# Bovine rotavirus type detection
# by neutralizing monoclonal antibodies

## Brief Report

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**Summary.** A series of monoclonal antibodies were developed against bovine rotavirus $Q_{17}$. Among the five high affinity antibodies characterized, two (RQ 31 and RQ 4) were able to neutralize type G 6 viruses and may be specific for PB 1 type virus. Seventy seven feces from diarrheic calves were tested by "double sandwich" ELISA using four monoclonal and one polyclonal anti-rotavirus antibodies. The combination of mono- and polyclonal antibodies thus appears to be a more efficient strategy for detection and typing of bovine rotaviruses.

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The rotaviruses, classified as members of the family *Reoviridae*, are the major cause of infectious diarrhea in humans and other animals [15]. Currently, 11 types of rotavirus have been defined [10] and genetic studies clearly demonstrate that the genes which code for viral proteins VP 4 and VP 7 cosegregate with neutralization specificities [13, 21]. VP 7 is a glycosylated outer capsid protein which is the product of genome segment 7, 8 or 9. VP 4 is the product of genome segment 4, and is a nonglycosylated outer capsid protein [10].

A binary system for serotyping rotavirus strains has been proposed [10]. The VP 4 type would be called P (for protease-sensitive protein) type and the VP 7 type be called G (for glycoprotein) type [10]. At least three G types: G 6, G 8 and G 10 and three P types: PB 1 (NCDV-like), PB 2 (UK-like) and PB 3 (KK 3-like) are present among bovine rotaviruses [2, 19, 26]. The vast majority of rotaviruses isolated from calves belongs to G 6 serotype [2, 7, 25, 26], but the epidemiology of P-type strains is not well known so far. Development of VP 4-specific monoclonal antibodies (MAbs) is urgently needed to extend the current serological classification of rotaviruses.

In this paper we described neutralizing and non-neutralizing MAbs against the bovine rotavirus $Q_{17}$ VP 4, VP 6 and VP 7 proteins. These antibodies will be useful for detection and classification of rotavirus infections.

The purified [20] bovine rotavirus $Q_{17}$ strain, type G 6, was originally isolated and characterized in this laboratory [1] and was used as immunizing antigen for BALB/c mice. The cell fusion or hybridoma production was performed as described previously [5]. The MAbs were tested by enzyme-linked immuno-sorbent assay (ELISA) [5], immunofluorescence (IF) and fluorescent focus (FF) neutralization test [8]. The isotype analysis was carried out by ELISA using a "Mouse Type" kit from Bio-Rad, Richmond, CA. The antibody affinities were assessed by ammonium thiocyanate elution ELISA [5].

The protein specificity of MAbs was determined by immunoprecipitation and SDS-PAGE using [$^{35}$S]methionine-labelled $Q_{17}$ rotavirus [6].

The binding patterns of MAbs against a panel of rotavirus strains were evaluated by immunoprecipitation [6], Western blotting [5] or F(ab')$_2$ ELISA.

The rotavirus NCDV (G 6 type) and OSU (G 5 type) strains were obtained from Dr. Linda Saif, Ohio State University, Ohio. Strain 678 (G 8 type) was obtained from Dr. D. Snodgrass, Moredun Research Institute, Scotland. All viruses were propagated in monkey kidney (MA-104) cells maintained in serum-free cell culture medium containig 10 μg of trypsin (Sigma) per ml.

The F(ab')$_2$ ELISA was performed using F(ab')$_2$ MAb fragments obtained as described by Parham [22] as capture antibody. The bound MAb F(ab')$_2$ was incubated with the antigens, the corresponding MAb was added and bound antibody was detected by Fc fragment-specific peroxidase-conjugate goat anti-mouse IgG (Jackson Immunoresearch, West Grove, PA).

