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Outbreak of Human Calicivirus Gastroenteritis in a Day-Care Center in Sydney, Australia

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Between January and March 1988, an outbreak of gastroenteritis occurred among children and staff at a day-care center in Sydney, New South Wales, Australia. Over an 11-week period, 53 persons had 101 episodes of gastroenteritis; some patients had 5 separate episodes. The principal etiologic agent in the outbreak, human calicivirus (HCV), was detected by electron microscopy in 32% of fecal specimens from children and staff members with symptoms but in only 8% of asymptomatic individuals ($P < 0.01$). HCV was confirmed by both an enzyme immunoassay and solid-phase immune electron microscopy. HCV infection was a particular problem in infants, who had the highest age-specific attack rates, had the greatest symptomatic/asymptomatic infection ratio, and were most likely to have a second symptomatic episode. The mode of transmission of this virus was not identified, and extensive efforts to control the 11-week outbreak had little effect. Prolonged excretion of HCV by some symptomatic patients and high rates of asymptomatic infection may have contributed to the extended duration of the outbreak. HCV may be a common cause of gastroenteritis in children that is underrecognized because of insensitive methods of detection.

Human caliciviruses (HCVs) were first detected in fecal specimens by Madeley and Cosgrove in 1976 (18) and appear to cause diarrhea in people of all ages and on all continents (3). These viruses have been detected by electron microscopy (EM) in only 0.2 to 6% of fecal specimens screened from persons with diarrhea, which suggests that HCV-associated diarrhea is uncommon (5, 11, 21, 22, 26, 29, 30). The apparently low prevalence of this disease is also indicated by the relatively few outbreaks of HCV-associated gastroenteritis reported among infants and young children and the elderly (1, 7, 25) compared with the many outbreaks of gastroenteritis that occur yearly. Several serosurveys have found that antibody to HCV is present in most older children and adults in Asia, Australia, Africa, Europe, and North America (6, 23), which demonstrates that exposure to HCV is in fact common, even though HCV-associated diarrhea is rarely detected.

Recently, a sensitive enzyme immunoassay (EIA) that can detect HCV antigen when intact virions are not visible by direct EM has been developed (24). Since the characteristic and diagnostic morphology of HCV can be obscured by coproantibody or degradation of virus in the specimen, the EIA can also be used to identify HCV in specimens with small round structured viruses (SRSVs) whose surface morphologic features are unclear (19). The reagents used for EIA can also be used for immunosorbent EM (ISEM) to confirm the identities of HCVs whose characteristic features are indistinct.

We report here an investigation of the first large outbreak in Australia of HCV gastroenteritis which occurred and spread in a child care center. Application of EM, EIA, and ISEM allowed us to confirm HCV as the etiologic agent of this outbreak and to demonstrate asymptomatic infection. This investigation yielded new information on the epidemiology of HCV infection. At the same time, however, new questions were raised about the mode of transmission of this agent because attempts to control the outbreak through traditional measures—even when applied early—had little effect on the spread of infection in the day-care center.

MATERIALS AND METHODS

Description of the outbreak. On 11 January 1988, the director of a day-care center in Bondi Junction, an eastern suburb of Sydney, New South Wales, Australia, notified the regional Division of Environmental Health that a number of children who returned to the center on 2 January after the New Year's recess had been ill with gastroenteritis. Some children had gastroenteritis during the week before Christmas, and it was presumed that this problem would resolve itself during the 1-week holiday recess. The day-care center, located in a renovated church building, had an enrollment of 73 children who were divided into four classes by age: a nursery (<14 months old; $n = 14$), a toddler class (14 to 29 months old; $n = 14$), a little class (30 to 42 months old; $n = 22$), and a big class (43 to 60 months old; $n = 23$). The children were cared for by the center's 22 staff members, each of whom was generally assigned to one group of children, although this procedure changed during the outbreak period because of staff absenteeism due to illness. Lunch meals and snacks were served in the facility. The children and staff members used separate bathrooms, and the nursery and toddler groups had designated diaper changing areas.

The outbreak began in the nursery, spread throughout the day-care center, and continued for 11 weeks despite continuous and often aggressive infection control interventions.

