Isolation of a Serotype G6P11 Bovine Rotavirus Showing Two-Way Cross-Neutralization with the Serotype G10P11 Virus

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ABSTRACT. Four strains designated as OB94–1 to OB94–4 of group A bovine rotavirus (BRV) were isolated from 35 fecal samples of calves with diarrhea in sporadic outbreaks. In VP7 (G) and VP4 (P) serotyping of these isolates, OB94–1 to OB94–3 were determined as G6P5, G6P5 and G10P5, respectively, by cross neutralization (NT) test and the G- and P- serotyping polymerase chain reaction (PCR) analysis. OB94–4 showed a one-way antigenic relation with the Lincoln strain (G6P1) and a weak antigenic relationship with the KK3 strain (G10P11), and was determined as G6P11 by the PCR method. Thus, OB94-4 was shown to be a new G6 BRV with different antigenic properties from the others in the NT test.—KEY WORDS: bovine rotavirus, diarrhea, serotype.

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Group A rotavirus is the most frequent cause of diarrhea in young mammals and birds [28, 29]. A virion consists of two layers of capsid enclosing 11 segmented double strand RNA genomes. Outer capsid proteins VP4 (encoded in gene 4) and VP7 (encoded in gene 7, 8 or 9 depending on the strain) are involved in virus neutralization (NT). Recently rotaviruses have been classified by using VP4 (P serotype) and VP7 (G serotype) serotyping [7, 14, 29]. To date, group A rotaviruses have been shown to have 14 G [1, 3, 4, 7] and 12 P [7–9, 21] serotypes. Four serotypes (G1, G6, G8 and G10) [2, 6, 23, 27] of group A bovine rotavirus (BRV) have been confirmed so far. G6 and G10 of them are predominant among cattle populations [5, 11, 18, 19, 23].

Diarrheal feces were obtained from 35 dairy and beef calves aged between 7 days and 4 months in 7 herds from February to September, 1992 in Okayama Prefecture. A 10% emulsion of the diarrheal feces was centrifuged and filtrated through a 450 nm pore sized membrane, and a supernatant was inoculated in Vero cells according to the method described by Sanekata *et al.* [22]. We used Rotalex dry (Daiichi Kagaku, Japan) to detect BRV antigen from the fecal specimens and infectious fluids in cell cultures. The isolates were purified by either the plaque formation method described by Matsuno *et al.* [16] or the terminal dilution method.

The Lincoln strain (G6P1) [17, 24] and the KK3 strain (G10P11) [20, 23, 24] were used as the reference strains of BRV in this study.

A hyperimmune antiserum to each isolate was prepared according to the method described by Sanekata *et al.* [22]. An immune serum to each reference strain of BRV was kindly supplied by Dr. Hiroshi Tsunemitsu, Ohio Agricultural Research and Development Center.

By the latex agglutination test, four fecal specimens collected in three herds showed positive in the agglutination. Four strains of BRV (OB94–1 to OB94–4) were isolated from three positive specimens and one negative specimen in the agglutination. Paired sera collected from the BRV-positive calves were tested for neutralizing antibodies to the isolates and the reference strains of BRV to confirm the infection of the isolates. A significant increase in neutralizing antibodies to the homologous isolate and the Lincoln strain was observed on the paired sera from OB94–1 and OB94–2-isolated calves. On the other hand, OB94–4-isolated calf showed a four times increase in the antibodies to the homologous isolate and the KK3 strain, respectively, but not to the Lincoln strain (data not shown).

To confirm that the isolates really came from the diarrheal feces, polyacrylamide gel electrophoresis (PAGE) was performed on viral RNA extracted from the isolate-infected culture fluid and its original diarrheal feces. Both samples from the virus-isolated calves, except one from which OB94–3 was isolated, had 11 segments with exactly the same electrophoretic RNA pattern (Fig. 1). Although RNA extracted from the diarrheal feces in which OB94–3 was isolated had more than 11 bands, showing mixed-infection by two or more viruses, only one BRV strain has been isolated. The RNA-PAGE pattern of OB94–4 was similar to those of OB94–3 and the KK3 strain rather than OB94–1, OB94–2 and the Lincoln strain (data not shown).

To determine the G serotype of isolates, cross NT test was performed with antisera against the isolates, the Lincoln strain (G6) and the KK3 strain (G10) by the method of Murakami *et al.* [18]. The results of the cross NT test are shown in Table 1. There is a close antigenic relationship among the Lincoln strain, OB94–1 and OB94–2, and between the KK3 strain and OB94–3. OB94–1 and OB94–2 were thus serotyped as G6 and OB94–3 as G10. OB94–4, however, was observed to have a one-way antigenic relation with the Lincoln strain and a week cross relation with the KK3 strain.

