A Minor Prevalent Strain in a Severe Outbreak of Foal Diarrhea Associated with Serotype 3 Rotavirus

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ABSTRACT. An epizootic of foal diarrhea due to serotype 3 rotavirus (RV) was observed in 89 of 168 cases (53%) during the period from March to July in 1987. A total of 51 strains of RV were isolated from the 62 diarrheal feces examined, and one isolate (CH-3) showed a unique electropherotype of viral RNA which differed from the others that widely prevailed on this farm. No positive reaction was observed between strain CH-3 and each of the antisera against serotypes 1 to 12 of human and animal RV in neutralization tests. However, dsRNAs of the CH-3 virus were hybridized with a probe prepared from a strain of equine serotype 3 RV.—**KEY WORDS:** epizootic foal diarrhea, minor strain, rotavirus.

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Rotavirus (RV) has been recognized as a major agent causing acute diarrhea in the young of many mammals, including humans, and avian species [3, 4]. It is a well-known fact that the occurrence of RV infection in young animals is affected by such factors as the age, colostrum intake, antibody levels in the colostrum, stresses due to animal transfer, introduction of animals to the farm, hygienic conditions in farms, cold temperature, and relative humidity [6–8, 11, 14]. However, little is known about the ecological nature of RV in acute diarrhea in an animal herd. This paper is concerned with the characterization of a minor RV strain in an outbreak of foal diarrhea due mainly to serotype 3 of equine RV on a light horse farm in Hokkaido in 1987.

The light horse farm consisted of 3 foal-raising locations in the Iburi district of Hokkaido, where a total of 160 to 200 foals were born from February to June in each year. Epizootics of foal diarrhea due to serotype 3 RV were observed during the period from April to August in the years 1986 to 1989, with 40 to 75% incidence of the diarrhea. Isolation of RV was confirmed by the observation of cytopathic effects in cell culture and enzyme-linked immunosorbent assay [10]. Strains SH-31 (1986), SH-52 (1987), SH-86 (1988), and SH-98 and HH-22 (1989) were designated as representative strains in each epizootic.

In the present study, 60% plaque reduction neutralization (PRN) test [10], fluorescent focus neutralization (FFN) in 60% FF reduction [12], 10% polyacrylamide slab gel electrophoresis (PAGE) of viral RNA [5, 10] and RNA-RNA hybridization were performed as described previously [9, 13]. The strains of RV and its antisera used in the FFN tests were the same as those described elsewhere [10, 12]. Strain PO-13 (serotype 7) used in the RNA-RNA hybridization was supplied by courtesy of the Department of Veterinary Public Health, Faculty of Agriculture, Gifu University, Gifu.

In the outbreak in 1987, 51 strains of RV were isolated from the 62 diarrheal feces examined, and the viral RNA of the 51 isolates showed two electropherotypes by PAGE as reported in our previous study [10]. One type was dominant in the 49 strains which were represented by strain SH-52, and the other was observed only in the 2 strains designated as CH-2 and CH-3. The strain SH-31

(1986) showed a titer of 1:12,500 to anti-SH-52 serum with homologous titer of 1:9,000, and the strain SH-52, 1:31,500 to anti-SH-31 serum with 1:36,000, respectively, in the PRN test. The strain CH-3 also showed a titer of <1:100 to anti-SH-52, and 1:18,000 to the homologous atiserum. The electrophoretic pattern of viral RNA of the dominant strain, SH-52, was the same as that of the SH-31, and almost identical to those of the SH-86 (1988), SH-98 and HH-22 (1989) strains isolated on this farm (pictures not shown). The minor strains were isolated from only the feces collected in one of the 3 foal-raising locations. No positive reaction was observed between strain CH-3 and each of the 13 specific antisera against serotypes 1 to 12 of human and animal RV in FFN tests (Table 1).

Co-electrophoresis of strain CH-3 with strains SH-31 (1986) and HH-22 (1989) was performed (Fig. 1). The migration of CH-3 revealed considerable differences in segments 1, 4, 7, 8, 9, 10 and 11 from SH-31, and in segments 1, 3, 4, 5, 7, 8, 9, 10 and 11 from HH-22. RNA-RNA hybridization was carried out in order to examine the genome homology between strain CH-3 and

Table 1. Neutralization of the strain CH-3 of equine RV by FFR method with serial type specific antisera against human and animal RV

Type specific antisera (Serotypes)	Subgroups	FFR antibody titers in antisera to indicated RV	
		CH-3	homol. virus
(Human RV)			
Anti-K U (1)	II	<512	8,192
Anti-S 2 (2)	I	<512	8,192
Anti-Y O (3)	II	<512	16,384
Anti-Hochi (4)	II	<512	16,384
Anti-69M (8)	I	<512	16,384
Anti-WI61 (9)	II	256	32,768
Anti-L26 (12)	I	<512	16,384
(Animal RV)			
Anti-OSU (5)	I	256	65,536
Anti-NCDV (6)	I	<512	65,536
Anti-TY-1 (7)	I?	<512	16,384
Anti-KK3 (10)	I	256	32,768
Anti-61A (10)	I	256	32,768
Anti-Y M (11)	I	512	65,536

Fig. 1. Comparison of RNA electropherotypes between the minor (CH-3) and the dominant (SH-31 or HH-22) strains of RV by co-electrophoresis on a 10% polyacrylamide gel. RNAs from RV strains indicated above each lane were loaded onto the gel singly or mixed (+). Approximate positions of RNA segments of RV are indicated at the left.

other equine or animal RVs (Fig. 2). When dsRNAs of RV strains were hybridized with a probe prepared from strain HI-23 of equine serotype 3 RV [10], many hybrid bands were recognized for strains SH-31, CH-3 and HH-22. However, the probe produced only one band with the simian RV strain SA-11 and no hybrid band with the avian strain PO-13 which served as a negative control.

In conclusion, it must be pointed out that strain CH-3 was genotypically derived from equine RV. Serotyping of strain CH-3 remains to be determined, since two new serotypes of equine RV were reported recently [1, 2]. Although strain CH-3 temporarily invaded this light horse farm, the strain did not remain for a long time as compared with the dominant strains on the farm. The same finding was described as the occurrence of minor genomic variations of the virus at different places or times

Fig. 2. Hybridization patterns between genomic RNAs from RV strains indicated above each lane and the ^{32}P -labeled probe prepared from strain HI-23. The left 6 lanes of the picture show ethidium bromide-stained gel under UV light illumination; the right lanes the corresponding autoradiograms. RNA segments are indicated on the left.

in outbreaks of RV diarrhea in young children [9] and calves [13], although the major prevalence of the same genomic RV was observed during several outbreaks of diarrhea in these populations. For the strain CH-3, further studies on the antigenic and genetic analyses of virus are necessary and are now in progress.

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