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Veterinary Microbiology, 13 (1987) 27—34
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHARACTERISATION OF ROTAVIRUS ISOLATES FROM SUB-CLINICALLY INFECTED CALVES BY GENOME PROFILE ANALYSIS

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(Accepted for publication 4 February 1986)

ABSTRACT

Pocock, D.H., 1987. Characterisation of rotavirus isolates from sub-clinically infected calves by genome profile analysis. *Vet. Microbiol.*, 13: 27—34.

Rotaviruses isolated from 43 sub-clinically infected calves from a single farm were analysed by genome profile analysis. The isolates showed genomic variation and eight different profiles were observed, including one which was atypical for Group A rotaviruses.

The 3′ terminal labelling method for the analysis of genome profiles used in this study required only 1 ng of viral RNA, an increase of 1000-fold in sensitivity over ethidium bromide staining for detecting all rotavirus genome segments. However, dual infections involving two rotaviruses with distinct profiles could not be detected if the concentrations of the viruses differed by > 10-fold.

INTRODUCTION

Although rotaviruses are frequently associated with acute diarrhoea in man and animals, sub-clinical infections are common (Chrystie et al., 1978; de Leeuw et al., 1980). In a recent longitudinal survey McNulty and Logan (1983) reported the detection of rotavirus in 79% of calves during the first 3 weeks of life and of these 80% showed no clinical signs. A similar survey carried out on children found that rotavirus shedding was detected to the same degree in children with or without diarrhoea (Barron-Romero et al., 1985). The reasons for sub-clinical reactions to infection have not been fully elucidated, but there is experimental evidence that oral administration of antibody during virulent rotavirus infection eliminates any clinical signs of the infection (Snodgrass and Wells, 1976; Bridger and Brown, 1981). Differences in virulence among bovine rotaviruses have also been reported (Carpio et al., 1981) but naturally occurring avirulent strains have not yet been isolated and characterised.

Since genomic diversity among rotaviruses was found to be extensive

0378-1135/87/$03.50

(Kalica et al., 1978; Rodger and Holmes, 1979), genome profile analysis has been used to characterise virus isolates. To improve the sensitivity of this technique Clarke and McCrae (1981) described a method in which the viral RNA was 3′ terminal labelled prior to electrophoresis, so that the RNA could then be detected in the polyacrylamide gels by autoradiography instead of ethidium bromide staining. This report investigates the use of this technique for the characterisation of rotaviruses isolated from sub-clinically infected calves, as the first stage in a study of asymptomatic rotavirus infections in calves.

MATERIALS AND METHODS

Viruses

The UK strain of bovine rotavirus (Bridger and Woode, 1975) was grown in MA104 cells with serum-free Eagles MEM containing 0.5 μg ml^{-1} trypsin (Sigma Chemical Co. Ltd., Type 3).

Isolates of bovine rotavirus were obtained from 43 sub-clinically infected calves on a farm where such infections were common (Reynolds, 1983). Rotavirus was detected in the faecal samples of these animals by enzyme-linked immunosorbent assay (ELISA) (Reynolds et al., 1984).

Extraction of rotavirus RNA

Ten ml of a 10% faecal suspension were extracted twice with 5 ml fluorocarbon (Arklone P, ICI Ltd.) and the virus pelleted from the aqueous phase by centrifugation at 75 000 × *g* for 1 h. Cell culture grown virus was similarly pelleted from supernatant fluids after clarification at 5000 × *g* for 20 min. Virus pellets were resuspended in buffer (20 mM NaCl; 2 mM $CaCl_2$; 0.5% SDS; 25 mM Tris/HCl pH 7.4) and Proteinase K (Sigma Chemical Co. Ltd., Protease Type XI) was added to give a final concentration of 500 μg ml^{-1}. The mixture was incubated at 37°C for 30 min and extracted twice with 2 vol phenol:chloroform (1:1 v/v). Sodium acetate (pH 5.5) was added to the aqueous phase to give a final concentration of 0.3 M and the RNA was precipitated with 67% ethanol. After centrifugation at 10 000 × *g* for 30 min, the RNA pellet was dissolved in 0.1 ml distilled water and stored at −20°C. RNA concentrations were determined by spectrophotometry: $10D_{260\ nm}$ was assumed equivalent to 50 μg ml^{-1} (Gaillard and Joklik, 1982).

