

Rotavirus 993/83, isolated from calf faeces, closely resembles an avian rotavirus

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Polypeptides from purified virions of the calf rotavirus (RV) isolate 993/83 and those from the pigeon RV isolate PO-13 comigrated on SDS–polyacrylamide gels. Two polypeptides of 45K and 47K were detected at the position of VP6. Both proteins behaved like authentic VP6 protein with EDTA and heat treatment.

RV 993/83 and PO-13 showed identical one-dimensional peptide maps for VP2, and the 45K and 47K proteins. More than 70% of sera from German cattle older than 1 year showed neutralizing serum antibodies to RV 993/83 and RV PO-13.

Rotaviruses (RVs) are a major infectious cause of diarrhoea in calves (McNulty, 1978). In a survey of 1430 faecal samples from calves with diarrhoea conducted in Germany (Eichhorn *et al.*, 1985) we identified a group A RV that differed from conventional bovine RV in its subgroup, serotype and genogroup (Brüssow *et al.*, 1992). This isolate, RV 993/83, was a serotype 7 RV and it showed RNA sequence homology with a pigeon RV (Minamoto *et al.*, 1988; Brüssow *et al.*, 1992). In the present report we extend the biochemical and serological comparison of RV 993/83 with pigeon RV PO-13.

MA104 cells were labelled throughout the entire infection cycle with [³⁵S]methionine (15 µCi/ml; Dupont, NEN) in a medium containing one-tenth the normal methionine content. RV particles were isolated from the cell-free culture supernatant by high-speed centrifugation (90000 g, 2 h, 4 °C) through a 20% sucrose cushion followed by equilibrium centrifugation on preformed CsCl gradients (1.25 to 1.42 g/ml). Protein electrophoresis was carried out in slab gels using the method of Laemmli (1970) with 13% running and 3% stacking gels. Gels were fluorographed with Enlightning (Dupont, NEN), dried and exposed on X-ray films (YAR-5; Eastman Kodak).

Putative VP1, VP2, VP3, VP5* (the major tryptic cleavage product of VP4), VP6 and VP7 (37K and 35K bands) were detected in double-shelled RV 993/83 purified by CsCl density gradient centrifugation (Fig. 1a). Pigeon RV PO-13 showed a very similar polypeptide migration pattern. In addition, RV 993/83 and RV PO-

13 contained two polypeptides that migrated to near the position of authentic VP6: a major 47K band which migrated slower than bovine RV VP6 and a minor 45K band which migrated at approximately the same position as bovine RV VP6. The 45K and 35K bands varied in intensity between different preparations from identical virus stocks (data not shown).

Rotavirus particles banding at 1.36 g/ml (double-shelled particles) were recovered from the CsCl gradients using a Pasteur pipette, diluted with TNC (50 mM-Tris HCl pH 7.5 containing 150 mM-NaCl and 10 mM-CaCl₂) or alternatively with TNE (containing 10 mM-EDTA in place of 10 mM-CaCl₂) and pelleted by high-speed centrifugation (130000 g, 1 h, 4 °C). EDTA treatment resulted in loss of putative VP5* and VP7, whereas both polypeptide bands at the approximate VP6 position (the 47K and 45K bands) remained associated with single-shelled particles (Fig. 1b).

When RV 993/83 was loaded onto SDS–polyacrylamide gels without prior heating (2 min at 100 °C) in sample buffer (containing 2-mercaptoethanol), it was found that the autoradiographic intensity of the 47K and 45K polypeptide bands decreased and a polypeptide band with an *M_r* of approximately 140K appeared (Fig. 1c). Thus, with EDTA treatment and omission of sample boiling, the 47K and 45K polypeptides behave like authentic VP6 (Cohen *et al.*, 1979; Gorziglia *et al.*, 1985; McCrae & Faulkner-Valle, 1981; Sabara *et al.*, 1987).

