

# National Laboratory Surveillance of Viral Agents of Gastroenteritis in Brazil<sup>1,2</sup>

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*In 1984 an enzyme immunoassay for rotavirus and adenovirus developed in the Virology Department of the Oswaldo Cruz Foundation was distributed to laboratories in 14 Brazilian states as part of a project to survey viral agents in fecal specimens from children with diarrhea. The ensuing surveillance continued for 3 to 5 years, and in 1991 the results obtained by all laboratories that tested 300 or more fecal specimens were reviewed at a workshop held in Belém. These results showed that rotavirus had been detected in 13% to 20% of the specimens tested, with positive specimens appearing to peak in the May to September "winter" or dry season in Brazil's central and southern states but not in the more tropical northern areas. Adenovirus, detected in 0.7% to 5.5% of the specimens tested for it, showed no seasonal variations. Many other viral agents known to cause gastroenteritis (e.g., astrovirus, small round-structured viruses, calicivirus, and group C rotavirus) were detected at centers that used electron microscopy. Picobirnavirus, a novel agent not yet associated with gastroenteritis in humans, was found by polyacrylamide gel electrophoresis in specimens from a number of centers using this technique. Vaccines to prevent rotavirus that are currently under development would be of great use in Brazil, where rotavirus is the most common cause of childhood diarrhea. Improved diagnostics will be required to assess the importance of the other viral agents.*

**A**cute gastroenteritis is a major cause of human morbidity throughout the world and of infant mortality in developing countries (1). Over the past two decades, a number of viruses have been

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<sup>1</sup>Report of a workshop held by the Pan American Health Organization in conjunction with the Annual Meeting of The Brazilian Society of Virology in Belém, Pará, Brazil on 16 November 1991. Correspondence should be addressed to Dr. Roger Glass, Viral Gastroenteritis Section (G04), Centers for Disease Control and Prevention, 1600 Clifton Rd. NE, Atlanta, Georgia 30333, USA (tel. 404-639-3577, fax 404-639-3645). This article will also be published in Spanish in the *Boletín de la Oficina Sanitaria Panamericana*, vol. 115, no. 5, 1993.

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identified that cause diarrhea in children. Of particular importance are the group A rotaviruses, which are estimated to cause about 870 000 diarrheal deaths each year in developing countries (2, 3). Second in importance are the enteric adenoviruses, which have been associated with 7% to 17% of diarrhea cases in children (3). Other viruses associated with gastroenteritis include the astroviruses; caliciviruses; non-group A rotaviruses; and a number of small round-structured viruses, the prototype of which is the Norwalk virus (4-6).

Gastroenteritis viruses were first detected by electron microscopy, and their discovery has been followed by the development of more accessible assays for virus-specific antigens or nucleic acids and for the corresponding antibodies. Application of these assays to diagnostic and epidemiologic studies has given rise to abundant information establishing the importance of at least some of these viruses as causes of diarrhea.

Although acute diarrhea can be effectively treated with oral rehydration, most diarrheal illnesses are difficult to prevent. Thus, at least for diarrhea caused by rotavirus, vaccines appear to offer the most likely avenue of prevention (7). A number of candidate rotavirus vaccines are currently under field investigation. Inconsistent reports of their efficacy may be due to variable epidemiologic conditions prevailing in different regions. Epidemiologic information is particularly scarce in developing countries, where these vaccines could have their greatest impact.

The present study was undertaken with the aim of (a) establishing the capability to diagnose viral gastroenteritis in Brazilian public health and hospital laboratories, (b) searching for the occurrence of different viruses in children with diarrhea, and (c) examining in pilot fashion the prevalence, seasonality, and distri-

bution of rotaviruses and enteric adenoviruses in different parts of Brazil.

## MATERIALS AND METHODS

### Enzyme Immunoassays for Rotavirus and Adenovirus

During the period 1981-1983, assays for rotavirus and adenovirus were developed and tested at the Oswaldo Cruz Foundation (FIOCRUZ). The present study was started in 1984 with the participation of 25 public health and university laboratories in 14 states of Brazil. All participants were trained in the use of enzyme immunoassays (EIA), polyacrylamide gel electrophoresis (PAGE) with silver staining, and electron microscopy for the diagnosis of viral gastroenteritis and were supplied with kits for combined group A rotavirus and adenovirus enzyme immunoassays (EIARA) and also with equipment and reagents for PAGE (8). The EIARA and PAGE assays were used routinely in most laboratories; electron microscopy was used in a few laboratories, either to confirm results or to detect viruses other than rotaviruses and adenoviruses. Besides the EIARA kits, some laboratories also used rotavirus EIA kits from commercial or World Health Organization sources.

