BIKEN JOURNAL Vol. 22 21-24, 1979

SHORT COMMUNICATION

AN OUTBREAK OF DIARRHEA IN SCHOOL CHILDREN ASSOCIATED WITH COXSACKIE VIRUS TYPE B-3 INFECTION

ISAO OISHI, AKIKO MAEDA, KEIJI OTSU, YOSHIICHI MINEKAWA and TOSHIYUKI KITAURA

Laboratory of Virology and Division of Public Health, Osaka Prefectural Institute of Public Health, Nakamichi, Higashinari-ku, Osaka 537, Japan

(Received October 3, 1978)

ECHO, Coxsackie, polio or adenoviruses are often cited as causal agents of acute non-bacterial gastroenteritis in children (Ramos-Alvarez and Olarte, 1964; Yow et al., 1970; Steinhoff, 1978). In addition rotavirus, originally reported independently by Bishop et al. (1973) and by Flewett et al. (1973), and parvovirus-like agents (Norwalk, Hawaii, MC and W), investigated by many workers (Adler and Zickl, 1969; Dolin et al., 1972; Kapikian et al., 1972; Schreiber et al., 1974; Wyatt et al., 1974), have also been mentioned. There have also been some unexplained cases of acute gastroenteritis of children. Epidemics or mass outbreaks of diarrhea in children caused by Coxsackie virus type B-3 (Cox B-3) are very rarely reported (Felici et al., 1962).

This communication describes an outbreak of diarrhea accompanied with fever in primary school children, the isolation of Cox B-3 from a patient and seroepidemiological evidence of Cox B-3 infection among these children.

Early in June, 1978, there was a mass outbreak of vomiting and diarrhea accompanied with moderate fever in 13 primary schools in Tondabayashi City of Osaka Prefecture. During the outbreak, the rate of absence of children from these 13 schools reached a mean of 6.9% and a maximum of 13.7%, while it was usually between 1 and 2%. Kishi Primary School, which had the highest absence rate among the 13 schools (Fig. 1) was chosen for etiological examination. The other 12 schools had similar patterns of absence rates to that shown in Fig. 1.

The clinical symptoms of the children in this school are summarized in Table 1. These values were calculated from retrospective answers to written inquiries to the patients.

TABLE 1. *Frequency of clinical symptoms of the patients*

Symptom	Frequency (%)[a]
Abdominal pain	86.1
Diarrhea	69.4
Appetite loss	63.9
Nausea	30.6
Vomiting	11.1
Fever	83.3
Headache	69.4
Catarrhal signs of upper respiratory tract	52.8

[a] No. of positive symptoms/37 patients with acute gastroenteritis (×100).

The most common abdominal signs were abdominal pain followed by diarrhea and loss of appetite, while nausea and vomiting were not so common. This latter phenomenon, as well as the appearance of fever, headache and catarrhal signs of the upper respiratory tract, like those of a common cold in summer time, were unlike the signs of the usual so-called infectious vomiting and diarrhea syndrome of school children. The duration of the clinical symptoms was 2 to 3 days. The outbreak was not restricted to any particular grade or class.

Attempts were made to isolate some virus from throat swabs and stools of 16 patients.

TABLE 2. *Antibody titers of the patients against human adenovirus type 4, rotavirus and Coxsackie virus type B-3*

Code No.	Patient	Sex	School year	CF antibody titer vs				NT antibody titer vs	
				Adenovirus type 4		Rotavirus[a]		Coxsackie virus B-3	
				Acute	convalescent	Acute	convalescent	Acute	convalescent
1	K. K.	M	3	16	16	8	4	< 4	< 4
2	M. K.	M	4	8	8	< 4	< 4	< 4	< 4
3	M. M.	M	5	< 4	< 4	< 4	< 4	≧1024	≧1024
4	T. K.	M	5	8	8	8	8	< 4	< 4
5	K. H.	M	5	8	8	8	8	512	512
6	Y. Y.	M	5	8	8	8	8	512	512
7	S. I.	F	5	< 4	< 4	4	4	512	512
8	H. I.	F	5	16	16	8	8	≧1024	≧1024
9	M. K.	F	5	8	8	4	4	< 4	< 4
10	K. S.	F	5	8	8	4	4	512	512
11	J. S.	F	5	32	32	8	8	128	≧1024
12	M. A.	F	5	8	8	8	8	< 4	< 4
13	N. M.	F	5	< 4	< 4	< 4	< 4	128	≧1024
14	H. Y.	F	5	8	8	8	8	< 4	8
15	K. Y.	F	5	4	4	4	4	512	≧1024
16	H. A.	M	5	4	4	8	8	512	512
17	H. O.	M	5	8	8	< 4	< 4	128	128
18	T. T.	F	5	8	8	4	4	≧1024	512
19	Y. T.	F	5	≧64	≧64	< 4	< 4	512	512
20	M. T.	F	5	32	32	< 4	< 4	512	512
21	H. Y.	F	5	16	16	8	8	< 4	< 4
22	M. Y.	F	5	32	16	8	8	≧1024	512
23	M. T.	M	6	< 4	< 4	< 4	< 4	128	512
24	S. M.	M	6	16	16	4	4	< 4	< 4
25	Y. Y.	M	6	8	8	< 4	< 4	512	512
26	M. Y.	M	6	8	8	8	8	≧1024	≧1024
27	S. S.	F	6	8	8	8	8	< 4	< 4
28	H. T.	F	6	16	16	16	16	≧1024	512
29	Y. Y.	F	6	8	8	< 4	< 4	512	≧1024

