JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1993, p. 3046–3049
0095-1137/93/113046-04$02.00/0

Vol. 31, No. 11

Relative Frequencies of G (VP7) and P (VP4) Serotypes Determined by Polymerase Chain Reaction Assays among Japanese Bovine Rotaviruses Isolated in Cell Culture

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Received 2 June 1993/Returned for modification 28 July 1993/Accepted 13 August 1993

The relative frequencies of both the G (VP7) and P (VP4) serotypes of 40 bovine rotaviruses isolated in cell culture from diarrheaic calves in Japan between January 1983 and February 1991 were determined by recently developed polymerase chain reaction assays. Isolates with G serotype 6 and P serotype 5 (UK-like strains) were most frequently found (42.5%) followed by isolates with G6P11 (17.5%), G6P1 (10%), or G10P5 (10%). Isolates with G10P11 (B223-like strains) were least frequently found (7.5%). The presence of various combinations of G and P serotypes suggests frequent reassortment in nature among bovine rotaviruses.

Group A rotaviruses, members of the genus *Rotavirus* in the family *Reoviridae*, have been implicated as the major infectious agents inducing diarrhea in young calves. The virions possess two outercapsid proteins, VP4 (encoded by gene segment 4) and VP7 (encoded by gene segment 7, 8, or 9 depending on the strain) (3). Both proteins are involved in virus neutralization (9, 19). The neutralization specificity carried on VP7 is referred to as the G serotype (for glycoprotein), and that carried on VP4 is referred to as the P serotype (for protease-sensitive protein) (3).

There are currently 14 G serotypes (2) and 12 P serotypes (3, 6, 7, 22) among mammalian and avian rotaviruses. Among group A bovine rotaviruses, four G serotypes (G1, G6, G8, and G10) are present, and G6 and G10 have been shown to predominate in cattle (23). The G serotype 1, which has been almost exclusively found in human rotaviruses, has recently been described for bovine rotavirus on the basis of serologic and molecular characterization of an Argentine strain, T449 (1). As for the P serotype, Matsuda et al. (14) have discriminated by cross-neutralization assays with reassortants three P serotypes, which they tentatively designated PB1 (for bovine P serotype 1), PB2, and PB3. Serotype PB1 is carried by strains NCDV and C486 and corresponds to P1 according to the numbering system described by Estes and Cohen (3). Serotype PB2 is carried by strains UK, B641, and 0510 and corresponds to P5 of the Estes and Cohen system. Serotype PB3 is carried by strains B223, KK-3, and B11 and has recently been proposed to be designated P11 (24). Although conventional wisdom is that the serotype defined by plaque-reduction neutralization assays with hyperimmune antisera primarily reflects the antigenicity of the VP7 protein (3), recent studies have revealed the importance of not only VP7 but also VP4 in mediating complex antigenic relationships among some natural isolates of bovine rotaviruses (12, 24). Consequently, these studies call for the adoption of a binary system to describe bovine rotavirus serotypes.

Although the primary definition of any serotype must be based on serological reactions (24), nucleic acid probes and polymerase chain reaction (PCR)-based assays have become widely accepted as surrogates for the identification of G and P serotypes. Thus, Parwani et al. (20, 21) developed cDNA probe methods for the determination of bovine rotavirus G and P serotypes. On the basis of the same nested-PCR strategy taken by Gouvea et al. (5) to develop a PCR-based G-typing method for human rotaviruses, we have recently developed PCR-based G- and P-typing assays for bovine rotaviruses (10). By using these assays we have determined the relative frequencies of both G and P serotypes of Japanese bovine rotavirus strains isolated in cell culture.

A total of 40 rotavirus field isolates used in this study were obtained as diarrhea survey samples from calves in several farms breeding a small number of cattle in Tochigi prefecture, Japan, between January 1983 and February 1991. They were grown in MA104 cells in the presence of 0.5 μg of trypsin per ml. Their genomic RNAs were extracted with phenol-chloroform from the infected cell culture harvest, which was concentrated for virus particles by centrifugation for 1 h at 80,000 rpm in a Beckman's 100.3 rotor.

Genomic RNAs from each of the 40 field rotaviruses were analyzed on 10% polyacrylamide gels by the method described previously (18).