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These traditional measures were directed to three public health objectives: (i) control of person-to-person spread—i.e., mandatory wearing of gloves for diaper changes, strict supervision of toilet practices, strict handwashing procedures, restriction of staff member movements to their own classes, isolation of children who were ill from those who were well, and elimination of shared articles (e.g., toys and books); (ii) prevention of foodborne contamination by closing the kitchen and arranging for meals to be brought from home; and (iii) quarantine by temporarily excluding ill children and staff members from the center until 24 h after their last episode of gastroenteritis. Several meetings of the investigators, day-care staff, and parents led to reinforcement of the control measures and expansion of efforts to identify the causative agent through additional screening by EM of fecal specimens collected from both ill and well children and staff members. The outbreak subsided after 11 weeks, apparently independently of all of the public health measures that had been taken.

Epidemic investigation. Public health officials were notified of the outbreak on 11 January 1988, and they initiated an investigation on the next day. All parents were given questionnaires inquiring about their childrens symptoms of gastrointestinal illness during the previous 2 weeks. Fecal specimens were requested from all children and staff members regardless of their history of recent illness. Because the initial investigation failed to identify a mode of transmission, the nursery was closed on 14 January and other control measures were taken. However, the nursery was reopened on 25 January because parents began enrolling their children in other day-care centers, increasing the risk of spread. Some episodes of gastroenteritis were reported in a different day-care center where an infant had been transferred. The focus of the outbreak continued in the nursery and with less intensity among children in the other three groups at the day-care center. On 5 February, a second investigation was conducted with a second mass collection of fecal specimens. During the 11-week period of surveillance, fecal specimens and clinical information were obtained from all children or staff members who developed gastrointestinal symptoms.

Three case definitions were adopted for analysis. These were dictated, in part, by the need to link both retrospective and surveillance data on symptoms with the large number of stool specimens collected at the time of the two mass screenings. HCV-associated gastroenteritis was defined as an episode of illness for which a fecal specimen collected 1 day before or within 7 days after the onset of symptoms was positive for HCV. This definition is consistent with both the report of Chiba et al. (2) on the duration of fecal shedding of HCV among children in a day-care center in Japan and our recent observation of a 9-month-old child with HCV-associated gastroenteritis who excreted HCV daily for 8 days, even though the child recovered in 24 h. An asymptomatic HCV infection was defined as one in which a person's fecal specimen tested positive for HCV without symptoms of gastroenteritis in the previous week or in the subsequent 2 days. Many children and staff members had multiple episodes of gastroenteritis during the period of the outbreak; therefore, a separate episode was defined as the new onset of gastroenteritis in a person who had a 1-week period free of symptoms after the preceding episode.

Several sanitary investigations were conducted at the day-care center. At the beginning of the outbreak, two samples of drinking water and 10 samples of food were collected, tested for common foodborne pathogens, and analyzed for chemical contamination at the Division of Analytical Laboratories, Lidcombe, Sydney.

Specimen collection. Over the 11-week period, a total of 465 fecal specimens were collected from the 95 children and staff members at the day-care center. The 53 persons who became ill during the outbreak provided 75 stool specimens within 8 days of their illness (from 1 day before to 7 days after onset of symptoms) and an additional 214 specimens when they were well, outside of the 8-day window. Another 42 individuals who never became ill provided 176 stool specimens. Paired acute- and convalescent-phase sera were collected from all 22 staff members within 1 week of the onset of illness and again 3 to 6 weeks later.

Laboratory methods. Fecal specimens were examined by microscopy for parasites and cultured for bacterial pathogens, including *Salmonella*, *Shigella*, and *Campylobacter* spp., in the Microbiology Laboratory, St. Vincent's Hospital, Sydney. Specimens kept at 4°C were tested in the Virology Unit, Westmead Hospital, Sydney, by direct EM for enteric viruses (12); by cultivation in primary monkey kidney, HEp-2, and MRC5 cells for enteroviruses and adenoviruses (10); and by commercially available EIAs (Pathfinder [Kallestad] and Adenoclone [Cambridge Biosciences]) for rotaviruses and enteric (adenovirus 40/41) and nonenteric (adenovirus 1-39) adenoviruses. All of the nonenteric adenoviruses were inoculated into cell cultures (cultured), and those that did not grow were designated noncultivable adenoviruses that were nontypeable and possible enteric strains (13). Selected frozen specimens were examined in the Viral Gastroenteritis Unit, Centers for Disease Control, Atlanta, Ga., for HCV by using an HCV-specific EIA and ISEM (19). Paired sera were tested for antibody to the Norwalk virus, and the 75 fecal specimens from symptomatic patients were tested for Norwalk antigen by EIA by using methods described by Gary et al. (8) with reagents from Australia (12). Selected paired sera were also tested for antibody to HCV from the fecal specimens collected during the outbreak by immune EM (IEM) by using methods described by Kapikian et al. (15).