[a] NCDV was used as antigen.

Specimens were inoculated into suckling mice and cultured cells (FL, HEp-2 or primary African green monkey kidney cells). A throat swab specimen from one patient who had a typical common cold with diarrhea had cytopathic effects (cp) in all three kinds of cultured cells but also a lethal effect in suckling mice. This cp agent was identified as Cox B-3 by a neutralization (NT) test by the microtiter method with known standard immune sera against Cox B-3 (data not shown).

Stool specimens from patients were also examined by electron microscopy after partial purification of the material by fluorocarbon treatment and centrifugation. No virus was detected in any specimen.

Paired blood specimens were taken from 29 patients at 3 weeks intervals. They were examined by the complement fixation (CF) or NT test for antibody against human adenovirus type 4, NCDV (neonatal calf diarrhea virus) or newly isolated Cox B-3, as shown in Table 2. NCDV was used as a substitute for human rotavirus in CF tests, because of its close antigenic relation with both viruses (Kapikian et al., 1975). Unusually high antibody titers against Cox B-3 were detected in both acute and convalescent sera of all except 8 patients, while no significant antibody responses to adenovirus or rotavirus were seen.

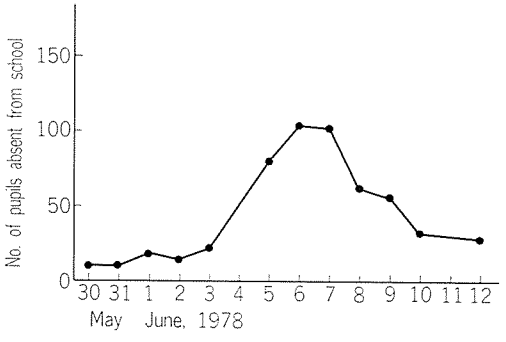

FIGURE 1. Number of pupils absent from Kishi Primary School. This school has 751 pupils.

The reason why high antibody titers against Cox B-3 were already present in the acute phase is that the first blood specimens were taken on June 10 and especially on June 12, when the outbreak had already passed its peak, as can be seen from Fig. 1. These high antibody titers may indicate that the present outbreak of diarrhea accompanied with fever was associated with Cox B-3 infection. The reason why 8 patients did not show an antibody response against Cox B-3 may be that the blood specimens were taken from children who had been absent from school during the outbreak, since they were thought to be patients irrespective of the extent of their clinical symptoms.

Epidemics or mass outbreaks of diarrhea in children caused by Cox B-3 infection seem to be very rare and only one has been reported by Felici et al. (1962). But sporadic cases of infection (Sato et al., 1972) and virus isolation from sewage (Otsu et al., 1977; Yamazaki and Otsu, 1978) or from stool specimens (Toyoshima et al., 1964; Oishi et al., 1968; Moritsugu et al., 1970) of either patients or healthy persons have been reported. Thus Cox B-3 seems to be detectable everywhere, especially during the summer, and the possibility of mass outbreak of Cox B-3 infection in schools or kindergartens is always present. Antigenic analysis of Cox B-3 isolated at this time from a patient with diarrhea is necessary to see if it differs from the standard strain.

ACKNOWLEDGMENTS

We wish to thank Dr. K. Harada, Laboratory of Microbiology of this Institute, for helping in taking blood specimens from the children and Mr. H Nishimura, Laboratory of Pathology of this Institute, for excellent technical assistance in electron microscopic observations. This work was supported by a Grant-in-Aid for Research on Epidemic Vomiting and Diarrhea from the Department of Hygiene of Osaka Prefecture, Japan.

