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Virus Research 114 (2005) 167–171
Virus Research
www.elsevier.com/locate/virusres

Short communication

Serological characterization of novel P11[14],G8 bovine group A rotavirus, Sun9, isolated in Japan

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Received 26 January 2005; received in revised form 19 May 2005; accepted 19 May 2005
Available online 5 July 2005

Abstract

In this study, a novel bovine group A rotavirus (BoRV-A), Sun9, isolated from calf diarrhea in Tochigi Prefecture, Japan, was serologically characterized by a cross-neutralization assay, and serological surveillance by using its reassortant was performed on cattle bred in Japan. The G serotype of Sun9 was identified as G serotype 8 based on the one- or two-way serological relationships observed in Sun9 and other G8 strains. The P serotype of Sun9 was identified as P serotype 11 based on the one- or two-way serological relationships observed in Sun9, its reassortants, and the P11 lapine group A rotavirus R-2. The serological surveillance data indicated that 2.4% of the specimens appeared to possess antibodies against the P11[14] antigen. Few P11[14] bovine group A rotaviruses may exist in the Japanese cattle population.

A bovine group A rotavirus (BoRV-A) is an important pathogen of neonatal calf diarrhea (Suzuki et al., 1993; Ishizaki et al., 1996; Fukai et al., 1998a; Falcone et al., 1999; Alfieri et al., 2004). BoRV-A possesses two independent neutralization antigens VP4 and VP7 on its outer capsid, and they establish P type (for protease-sensitive protein) and G type (for glycoprotein), respectively (Estes, 2001). To date, at least six P (P6[1], P7[5], P8[11], P[14], P[17], and P[21]) and 10 G (G1, G2, G3, G5, G6, G7, G8, G10, G11, and G15) types have been reported among BoRV-A (Matsuda et al., 1990; Blackhall et al., 1992; Brüssow et al., 1992; Hüssein et al., 1993; Isegawa et al., 1994; Fukai et al., 1999, 2002, 2004a; Rao et al., 2000; Alfieri et al., 2004). However, studies on their distribution have demonstrated that P7[5], P8[11], G6, and G10 are the most commonly occurring types (Ishizaki et al., 1996; Fukai et al., 1998a; Falcone et al., 1999; Okada and Matsumoto, 2002; Alfieri et al., 2004). G8 was the third most common G type among BoRV-A, and many G8 strains reported to date have been detected and/or isolated sporadically (Taniguchi et al., 1991; Parwani et al., 1993; Sato et al., 1997; Falcone et al., 1999; Okada and Matsumoto, 2002). The P11[14] human group A rotavirus (HuRV-A) and the lapine group A rotavirus (LaRV-A) have been reported sporadically in various districts of the world (Sato et al., 1982; Thouless et al., 1986; Gerna et al., 1992, 1994; Urasawa et al., 1992; Palombo and Bishop, 1995; Palombo et al., 2000; Ciarlet et al., 1997; Holmes et al., 1999; Mphahlele et al., 1999; Cooney et al., 2001; Banyai et al., 2003; Martella et al., 2003). On the other hand, we have previously isolated a novel P[14],G8 BoRV-A, Sun9, from calf diarrhea in Japan and genetically characterized its genome segments that encoded the proteins VP4 and VP7 (Fukai et al., 2004a). However, the serological characterization of Sun9 has not yet been performed, and the distribution of P[14] BoRV-A in a field has not yet been examined. The purpose of this study was to serologically characterize the novel P[14],G8 BoRV-A Sun9 by cross-neutralization assay and to perform serological surveillance by using its reassortant on the cattle bred in Japan.