3′ terminal labelling of rotavirus RNA

The method of RNA labelling was essentially that described by Clarke and McCrae (1981), except that 2 μg RNase-free bovine serum albumin (Miles Laboratories Ltd.) was added to the reaction mixture to prevent adsorption

of the T_4 RNA ligase to the reaction vessel. Unincorporated radioactivity was removed by chromatography on Whatman CF11 cellulose columns.

Electrophoresis of rotavirus RNA

Electrophoresis was carried out on 10% polyacrylamide gels with a 3% stacking gel using the discontinuous buffer system described by Laemmli (1970), at either 40 mA constant current for 5—6 h or overnight at 20 mA. The gels were either stained with a 1 μg ml^{-1} aqueous solution of ethidium bromide (EB) for 1 h to detect unlabelled RNA or dried onto filter paper and autoradiographed when ^{32}P-labelled RNA was used.

Analysis of mixtures of two rotaviruses with distinct profiles

RNA extracted from the UK strain and from an isolate with a C7 profile was adjusted to give equal concentrations as estimated by spectrophotometry. Mixtures containing a constant amount of C7 RNA and varying amounts of UK strain RNA were prepared and 3′ terminally labelled as above. Samples of the labelled ds-RNA mixtures containing 10 000 c.p.m. were electrophoresed on a 10% gel.

RESULTS

Comparison of 3′ terminal labelling and ethidium bromide staining methods for detecting rotavirus RNA

By 3′ terminal labelling of 5 ng of viral RNA, all 11 rotavirus segments were detected after autoradiographic exposure for 16 h (Fig. 1a). The amount of RNA could be reduced to 1 ng if the exposure time was extended to 3—4 days (data not shown). A comparable gel stained with ethidium bromide (Fig. 1b) showed that the smallest amount of RNA which gave a complete pattern was 1 μg, i.e. there was a 1000-fold difference in sensitivity between the two methods.

Genome profile analysis of field isolates

Genome profiles were obtained from the rotavirus-positive faecal samples of all 43 calves, and the patterns fell into eight types, C1—C8 (Fig. 2). There was clear evidence of genomic variation and this variation was not confined to any segment or group of segments. The profile type C7 differed from the normal pattern produced from Group A rotaviruses as no band was observed in the normal Segment 11 region of the gel but a band was evident between Segments 6 and 7. Viruses with these profiles were detected in the faecal samples of eight calves by an ELISA specific for Group A rotaviruses indicating that they must belong to this group. Profiles