### Surveillance

The patient populations consisted mainly of children under 5 years of age with acute gastroenteritis who were seen at hospitals being covered by the collaborator in each region. No single set of criteria was used for screening by these centers, aside from the fact that each child had diarrhea when seen at the hospital. Hence, this was a convenience sample

procured for surveillance purposes rather than a proper random sample.

Between 1984 and 1990 many of the participating investigators presented short reports of their surveillance results at regional scientific meetings or published those results in regional journals. Consequently, much information about this project was presented, even though the national experience had never been reviewed.

In order to develop an overall evaluation of the results, participants were asked to submit reports summarizing information for the period when they conducted surveillance—including the number of samples tested per month, the diagnostic methods employed, the results obtained, and when possible the age distributions and socioeconomic levels of the patients studied. In this vein, reports by 18 workers at 15 laboratories in nine states and the Federal District (Brasília) were presented at a meeting held in Belém, Pará, Brazil in November 1991 (see footnote 2 on p. 224).

## RESULTS

### Rotavirus and Adenovirus Prevalences

While 25 laboratories received the assays, many did not pursue screening for a complete year, and some centers recruited small numbers of patients. We therefore elected to limit the analysis to the nine centers that had examined 300 or more samples over periods of at least one year (see Table 1 and Figure 1).

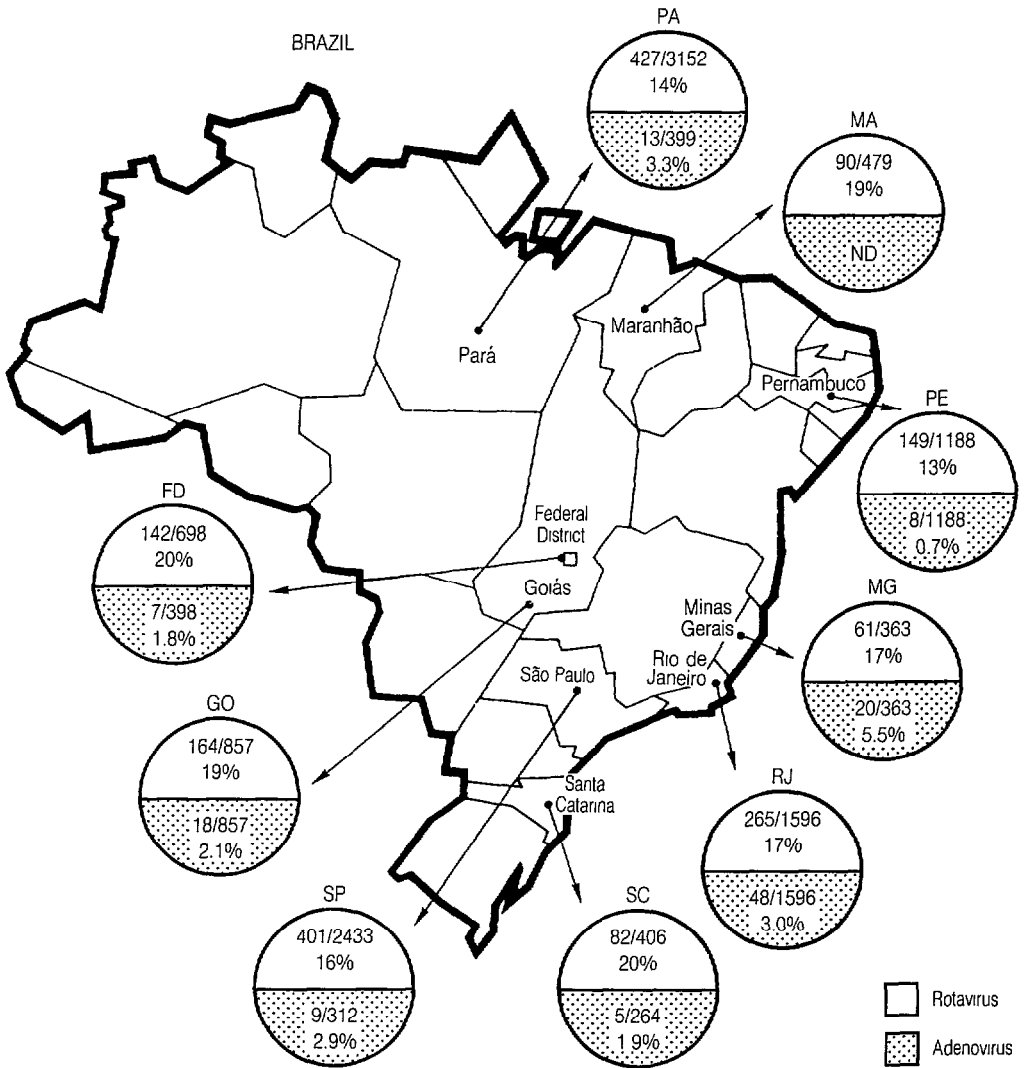
Rotavirus was the agent detected most frequently in the 11 772 specimens tested, being found in 1 781 (16%, with a range of 13% to 20%). Adenovirus was detected in 128 of 5 377 specimens tested (2.4%, with a range of 0.7% to 5.5%). No obvious trend was observed between the rate of detection of either virus and climatic conditions, which ranged from equatorial to subtropical. In a limited study (unpublished) in Rio de Janeiro, rotaviruses, but not adenoviruses, were detected significantly more often in chil-

