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Outbreak of acute gastroenteritis in young children with death due to rotavirus genotype G9 in Rio Branco, Brazilian Amazon region, 2005☆

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SUMMARY

Background: An epidemic of acute gastroenteritis occurred in Rio Branco City, Acre State, in Brazil's Amazon region in 2005. An investigation was conducted to confirm the etiology and identify possible risk factors for death.
Methods: Rio Branco municipality surveillance data for the period May to October 2005 were reviewed. In a case–control study, children who died following acute gastroenteritis were compared to age-matched controls with acute gastroenteritis who survived. Rotavirus A (RV-A) was investigated in 799 stool samples and genotyped by reverse transcription polymerase chain reaction (RT-PCR).
Results: The cumulative incidence of diarrhea in children aged <5 years was 21%. A fatal outcome was significantly associated with uncovered household water storage containers. RV-A was identified in 88% of samples and G9 was the prevalent genotype (71%).
Conclusions: Oral rehydration solution and boiling or chlorinating drinking water likely limited mortality. This epidemic was caused by RV-A genotype G9. After the outbreak, a rotavirus vaccine was introduced into the official childhood immunization schedule in Brazil.
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Introduction

Acute diarrheal disease (ADD) is one of the more challenging issues for public health globally, as it is the leading cause of morbidity and mortality in young children, mainly in less developed countries.[1] An estimated 125 million cases of rotavirus-related ADD in children occur annually, with 440 000 deaths.[2,3,4] Although possible infectious etiologies include numerous viral and bacterial agents and parasitic infestations, rotavirus remains the single most important enteropathogen in children under 5 years of age.[5,6] Rotaviruses are members of the *Reoviridae* family; seven distinct groups (A through G) have been described. Groups A through C normally infect humans, and among group A rotaviruses (RV-A) the principal genotypes with global epidemiological relevance include: G1P[8], G2P[4], G3P[8], and G4P[8], with genotype G9P[8] considered an emerging pathogen, ranked fifth in global importance.[4,7] Some authors have reported an increased severity of illness associated with the G9 serotype of rotavirus, with outbreaks occurring in multiple locations.[8]

During August 2005 in the State of Acre, in Brazil's Western Amazon region (see Figure 1), an increased number of ADD cases were reported, mainly among young children living in the capital city of Rio Branco. A total of 816 persons with acute gastroenteritis were reported from August 14 to August 19, an increase of approximately 70% compared to the same period in the previous year (data not shown). Rotavirus was identified in the stool samples of two patients early in the outbreak.

After two deaths occurred in children aged less than 2 years, a team of local health officials and epidemiologists from the

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Figure 1. A The location of Rio Branco City in the Western Amazon Region of Brazil.

Brazilian Field Epidemiology Training Program (FETP, or 'EPISUS' in Portuguese) began an investigation. The objectives of this epidemiological and laboratory-based investigation were to determine the magnitude of the outbreak, to confirm the cause, to determine possible risk factors for ADD-related childhood death, and to evaluate the knowledge and practices in households concerning the preparation of oral rehydration solution (ORS). We also sought to identify practices regarding personal hygiene and issues related to the storage and treatment of potable water, as well as factors related to the extensive drought which had caused water shortages at springs supplying the municipal water system.

1. Materials and methods

1.1. Data sources and case–control study design

Data on children with acute gastroenteritis, reported by clinicians from May 1 to September 30, 2005 to Rio Branco's surveillance system (SSSD) for the monitoring of acute diarrheal disease, were reviewed. In a matched case–control study, case patients were defined as children aged <5 years who died following the acute onset of gastroenteritis (defined as fever, vomiting and/or acute diarrhea (i.e., three or more watery stools per day)) reported to the SSSD. Controls were defined as children with acute gastroenteritis during the same time period who survived, selected from the nearest neighbor households of case patients. Controls were matched by age using an interval ranging from 2 months younger than the case patient to 6 months older.

We interviewed parents and care-givers of children enrolled in the case–control study using a standardized questionnaire. Information collected included: basic demographic characteristics, monthly household income, and selected exposure to factors including drinking water (source, storage, handling, and consumption), hygiene (hand washing, food preparation, toilet use, changing diapers), and breast feeding, and information about health care, history of treatment, and knowledge about the preparation and use of ORS. Data on clinical symptoms (diarrhea, fever, vomiting, anorexia, and abdominal pain) were also obtained.