Further characterization of the binding specificity of MAb was carried out by ELISA using purified fractions of $Q_{17}$ rotavirus or purified virus proteins (PP-ELISA) as antigens. The purified virus fractions 1,36 and 1,38 were obtained by density gradient centrifugation [20]. The 1,36 fraction was used before and after trypsin treatment [3]. The virus purified at a density of 1,36 contains both outer and inner capsid proteins but the virus at 1,38 density contains only the inner capsid proteins [20]. Individual viral proteins were purified by electro-elution of the appropiate band from SDS-PAGE gels of purified virus [23]. These proteins were used as antigen in the PP-ELISA. All the above techniques were performed using MAb supernatants; NS-1 mouse myeloma cell super-natant was included as control.

The epitope relationship between the neutralizing MAb (N-MAb) was de-termined by a competition test as previously described [24]. The immuno-globulin G (IgG) fraction from ascitic fluid of N-MAb RQ 4 and RQ 31, which was obtained by protein-A affinity chromatography, was biotinylated using the succinimide ester [12].

Double sandwich ELISA was performed using rabbit anti-rotavirus antibody as capture antibody. After incubation with the samples, 100 μl/well of the ap-propriate MAb supernatant or bovine anti-rotavirus antibody was added. An-

tibody binding was detected using a goat anti-mouse peroxidase conjugate (Jackson) or an anti-bovine peroxidase conjugate (Cappel).

Forty-eight hybridomas producing rotavirus-specific MAbs were isolated after two cell fusions from mice immunized with purified rotavirus $Q_{17}$. Five MAbs were selected for their high affinity as determined by ammonium thiocyanate elution ELISA [5]. The most common isotype detected was IgG2a (Table 1).

The RQ34 MAb detected a conformational epitope on VP6. This was defined since it was active by immunoprecipitation test not by SDS denatured Western blotting or PP-ELISA (Fig. 1 and Table 1). The reactivity of this MAb against an inner capsid protein is also demonstrated by its binding to the purified virus fractions 1,38 and 1,36 (Table 2). RQ34 is the only MAb which bound to all rotavirus strains (Table 3) and must therefore be specific for a group antigen. Similar MAbs against non-neutralizing group specific epitopes on VP6, and their use as diagnostic tools are widely cited in the literature [4, 9, 10]. The high affinity and the high cross-reactivity of the RQ34 using its $F(ab')_2$ fraction as capture antibody for ELISA clearly characterize this MAb as a useful diagnostic reagent to detect rotavirus. This was confirmed in a double sandwich ELISA, where the RQ34 detected 65.5% of positive samples and was able to detect rotavirus in 10% of samples that were negative when tested with polyclonal antibody (Fig. 3).

Of the other antibodies, RQ115 was the only MAb which recognized a non-conformational epitope as shown by its reactivity with VP7 both by Western blot (Fig. 2) and PP-ELISA (Table 1). The binding in the PP-ELISA produced an optical density over 1.0 in spite of the small quantity of antigen ($< 100$ ng/well) and the use of MAb cell culture supernatant. However, RQ115 bound only to the Q17 rotavirus strain indicating the presence of antigenic variation on VP7 of the same G type viruses (Table 3).

The RQ104 MAb was specific for a conformational epitope on VP4 (Fig. 1 and Table 1). The epitope was common to porcine and bovine rotavirus of G5

Table 1. Characterization of monoclonal antibodies against bovine rotavirus $Q_{17}$ (type G6)

| MAb | ELISA | IF | FF | Isotype | Protein specificity | |
|---|---|---|---|---|---|---|
| | | | | | conf.[a] | non-conf.[b] |
| RQ34 | + | + | − | IgG2a | VP6 | − |
| RQ115 | + | + | − | IgG1 | − | VP7 |
| RQ104 | + | + | − | IgG2a | VP4 | − |
| RQ4 | + | + | + | IgG2a | VP4 | − |
| RQ31 | + | + | + | IgG2a | VP4 | − |

