J. Hyg., Camb. (1983), 90, 117–125
Printed in Great Britain

Electrophoretic study of the genome of human rotaviruses from Rio de Janeiro, São Paulo and Pará, Brazil

H. G. PEREIRA, ROSETE S. AZEREDO, J. P. G. LEITE
Department of Virology, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

J. A. N. CANDEIAS, MARIA L. RÁCZ
Department of Biomedical Sciences, University of São Paulo, S. Paulo, S.P. Brazil

A. C. LINHARES, YVONNE B. GABBAY
Evandro Chagas Institute, Belem, Pará, Brazil

AND J. R. TRABULSI
Department of Microbiology, Immunology and Parasitology, Escola Paulista de Medicina, S. Paulo, S.P. Brazil

(*Received* 11 *June* 1982; *accepted* 23 *August* 1982)

SUMMARY

Human rotaviruses from the states of Rio de Janeiro, São Paulo and Pará of Brazil were analysed by RNA electrophoresis. At least some bands characteristic of rotavirus double-stranded RNA were detected in 138 (86·8%) of 159 faecal samples in which the presence of rotavirus had been demonstrated by enzyme immunoassay. Of the RNA-positive samples, 18 (13·0%) were classified as subgroup 1, 94 (68·1%) as subgroup 2, and 26 (18·8%) could not be classified due to absence of visible bands 10 and 11. Subgroup 2 was more frequent in the three states. All strains of subgroup 1 detected in Rio de Janeiro were associated with a single short-lived school outbreak. All strains of subgroup 1 resembled each other in electrophoretic pattern, irrespective of geographical origin, although minor differences could be detected by co-electrophoresis. Subgroup 2, on the other hand, showed a great degree of electrophoretic heterogeneity and could be divided into several sub-categories.

INTRODUCTION

The importance of rotaviruses in the aetiology of gastroenteritis of man and animals has been demonstrated in many parts of the world (see reviews by Holmes, 1979 and Wyatt *et al.* 1981). Although all rotaviruses from human and other animal hosts are morphologically and biochemically similar and nearly all share common antigens, strains derived from different host species and even from different individuals of a given species can be distinguised by serological (Thouless *et al.* 1977; Thouless, Bryden & Flewett, 1978; Flewett *et al.* 1978; Spence, Fauvel & Petro, 1978; Yolken *et al.* 1978; Zissis & Lambert, 1978, 1980; Bishai *et al.* 1979)

and physicochemical (Kalica *et al.* 1976, 1978; Espejo, Calderón & Gonzaléz, 1977; Espejo *et al.* 1979, 1980*a*; Verly & Cohen, 1977; Rodger & Holmes, 1979; Rodger *et al.* 1981; Todd, McNulty & Allan, 1980; Lourenço *et al.* 1981) methods. Analysis of the virus genome by polyacrylamide gel electrophoresis (PAGE) is the method most commonly used for biochemical differentiation. This method is based on the fact that the rotavirus genome consists of 11 separate segments of double-stranded RNA (ds RNA), some of which have varying electrophoretic mobilities in different strains. A correlation between genome electrophoresis pattern and antigenic behaviour of human rotavirus strains has recently been demonstrated (Kalica *et al.* 1981*a*, *b*; M. E. Thouless, G. M. Beards & T. H. Flewett, personal communication). A study of genetic reassortants derived from mixed infections of human rotavirus strains with temperature-sensitive mutants of bovine rotavirus allowed Kalica *et al.* (1981*b*) to assign the genome segments coding for antigens with sub-group and type-specificities. The same group of workers (Kalica *et al.* 1981*a*) divided human rotaviruses into two sub-groups (1 and 2) distinguishable by RNA electrophoretic patterns and by sub-group specific serological tests such as enzyme-linked immunosorbent assay (ELISA) and immune adherence haemagglutination assay (IAHA).

Rotavirus strain differentiation by RNA electrophoresis has been used by several workers (Espejo *et al.* 1977, 1978, 1979, 1980*a*, *b*; Kalica *et al.* 1978; Rodger *et al.* 1981; Lourenço *et al.* 1981) to study the temporal and geographic distribution of distinct rotaviruses in several countries.

In the present paper we describe a similar study of rotaviruses detected in faecal samples from children suffering from gastroenteritis in Rio de Janeiro, São Paulo and Pará, Brazil.