RESULTS

Epidemiology of the outbreak. With the information obtained from questionnaire surveys and ongoing surveillance, we constructed an epidemic curve with all of the episodes of gastroenteritis that occurred among children and staff members from 2 January, when the center reopened, through 15 March, when the outbreak subsided (Fig. 1). The outbreak began in the nursery between 2 and 5 January and peaked on 13 January, when four new cases were reported on a single day. When the nursery was closed on 14 January, 10 of the 14 infants in the unit had already experienced 14 episodes of diarrhea. Four staff members, three of whom worked in the nursery, had also become ill. During the 11-week period of the outbreak, 33 episodes of illness occurred among the 22 staff members, with six reported on a single day (20 January). Sporadic illness with no large clustering of cases was observed among the other groups of children. Fecal specimens were screened for enteric viruses by EM, and the 24 persons who met the case definition of HCV-associated diarrhea are indicated on the epidemic curve (Fig. 1).

Surveys conducted in January and February provided uniform data on the symptoms noted for 75 (60%) of the 101 episodes of gastroenteritis, although the period of recall for some early episodes was nearly 2 weeks. Diarrhea was the most common symptom in 57% of the patients, followed by

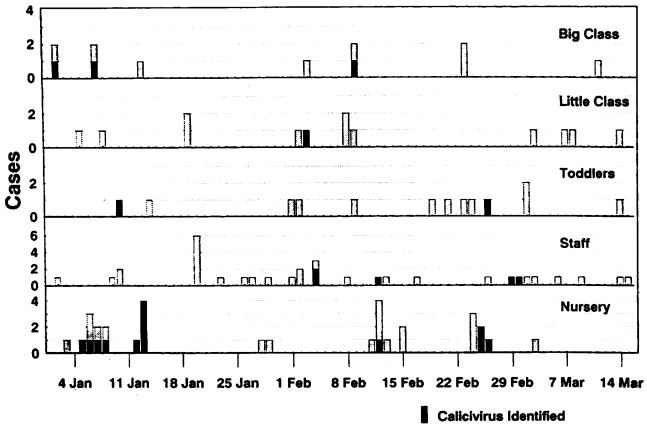

FIG. 1. Epidemic curves of the outbreak of gastroenteritis in the day-care center by groups. The center reopened after the holidays on 2 January, the investigation began on 12 January, and the nursery was closed from 14 to 25 January. On 5 February, a second investigation was conducted.

vomiting (31%), nausea (23%), abdominal cramps (8%), and fever (5%), and many patients had more than one symptom. The number of patients was not adequate for examination of differences in symptoms by age groups. The incubation period was estimated to be 24 to 36 h, and the duration of symptoms was 24 to 48 h.

A peculiar feature of this outbreak was the high frequency with which infants in the nursery and staff members became ill more than once during the 11-week period (Table 1). Four infants and one staff member each had four separate illnesses, and two staff members each experienced five illnesses. Among the 95 children and staff members at the day care center, 53 (56%) had at least one episode of gastroenteritis and of these, 26 (50%) had more than one episode, with a total of 48 repeat episodes. Among children, the attack rate of both first and total cases of gastroenteritis was highest in the nursery infants and declined as the ages of the children increased. The staff had attack rates that were comparable to those of infants in the nursery.

Laboratory analysis. Fecal specimens for 75 of the 101 episodes of gastroenteritis were examined in the laboratory (Table 2). No parasites, bacterial pathogens, or cultivable viruses were found. By EM, SRSVs that had some morphologic features of HCV were the predominant agent present in 24 (32%) of the specimens. Few pathogens were identified in the 390 fecal specimens collected from asymptomatic persons. HCV was the most common pathogen detected, but the rate of detection (31 [8%] of 390) was less than that found in symptomatic cases ($P < 0.001$).