Sun9 was isolated from diarrheal calf feces in Tochigi Prefecture, Japan, as described previously (Fukai et al., 2004a). Table 1 summarizes the reference strains as well as their P and G type specificities employed in this study. Sun9 and the reference strains were propagated and plaque-purified three times in MA-104 cells in the presence of trypsin (Matsuda et al., 1990). Antisera were raised in guinea pigs or mice by using

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doi:10.1016/j.virusres.2005.05.009

Table 1
Serological characterization of group A rotavirus strains by fluorescent focus reduction neutralization assay

Strain	Origin	P type	G type	Reciprocal of neutralization of antiserum to										
				Sun9 (P[14],G8)	BRV16 (P6[1],G8)	Tokushima9503 (P[11],G8)	Niigata9801 (P[14]-like,G8)	NCDV (P6[1],G6)	KK3 (P8[11],G10)	S9Wa-6/3/1 (P[14],G1)	Hochi (P1A[8],G4)	SA11 (P5B[2],G3)	OSU (P9[7],G5)	R-2 (P11[14],G3)
Sun9	Bovine	[14]	8	**800**[a]	800	1600	3200	1600	<100	800	<100	100	<100	400
BRV16	Bovine	6[1]	8	100	**3200**	1600	400	800	<100	<100	NT[b]	NT	NT	NT
Tokushima9503	Bovine	8[11]	8	800	800	**6400**	800	100	400	<100	NT	NT	NT	NT
Niigata9801	Bovine	[14]-like	8	200	800	800	**1600**	<100	<100	100	NT	NT	NT	200
NCDV	Bovine	6[1]	6	100	1600	200	<100	**6400**	<100	<100	NT	NT	NT	NT
UK	Bovine	7[5]	6	<100	200	<100	<100	800	<100	<100	NT	NT	NT	NT
KK3	Bovine	8[11]	10	<100	<100	6400	<100	<100	**3200**	<100	NT	NT	NT	NT
S9Wa-6/3/1	Reassortant	[14]	1	200	<100	100	400	<100	<100	**800**	<100	<100	<100	400
S9Wa-18/2/2	Reassortant	[14]	1	200	<100	<100	400	<100	<100	400	<100	<100	<100	200
S9Wa-3/1/1	Reassortant	[14]	1	400	<100	<100	400	<100	<100	400	<100	<100	<100	200
Wa	Human	1A[8]	1	<100	NT	NT	NT	NT	NT	200	200	NT	NT	NT
YO	Human	1A[8]	3	<100	NT	NT	NT	NT	NT	<100	100	1600	NT	800
Hochi	Human	1A[8]	4	<100	NT	NT	NT	NT	NT	<100	**3200**	NT	NT	NT
F45	Human	1A[8]	9	<100	NT	NT	NT	NT	NT	<100	<100	NT	NT	NT
S2	Human	1B[4]	2	<100	NT	NT	NT	NT	NT	<100	NT	NT	NT	NT
L26	Human	1B[4]	12	<100	NT	NT	NT	NT	NT	<100	NT	NT	NT	NT
69M	Human	4[10]	8	400	800	800	800	NT	NT	<100	NT	NT	NT	NT
HI23	Equine	4[12]	3	<100	NT	NT	NT	NT	NT	<100	NT	6400	NT	800
SA11	Simian	5B[2]	3	<100	NT	NT	NT	NT	NT	<100	NT	**6400**	NT	800
OSU	Porcine	9[7]	5	<100	NT	NT	NT	NT	NT	<100	NT	NT	**6400**	NT
R-2	Lapine	11[14]	3	100	NT	NT	400	NT	NT	100	NT	1600	NT	**3200**

[a] Homologous values are shown in boldface type.
[b] Not tested.

Sun9, BRV16, NCDV, KK3, and S9Wa-6/3/1 with the aid of Freund's adjuvant. The guinea pigs and mice were determined to be free of BoRV-A neutralizing antibodies by fluorescent focus neutralization (FFN) assay. The FFN assay was performed as described previously (Knowlton et al., 1991). The antibody titer was expressed as the reciprocal of the antiserum dilution ratio. The antisera were inactivated before use by heating at 56 °C for 30 min. The inactivated antisera were serially double diluted (1:100–1:12800). The antisera for Tokushima9503, Niigata9801, Hochi, SA11, OSU, and R-2 were kindly supplied by Dr. Nobutaka Okada (Division of Veterinary Microbiology, Kyoto Biken Laboratories, Kyoto, Japan) and Dr. Hiroshi Imagawa (Epizootic Research Station, Equine Research Institute, Japan Racing Association, Tochigi, Japan). Reassortant viruses between Sun9 and Wa were generated as described previously (Hoshino et al., 1998). Viral genomic double-stranded RNA (dsRNA) was extracted from the plaque-purified viruses by using TRIzol reagent (Invitrogen) base on the instructions provided by the supplier. The plaque-purified reassortant viruses were examined for P and G types by RT-PCR by using type-specific primers (Gouvea et al., 1990, 1993, 1994).

