens had the major impact.

DISCUSSION

The results presented here provide further evidence that astroviruses cause diarrhea in young children. The detection rate of the agents during episodes of diarrhea was 7.3%, while only 2.4% of the specimens collected from healthy individuals were positive. These figures, which are statisti-

TABLE 3. Mixed infections in astrovirus-positive cases

Parameter	Value for sample type	
	Diarrhea (n = 65)	Asymptomatic (n = 20)
Age (mo)		
Median	12	17
Mean ± SD	14.4 ± 7.5	18.9 ± 10.0
Coinfections with (n):		
Shigella sp.	9	0
Salmonella sp.	3	0
Enterotoxigenic *E. coli*		
Heat-labile toxin	10	0
Heat-stable toxin	6	2
Heat-labile heat-stable toxin	7	1
Enteroadherent *E. coli*		
Diffuse pattern	10	4
Localized pattern	3	1
Autoaggregative	4	1
Enteropathogenic *E. coli*	3	3
Campylobacter jejuni	16	3
Cryptosporidium sp.	5	1
Giardia sp.	7	7
Rotavirus	14	1
Adenovirus 40, 41	2	0
Two agents	42	16
Three agents	17	4
Four agents	3	0
Five agents	1	0
Six agents	2	0

cally different from each other, are almost identical to those reported for children under the age of 5 years from Thailand (9). Our longitudinal approach, with sample collection from healthy individuals, allowed us to examine the excretion of astroviruses shortly before the onset of diarrhea in 460 instances; less than 1% of the specimens taken 8 to 14 days before initiation of symptoms were positive, as were 4.9% of those taken within a week prior to illness, indicating that these latter infections were documented while in the incubation period. After a higher rate of positive findings in the days of diarrhea, the positive specimens obtained in the convalescent period decreased to 3.4%, again providing stronger evidence that astroviruses cause gastroenteritis.

TABLE 4. Clinical characteristics of the diarrhea episodes associated with astroviruses

Parameter	Value for[a]:		
	Astroviruses alone (n = 35)	Astroviruses + other pathogen(s) (n = 65)	Other pathogens (n = 715)
Duration (days)			
Median	5	9	8
Range	1–35	2–73	1–229
Cases with:			
Vomiting	8.6%	38.5%	21.7%
Fever	17.1%	30.8%	27.6%
Dehydration	5.7%	15.4%	11.7%
Loss of appetite	34.3%	69.2%	44.2%
Tiredness	20.0%	46.2%	27.6%
Change in wt (g)	0.100	−0.008	0.080
Change in ZWA[b]	−0.118	−0.235	−0.132
Change in ZWH[b]	−0.042	−0.217	−0.109

[a] P < 0.001 for all values.
[b] ZWA = Z score, weight for age; ZWH = Z score, weight for height.

TABLE 5. Astrovirus-associated diarrhea, according to nutritional status (Z score)

Z parameter	% (no.) of cases with nutritional status[a]	
	Adequate	Deficient
Weight for age	6.0 (547)	8.0 (650)
Height for age	5.5 (311)	7.7 (817)
Weight for height	6.7 (876)	8.9 (245)

[a] P values were not significantly different.

Furthermore, the pattern of astrovirus detection along the year correlated with the monthly incidence rate of diarrhea in the study population, suggesting that astroviruses indeed contribute to the diarrheal morbidity in the community.

In this ambulatory population, the astrovirus infection rate (38.6%) is higher than those of adenoviruses types 40 and 41 (22.3%) and of rotaviruses (10.3%) (4). The active surveillance of diarrhea by our field personnel contributed to the study of mild diseases, which seem to be common following astrovirus infections. Furthermore, the existence of five different serotypes of astroviruses (12) also permits repeated infections in the same individual. In Santa María de Jesús, 38% of the astrovirus excretors had more than one infection during the study period.

The disease that astroviruses induced in our population was mild. In our study, the median duration of disease was 5 days; Esahli et al. (6) reported that the median duration of symptoms in Swedish children was 4 days; vomiting and fever, nevertheless, were seen in 54% of community-acquired infections. Herrmann and colleagues (9) found that 61% of the diarrhea episodes seen in Thai children were accompanied by vomiting and 80% were accompanied by fever. In our Guatemalan children, vomiting and elevated temperature were seen in only a small proportion of the episodes, 8.6 and 17.1%, respectively. Additionally, dehydration was noted in only 5.7% of the diarrhea episodes associated with astroviruses alone, again identical to the 5% reported for Thailand (9) but much lower to the 39% reported for Sweden (6).