HCVs detected by EM often appeared to be covered with coproantibody that obscured some of their distinct morphologic features—the calices, star of David, and 6- and 10-pointed axes—that are diagnostic. To confirm the presence of HCV, 17 specimens believed to be positive for HCV on the basis of EM and 19 EM-negative specimens were tested with an HCV-specific EIA (Table 3). Of the 17 EM-positive specimens, 15 were positive for HCV by EIA (sensitivity, 88%), and of the 19 EM-negative specimens 12 were negative by EIA (specificity, 63%). Seven specimens that were positive by EIA but initially negative by EM were reexamined by ISEM. HCV was confirmed in each specimen.

Particles seen in this outbreak were masked with coproantibody, often making them appear to be SRSVs (Fig. 2A), but the characteristic features of HCV were observed by ISEM (Fig. 2B). We tried to reproduce this masking of structure by IEM. HCVs with good morphologic features were reacted with both low- and high-titer antisera to HCV. The HCVs treated with low-titer serum retained their distinct morphology (Fig. 2C), whereas those treated with high-titer serum lost their features and looked like SRSVs (Fig. 2D), indistinguishable from the particles seen in the outbreak (Fig. 2A).

Periodic collection of a large number of specimens on a single day, accompanied by active surveillance of acute cases throughout the period of the outbreak, allowed us to define the new onset of episodes that began a few days before or after routine specimen collection. HCV was ex-

TABLE 1. Attack rates of new and repeat episodes of gastroenteritis among children and staff members at the day-care center

Group	Age (mo)	No. of persons	No. of episodes of gastroenteritis (attack rate)[a]	
			First	Repeat
Nursery	<14	14	12 (0.86)	19 (1.58)
Toddlers	14–30	14	10 (0.72)	3 (0.30)
Little class	30–41	22	10 (0.45)	3 (0.30)
Big class	42–60	23	8 (0.35)	3 (0.38)
Staff	Adult	22	13 (0.59)	20 (1.54)

[a] Attack rate, Number of episodes per person.

TABLE 2. Pathogens identified from children and staff members with or without gastroenteritis

Pathogen	% (no.) of persons with pathogen		*P* value[c]
	WG[a]	NG[b]	
Calicivirus	32 (24)	8 (31)	<0.001
Rotavirus	1 (1)	1 (4)	NS
Noncultivable adenovirus[d]	5 (4)	3 (11)	NS
None[e]	60 (45)	86 (338)	<0.001

[a] WG, With gastroenteritis ($n = 75$).
[b] NG, No gastroenteritis ($n = 390$).
[c] Rate of agent detection in patients with or without gastroenteritis tested by the Fisher exact or chi-square test. NS, Not significantly different.
[d] Adenoviruses identified by EM were tested by EIA with monoclonal antibody and cultivated. Enteric strains classified as noncultivable adenoviruses were either adenovirus 40/41 or nontypeable. Adenovirus 40/41, one of the four noncultivable adenoviruses in children with gastroenteritis, accounted for 5 of the 11 noncultivable adenoviruses in those without gastroenteritis.
[e] Other agents detected in asymptomatic cases: *Yersinia enterocolitica* (one case), *Giardia lamblia* (one case), *Enterovirus* sp. (four cases).

TABLE 3. Comparison between EM and EIA for detection of calicivirus in selected stool specimens

EIA result	No. of EM results		
	Positive	Negative	Total
Positive	15	7[a]	22
Negative	2	12	14
All	17	19	36

[a] These seven were all confirmed as positive for HCV by ISEM.