The detailed protocol for the G- and P-typing method used in this study has been described elsewhere (10). The G and P types of a given strain were determined by two independent nested PCR assays. In brief, the G-typing assay consisted of three steps: (i) reverse transcription of genomic RNAs with a pair of generic primers (Bov9Com5 and Bov9Com3), (ii) the first PCR amplification of a nearly full-length VP7 gene with Bov9Com5 and Bov9Com3, and (iii) the second (nested) PCR amplification with the 5′ generic primer (Bov9 Com5) and a cocktail of typing primers specific for G6 and G10. Since these two typing primers were selected such that each primer is located at a different distance from the 5′ end of the gene, the size of the second PCR product indicated the G type of the strain tested. The P-typing assay consisted of essentially the same three steps with two generic primers

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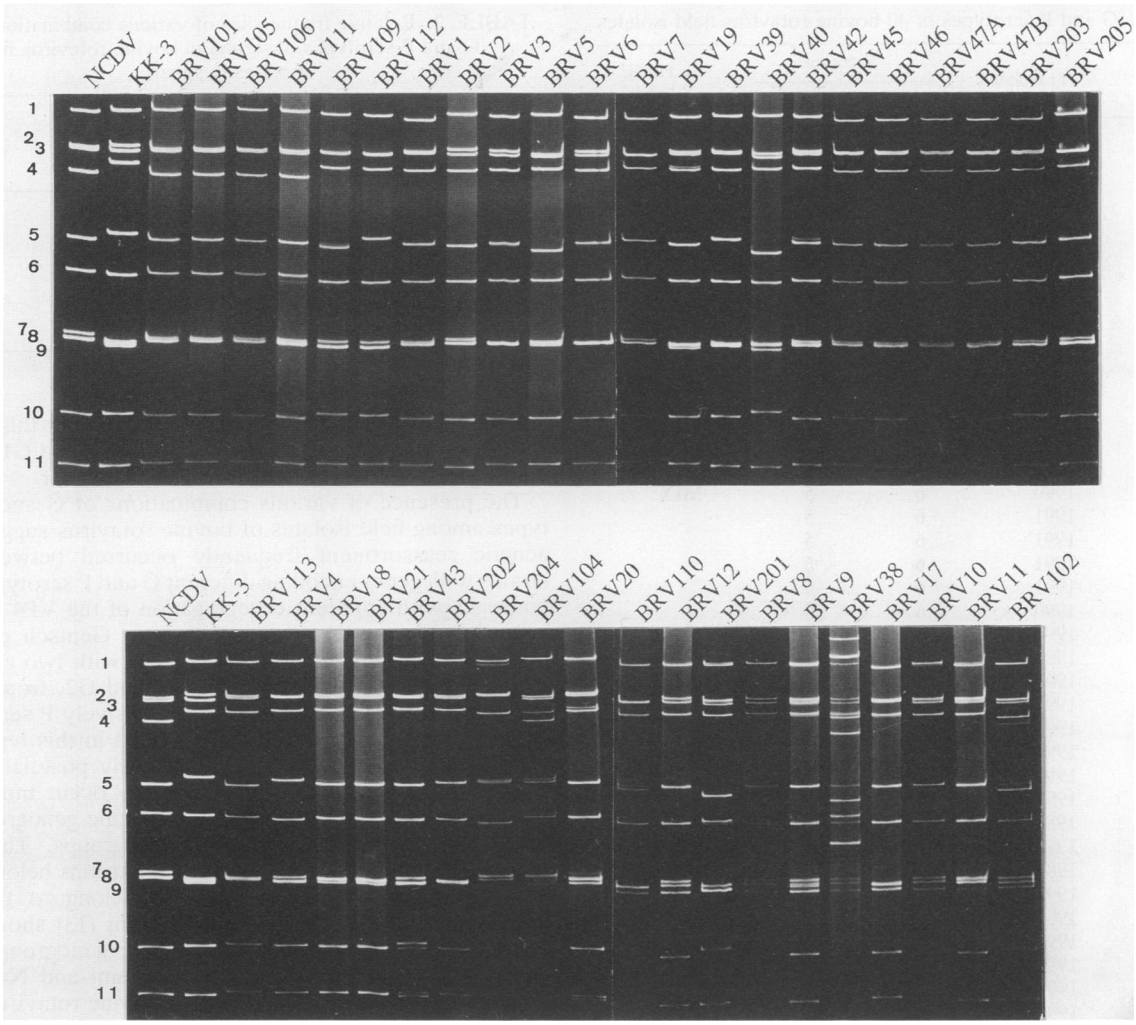

FIG. 1. Electropherotypes of 40 field bovine rotavirus isolates in reference to prototype strains NCDV and KK-3.