MATERIALS AND METHODS

Faecal samples

Rotavirus-positive faeces from Rio de Janeiro were obtained between June 1979 and August 1981, from out-patient departments (15 samples), hospital wards (5 samples), child care institutions (3 samples) and a day school (10 samples). In São Paulo they were obtained between June 1978 and August 1981 from hospital wards or outpatient departments (48 samples), general practices (9 samples) and a child care institution (3 samples). Those from Pará were obtained between February 1979 and November 1981 from general practices (61 samples) and from a hospital (5 samples).

ELISA techniques

Faecal samples from children with gastroenteritis were tested for the presence of rotavirus by different modifications of the double antibody sandwich ELISA (Voller, Bartlett & Bidwell, 1978) used routinely in each of the collaborating laboratories.

In Rio de Janeiro (Oswaldo Cruz Foundation), wells of polystyrene microtitration plates (Cooke Laboratories) were coated with rotavirus antibody by the addition of 100 μl of a 1 in 40000 dilution of a goat antiserum to human rotavirus (kindly supplied by Dr R. M. Chanock, National Institutes of Health, Bethesda, Md, USA)

in 0·05 M carbonate buffer, pH 9·7. Buffer alone was added to parallel wells. After standing overnight at 4 °C, wells were washed three times with phosphate-buffered saline, pH 7·4, containing Tween 20 (polysorbate) at a final concentration of 0·05 % (PBS/T). Faecal suspensions (approximately 10–20 %) in 0·01 M Tris-HCl (pH 7·4) buffer containing 15 mM $CaCl_2$ (Tris-Ca^{2+} buffer) were diluted to $10^{-0·5}$ and $10^{-1·0}$ in PBS/T containing 1 % bovine serum albumin (PBS/T/BSA) and 100 μl of each dilution added to an antibody-coated and to an uncoated well. After 2 h incubation at 37 °C, wells were washed three times with PBS/T and to each was added 100 μl of a standardized dilution (1/4000) of a guinea-pig antiserum to simian rotavirus SA 11 in PBS/T/BSA. After incubation at 37 °C for 2 h, the wells were washed as above and to each was added 100 μl of a standardized dilution (1/2500) of a peroxidase-labelled rabbit antiserum to guinea-pig IgG (Cappel Laboratories Inc., Cochranville, PA, USA) in PBS/T/BSA. After further incubation at 37 °C for 2 h followed by washing as above, each well received 200 μl of a substrate solution containing 40 mg of ortho-phenylenediamine 2 HCl (Sigma) in a citrate–phosphate buffer (24·3 ml of 0·1 M citric acid, 25·7 ml of 0·2 M disodium phosphate and 50 ml distilled water) to which 150 μl of 30 % hydrogen peroxide was added immediately before use. After 20–30 min at room temperature the reaction was stopped by the addition of 25 μl of 4 N sulphuric acid to each well. Optical densities were read in a Multiskan Titertek colorimeter (Flow Laboratories) fitted with a 492 nm filter. Samples with which any of the two dilutions tested gave optical densities at least twice as high in antibody-coated than in uncoated wells were considered positive.

The ELISA technique performed in Pará (Evandro Chagas Institute) differed from the above mainly in that guinea-pig antiserum to human rotavirus was used both as coating antibody and as conjugate. Wells of polystyrene plates were coated with 100 μl of a 1/800 dilution of guinea-pig anti-rotavirus serum in carbonate buffer, pH 9·6. After standing at room temperature for 2 h, plates were washed three times with PBS/T and 50 μl of 20 % faecal suspensions in saline were added to duplicate pre-coated wells; 50 μl of PBS/T containing 1 % fetal calf serum (FCS) and 1 % normal guinea-pig serum were also added to each well. The plates were reincubated at room temperature for 4 h, washed three times as above, and then each well received 100 μl of a 1/100 dilution of peroxidase-labelled guinea-pig anti-human rotavirus globulin in PBS/T with 1 % FCS and 1 % normal guinea-pig serum. After incubation at 4 °C overnight, plates were washed again as above and to each well were added 100 μl of ortho-phenylenediamine at a concentration of 0·34 mg/ml in citrate-phosphate buffer, containing 0·02 % hydrogen peroxide. After 30 min at room temperature the reaction was stopped by adding 50 μl of a 4 N sulphuric acid solution. Readings of optical density were performed with a Bausch–Lomb colorimeter, using a 492 nm filter. All samples yielding a positive/negative value (Yolken *et al.* 1977) of greater than 2·1 were regarded as positive.