**Table 1.** Rates of rotavirus and adenovirus detection at laboratories in eight Brazilian states and the Federal District.

State or district	Years	Samples tested for rotavirus		Samples tested for adenovirus	
		No. tested	Positive No. (%)	No. tested	Positive No. (%)
Federal District	1986–1990	698	142 (20)	398	7 (1.8)
Goiás	1986–1990	857	164 (19)	857	18 (2.1)
Maranhão	1986–1988	479	90 (19)	ND	— (—)
Minas Gerais	1986–1990	363	61 (17)	363	20 (5.5)
Pará	1981–1990 <sup>a</sup>	3 152	427 (14)	399	13 (3.3)
Pernambuco	1982–1990	1 188	149 (13)	1 188	8 (0.7)
Rio de Janeiro	1981–1984 <sup>a</sup>	1 596	265 (17)	1 596	48 (3.0)
Santa Catarina	1985–1989	406	82 (20)	264	5 (1.9)
São Paulo	1981–1988 <sup>a</sup>	2 433	401 (16)	312	9 (2.9)
Total (N)		11 772	1 781 (16)	5 377	128 (2.4)

<sup>a</sup>Collections began in the indicated areas before the main surveillance project began. However, data from these initial collections were included because the assays employed were the same as those used in the main project.

**Figure 1.** A map of Brazil showing the numbers of study samples tested for rotaviruses and adenoviruses, the numbers positive, and the percentages positive in eight states and the Federal District.