(Bov4Com5 and Bov4Com3) and three typing primers specific for P1 (PB1), P5 (PB2), and P11 (PB3). In this assay, the size of the second PCR product indicated the P type of the strain tested.

Figure 1 shows the electropherotype of each of 40 field isolates in reference to prototype strains NCDV (G6P1) and KK-3 (G10P11). While these electropherotypes were similar in that all belonged to long RNA patterns, two distinct migration patterns of gene segment 4 were noticed. A fast-migrating gene segment 4 resembling the one carried by strain NCDV was found in BRV101, BRV105, BRV106, and BRV111. Interestingly, these four strains possess serotype P1 (PB1) (Table 1). A slowly migrating gene segment 4, resembling the one carried by KK-3, was observed in all other strains. Of added interest was the finding that more than 11 gene segments were observed in 16 isolates (40%), suggesting that calves were frequently infected with multiple strains of rotaviruses. An RNA pattern characteristic of reovirus (in terms of both the relative positions and the number of gene segments) was superimposed on the rotavirus RNA pattern in the lane with BRV38 (Fig. 1), indicating that the calf from which BRV38 was isolated was also infected with a reovirus.

Tables 1 and 2 show the result of the G and P type analysis of 40 field isolates of bovine rotaviruses. The PCR-based typing assays described in this paper identified G types in 95% of the isolates, P types in 92.5% of the isolates, and both G and P types in 87.5% of the isolates. The P type was not determined for BRV104, BRV17, and BRV20 because of the appearance of two bands after the second PCR amplification. BRV104 produced two bands corresponding to P1 and P11, and both BRV17 and BRV20 produced two bands corresponding to P5 and P11. These typing results were apparently consistent with the mixed electropherotypes of these three isolates; i.e., BRV104 contained a fast-migrating and a slowly migrating gene segment 4, whereas both BRV17 and BRV20 contained a mixture of two different slowly migrating gene segments 4 (Fig. 1). Similarly, the G type was not determined for BRV11 and BRV102 because of the appearance of two bands corresponding to G6 and G10. Although both isolates contained more than one strain of rotavirus upon polyacrylamide gel electrophoresis, the presence of two VP7 genes was not clear because of closely migrating gene segments 7, 8, and 9 (Fig. 1).

The most frequently found combination of the G and P serotypes was G6P5, and these UK-like strains accounted for 42.5% of the isolates. This combination was followed in frequency by the combination of G6 and P11, accounting for

TABLE 1. G and P serotypes of 40 bovine rotavirus field isolates

Isolate	Yr isolated	Serotype		PAGE result[a]
		G	P	
BRV101	1985–1986	6	1	
BRV105	1983	6	1	
BRV106	1983	6	1	
BRV111	1986	6	1	MIX
BRV109	1986	6	5	MIX
BRV112	1986	6	5	
BRV1	1991	6	5	
BRV2	1990	6	5	
BRV3	1990	6	5	
BRV5	1990	6	5	
BRV6	1990	6	5	
BRV7	1990	6	5	
BRV19	1991	6	5	MIX
BRV39	1991	6	5	
BRV40	1991	6	5	
BRV42	1990	6	5	MIX
BRV45	1991	6	5	
BRV46	1991	6	5	
BRV47A[b]	1991	6	5	
BRV47B[b]	1991	6	5	
BRV203	1990	6	5	
BRV205	1990	6	5	
BRV113	1986	6	11	
BRV4	1990	6	11	
BRV18	1991	6	11	MIX
BRV21	1991	6	11	
BRV43	1990	6	11	MIX
BRV202	1990	6	11	
BRV204	1990	6	11	MIX
BRV104	1983	6	1, 11[c]	MIX
BRV20	1991	6	5, 11[c]	MIX
BRV110	1986	10	5	MIX
BRV12	1991	10	5	
BRV201	1989	10	5	MIX
BRV8	1991	10	11	
BRV9	1991	10	11	MIX
BRV38	1991	10	11	+ reovirus
BRV17	1991	10	5, 11[c]	MIX
BRV10	1991	10	5	MIX
BRV11	1991	6, 10[c]	5	MIX
BRV102	1983	6, 10[c]	11	MIX