In São Paulo (Department of Biomedical Sciences, University of São Paulo) the ELISA was performed with the Rotazyme diagnostic kit (Abbot Laboratories, North Chicago, Il., USA) according to the manufacturer's instructions.

Polyacrylamide gel electrophoresis

This was performed in one of the collaborating laboratories (Oswaldo Cruz Foundation) by the following techniques. Approximately 10–20% faecal suspensions in Tris-Ca^{2+} buffer were clarified by centrifugation at 5000 *g* for 15 min. Supernatants which remained cloudy were added to an equal volume of Freon 113, homogenized and centrifuged as above. To 1 ml of each clarified suspension was added 0·1 ml of a 10% (w/v) solution of sodium dodecyl sulphate (SDS) followed by incubation at 37 °C for 30 min. To each was then added 1 ml of a mixture of phenol and chloroform in equal parts. The mixtures were agitated at frequent intervals for 15 min at room temperature, followed by centrifugation at 5000 *g* for 15 min. The aqueous phases were transferred to tubes containing 0·1 ml of a 20% (w/v) solution of NaCl to which were subsequently added 2·5 ml of ethyl alcohol. The tubes were kept at −20 °C overnight and centrifuged at 20000 *g* for 30 min. The supernatants were decanted and the pellets dissolved in 0·1 ml of Laemmli's (1970) sample buffer (40% glycerol, 5% 2-mercaptoethanol, 3% SDS, 0·0625 M Tris, pH 6·8). Volumes ranging from 5 to 50 μl, according to expected RNA concentration, were used for electrophoresis.

Bovine (strain UK) and simian (strain SA11) rotaviruses supplied by Dr T. H. Flewett (Regional Virus Laboratory, Birmingham, U.K.) were cultivated in simian cell lines MA104 or LLCMK2 cells in the presence of trypsin. The total contents of infected cultures showing advanced cytopathic effect were frozen and thawed and spun at 100000 *g* for 1 h. The resulting pellet was homogenized in Tris-Ca^{2+} buffer in the presence of an equal volume of Freon 113 and centrifuged at 5000 *g* for 15 min. The aqueous phase was made up to 4 ml, layered on 1 ml of 45% (w/v) sucrose in Tris-Ca^{2+} buffer and centrifuged for 90 min at 100000 *g*. The resulting pellet was used for RNA analysis either directly or after isopycnic banding on CsCl. In the latter case 10–20 μl volumes of samples from virus-containing bands with densities of 1·38–1·40 were dissociated with an equal volume of 5 M urea, 2% SDS, 2% 2-mercaptoethanol and 0·002% bromophenol blue and placed on the gels.

Electrophoresis was carried out by Laemmli's (1970) techniques slightly modified as follows: The separating gel contained acrylamide either in constant concentration of 7·5% or in a gradient ranging from 3·5% to 10%. In both cases the stacking gel contained 3% acrylamide. Both stacking and separation gels, as well as the reservoir buffer contained, in addition to Laemmli's formula, 0·01 M ethylenediamino tetracetic acid (EDTA). Slab gels were 1·5 mm thick, 16 cm wide and 12·5 cm high. Electrophoresis was done at room temperature at constant current at 25–30 mA per slab, for 14–16 h. The gels were stained with ethidium bromide at a concentration of 0·5 μg/ml of distilled water for 30 min at room temperature followed by washing in distilled water for several hours. The gels were photographed in transmitted u.v. light.

RESULTS

In the following description of RNA patterns, the 11 bands are grouped, as by previous workers (e.g. Kalica *et al.* 1976, 1978; Lourenço *et al.* 1981) into four clusters shown in Fig. 1.