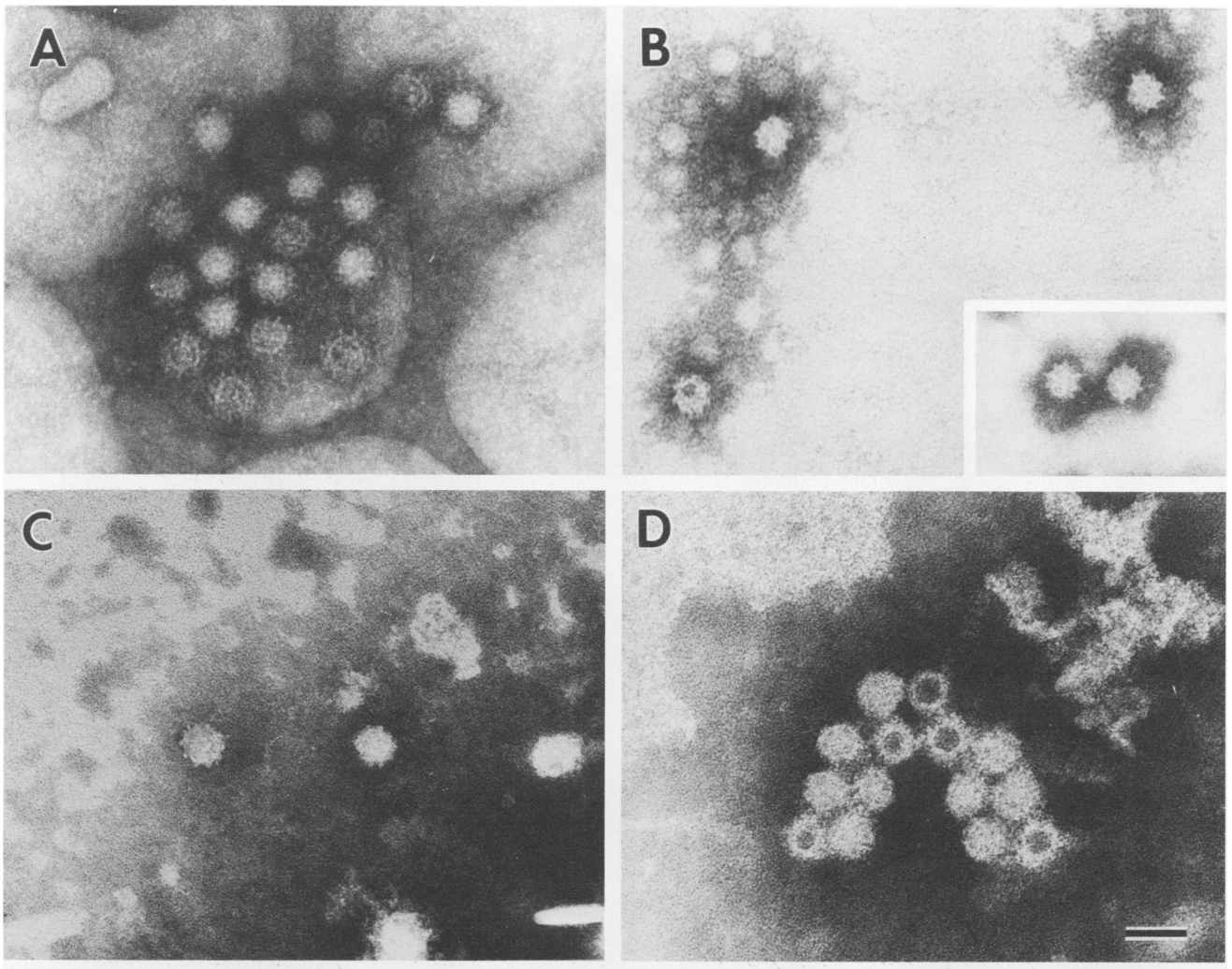

FIG. 2. Electron micrographs. (A) Original particles seen in the outbreak appeared to be SRSVs with some features of HCV obscured by coproantibody. (B) ISEM of particles in panel A using a rabbit antiserum to HCV for capture. Distinct calices of HCV are visible. (C and D) IEM of reference HCV particles reacted with low-titer (C) and convalescent-phase (D) sera from a patient with HCV diarrhea. The distinct morphology of HCV is lost when antibody is present and the coated particle is similar to the SRSV-like particle seen originally in the outbreak. Bar, 50 nm.

creted from the day before until 7 days after the onset of illness. Of the 24 HCV-positive stools from patients with gastroenteritis, we collected 6 on the day before the onset of symptoms, 5 on the day of onset, 10 from days 2 to 6 after onset of symptoms, and 3 on day 7. Prolonged excretion (more than 7 days) was found in two children from the nursery who each had symptoms for 3 to 4 days.