We used the matched odds ratio (mOR) as the measure of association. Mantel–Haenszel Chi-square, paired Student's *t*-tests, or Kruskal–Wallis tests were used, and 95% confidence intervals (95% CI) were calculated as appropriate. A p-value of <0.05 was considered significant.

1.2. Laboratory methods

Stool samples collected from children with acute gastroenteritis were processed for bacterial enteropathogens and evaluated by microscopy for parasites using standard procedures.[9] A total of 799 stool samples were collected from case patients; these were refrigerated and then screened for the presence of RV-A using a latex agglutination methodology (Bioclin®). Two hundred thirty-two fecal samples were randomly screened: samples were placed in 10% w/v fecal suspensions with Tris-Ca^{2+} buffer and the nucleic acid was extracted from clarified stool samples by the glass powder method[10] with modifications[11] and analyzed by polyacrylamide gel electrophoresis (PAGE) following a previously described protocol.[12] All nucleic acid extracted samples were also submitted to a complementary DNA synthesis using a random initiator (50 A_{260} units of hexamer pd$(N)_6$; Amersham Biosciences, Chalfont St Giles, Buckinghamshire, UK), as previously described.[13] The synthesized cDNA were submitted to nucleic acid amplification by PCR to investigate the presence of astroviruses (HAstVs) and noroviruses (NoVs), as previously described.[13,14] The molecular characterization of RV-A was performed with 153 fecal samples with sufficient fecal material using specific primers to recognize the VP4 and VP7 genes, as previously described.[15,16]

To avoid false-positive results, four different rooms were used and all PCR amplification procedures were applied as recommended. Negative (Milli-Q water) and positive control samples were included

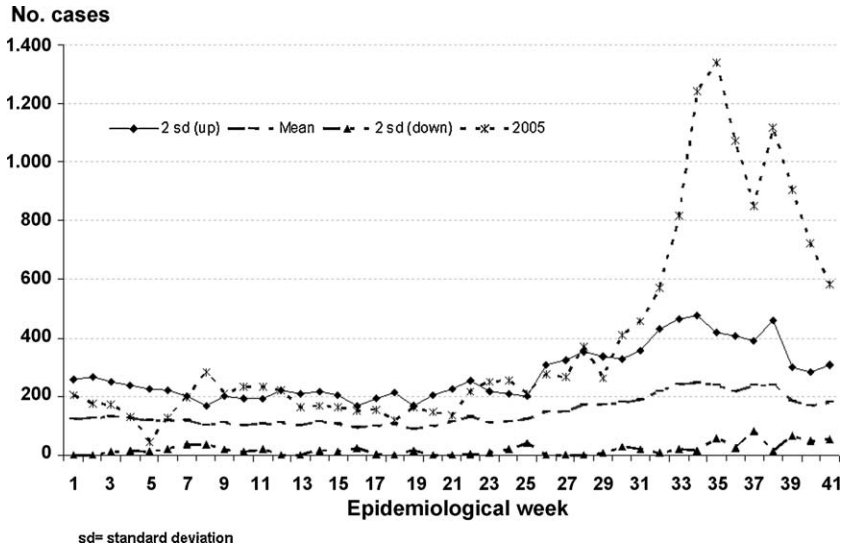

Figure 2. Control chart showing outbreak of ADD detected on 30th epidemiological week using comparison of observed rate in 2005 compared to background (expected) weekly rate (Mean ± 2 sd) during 2000–2004 period.
Source: SSSD surveillance system, Rio Branco Municipal Health Department, and State.Health Department, Acre.

for each set of nucleic acid extractions and amplifications. All methodologies were standardized with reference strains of RV-A (Wa and DS-1) kindly supplied by Dr Jon Gentsch, Centers for Disease Control and Prevention, Atlanta, USA, and for the present study, previously characterized virus strains obtained from fecal samples were used as positive controls. The PCR products were resolved on 1.0% electrophoresis grade agarose gels (Invitrogen, USA), followed by ethidium bromide staining (0.5 μg/ml); images were obtained using the image capture system (BioImaging Systems) with the Labworks 4.0 software program.

Figure 3. (A) Photo showing the open sewer around houses in Rio Branco City. (B) Photo showing the outside toilet at the house of a case patient. (C) Photo showing an example of an open storage container at the house of a case patient.