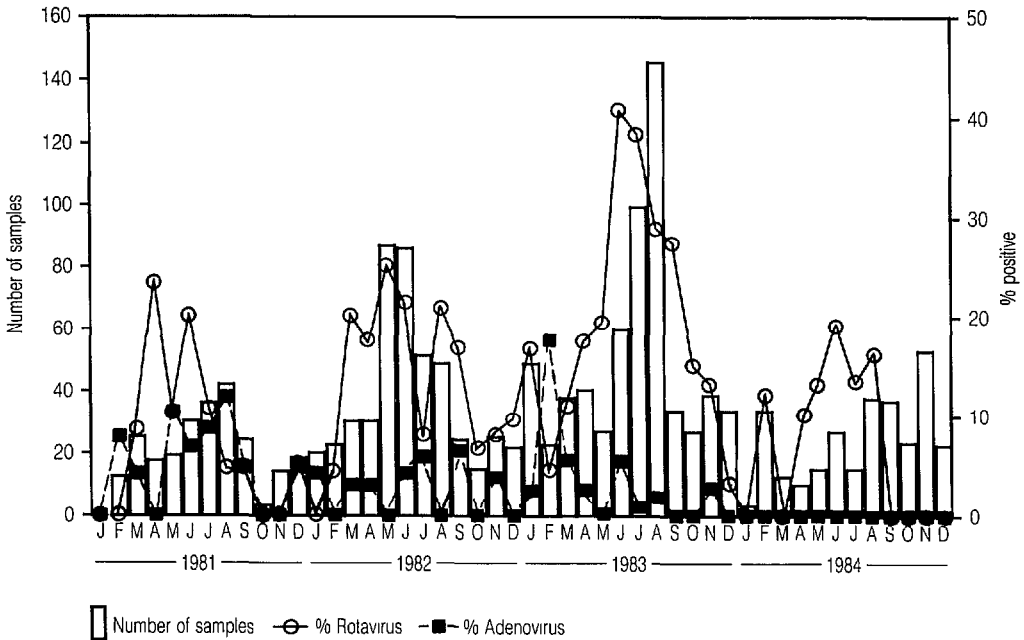


dren with acute gastroenteritis than in healthy controls. Studies in São Paulo (9) and Rio de Janeiro (unpublished) indicated that rotavirus was detected more frequently in stool specimens from pediatric gastroenteritis patients at private clinics than in stool specimens from pediatric patients at public clinics.

### Seasonal Distribution

The numbers of samples received for testing in Rio de Janeiro and the percentages positive for rotavirus over 3 consecutive years (1981–1983) both tended to peak in the winter months (Figure 2), while the percentages positive for aden-

**Figure 2.** A chart indicating the seasonality of rotaviruses in stool samples tested at the Oswaldo Cruz Foundation in Rio de Janeiro (1981–1984). The bars show the monthly numbers of stool samples tested while the lines show the percentages positive for rotaviruses and adenoviruses.



ovirus over the same period did not. These rotavirus peaks in the winter or dry season (May to September) were observed in the Federal District, Goiás, São Paulo, Santa Catarina, and Pernambuco, but were not observed in Minas Gerais, Maranhão, or Pará (Figure 3).

### Age Distribution

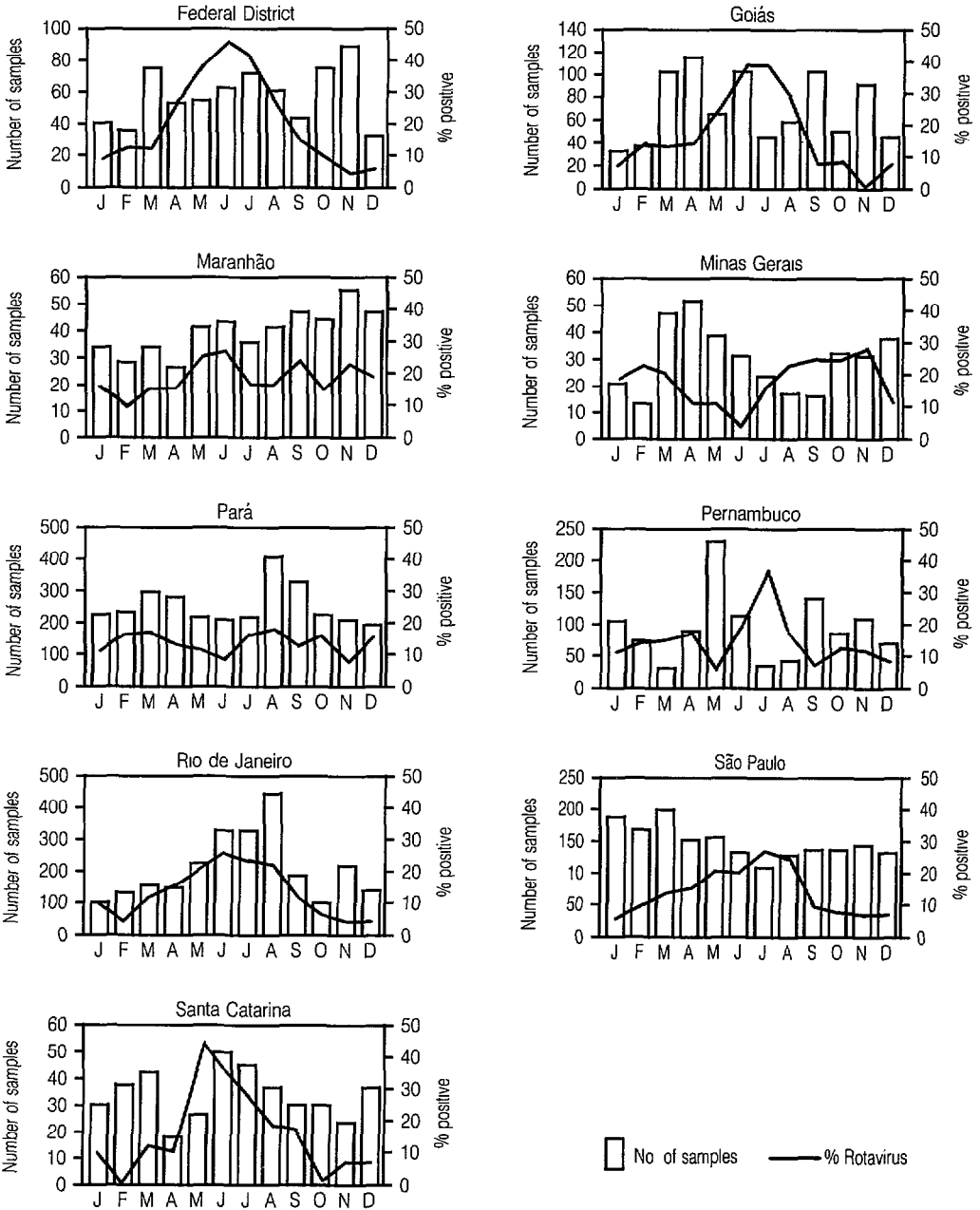
Several laboratories (in the Federal District, Maranhão, and São Paulo) that recorded their subjects' ages reported that the incidence of rotavirus was greatest in children 6 to 24 months old and was most likely to be pathogenic during the first 18 months of life. Well-defined rotavirus outbreaks involving adults as well as children were observed at a school in Rio de Janeiro (10) and among an indigenous population in Pará (11).