HCV was a particular problem for infants, whose rate of symptomatic infection (54%) was greater than the confirmed rates in toddlers and older children (26%) and staff members (18%) (Table 4). Similarly, the symptomatic/asymptomatic infection ratios, an indirect index of pathogenicity, were greatest in infants (9:1; $P < 0.001$), intermediate in toddlers and older children (4.3:1; $P < 0.01$), and lowest in staff members (1.6:1; not significantly different). This trend with age was highly significant ($P < 0.01$; chi-square test for

TABLE 4. Rate of detection of HCV by age in persons with symptomatic or asymptomatic HCV infections

Group	No. of persons	No. (%) symptomatic[a]		No. (%) asymptomatic		Symptomatic/asymptomatic infection	*P* value
		Tested	HCV positive	Tested	HCV positive		
Nursery	14	24	54 (13)	50	6 (3)	9.0	<0.001
Toddlers, older children	59	23	26 (6)	191	6 (12)	4.3	<0.01
Staff	22	28	18 (5)	149	11 (16)	1.6	NS[b]

[a] Of the total of 101 episodes, 75 were screened by EM. Three infants and one staff member had two symptomatic infections more than 2 weeks apart.
[b] NS, Not significantly different.

TABLE 5. Presence of symptoms among 13 persons with Repeat HCV infections by group[a]

Infection 1	Infection 2	No.		
		Nursery children	Older children	Staff
Ill	Ill	3		1
	Well	1	1	2
Well	Ill		1	
	Well		1	3

[a] The hypothesis that a first infection is associated with immunity leading to an asymptomatic second infection was tested by using Fisher exact test (one tailed). Nursery infants were more likely to have a symptomatic second infection than were staff members ($P = 0.12$) or staff members plus older children ($P = 0.12$).

trend). Of note, seven persons who never developed gastroenteritis (i.e., two toddlers, two older children, and three staff members) excreted HCV.

During the period of the outbreak, HCV was detected twice at intervals of 2 weeks or more in 13 persons (Table 5). Of the four infants who had two separate HCV infections, three were symptomatic on both occasions. In contrast, five of the six staff members who had two HCV infections were without symptoms with their second infection ($P = 0.12$; Fisher exact test).

The water and food samples tested had no fecal coliforms to incriminate them as vehicles of infection. Norwalk virus was not implicated as a cause of this outbreak. Seven paired sera showed no rise in titer indicative of infection, and 75 acute-phase fecal specimens tested for Norwalk antigen were negative. Paired sera from staff members and HCV found in fecal extracts were used in IEM tests for seroconversion to HCV, which were inconclusive because much of the virus was already coated with coproantibody and because virus titers were generally low.

DISCUSSION

This report documents the first outbreak of HCV-associated gastroenteritis in Australia. The investigation highlights some unusual features of the disease, as well as difficulties with its control. HCV was the prime agent of the outbreak, identified by EM in 32% of patients with gastroenteritis but in only 8% of those with no symptoms ($P < 0.01$). HCV was a particular problem for infants, who had the highest attack rate of symptomatic HCV infection, whose infection was more likely to be symptomatic than asymptomatic, and who occasionally were documented to have two symptomatic infections, an observation rare in older children and staff members. This age distribution is similar to that observed among children in an orphanage in Sapporo (1) and in day-care centers in Phoenix, Ariz. (19), and Houston, Tex. (20), where infants under 6 months of age had the highest rate of HCV-associated gastroenteritis. Furthermore, data from EM surveillance of viral agents of gastroenteritis in the United Kingdom (21), the United States (17), and Australia (11) indicated that 80 to 90% of HCV detections were in children under 5 years of age and that most of these occurred in the first year of life. Cubitt and McSwiggan and Nakata et al. observed that children acquire antibodies to HCV in their first years of life and 80% have antibody by 5 years of age (6, 23). While HCV can cause disease in adults, infants appear to be at greatest risk of disease, and young children in day-care centers or institutional settings appear to be at a particularly high risk.