Table 1
Exposures analyzed in the matched case–control study, Rio Branco, Acre State, Brazil 2005

Exposures	Case patient, *n* (%)	Control, *n* (%)	mOR	95% CI	*p*-Value
Containers without cover					
Yes	4 (57)	3 (11)	11.2	1.02–551.3	<0.03
No	3 (43)	24 (89)			
Household reservoir					
Yes	3 (43)	17 (63)	0.4	0.0–3.5	0.5
No	4 (57)	10 (37)			
Municipal water at residence					
Yes	5 (71)	17 (61)	2.5	0.2–114.7	0.9
No	2 (29)	11 (39)			
Interruption of water supply					
Yes	1 (20)	10 (48)	0.2	0.0–2.7	0.1
No	4 (80)	11 (52)			
Well water					
Yes	2 (29)	9 (32)	∞	∞	0.4
No	5 (71)	19 (68)			
No sewer connection					
Yes	7 (100)	17 (63)	∞	∞	0.1
No	0 (0)	10 (37)			
Infant used pacifier					
Yes	4 (57)	15 (54)	1.1	0.1–10.7	0.7
No	3 (43)	13 (46)			
Dilution ORS (correct)					
Yes	5 (83)	13 (54)	4.0	0.35–196.8	0.2
No	1 (17)	11 (46)			

mOR, matched odds ratio; CI, confidence interval; ORS, oral rehydration solution.

1.3. Municipal water quality testing

Samples of drinking water collected from the municipal system in Rio Branco, as well as from individual residences were evaluated for color, turbidity, and chlorine concentration. In addition, these samples were tested for the presence of bacterial contamination. However, detailed results of this water quality testing were frequently unavailable from the municipality, limiting our ability to carry out a detailed analysis of water quality during the outbreak.

2. Results

A total of 12 145 persons aged <5 years with acute diarrhea were reported to the SSSD between May and October 2005 (Figure 2). From May to October 2005, the cumulative incidence in children aged under 5 years (7757/37 688) was 21 per 100 inhabitants, significantly higher than that of those aged over 5 years (OR = 12.6 95% CI 12.1–13.0; $p < 0.0001$).

Among eight case patient children aged <5 years with a fatal outcome (mortality rate = 0.1%), data were available for only seven. Their median age was 7 months (range 2–16 months) and four were male; all resided in urban neighborhoods of Rio Branco City with poor sanitation (e.g., open sewers) (see Figure 3, A and B). Six of the case patients who died had a triad of the symptoms fever, vomiting, and diarrhea and 100% were dehydrated. All of these case patients had used ORS at home, however one reported ORS not prepared with the correct volume of water per package ('sachet'), likely resulting in solutions either too concentrated or too dilute (serum electrolyte values were not available). In the seven case patients for whom data were available, five (71%) reported consuming water from the official municipal system of Rio Branco (SAERD) and two (29%) reported consuming well water. Interruption of the municipal water supply was reported by one family.

Regarding storage and treatment of potable water in households, four (57%) case patients reported use of water stored in open storage containers. Three (43%) referred to having added 2.5% sodium hypochlorite to drinking water. Seven (100%) referred to having latrines discharging at open sewers (Figure 3B).

2.1. Risk factor analysis

In bivariate analysis, case patients with a fatal outcome were 11 times more likely to have household drinking water stored in an open storage container (Table 1; mOR 11.2, 95% CI 1.02–551.3; $p < 0.03$) (example in Figure 3C). Characteristics not found to be significantly associated with a fatal outcome in the case–control study included: prior history of disease, type of housing, origin or type of potable water treatment, hygiene practices, type of diapers used for children, medication, and methods and time used to prepare ORS.

2.2. Laboratory results

A total of 799 stool samples from case patients were processed. RV-A was identified in 78% (627) using latex agglutination. Of 232 fecal samples submitted to nucleic acid extraction and analyzed by PAGE, 205 (88%) presented an electropherotype characteristic of

Table 2
G and P rotavirus A genotype strains characterized from children with acute gastroenteritis, Acre State, Brazil, 2005

Genotype P	Genotype G, *n* (%)								
	G1	G2	G4	G9	G2+G9	G4+G9	G5+G9	NT	Total
P[4]	-	10	-	1	-	-	-	-	11
P[8]	3	-	2	104	-	28	1	-	138
P[4+8]	-	-	-	9	1	1	-	1	12
NT	-	-	-	2	-	-	-	1	3
Total	3 (2)	10 (6)	2 (1)	116 (71)	1 (0.6)	29 (18)	1 (0.6)	2 (1)	164

NT, not genotyped.