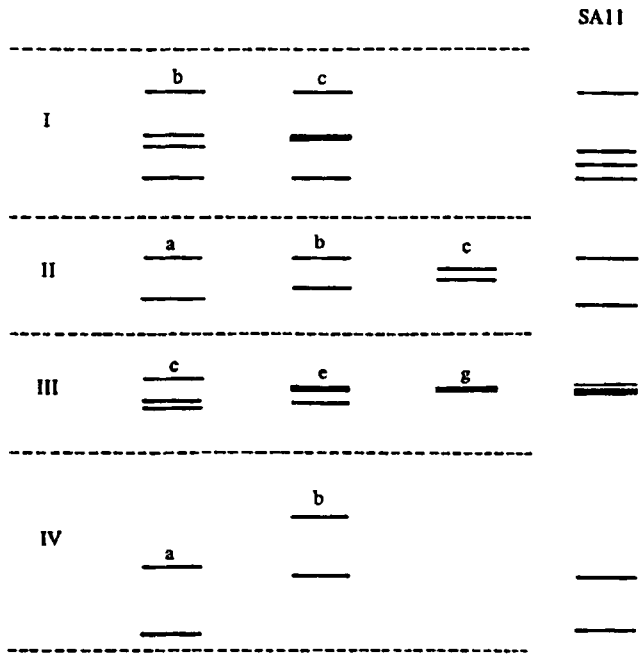


Fig. 1. Diagram of RNA cluster patterns (modified from Lourenço *et al.* 1981). RNA bands are divided into four clusters represented by bands 1–4 (cluster I), bands 5 and 6 (cluster II), bands 7–9 (cluster III) and bands 10 and 11 (cluster IV). Distinct patterns within each cluster are referred to as a, b, c, etc. Cluster patterns not found in the present study are not included in the diagram. The pattern obtained with the simian rotavirus (SA11) is also shown.

RNA patterns of bovine and simian rotaviruses are compared with that of a subgroup 2 human strain in Plate 1. Electrophoresis of a random selection of ELISA-positive faecal samples from São Paulo are shown in Plate 2 where a number of different patterns can be distinguished. Samples in channels E and H closely resemble each other and are identified as subgroup 1 strains (slow moving bands 10 and 11) as opposed to all others which belong to subgroup 2. Several differences are also apparent in the relative positions of bands other than 10 and 11. Thus, the distance between bands 5 and 6 of E and H is shorter than that of D and longer than those of all other subgroup 2 strains. In cluster III, the two subgroup 1 samples show a relatively slow band 7 with bands 8 and 9 close to each other, in contrast to all others where bands 7 and 8 are almost superimposed, whereas band 9 runs faster. Under the condition in which the gel shown in Plate 2 was run (7·5% polyacrylamide throughout the separating gel), no clear differences could be seen in band cluster I. To improve resolution of this cluster, all samples were run in gels with a polyacrylamide gradient ranging from 3·5% to 10%. Under these conditions, two distinct patterns of band cluster I could be distinguished: in one of them bands 2 and 3 were resolved (as in Fig. 1, channel C) whereas in the other they were superimposed.

An attempt was made to classify the electrophoretic patterns observed in this

https://doi.org/10.1017/S0022172400063919 Published online by Cambridge University Press

Table 1. *RNA electrophoresis results of 159 rotavirus-positive human faeces from Brazil*

Subgroup	Electrophoresis cluster pattern I	II	III	IV	Origin Rio de Janeiro	São Paulo	Pará	Total
1	c	b	c	b	9	5	3	17
	c	b	*	b			1	1
	Total				9	5	4	18
2	b	c	e	a	5	12	25	42
	c	a	e	a		1		1
	c	b	e	a	2			2
	c	c	e	a	9	10	5	24
	b	b	g	a			1	1
	b	c	g	a		3	1	4
	c	c	g	a		1		1
	b	e	*	a			5	5
	c	c	*	a			5	5
	b	*	e	a		2		2
	c	*	e	a	2			2
	*	c	e	a		5		5
	Total				18	34	42	94
Indeterminate	b	c	e	*		3	1	4
	c	c	e	*		3	1	4
	c	b	e	*		1		1
	c	c	g	*		1		1
	b	c	*	*	1	4	2	7
	c	b	*	*	1			1
	c	c	*	*	1		1	2
	c	*	e	*		1		1
	b	*	*	*		1		1
	c	*	*	*		1		1
	*	c	*	*			3	3
	Total				3	15	8	26
Total								
Positives					30	54	54	138
Negatives					3	6	12	21

* Indistinct or invisible.

study according to the system proposed by Lourenço *et al.* (1981). All strains in which all 11 bands were visible in gradient gels could be allocated to one of the combinations of RNA cluster patterns shown in Fig. 1 which is an adaptation of the diagram published by Lourenço *et al.* (1981). An additional pattern of cluster III, differing from those described by the above authors and here designated as IIIg, was observed in some of our strains and was characterized by superimposition of bands 7, 8 and 9.