REFERENCES

Adler, J. L. and R. Zickl. 1969. Winter vomiting disease. J. Infect. Dis. 119: 668–673.

Bishop, R. F., G. P. Davidson, I. H. Holmes and B. J. Ruck. 1973. Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. Lancet 2: 1281–1283.

Dolin, R., N. R. Blacklow, H. DuPont, R. F. Buscho, R. G. Wyatt, J. A. Kasel, R. Hornick and R. M. Chanock. 1972. Biological properties of Norwalk agent of acute infectious nonbacterial gastroenteritis. Proc. Soc. Exp. Biol. Med. 140: 578–583.

Felici, A., I. Archetti, F. Russi, C. Bellocchi and F. Marzi. 1962. Contribution to the study of diseases caused by the Coxsackie B group of viruses in Italy. III. Role of Coxsackie B virus type 3 in summer diarrheal infections in infants and children. Arch. Gesamte Virusforsch. 11: 592–598.

Flewett, T. H., A. S. Bryden and H. Davies. 1973. Virus particles in gastroenteritis. Lancet 2: 1497.

Kapikian, A. Z., W. L. Cline, C. A. Mebus, R. G. Wyatt, A. R. Kalica, H. D. James, Jr., D. VanKirk and R. M. Chanock. 1975. New complement-fixation test for the human reovirus-like agent of infantile gastroenteritis: Nebraska calf diarrhea virus used as antigen. Lancet 1: 1056–1061.

Kapikian, A. Z., R. G. Wyatt, R. Dolin, T. S. Thornhill, A. R. Kalica and R. M. Chanock. 1972. Visualization by immune electron microscopy of a 27 nm particle associated with acute infectious nonbacterial gastroenteritis. J. Virol. 10: 1075–1081.

Moritsugu, Y., T. Komatsu, T. Karasawa, M. Hara, K. Tsuchiya, Y. Tsuchiya, C. Nakao, K. Soda, I. Tagaya, Y. Miki, S. Komiyama, H. Mizutani, Y. Yamazaki and S. Asada. 1970. A longitudinal study of enterovirus infections in Kawasaki, Japan. I. Virus isolations from 1962 to 1966. Jpn. J. Med. Sci. Biol. 23: 147–160.

Oishi, I., T. Yamada, B. Mitsuda, A. Kawai, T. Kimoto, K. Oyagi, F. Nakajyo and K. Shimada. 1968. Epidemiological studies on aseptic meningitis in Osaka, 1964–1967. Proc. Osaka Pref. Inst. Public Health 6: 38–44 [In Japanese].

Otsu, K., K. Yamazaki and T. Okabe. 1977. Virus isolation from sewage and the specimens from tertiary treatment. Proc. Osaka Pref. Inst. Public Health 15: 19–23 [In Japanese].

Ramos-Alvarez, M. and J. Olarte. 1964. Diarrheal diseases in childhood. Am. J. Dis. Child. 107: 218–231.

Sato, N., H. Sato, R. Kawana and M. Matumoto. 1972. Ecological behavior of 6 Coxsackie B and 29 ECHO serotypes as revealed by serologic survey of general population in Aomori, Japan. Jpn. J. Med. Sci. Biol. 25: 355–368.

Schreiber, D. S., N. R. Blacklow and J. S. Trier. 1974. The small intestinal lesion induced by Hawaii agent acute infectious nonbacterial gastroenteritis. J. Infect. Dis. 129: 705–708.

Steinhoff, M. C. 1978. Viruses and diarrhea—a review. Am. J. Dis. Child. 132: 302–307.

Toyoshima, K., B. Mitsuda, T. Yamada, M. Shioji, N. Kunita and S. Yakushigawa. 1964. Enterovirus survey in Osaka Prefecture from 1960 to 1963. Proc. Osaka Pref. Inst. Public Health 1: 56–59 [In Japanese].

Wyatt, R. G., A. Z. Kapikian, T. S. Thornhill, M. M. Sereno, H. W. Kim and R. M. Chanock. 1974. In vitro cultivation in human fetal intestinal organ culture of a reovirus-like agent associated with nonbacterial gastroenteritis in infants and children. J. Infect. Dis. 130: 523–528.

Yamazaki, K. and K. Otsu. 1978. Virus isolation from sewage. On their seasonal prevalencies. Proc. Osaka Pref. Inst. Public Health 16: 9–13 [In Japanese].

Yow, M. D., J. L. Melnick, R. J. Blattner, W. B. Stephenson, N. M. Robinson and M. A. Burkhardt. 1970. The association of viruses and bacteria with infantile diarrhea. Am. J. Epidemiol. 92: 33–39.