[a] Conformational epitope determined by immunoprecipitation test

[b] Non-conformational epitope determined by Western blotting and ELISA using as capture antigens rotavirus purified proteins
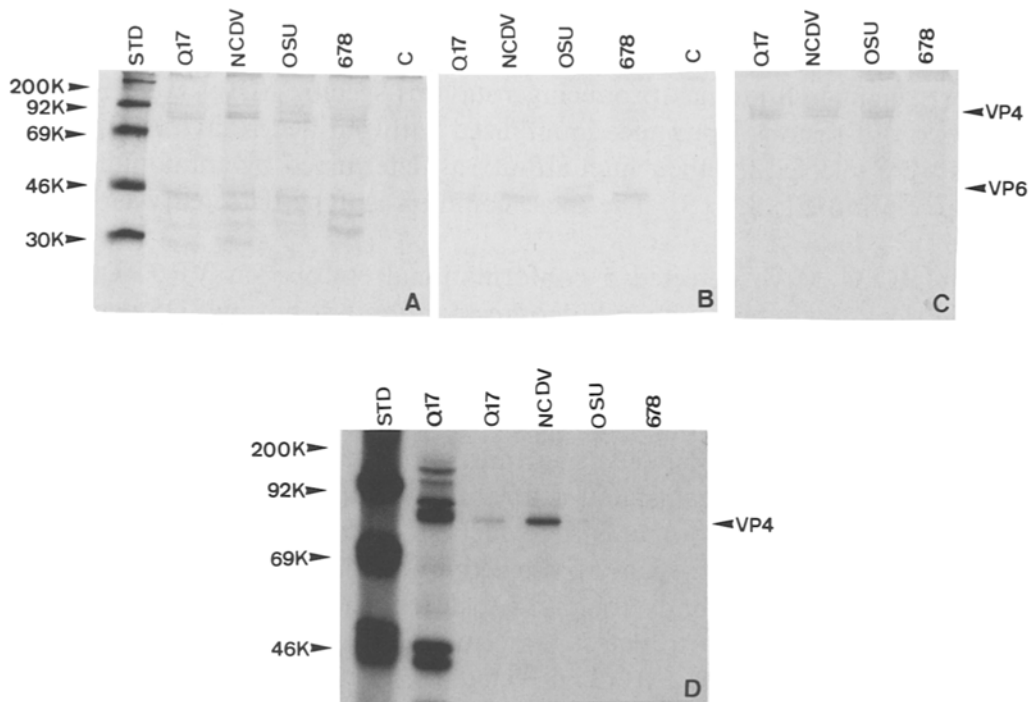
**Fig. 1.** Specificity for porcine (OSU, strain), and bovine (NCDV, 678, $Q_{17}$ strains) rotavirus proteins of MAb by immunoprecipitation test. **A** SDS-PAGE analysis of $[^{35}S]$-labelled proteins immunoprecipitated from cell lysate by a polyclonal anti-$Q_{17}$ antibody. **B** $[^{35}S]$-labelled proteins immunoprecipitated by MAb RQ 34. **C** $[^{35}S]$-labelled proteins immuno-precipitated by MAb RQ 104. **D** $[^{35}S]$-labelled proteins immunoprecipitated by MAb RQ 31. *C* Control cell lysate; *$Q_{17}$* Quebec 17 bovine G 6 rotavirus; *NCDV* neonatal calf diarrhoea virus, G 6 type; *OSU* porcine G 5 rotavirus; *678* bovine G 8 rotavirus

**Table 2.** Reactivities of MAbs against different preparations of $Q_{17}$ rotavirus by ELISA test

| MAb | PR 1,36[a] | PR 1,38[b] | PR 1,36-T[c] | PPR[d] |
|---|---|---|---|---|
| RQ 34 | + | + | + | − |
| RQ 115 | + | + | + | + |
| RQ 104 | + | + | + | − |
| RQ 4 | + | − | + | − |
| RQ 31 | + | − | + | − |