This outbreak persisted for 11 weeks, its mode of transmission was never identified, and the disease continued to spread despite aggressive infection control measures. Control efforts were directed at preventing foodborne spread by closing the kitchen and having meals brought from home and by interrupting person-to-person transmission by encouraging handwashing, proper toilet behavior, and diaper changing practices. The failure of these measures to interrupt disease spread leaves open the possibility that droplets from feces or vomitus are part of a chain of airborne transmission, a hypothesis that has been advanced to explain several recent outbreaks of disease associated with SRSVs (14, 28). Excretion of HCV for 5 to 7 days after the onset of and recovery from illness and high rates of asymptomatic infection in the staff and older children may have helped prolong the circulation of HCV in this outbreak. In retrospect, closing the nursery was of dubious value, since most children had already been infected and at least one subsequent outbreak in another day-care center was traced to an infant who had been transferred to this facility from the day-care center where the initial outbreak occurred.

The high rates of HCV infection and disease among the staff are similar to adult rates for other enteric diseases, like rotavirus and endemic cholera, that are common in children and for which immunity is acquired early in life. In a study of cholera, for example, mothers with high titers of vibrocidal antibodies, who should have been immune to disease, developed cholera when their infants became ill, possibly because they had extensive contact with the large numbers of vibrios present in their children's stools (9). Similarly, mothers who should have been immune to rotavirus infection developed mild disease when their children became ill (16). In the present study, the staff at the day-care center appeared to have some immunity to HCV by virtue of the higher rates of asymptomatic infections, but they were still at risk for disease despite the precautions taken. Day-care center personnel, like parents of small children, remain at risk for these enteric diseases of children despite their age and immunity.

This investigation raises several issues in the epidemiology of HCV infection. (i) Mass screening of stool specimen from persons who were either ill or well led to clear documentation of asymptomatic infection and the prolonged duration of HCV shedding. Moreover, some patients were documented to have HCV in their feces in the 24 h prior to development of symptoms, a pattern similar to that observed for rotavirus infection by Pickering et al. (27). (ii) The prolonged duration of the outbreak and intense EM screening allowed us to identify 13 persons who had two separate HCV infections. Three infants had symptoms documented for both infections which were more than 2 weeks apart, raising questions about immunity to HCV. Was HCV the pathogen both times, and did immunity not occur? Does HCV produce immunity which was overwhelmed by the large challenge inoculum? Or, since four serotypes of HCV have been identified (3), was the outbreak caused by different serotypes that did not confer heterotypic protection? Because serum was not drawn from the children and IEM could not be performed, we have no answers to these questions. (iii) While HCV was identified in 32% of the stool specimens of those with gastroenteritis and while EIA detected an additional fraction of HCV-positive cases, we do not know whether another pathogen or multiple serotypes of HCV were involved in this prolonged outbreak.

The laboratory investigation of this outbreak demonstrates the importance of using different methods to detect and confirm HCV. The first particles seen by EM looked like SRSVs with antibody obscuring the distinct features of HCV. The suspicion that the SRSVs were HCV was tested and confirmed by EIA, which also indicated that some EM-negative specimens contained HCV. In every case, the EIA results were confirmed by ISEM, which also clarified the morphologic features of the HCVs. Consequently, the finding of 30- to 35-nm SRSVs with a coating of antibody requires further clarification of their possible identity as HCVs. The HCVs did not react in an assay for Norwalk antigen, and paired sera from the staff did not show a fourfold rise in titer in a Norwalk assay, an observation reported by Cubitt et al., who used paired sera from previous outbreaks of HCV gastroenteritis (4). Finally, we were unable to document seroconversion to HCV by IEM because of the heavy antibody coating on the particles, the small number of particles present in most specimens, and the small volumes of fecal specimens collected early in the outbreak, when HCV shedding was greatest. Further investigations of viral gastroenteritis in day-care center studies will require paired sera from patients of all ages and larger volumes of acute-phase stool specimens to achieve definitive answers to some of the issues raised in this investigation.

Although this outbreak of HCV was undoubtedly not the first to occur in Australia, it was the first to be detected and documented. HCV is a common infection in infants and children, particularly in those of day-care age, but methods to detect the agent are insensitive and not commonly available. Improvements in assay methods and increased surveillance will be needed to appreciate the full extent of the disease caused by this agent.

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