[a] PAGE, Polyacrylamide gel electrophoresis. MIX indicates the presence of more than 11 gene segments.

[b] These two strains originated from different starting materials from the same animal. They were regarded as a single strain in this study.

[c] There were two bands whose migrations upon agarose gel electrophoresis were compatible with the sizes expected for strains carrying the two serotypes listed.

TABLE 2. Relative frequencies of various combinations of the G and P serotypes observed in bovine rotavirus field isolates in Japan

Serotype	No. of isolates (%) identified as indicated			Total no. of isolates (%)
	G6	G10	Untypeable	
P1	4 (10.0)	0 (0)	0 (0)	4 (10.0)
P5	17 (42.5)	4 (10.0)	1 (2.5)	22 (55.0)
P11	7 (17.5)	3 (7.5)	1 (2.5)	11 (27.5)
Untypeable	2 (5.0)	1 (2.5)	0 (0)	3 (7.5)
Total	30 (75.0)	8 (20.0)	2 (5.0)	40 (100)

17.5% of the isolates. B223-like strains (G10P11) were least frequently found (7.5%). Although variable combinations of the G and P serotypes were encountered, the combination of G10P1 was not found among Japanese bovine rotaviruses. The possibility of a selection bias during cell culture adaptation could not be excluded because certain strains are known to be more difficult than others to adapt to growth in cell culture (23).

The presence of distinct P types within the same G serotype (G6) has been documented for laboratory strains of bovine rotavirus. Both strains NCDV and UK possess G serotype 6 while their P serotypes are different (P1 and P5, respectively) (11). Strain KN-4 has recently been shown to possess P11 (PB3), while its G serotype is 6 (12). This strain has captured attention because KN-4 (G6P11) and KK-3 (G10P11) exhibited two-way cross neutralization despite distinctness in their G serotypes (12). Whether this unusual antigenic relationship holds true for all G6 and G10 strains bearing P11 has yet to be determined, however.

The presence of various combinations of G and P serotypes among field isolates of bovine rotavirus suggests that genetic reassortment frequently occurred between viral strains with genes encoding different G and P serotypes. This contrasts with apparent cosegregation of the VP4 and VP7 genes observed in human rotaviruses. Gentsch et al. (4) observed that human rotavirus isolates with two epidemiologically important G serotypes, G1 and G2, from several parts of the world possess almost exclusively P serotypes 8 and 4, respectively. It deserves mention in this regard that Nakagomi and Nakagomi (17) previously postulated a hypothesis that genetic reassortment may occur much more frequently between the strains of the same genogroup than between the strains of different genogroups. They have shown that, with a few exceptions, G1 strains belong to the Wa genogroup whereas G2 strains belong to the DS-1 genogroup (16). Matsuda and Nakagomi (13) showed that bovine rotaviruses constitute a single genogroup. Thus, according to the hypothesis of Nakagomi and Nakagomi, mixed infections with two strains of bovine rotavirus would more frequently result in the emergence of viable reassortants than mixed infections with two strains of human rotaviruses belonging to different genogroups. The apparent random segregation of the G and P serotypes of bovine field isolates observed in this study is consistent with the aforementioned hypothesis. It has become clear that the VP4 protein plays an important role in virus neutralization and resistance to disease (8, 15, 25). Thus, the currently available bovine rotavirus vaccine strain (NCDV) may not be an ideal one because its P serotype (P1) is different from the P serotype most frequently found among field isolates (P5) and because, through genetic reassortment, new strains bearing the combination of the G and P serotypes that can escape from the vaccine-induced herd immunity are likely to emerge and prevail among calves in nature. Future and ongoing vaccine programs should therefore take these points into consideration.

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