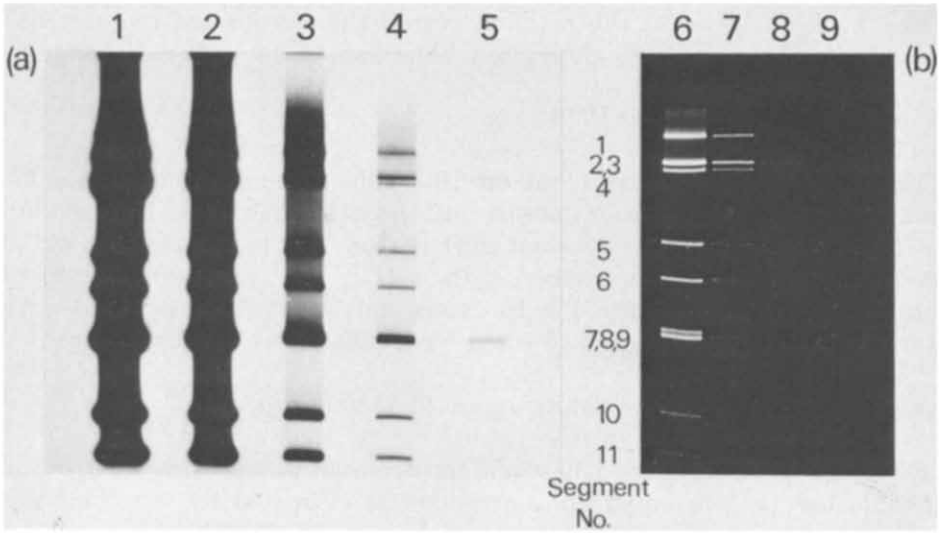

Fig. 1. Detection of different amounts of rotavirus genomic ds-RNA after separation of individual segments by electrophoresis through 10% polyacrylamide gels. Samples containing known amounts of ds-RNA were: (a) terminally labelled with [^{32}P] p-C-p and after PAGE the bands were detected by autoradiography for 16 h, or (b) electrophoresed and the bands visualised by UV illumination of the gel after staining with 1 μg ml^{-1} aqueous ethidium bromide. Lanes 1—5 contained 3.125 μg, 625 ng, 125 ng, 25 ng and 5 ng, and Lanes 6—9 contained 5 μg, 1 μg, 200 ng and 40 μg ds-RNA, respectively.

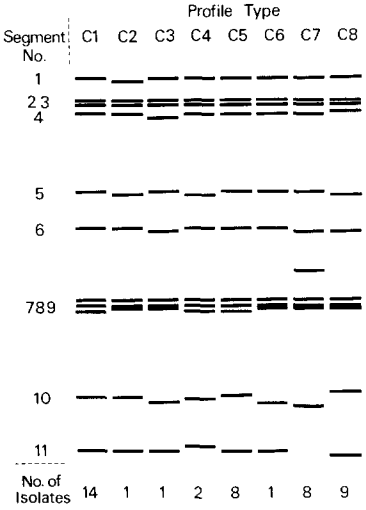

Fig. 2. Diagrammatic representation of the genome profiles of isolates identified during the study.

containing the expected 11 segments of ds-RNA were found from 42 samples. One profile, however, had an extra four bands suggesting that this calf had been infected with two viruses having C4 and C5 type profiles.

Dectection of mixed infections with bovine rotavirus

In this study only one sample showed evidence of mixed infection with two rotaviruses. It was therefore important to determine whether mixed infections were rare or whether the technique simply could not detect them. To investigate the latter, mixtures containing known amounts of ds-RNA from two rotavirus isolates with disparate genome profiles (UK strain and a C7 type) were used to simulate the type of samples that might be recovered from calves concurrently infected with these two viruses. After 3′ terminal labelling the RNA segments were analysed by electrophoresis (Fig. 3). Only samples b to f showed evidence of being mixed