Electrophoretic patterns and subgroup classification of strains from different parts of Brazil are shown in Table 1. Within the total of 159 rotavirus-positive samples tested, at least some of the characteristic rotavirus RNA bands were

https://doi.org/10.1017/S0022172400063919 Published online by Cambridge University Press

detected in 138 (86·8%). Of these, 112 could be classified, on the basis of the mobility of bands 10 and 11, into subgroups 1 (18 samples) or 2 (94 samples) and the remaining 26 could not be subgrouped due to the absence of visible bands 10 and 11.

Subgroup 2 predominated in all areas investigated and its occurrence was spread out both in time and in space. In contrast, all subgroup 1 strains from Rio de Janeiro originated from an outbreak of short duration during May 1980 in a day school (Sutmoller *et al.* 1982). In São Paulo, however, four subgroup 1 strains were detected in one hospital in January, February, March, and July 1979 and one in another hospital in May 1981. In Pará, subgroup 1 strains were detected in patients from general practices, two in December 1980 and two in January 1981. A notable feature of subgroup 1 strains was that they all resembled each other irrespective of geographical origin, and differed from those of subgroup 2 in regard to several bands other than 10 and 11 (Table 1). To investigate this point in more detail, a comparison of subgroup 1 strains was carried out by co-electrophoresis. This revealed the electrophoretic identity of three strains from the outbreak in Rio de Janeiro but demonstrated small differences limited to bands 10 and 11 between strains from the three geographical areas under study. Thus, strains from Rio de Janeiro and Pará could be distinguished by a slightly slower migration of band 10 of the former, and strains from Pará and São Paulo could be differentiated by a slightly faster migration of band 11 in the latter.

In contrast to subgroup 1, strains of subgroup 2 showed easily distinguishable patterns indicating a greater degree of heterogeneity. Difficulty in distinguishing between some of the patterns was sometimes found, especially in cluster II in which the bands (5 and 6) sometimes occupied intermediary positions in an apparently continuous variation. Furthermore, it was not always easy to relate some of the patterns to those described by Lourenço *et al.* (1981), especially as the pattern shown by the simian rotavirus SA11 used in our laboratory as the reference strain in all runs differs in some respects, for instance in cluster III, from the one described by those authors.

It must be stressed that our results probably represent an oversimplification, as only a small number of strains could be compared with each other by co-electrophoresis which would be a more discriminatory method for strain differentiation.

DISCUSSION

The present findings show that, as in other parts of the world, human rotaviruses detected in Brazil are very heterogeneous in the electrophoretic behaviour of their genome segments. Polyacrylamide gel electrophoresis proved to be a simple and sensitive method capable of detecting virus RNA in a high proportion (86·8%) of faeces in which the presence of rotavirus had been demonstrated by enzyme immunoassay. Furthermore, nearly 60% of the samples investigated could be classified into subgroup 1 or 2 although the latter could be subdivided into several electrophoretic categories.

The relation between electrophoretic characters and epidemiological behaviour was clearly demonstrated in Rio de Janeiro, where all strains of subgroup 1 were

associated with a school outbreak of short duration. This supported the evidence previously presented by Sutmoller *et al.* (1982) for a common source of infection in that episode. There was also a suggestion that the occurrence of subgroup 1 was limited in time in Pará where the three strains of this subgroup were all recorded between 18 December 1980 and 20 January 1981, whereas in São Paulo this subgroup was detected over a period of 3 months (January–March) in 1979 and on single occasions in July of the same year and in May 1981. The occurrence of subgroup 2 strains was more widespread both in time and space, but this may merely reflect a higher incidence of this subgroup. No clear evidence was obtained to indicate that electrophoretically distinct strains of subgroup 2 were associated with particular epidemiological episodes, but this possibility cannot be excluded.

Study of a larger number of samples, preferably in longitudinal surveys of given populations will be required to evaluate the epidemiological significance of viral variation as reflected by RNA electrophoresis.

The authors gratefully acknowledge the support of the Mérieux Foundation, Lyon, France for the Oswaldo Cruz Foundation, Rio de Janeiro.

REFERENCES

BISHAI, F. R., SPENCE, L., GOODWIN, D. & PETRO, R. (1979). Use of antisera against bovine (NCDV) and simian (SA11) rotaviruses in ELISA to detect different types of human rotaviruses. *Canadian Journal of Microbiology* **25**, 1118–1124.