### Rotavirus Groups, Subgroups, and Serotypes

In addition to group A rotavirus detected by EIA and/or PAGE, group C rotavirus was occasionally detected by PAGE and/or electron microscopy in Rio de Janeiro (12), São Paulo (unpublished), and Santa Catarina (unpublished) (one positive sample each), as well as in Pará (13) (two positive samples).

A majority of the group A strains detected had long electrophoretic profiles indicative of subgroup II; but a small proportion of group A strains with short profiles were found in most states (14–17), and these findings were sometimes associated with well-defined outbreaks. In general, except among samples from localized outbreaks, electrophoretic profiles showed considerable heterogeneity.

**Figure 3.** Charts showing the numbers of stool samples tested for rotaviruses in nine states over the course of the study and the percentages yielding positive results, by month. Clear seasonal trends are evident in several states and the Federal District.



A number of rotavirus samples from three centers were serotyped (Table 2). Serotype 1 was the most common, followed in order of decreasing frequency by types 2, 4, and 3. One sample from Pará was considered to be a new serotype. Of the type 2 strains, one of six from São Paulo and four of nine from Pará had long electrophoretic profiles not usually associated with this serotype.

### Adenovirus Grouping and Serotyping

Adenovirus grouping and serotyping was reported only in Rio de Janeiro (18) and São Paulo (unpublished). Fourteen of 39 adenovirus strains isolated from children with diarrhea in Rio de Janeiro and six of eight strains from São Paulo grew fastidiously in tissue culture, reacted positively in an EIA specific for enteric adenovirus types 40 and 41, and were not neutralized by antisera to adenovirus types 1 through 31. The remaining 25 strains from Rio de Janeiro were not fastidious and were neutralized by antisera to adenovirus types 1 through 18; while the remaining two São Paulo strains were found to belong to adenovirus types 1 and 7.

### Other Viruses

Astroviruses, caliciviruses, coronavirus-like viruses, parvoviruses, and small round-structured viruses were detected by electron microscopy at a number of laboratories in Rio de Janeiro (19), São

Paulo (20), Pará, Pernambuco, and Maranhão (unpublished); but the number of samples tested was too small to estimate prevalences or seasonal distributions. The identity of the astroviruses detected in Rio de Janeiro, São Paulo, and Maranhão was confirmed by immune electron microscopy (IEM) or EIA performed at the Viral Gastroenteritis Section, United States Centers for Disease Control and Prevention (CDC), or at the Virus Reference Laboratory of the United Kingdom's Public Health Laboratory Service. Picobirnavirus was detected by PAGE in a small proportion (about 0.5%) of the samples tested in most of the laboratories where this technique was used extensively, but no conclusions can be reached regarding its association with disease.