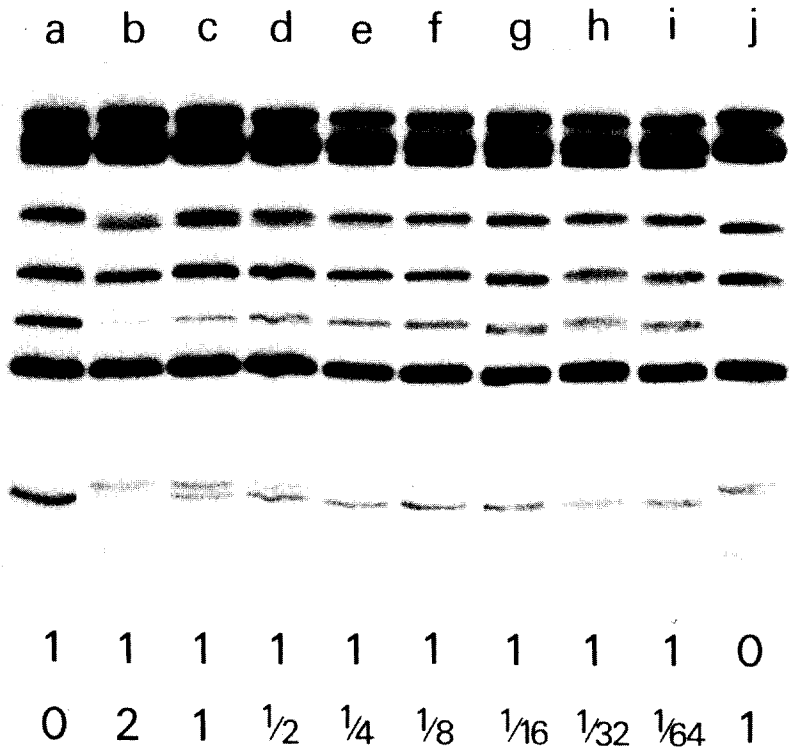

Fig. 3. Evaluation of genome profile analysis for the detection of heterogeneous mixtures of rotavirus RNA. Lanes a—j contained mixtures of C7 type and UK bovine rotavirus ds-RNA mixed in the proportions shown. The mixtures were terminally labelled and samples containing equal numbers of counts were electrophoresed.

profiles, and meant that in a faecal sample containing two rotaviruses if one virus was present at a concentration 10-fold less than the other, i.e. 1 $\log_{10}$ dilution, it would not be detected.

DISCUSSION

Rotaviruses isolated from 43 sub-clinically infected calves were characterised by genome profile analysis. The 3′ terminal labelling of rotavirus ds-RNA used in this study was found to require only 1 ng of viral RNA to give a genome profile after electrophoresis. Comparison of this technique with ethidium bromide staining for the detection of rotavirus ds-RNA segments showed it to be 1000-fold more sensitive, allowing the successful analysis of all the ELISA positive samples used. Herring et al. (1982) used silver staining to visualise the genome segments and reported an increase of 20-to 40-fold over ethidium bromide. However this method suffers the same problem as ethidium bromide staining in that low molecular weight segments are difficult to detect, because they both depend on the total weight of RNA present in each band whereas the 3′ terminal labelling method relies on the number of molecules of each segment.

Genomic variation was observed between isolates, and this was not confined to any particular genome segment or group of segments, a finding similar to that reported by Rodger and Holmes (1979) for rotaviruses from calves with enteric disease. Eight different profiles were observed and all were typical for Group A rotaviruses (Pedley et al., 1983), except for the C7 type which was typified by the lack of a band in the Segment 11 region and the appearance of a band between Segments 6 and 7. Variation in the position of Segment 11 has been reported for Subgroups 1 and 2 of human Group A rotaviruses (Kalica et al., 1981), although not to the extent seen with the C7 isolates. Rotaviruses with unusual genome profiles have been detected in man and animals (Pedley et al., 1983; Snodgrass et al., 1984), but these isolates have distinct group antigens and their genome profiles lack a triplet of Segments 7, 8 and 9, having similar electrophoretic mobilities, characteristic of Group A viruses. Therefore, the detection of C7 profile viruses by a Group A specific ELISA and the presence of the 7, 8 and 9 segment triplet in their genome profiles both confirm their assignment to Group A. The detection of these viruses highlights the caution needed if genome profile analysis alone is used to assign isolates to a particular rotavirus group.

The detection of calves dually infected with rotaviruses by the appearance of extra bands in genome profiles was found to depend on the relative rates of excretion of the two virus strains. It is therefore possible that other rotavirus variants were present in the samples but remained undetected, and underlines the requirement of cloning these isolates before further experimental work, e.g. animal inoculation, is undertaken.

Work is now in progress to adapt these genetic variants of rotavirus

for growth in cell culture so that further antigenic and virulence characterisation can be carried out. One isolate, with a C3 profile, has been isolated and cloned in cell culture and shown by inoculation into gnotobiotic calves to be avirulent (Bridger and Pocock, 1986).