RV-A, the majority being of long profile (Wa-like) and a few with a short profile (DS-1-like). One hundred sixty-four fecal samples were submitted to genotyping and the results are presented in Table 2. RV-A genotype G9 was the prevalent genotype representing 71% (116 case patients) of single infections and 19% (31 case patients) of mixed infections. NoVs were detected in 12 (5%) fecal samples (including two co-infected with RV-A) and HAstVs were detected in 22 (9%) fecal samples (including seven co-infected with RV-A).

2.3. Water sample testing

Among 75 samples from the municipal water system, 25% reportedly did not meet local drinking water standards. Among 64 samples from the alternative water system, 50% showed evidence of fecal contamination.

3. Discussion

The epidemic of acute gastroenteritis associated with RV-A, mainly of serotype G9P[8], caused widespread illness in children living in Rio Branco of Acre State in Brazil's Western Amazon region, affecting more than 20% of children aged < 5 years. This genotype was first described in the USA in 1995–1996,[17] and subsequently in outbreaks in Brazil, India, Italy, Malawi, Bangladesh, Australia, France, and the UK.[4] The magnitude of infection by genotype G9P[8] in the outbreak reported in our study is similar to that reported during an outbreak in Australia, 2001.[8] Other viruses, including NoVs and HAstVs, were isolated infrequently in the epidemic investigated in Acre State, Brazil during 2005. Although stool specimens were only collected from a small proportion of those with symptoms, and unreported cases likely occurred in the community, this is Brazil's largest known outbreak of diarrhea mainly due to rotavirus.

The magnitude of this outbreak in Brazil is consistent with the findings in other countries suggesting that G9P[8] is an emerging rotavirus genotype and an important cause of diarrhea-related morbidity in childhood.[4]

Eight deaths occurred in children between two and 16 months of age, consistent with the concept that younger children are at higher risk of fatal outcomes during acute gastroenteritis.[2,3] Six (86%) of the seven fatal cases with available data had a triad of symptoms including fever, vomiting, and diarrhea.

Overall, mortality was low, most likely due to the prompt adoption of prevention and control measures (including recommendations to boil and/or to chlorinate drinking water), and other timely actions taken by local public health officials. These actions included training of health staff and conducting health education for community organizations about the proper preparation and use of ORS to prevent diarrhea-related dehydration. Shortages of ORS packets and chlorine did occur in the initial weeks of the outbreak, so measures such as planning the stockpiling of materials in preparation for large outbreaks may be useful for municipalities. Although the majority of fatal cases reportedly had ORS packets available in their households, mothers were not always aware of the proper preparation method for mixing with water to obtain the correct final concentration. Although all of the fatal cases were hospitalized with severe dehydration and/or hypovolemic shock, information on serum electrolyte concentrations was not available.

The only risk factor identified in the case–control study was the use of open, uncovered water storage containers, suggesting that potable water supplies in households may have been contaminated.

The extensive transmission observed was likely multifactorial, influenced by factors such as repeated interruptions of the municipal drinking water system. Unfortunately, the requisite regular collection and testing to determine the quality of drinking water samples (Ordinance 518, March 25, 2004) was not routinely carried out, and test results were not readily available to investigators.

Daily notification of acute gastroenteritis in the SSSD helped provide information to managers about the extent of the outbreak. In addition, active surveillance identified additional outbreaks in municipalities outside of Rio Branco, and laboratory capacity was enhanced to process stool samples to identify causal infectious agents. Data reported to the SSSD need to be routinely provided to clinicians, hospitals, and health centers to provide feedback and keep staff informed of developments. Laboratory supplies should be made available to regularly identify agents circulating in children with acute diarrhea. Prevention remains of key importance in limiting rotavirus transmission, and following this outbreak the leadership of Brazil's Ministry of Health included rotavirus vaccine in the national schedule of childhood vaccination.

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Ethical approval: This paper is the product of an investigation conducted into an unexpected public health emergency in a metropolitan city in the Brazilian Amazon Region. For this situation, and following national laws (official document No. 196/1996), ethical approval was not required.

Conflict of interest: We declare that there are no other potential conflicts of interest.

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