Limited proteolysis analysis was performed using the procedure described by Cleveland *et al.* (1977). *Staphylo-*

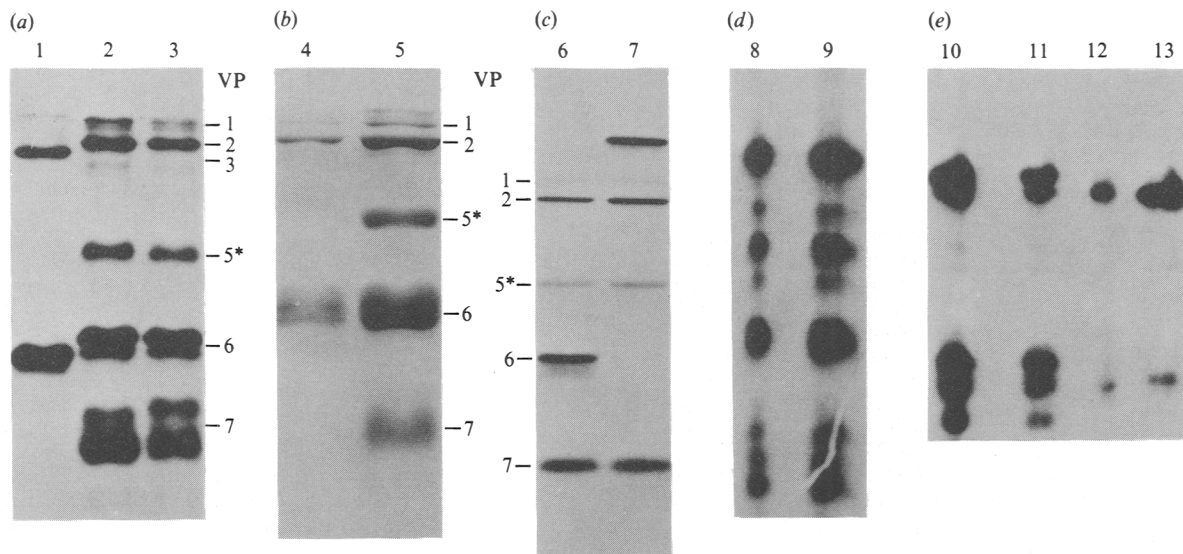


Fig. 1. Polypeptide analysis of RV 993/83. (a) SDS-PAGE of [³⁵S]methionine-labelled structural polypeptides of single-shelled bovine RV NCDV (lane 1), and double-shelled virions of RV 993/83 (lane 2) and pigeon RV PO-13 (lane 3). (b) SDS-PAGE of double-shelled virions of RV 993/93 after sedimentation in the presence of 10 mM-EDTA (lane 4) or 10 mM-CaCl₂ (lane 5). Polypeptides were stained with Coomassie blue. (c) SDS-PAGE of [³⁵S]methionine-labelled structural polypeptides of double-shelled virions of RV 993/83 with (lane 6) and without (lane 7) boiling for 2 min prior to electrophoresis. (d) Limited proteolysis analysis of VP2 from RV 993/83 (lane 8) and RV PO-13 (lane 9). (e) Limited proteolysis analysis of the RV PO-13 47K band (lane 10), the RV 993/83 47K (lane 11) and 45K bands (lane 12), and the RV PO-13 45K band (lane 13).

Table 1. Prevalence and geometric mean titre of neutralizing antibodies to rotavirus 993/83, pigeon RV PO-13 and chicken RV Ch-2

Age group	n*	Sera neutralizing the indicated rotavirus (%)†		
		993/83	PO-13	Ch-2
0-1 month	22	41 (44)‡	27 (24)	0 (10)
1-2 months	19	37 (47)	32 (17)	5 (15)
2-4 months	18	22 (31)	28 (14)	0 (10)
4-12 months	16	31 (21)	25 (17)	6 (13)
1-3 years	27	67 (100)	81 (83)	11 (16)
3-5 years	26	88 (165)	73 (100)	0 (11)
>5 years	13	100 (197)	92 (176)	8 (12)

* n, Number of serum samples tested.

† A serum was counted as neutralizing if a 1:90 dilution neutralized 100 TCID₅₀ of the rotavirus indicated in the peroxidase focus reduction test (Brüssow *et al.*, 1991b).