REFERENCES

Barron-Romero, B.L., Barreda-Gonzalez, J., Doval-Ugalde, R., Zermeno-Eguia Liz., J. and Huerta-Pena, M., 1985. Asymptomatic rotavirus infections in day care centers. J. Clin. Microbiol., 22: 116—118.

Bridger, J.C. and Brown, J.F., 1981. Development of immunity to porcine rotavirus in piglets protected from disease by bovine colostrum. Infect. Immun., 31: 906—910.

Bridger, J.C. and Pocock, D.H., 1986. Variation in virulence of bovine rotaviruses. J. Hyg., Camb., 96: 257—264.

Bridger, J.C. and Woode, G.N., 1975. Neonatal calf diarrhoea: identification of a reovirus-like (rotavirus) agent in faeces by immunofluorescence and immune electron microscopy. Br. Vet. J., 131: 528—535.

Carpio, M., Bellamy, J.E.C. and Babiuk, L.A., 1981. Comparative virulence of different bovine rotavirus isolates. Can. J. Comp. Med., 45: 38—42.

Chrystie, I.L., Totterdell, B.M. and Banatvala, J.E., 1978. Asymptomatic endemic rotavirus infections in the newborn. Lancet, i: 1176—1178.

Clarke, I.N. and McCrae M.A., 1981. A rapid and sensitive method for analysing the genome profiles of field isolates of rotavirus. J. Virol. Methods, 2: 203—209.

De Leeuw, P.W., Ellens, D.J., Straver, P.J., Van Balken, J.A.M., Moerman, A. and Baanvinger, T., 1980. Rotavirus infections in calves in dairy herds. Res. Vet. Sci., 19: 135—141.

Gaillard, R.K. and Joklik, W.K., 1982. Quantitation of the relatedness of Reovirus serotypes 1,2 and 3 at the gene level. Virology, 123: 152—164.

Herring, A.J., Inglis, N.F., Ojeh, C.K., Snodgrass, D.R. and Menzies, J.D., 1982. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. J. Clin. Microbiol., 16: 473—477.

Kalica, A.R., Sereno, M.M., Mebus, C.A., Chanock, R.M. and Kapikian, A.Z., 1978. Comparison of human and animal rotaviruses by gel electrophoresis of viral RNA. Virology, 87: 247—255.

Kalica, A.R., Greenberg, H.B., Espejo, R.T., Flores, J., Wyatt, R.G., Kapikian, A.Z. and Chanock, R.M., 1981. Distinctive ribonucleic acid patterns of human rotavirus subgroups 1 and 2. Infect. Immun., 33: 958—961.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature, 227: 680—685.

McNulty, M.S. and Logan, E.F., 1983. Longitudinal survey of rotavirus infection in calves. Vet. Rec., 113: 333—335.

Pedley, S., Bridger, J.C., Brown, J.F. and McCrae, M.A., 1983. Molecular characterization of rotaviruses with distinct group antigens. J. Gen. Virol., 64: 2093—2101.

Reynolds, D.J., 1983. The epidemiology of enteric virus infections of calves. Ph.D. thesis, University of Reading, Reading, U.K.

Reynolds, D.J., Chasey, D., Scott, A.C. and Bridger, J.C., 1984. Evaluation of ELISA and electron microscopy for the detection of coronavirus and rotavirus in bovine faeces. Vet. Rec., 114: 397—401.

Rodger, S.M. and Holmes, I.H., 1979. Comparison of the genomes of simian, bovine and human rotaviruses by gel electrophoresis and detection of genomic variation among bovine isolates. J. Virol., 30: 839—849.

Snodgrass, D.R. and Wells, P.W., 1976. Rotavirus infection in lambs: studies on passive protection. Arch. Virol., 52: 201—205.
Snodgrass, D.R., Herring, A.J., Campbell, I., Inglis, J.M. and Hargreaves, F.D., 1984. Comparison of atypical rotaviruses from calves, piglets, lambs and man. J. Gen. Virol., 65: 909—914.