ESPEJO, R. T., AVENDAÑO, L. F., MUÑOZ, O., ROMERO, P., ETERNOD, J. G., LOPEZ, S. & MONCAYA, J. (1980*a*). Comparison of human rotaviruses isolated in Mexico City and in Santiago, Chile, by electrophoretic migration of their double-stranded ribonucleic acid genome segments. *Infection and Immunity* **30**, 342–348.

ESPEJO, R. T., CALDERÓN, E. & GONZÁLES, N. (1977). Distinct reovirus-like agents associated with acute infantile gastroenteritis. *Journal of Clinical Microbiology* **6**, 502–506.

ESPEJO, R. T., CALDERÓN, E., GONZÁLEZ, N., SALOMON, A., MATRUSCELLI, A. & ROMERO, P. (1978). Rotavirus gastroenteritis in hospitalized infants and young children in Mexico City. *Revista Latino-Americana de Microbiologia* **20**, 239–246.

ESPEJO, R. T., CALDERÓN, E., GONZÁLEZ, N., SALOMON, A., MATRUSCELLI, A. & ROMERO, P. (1979). Presence of two distinct types of rotavirus in infants and young children hospitalized with acute gastroenteritis in Mexico City, 1977. *Journal of Infectious Diseases* **139**, 474–477.

ESPEJO, R. T., MUÑOZ, O., SERAFIN, F. & ROMERO, P. (1980*b*). Shift in the prevalent human rotavirus detected by ribonucleic acid segment differences. *Infection and Immunity* **27**, 351–354.

FLEWETT, T. H., THOULESS, M. E., PILFOLD, J. N., BRYDEN, A. S. & CANDEIAS, J. A. N. (1978). More serotypes of human rotavirus. *Lancet* ii, 632.

HOLMES, I. H. (1979). Viral gastroenteritis. *Progress in Medical Virology* **25**, 1–36.

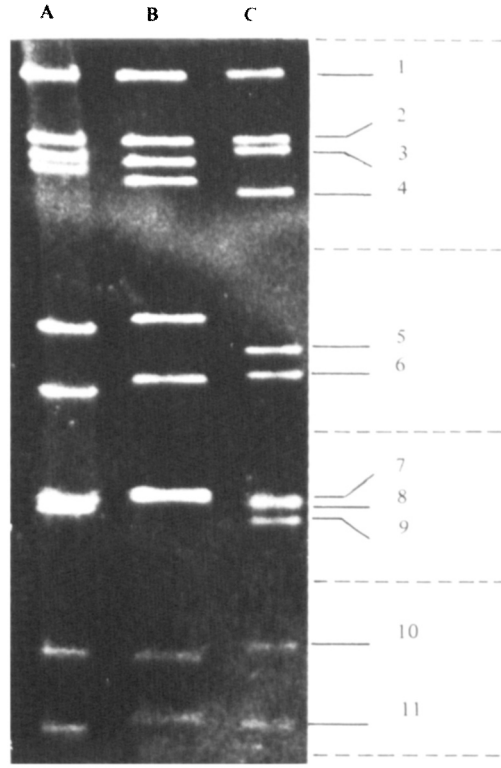
KALICA, A. R., GARON, C. F., WYATT, R. G., MEBUS, C. A., VAN KIRK, D. H., CHANOCK, R. M. & KAPIKIAN, A. Z. (1976). Differentiation of human and calf rotavirus-like agents associated with diarrhoea using polyacrylamide gel electrophoresis of RNA. *Virology* **74**, 86–92.

KALICA, A. R., GREENBERG, H. B., ESPEJO, R. T., FLORES, J., WYATT, R. G., KAPIKIAN, A. Z. & CHANOCK, R. M. (1981*a*). Distinctive ribonucleic acid patterns of human rotavirus subgroup 1 and 2. *Infection and Immunity* **33**, 958–961.