To analyse these unique serological characteristics, G and P serotypes were determined by the polymerase chain reaction (PCR) method described by Isegawa *et al.* [12]. G serotypes of OB94–1 to OB94–4 were determined as G6, G6, G10 and G6, respectively, by using G6- and G10-specific primers [12]. Moreover, from the result of PCR amplification with P1-, P5- and P11- specific primers [12], OB94–1 to OB94–4 were determined as P5, P5, P5 and P11, respectively (Fig 2).

Serological characterization of rotavirus reveals that VP4 and VP7 independently induce type-specific neutralizing antibodies, but serotypes are largely defined on VP7 [7, 13, 26, 30]. Recently G6P11 BRV was frequently

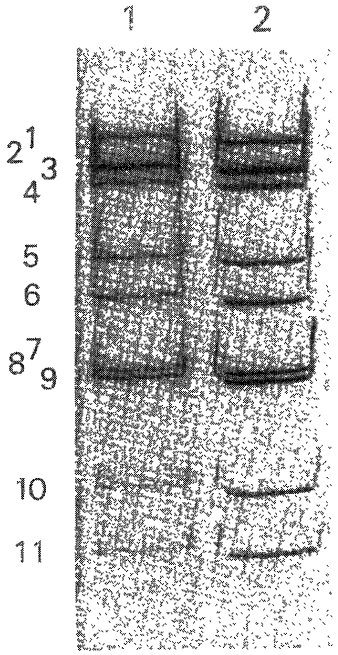

Fig. 1. The electrophoretic pattern of viral RNA extracted from the original fecal specimen (Lane 1) and the present isolate OB94–4 (Lane 2).

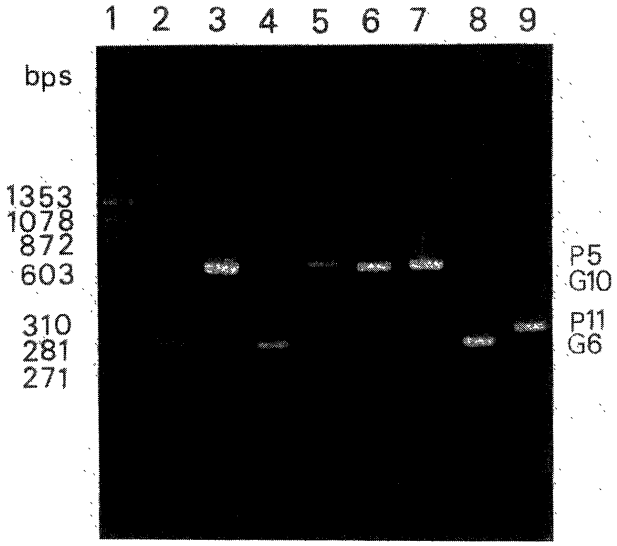

Fig. 2. Identification of the G-and P-serotypes of the present 4 isolates by the G-and P-serotyping PCR method. The PCR products were separated on a 2% agarose gel and stained with ethidium bromide. Lane 1 shows size markers (Hae III-digested øX174 DNA; marker molecular sizes are indicated on the left in base pairs). Lanes 2, 4, 6 and 8 reveal G-typing of the isolates (lane 2, OB94-1; lane 4, OB94-2; lane 6, OB94-3; lane 8, OB94-4). Lanes 3, 5, 7 and 9 reveal P-typing of the isolates (lane 3, OB94–1; lane 5, OB94–2; lane 7, OB94–3; lane 9, OB94–4).

Table 1. Serological characterization of 4 bovine rotavirus field isolates by cross-neutralization test

Strain (serotype)	Neutralizing antibody titer of antiserum to					
	OB94-1	OB94-2	OB94-3	OB94-4	Lincoln	KK-3
OB94-1	2,560	2,560	20	<20	1,280	<20
OB94-2	1,280	1,280	80	<20	1,280	<20
OB94-3	40	<20	2,560	<20	<20	2,560
OB94-4	2,560	1,280	<20	1,280	2,560	320
Lincoln (G6P1)	1,280	1,280	<20	<20	2,560	<20
KK3 (G10P11)	<20	<20	2,560	1,280	<20	≧2,560

found among field isolates in Japan [25], and an isolate with G6P11 showed two-way cross NT not only with the Lincoln strain but also the KK3 strain [15]. Although the serotype of OB94–4 was determined as G6P11, antiserum to OB94–4 could not neutralize the Lincoln strain (G6P1), but neutralized the KK3 strain (G10P11) strongly. This suggests that the antiserum to OB94–4 reacted with the VP4 antigen rather than VP7. Urasawa *et al.* [30] and Hoshino *et al.* [10] reported that VP4 antigenicity of gene reassortant is considerably stronger than that of field isolates. OB94–4 might be a naturally occurring reassortant. Even so, the reason why the VP4 antigen of OB94–4 is more active than VP7 is still unknown. To analyse the antigenic function of VP4, further analysis of OB94–4 will be needed.

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