Other reports (6, 9, 14) have shown that mixed infections with astroviruses are common. We found 54 and 65% of inapparent and symptomatic infections by astroviruses to be mixed. Poor hygienic conditions in the community would seem to favor multiple enteric infections. We have previously reported (4) that 50% of symptomatic infections by adenoviruses types 40 and 41 and 35% of rotavirus-associated diarrhea episodes are also accompanied by other enteric pathogens. In the astrovirus-mixed infections, the disease not only lasted longer but also was more severe than when only astroviruses or the other pathogens were documented (Table 4). The duration and the severity of the diarrheal illnesses played an important role in the detrimental nutritional impact of the episode.

Our data provide further evidence that astroviruses are commonly associated with gastroenteritis among young children living in poor areas of the world, among whom the viruses induce a generally mild disease. Astroviruses, however, may contribute to increase the severity of illnesses caused by other enteropathogens, with important nutritional consequences for the child. Only interventions aimed at improving the hygienic conditions under which large segments of the world population currently live will have an

impact on diarrheal morbidity and, therefore, on the general well-being of the young.

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REFERENCES

1. **Appleton, H., and P. G. Higgins.** 1975. Viruses and gastroenteritis in infants. Lancet i:1297.
2. **Ashley, C. R., E. O. Caul, and W. K. Paver.** 1978. Astrovirus-associated gastroenteritis in children. J. Clin. Pathol. 31:939–943.
3. **Cruz, J. R., A. V. Bartlett, H. Méndez, and R. Sibrián.** Epidemiology of persistent diarrhea among Guatemalan rural children. Acta Paediatr. Scand., in press.
4. **Cruz, J. R., P. Cáceres, F. Cano, J. Flores, A. Bartlett, and B. Torún.** 1990. Adenovirus types 40 and 41 and rotaviruses associated with diarrhea in children from Guatemala. J. Clin. Microbiol. 28:1780–1784.
5. **Cruz, J. R., F. Cano, P. Cáceres, F. Chew, and G. Pareja.** 1988. Infection and diarrhea caused by *Cryptosporidium* sp. among Guatemalan infants. J. Clin. Microbiol. 26:88–91.
6. **Esahli, H., K. Breback, R. Bennet, A. Ehrnst, M. Eriksson, and K. Hedlund.** 1991. Astroviruses as a cause of nosocomial outbreaks on infant diarrhea. Pediatr. Infect. Dis. J. 10:511–515.
7. **Fleiss, J.** 1973. Statistical methods for rates and proportions. John Wiley & Sons, Inc., New York.
8. **Herrmann, J. E., N. A. Nowak, D. M. Perron-Henry, R. W. Hudson, W. D. Cubitt, and N. R. Blackow.** 1989. Diagnosis of astrovirus gastroenteritis by antigen detection with monoclonal antibodies. J. Infect. Dis. 161:226–229.
9. **Herrmann, J. E., D. N. Taylor, P. Echeverría, and N. R. Blacklow.** 1991. Astroviruses as a cause of gastroenteritis in children. N. Engl. J. Med. 324:1757–1760.
10. **Jordan, M. D.** 1986. Anthropometric software package tutorial guide and handbook. Centers for Disease Control, Atlanta.
11. **Kjeldsberg, E.** 1977. Small spherical viruses in faeces from gastroenteritis patients. Acta Pathol. Microbiol. Scand. Sect. B 85:351–354.
12. **Kurtz, J. B., and T. W. Lee.** 1984. Human astrovirus serotypes. Lancet ii:1405.
13. **Madeley, C. R., and B. P. Cosgrove.** 1975. Viruses in infantile gastroenteritis. Lancet ii:124.
14. **Madeley, C. R., B. P. Cosgrove, E. J. Bell, and R. J. Fallon.** 1977. Stool viruses in babies in Glasgow. J. Hyg. Camb. 78:261–273.
15. **Middleton, P. J., M. T. Szymanski, and M. Petric.** 1977. Viruses associated with acute gastroenteritis in young children. Am. J. Dis. Child. 131:733–737.