[a] 1,36 fraction of purified $Q_{17}$ rotavirus (double capside)
[b] 1,38 fraction of purified $Q_{17}$ rotavirus (single capside)
[c] 1,36 fraction of purified $Q_{17}$ rotavirus treated with trypsin
[d] VP 1 to VP 4, VP 6 or VP 7 purified proteins of $Q_{17}$ rotavirus

**Table 3.** Reactivities of MAbs against porcine (OSU, strain), and bovine (NCDV, 678, $Q_{17}$ strains) rotaviruses by ELISA-F(ab')$_2$, immunoprecipitation and immunoblot

| MAb | $Q_{17}$ | | | NCDV | | | 678 | | | OSU | | |
|-----|---------|---------|---------|---|---|---|---|---|---|---|---|---|
|     | $E^a$ | $P^b$ | $B^c$ | E | P | B | E | P | B | E | P | B |
| RQ 34 | + | + | − | + | + | − | + | + | − | + | + | − |
| RQ 115 | − | + | + | − | − | − | − | − | − | − | − | − |
| RQ 104 | + | + | − | + | + | − | − | − | − | + | + | − |
| RQ 4 | + | + | − | + | + | − | − | − | − | − | − | − |
| RQ 31 | + | + | − | + | + | − | − | − | − | − | − | − |

[a] Determined by F(ab)'$_2$-ELISA
[b] Determined by immunoprecipitation test
[c] Determined by immunoblot

PAB   RQ115

VP6 ►

VP7 ►

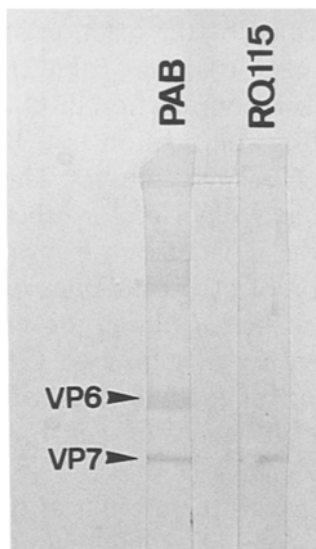**Fig. 2.** Specificity for bovine $Q_{17}$ rotavirus proteins of RQ 115 MAb by Western blotting. *PAB* Proteins detected by a polyclonal anti-$Q_{17}$ antibody; *RQ 115* protein detected by MAb RQ 115

and G 6 types respectively, but not to the bovine G 8 type (Table 3). This confirms the variability of the imunogenicity of VP4 among G types [11].

In the case of RQ 104, as for RQ 115, the epitope was located on a surface protein found in the 1,38 purified virus fraction (Table 2). The binding of both RQ 115 and RQ 104 to 1,38 indicates that fraction 1,38 contains both VP 4 and VP 7. However, this binding produced an optical density three times lower than in the case of 1,36 fraction. Neither VP 4 nor VP 7 were detected by SDS electrophoresis of the 1,38 fraction. This implies that fragments of VP 4 and VP 7 are present on the inner capsid of the virus purified by density gradient. Such results suggest that these proteins have outer and inner capsid domains. The epitopes for RQ 115 and RQ 104 must be on these inner capsid domains of VP 4 and VP 7.
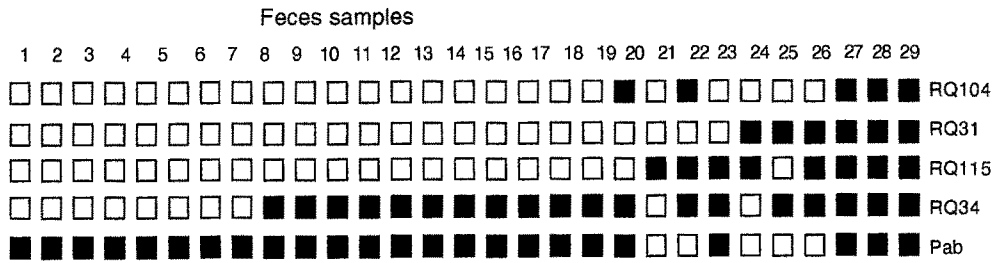
Feces samples