We generated three Sun9 × Wa reassortant viruses (S9Wa-6/3/1, S9Wa-18/2/2, and S9Wa-3/1/1). Each of the three reassortant viruses was confirmed by RT-PCR to carry P[14] and G1 specificities (data not shown).

Serum specimens were obtained from blood collected between April and December, 2003 from 167 individual cattle bred in 29 cattle farms in 16 cities within Tochigi Prefecture, Japan. Among the tested cattle, 43 were beef cattle and 124 were dairy cattle. Serum specimens were collected from 1 to 27 cattle in each farm. The cattle were below 8 years of age. Tochigi Prefecture is located in the northeastern part of Japan and is an eminent area for the production of dairy cattle in Japan. The antibody titers of these specimens were determined by FFN assay by using S9Wa-6/3/1 and Wa in separate assays. They were statistically analyzed as described previously (Fukai et al., 1998b).

The serological relationships between Sun9 and the reference strains are listed in Table 1. Hoshino and Kapikian (1996) established P and G serotypes based on the criterion of a 20-fold or greater difference between homologous and heterologous neutralizing antibody titers. However, in the FFN assay performed in this study, the antiserum against BRV16 (P6[1],G8) neutralized the VP4- and VP7-heterotypic UK (P7[5],G6) to a titer 16-fold lower than that of the homologous BRV16. Therefore, in this study, a 16-fold or greater difference was considered to distinguish between the P and G serotypes.

The antiserum against Sun9 neutralized the VP4-heterotypic BRV16, Tokushima9503, and 69M to titers that were between eight-fold lower and same than that of the homologous Sun9. The antisera against BRV16 and Tokushima9503 neutralized Sun9 to a titer four-fold lower than those of the homologous strains. BRV16, Tokushima9503, and 69M have been identified as G serotype 8 (Matsuno et al., 1985; Sato et al., 1997; Fukai et al., 2004b). The G serotype of Sun9 was identified as G serotype 8 based on the one- or two-way serological relationships observed in this study in Sun9, BRV16, Tokushima9503, and 69 M.

Hoshino et al. (2002) proposed that the P serotype of the strains bearing the P[14] genotype should be assigned as the P11 serotype. The antisera to Sun9 and S9Wa-6/3/1 neutralized the VP7-heterotypic R-2 to a titer eight-fold lower than those of the homologous strains; the antiserum to R-2 neutralized Sun9, S9Wa-6/3/1, S9Wa-18/2/2, and S9Wa-3/1/1 to titers that were between 8- and 16-fold lower than that of the homologous R-2. R-2 has been identified as P serotype 11 (Hoshino et al., 2002). In this study, the P serotype of Sun9 was identified as P serotype 11 based on the one- or two-way serological relationships found in Sun9, its reassortants, and R-2.

Okada and Matsumoto (2002) have reported that the partial nucleotide (nt) and deduced amino acid (aa) sequences of the genome segment encoding VP4 of BoRV-A Niigata9801 showed high identity with P[14] HuRV-A HAL1166 and PA169 (86.9 and 85.3% at the nt level and 86.8 and 86.7% at the aa level). However, they have not classified Niigata9801 into P[14] because P genotypes tentatively show a VP4 aa sequence identity that is greater than 89% (Gorziglia et al., 1990). Thus far, the nt and aa sequences of the genome segment encoding the VP4 of Niigata9801 have not been entered in the databases, and a further characterization of its genome segment encoding VP4 has not been published. Therefore, the genome segment encoding VP4 of Niigata9801 could not been compared with that of Sun9. In contrast, the antisera against Sun9, S9Wa-6/3/1, and R-2 neutralized Niigata9801 to titers that were between 4- and 16-fold lower than those of the homologous strains, while the antiserum against Niigata9801 neutralized Sun9, S9Wa-6/3/1, S9Wa-18/2/2, S9Wa-3/1/1, and R-2 to titers that were between four-fold lower and two-fold higher than those of the homologous Niigata9801. R-2 has been identified as P serotype 11 (Hoshino et al., 2002), and Sun9 was identified as P serotype 11 in this study. These results suggest that Niigata9801 VP4 is serologically related to P serotype 11 VP4 based on the one- or two-way serological relationships observed in Niigata9801, Sun9, its reassortants, and R-2.