### DISCUSSION AND CONCLUSIONS

It has been demonstrated that most groups of viruses previously described in association with diarrhea occur in several regions of Brazil. Rotaviruses, the most common agents of viral diarrhea and the easiest to detect, were found in 13% to 20% of the specimens tested. This finding is consistent with results from other studies in Brazil and elsewhere documenting the large disease burden associated with rotavirus (9–12, 15). Indeed, the prevention of rotavirus infection has become a recognized goal for diarrheal disease control, and development of a rotavirus vaccine has become a global priority (21). Adenoviruses were detected in 0.7% to

**Table 2.** Rotavirus serotypes detected at laboratories in three states.

State	Total	Serotype				
		1	2	3	4	Not typable
Pará	51	15	9	1	5	21
São Paulo	24	5	6	0	4	9
Maranhão	22	6	0	9	2	5
Total (%)	97 (100)	26 (27)	15 (15)	10 (10)	11 (11)	35 (36)

5.5% of the stool samples from children with diarrhea; however, no attempt was made to serotype these strains to determine the proportion of them associated with specific enteric serotypes. The number of samples tested for other viruses was too small to be meaningful.

This study involved the voluntary collaboration of many centers on a project of national interest. The results have some limitations in terms of patient selection and quality control of laboratory results. Each center had its own catchment area of patients and its own criteria for submitting specimens for diagnosis. While the detection rates obtained for rotaviruses and adenoviruses partly reflected patient age and disease severity, these rates are consistent with rates found by other hospital-based surveys in other countries (22).

Quality control efforts consisted mainly of providing hands-on laboratory-based instruction to all laboratories and providing confirmation testing for specimens yielding positive or ambiguous results. The procedures employed were both sensitive and specific, and minor variations in the results at any one center could only have marginally affected the ultimate interpretation. Of course, our intent was to assess the descriptive epidemiology of rotavirus and adenovirus in the sentinel centers rather than to arrive at precise prevalences for the nation. An important advantage of the approach employed is that laboratories from many parts of Brazil could be included and laboratory personnel could be motivated to extend their understanding of viral gastroenteritis agents as well as their repertoire of diagnostic tests.

The study findings indicate that the prevalence of rotavirus exhibited distinct peaks in the May to September "winter" or dry season in some central and southern areas of Brazil, but not in the country's northern tropical areas. These find-

ings are in agreement with an observation made by Cook et al (22) that rotavirus was more seasonal in temperate climates, but occurred year-round in the tropics. This pattern, the reason for which is unclear, is similar to the global pattern seen for measles.

In contrast, no seasonal variations were found in adenovirus prevalences. This finding is in agreement with reports from a number of countries reviewed by Wadell (3), while differing from the results of a South African study describing a mid-summer peak of enteric adenovirus infections (23).

A novel group of small viruses with bisegmented double-stranded RNA, for which the name picobirnavirus has been proposed, was detected in a small proportion of human stools (24). These viruses have also been found in several other species of vertebrates (25-28). Clearly, detection of these viruses demonstrates an unanticipated result of screening fecal specimens by PAGE, but their association with human disease has not been determined (24).

The combination of enzyme immunoassay for group A rotaviruses and adenoviruses (EIARA) (8) and PAGE with silver staining were the diagnostic methods routinely used in most laboratories. The PAGE assay, particularly adaptable for use in laboratories with moderate facilities, proved useful in studying rotavirus electrophoretic variation (14-16), detecting non-group A rotaviruses (12, 13), and discovering picobirnaviruses (24-28). The use of electron microscopy, while more restricted, was of value as the only method available in any of the participating laboratories for the detection of viruses other than those just mentioned. Continuation of the project with inclusion of new methods for viral diagnosis and further characterization of viruses associated with diarrhea is likely to provide valuable information required for under-



standing the epidemiologic patterns of this most important cause of infant morbidity and mortality in Brazil.

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## WHO Initiative on Cocaine

The multi-billion dollar cocaine trade became the world's fastest growing industry in the 1980s. Although North America remains the most lucrative market for the drug barons, there is mounting evidence that cocaine use is increasing in other parts of the world. In Latin America, traditional use of coca products is being replaced by "Western" style consumption.

Owing to concern over the rapidly changing patterns of cocaine use, in April 1993 the World Health Organization Program on Substance Abuse held three international meetings as part of a project, involving 24 centers in 20 countries, that aims to map the global extent and nature of the cocaine problem. The WHO Initiative on Cocaine Abuse will also spotlight the negative health and social consequences associated with the global spread of cocaine.

This initiative stems from the international seminar "Cocaine Today: Its Effects on the Individual and Society" held in Rome in 1991. WHO is implementing the project with the financial support of the United National Inter-regional Crime and Justice Research Institute (UNICRI).

Source: World Health Organization, Press Release WHO/24, 1 April 1993.