1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16  17  18  19  20  21  22  23  24  25  26  27  28  29

RQ104

RQ31

RQ115

RQ34

Pab

Fig. 3. Rotavirus detection in a double sandwich ELISA using bovine anti-rotavirus poly-clonal antibody and RQ 34, RQ 115, RQ 31 and RQ 104 MAb. *RQ 104* IgG2 a anti-VP4 MAb; *RQ 31* IgG2 a anti-VP4 MAb; *RQ 115* IgG1 anti-VP7 MAb; *RQ 34* IgG2 a anti-VP6 MAb; *Pab* polyclonal anti-$Q_{17}$ antibody. ■ positive sample; □ negative sample

RQ 4 and RQ 31 MAb had viral neutralizing activity and reacted with a conformational epitope on VP 4 (Fig. 1 and Table 1). This epitope was trypsin resistant and only present on the outer surface as indicated by the reaction of the MAbs with the 1,36 but not with the 1,38 purified virus fraction (Table 2). These MAbs were also unable to inhibit the hemagglutination reaction of $Q_{17}$ rotavirus and showed 80% cross-inhibition by a binding competition test. It thus appears that they react with the same or with closely related epitopes. The failure to inhibit the hemagglutination has been previously reported for other neutralizing VP 4-specific MAb [8]. This failure and the resistance to trypsin indicates that this site may be involved in determination of other functions of the protein (such as host cell tropism) but not in viral hemagglutination or trypsin-enhanced viral infection. RQ 4 and RQ 31 were specific for two G 6 bovine roatviruses ($Q_{17}$ and NCDV, PB 1 type) but not for the 678 rotavirus which belong to G 8 and PB 2 (UK-like) types (Table 3). This result suggests that RQ 4 and RQ 31 may be specific for the PB 1 type.

A second series of experiments was carried out to assess the usefulness of these antibodies for detection of rotavirus in clinical samples. A "double sand-wich" ELISA was performed using rabbit anti-rotavirus antiserum as the cap-ture antibody and a series of 77 feces samples from diarrheic calves. Fourty-eight negative and 29 positive samples were detected. Fifteen of the 29 positive samples were positive by both mono- and polyclonal antibodies (Fig. 3). Each of the antibodies to outer capsid proteins, RQ 115 (VP 7), RQ 104 (VP 4) and RQ 31 (VP 4) detected less than 50% of the samples that were positive with RQ 34 or the polyclonal anti-serum. However, when the results are pooled, the combined MAbs identified 5 positive samples that were negative for the poly-clonal antibody and the polyclonal antibody detected 8 positive samples that were negative for the MAbs. It is clear that for detection of rotavirus by ELISA a mixture of mono- and polyclonal antibodies is preferable.

When the results were analysed for the individual MAb it appears that VP 4 and VP 7 epitopes vary considerably in these virus samples. The binding of RQ 31 to 21% of the positive samples might indicate that this is the proportion

of PB 1 in the rotavirus population of Quebec. The results demonstrate at least 4 different VP 4 types for the same VP 7 epitope (RQ 115) which would confirm that there is considerable antigenic variations in this protein [17].

Until now, the P types of rotaviruses (VP 4 type) have been determined by genetic approaches, reassorting of virus, N-MAbs against rhesus and human rotavirus and by hyperimmune antisera [3, 11, 14, 16–18, 21, 24, 27]. Yet N-MAb with P type specificity against bovine rotavirus have not been described. The detection of 21% of rotavirus positive feces samples by the MAb RQ 31 and its specificity for a neutralizing epitope on VP 4 of Q 17 and NCDV rotavirus strains (G 6, PB 1 types) suggest that this N-MAb may be useful for detecting P-type of bovine rotaviruses.

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