‡ Figures in parentheses indicate the geometric mean titre.

coccus aureus V8 protease (10 µl, type XVII; Sigma) at a concentration of 1 µg/µl was added to each slot. Putative VP2 from RV isolate 993/83 showed a peptide map indistinguishable from that of pigeon RV PO-13 VP2 (Fig. 1d). The 47K band from RV 993/83 showed a one-dimensional peptide map identical to that of the corresponding band from avian RV PO-13 (Fig. 1e). The

peptide maps of the 45K bands from both RV 993/83 and avian RV PO-13 showed partial identity to the peptide map of the corresponding 47K bands.

In about 30% of German calves and in more than 70% of German cattle older than 1 year we detected serum neutralizing antibody to RV 993/83 (Table 1). Neutralizing antibodies against pigeon RV PO-13 were detected with similar frequency and comparable titres, whereas serum antibodies neutralizing chicken RV Ch-2 were found only infrequently (Table 1). However, this high prevalence of serum antibody does not prove that German cattle have been exposed to pigeon RV. About 50% of German cattle older than 1 year also show serum antibody neutralizing turkey RV Ty-3 (Brüssow *et al.*, 1991a), and serum antibodies to other RV serotypes of human and animal origin have also been detected (Brüssow *et al.*, 1991a).

In conclusion, RV 993/83 isolated from the faeces of a German calf with diarrhoea closely resembles a pigeon RV. We do not believe that RV 993/83 is a laboratory contaminant (Brüssow *et al.*, 1992). However, contamination of the original bovine faeces with avian faeces in the field remains a possibility that we cannot exclude. Only infecting a calf with RV 993/83 to determine whether it replicates in the seemingly heterologous host can provide experimental evidence that RV 993/83 has the capacity to cross the species barrier.

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References

- BRÜSSOW, H., EICHHORN, W., ROHWEDDER, A., SNODGRASS, D. & SIDOTI, J. (1991*a*). Cattle develop neutralizing antibodies to rotavirus serotypes which could not be isolated from faeces of symptomatic calves. *Journal of General Virology* **72**, 1559–1567.
- BRÜSSOW, H., OFFIT, P. & SIDOTI, J. (1991*b*). Neutralizing antibodies to heterologous animal rotavirus serotypes 5, 6, 7, and 10 in sera from Ecuadorian children. *Journal of Clinical Microbiology* **29**, 869–873.
- BRÜSSOW, H., NAKAGOMI, O., GERNA, G. & EICHHORN, W. (1992). Isolation of an avianlike group A rotavirus from a calf with diarrhea. *Journal of Clinical Microbiology* **30**, 67–73.
- CLEVELAND, D. W., FISCHER, S. G., KIRSCHNER, M. W. & LAEMMLI, U. K. (1977). Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. *Journal of Biological Chemistry* **252**, 1102–1106.
- COHEN, J., LAPORTE, J., CHARPILLENNE, A. & SCHERRER, R. (1979). Activation of RNA polymerase by calcium chelation. *Archives of Virology* **60**, 177–186.
- EICHHORN, W., KRAUSS, M., BACHMANN, P. A. & MAYR, A. (1985). Vorkommen und Verbreitung atypischer Rotaviren bei Kälbern in Deutschland. *Tierärztliche Umschau* **40**, 435–436.
- GORZIGLIA, M., LARREA, C., LIPRANDI, F. & ESPARZA, J. (1985). Biochemical evidence for the oligomeric (possibly trimeric) structure of the major inner capsid polypeptide (45K) of rotaviruses. *Journal of General Virology* **66**, 1889–1900.
- LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, London* **227**, 680–685.
- MCCRAE, M. A. & FAULKNER-VALLE, G. P. (1981). Molecular biology of rotaviruses. I. Characterization of basic growth parameters and pattern of macromolecular synthesis. *Journal of Virology* **39**, 490–496.
- MCNULTY, M. S. (1978). Rotaviruses. *Journal of General Virology* **40**, 1–18.
- MINAMOTO, N., OKI, K., TOMITA, M., KINJO, T. & SUZUKI, Y. (1988). Isolation and characterization of rotavirus from feral pigeon in mammalian cell cultures. *Epidemiology and Infection* **100**, 481–492.
- SABARA, M., READY, K. F. M., FRENCHICK, P. J. & BABIUK, L. A. (1987). Biochemical evidence for the oligomeric arrangement of bovine rotavirus nucleocapsid protein and its possible significance in the immunogenicity of this protein. *Journal of General Virology* **68**, 123–133.

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