Interestingly, the antiserum against Sun9 neutralized the VP4- and VP7-heterotypic NCDV to a titer that was eight-fold lower than that of the homologous Sun9, while the antiserum against NCDV neutralized Sun9 to a titer that was four-fold lower than that of the homologous NCDV. On the other hand, the antiserum against S9Wa-6/3/1 did not neutralize VP4- and VP7-heterotypic NCDV, and the antiserum against NCDV did not neutralize S9Wa-6/3/1, S9Wa-18/2/2, and S9Wa-3/1/1. These results suggest that Sun9 VP7 is serologically related to G serotype 8 VP7 and G serotype 6 VP7. Nagesha et al. (1990) have reported that the porcine group A rotavirus MDR-13 exhibited cross reactivity against G3 strains and G5 strains in the neutralization assay and that its strain possessed dual G serotype specificity. Timenetsky et

Table 2
Distribution of fluorescent focus neutralization antibody titers to S9Wa-6/3/1 and Wa rotavirus strains among 167 cattle in Tochigi Prefecture, Japan

Antibody titers to Wa	Antibody titers to S9Wa-6/3/1							Total
	<100	100	200	400	800	1600	3200	
<100	51	22	11	3	*2*[a]	*1*	*1*	91
100	4	9	12	5	2			32
200	1	4	10	7	3	1		26
400		1	7	2	2			12
800							1	1
1600						1	3	4
3200							1	1
Total	56	36	40	17	9	3	6	167

[a] Specimens in which the difference between anti-S9Wa-6/3/1 antibody titer and anti-Wa antibody titer was 16-fold or greater are shown in italics.

al. (1997) have also reported that HuRV-A IAL28 possessed dual G5 and G11 specificities. Similarly, Sun9 may be a strain with dual G serotype specificities, G6 and G8. Further characterization of Sun9 VP7 is required to be performed in the future.

In the serum survey, anti-S9Wa-6/3/1 and Wa antibody titers ranged from <100 to 3200 among the 167 field specimens, and 111 (66.5%) and 76 (45.5%) specimens possessed anti-S9Wa-6/3/1 and Wa antibodies, respectively (Table 2). The difference between the anti-S9Wa-6/3/1 and anti-Wa antibody titers of 163 (97.6%) specimens was eight-fold or lower. The distribution of anti-S9Wa-6/3/1 and Wa antibodies of these 163 specimens could be represented by the single regression line $Y = 0.641X - 0.004$ (correlation coefficient: 0.749, $p < 0.01$). Therefore, a majority of the anti-S9Wa-6/3/1 and Wa antibodies detected in this study appeared to be against the G1 antigen. G1 BoRV-A T449 has been isolated only in Argentina (Blackhall et al., 1992). These results suggest that G1 BoRV-A may moderately exist in the Japanese cattle population. The existence of G1 BoRV-A should to be considered in future studies on the distribution of the G types of BoRV-A field isolates.

In contrast, the difference between the anti-S9Wa-6/3/1 and anti-Wa antibody titers of 4 (2.4%) of the 167 specimens was 16-fold or greater. Therefore, these specimens appeared to possess antibodies against the P11[14] antigen. In a previous study, we have hypothesized that P[14],G8 BoRV-A might exist in the cattle population (Fukai et al., 2004a). These results suggest that few P11[14] BoRV-A exist in the Japanese cattle tested from Tochigi Prefecture, Japan.

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