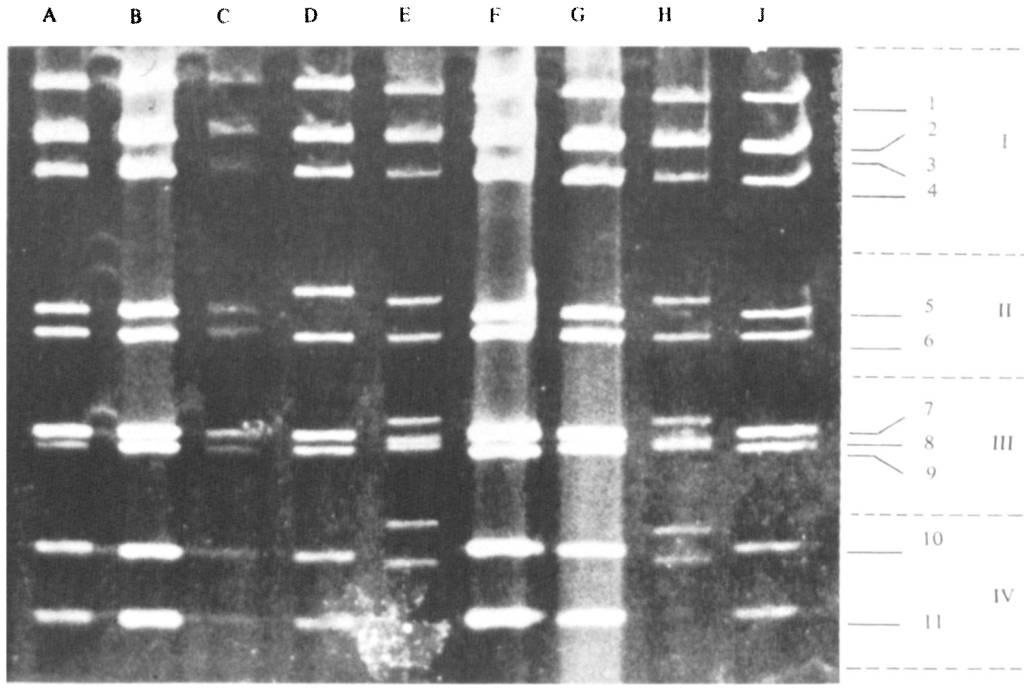
KALICA, A. R., GREENBERG, H. B., WYATT, R. G., FLORES, J., SERENO, M. M., KAPIKIAN, A. Z. & CHANOCK, R. M. (1981*b*). Genes of human (strain Wa) and bovine (strain UK) rotaviruses that code for neutralization and subgroup antigens. *Virology* **112**, 385–390.

KALICA, A. R., SERENO, M. M., WYATT, R. G., MEBUS, C. A., CHANOCK, R. M. & KAPIKIAN, A. Z. (1978). Comparison of human and animal rotavirus strains by gel electrophoresis of viral RNA. *Virology* **87**, 247–255.

LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature London* **227**, 680–685.



https://doi.org/10.1017/S0022172400063919 Published online by Cambridge University Press



https://doi.org/10.1017/S0022172400063919 Published online by Cambridge University Press

Lourenço, M. H., Nicolas, J. C., Cohen, J., Scherrer, R. & Bricout, F. (1981). Study of human rotavirus genome by electrophoresis: attempt of classification among strains isolated in France. *Annals of Virology* (Inst. Pasteur) **132E**, 161–173.

Rodger, S. M., Bishop, R. F., Birch, C., McLean, B. & Holmes, I. H. (1981). Molecular epidemiology of human rotaviruses in Melbourne, Australia, from 1973 to 1979 as determined by electrophoresis of genome ribonucleic acid. *Journal of Clinical Microbiology* **13**, 272–278.

Rodger, S. M. & 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. *Journal of Virology* **30**, 839–846.

Spence, L., Fauvel, M. & Petro, R. (1978). Comparison of rotavirus strains by hemagglutination inhibition. *Canadian Journal of Microbiology* **24**, 353–356.

Sutmoller, F., Azeredo, R. S., Lacerda, M. D., Barth, O. M., Pereira, H. G., Hoffer, E. & Schatzmayr, H. G. (1982). An outbreak of gastroenteritis caused by both rotavirus and *Shigella sonnei* in a private school in Rio de Janeiro. *Journal of Hygiene* **88**, 285–293.

Thouless, M. E., Bryden, A. S. & Flewett, T. H. (1978). Serotypes of human rotavirus. *Lancet* i, 39.

Thouless, M. E., Bryden, A. S., Flewett, T. H., Woode, G. N., Bridger, J. C., Snodgrass, D. R. & Herring, J. A. (1977). Serological relationship between rotaviruses from different species as studied by complement fixation and neutralization. *Archives of Virology* **53**, 257–294.

Todd, D., McNulty, M. S. & Allan, G. M. (1980). Polyacrylamide gel electrophoresis of avian rotavirus RNA. *Archives of Virology* **63**, 87–97.

Verly, E. & Cohen, J. (1977). Demonstration of size variation of RNA segments between different isolated of calf rotavirus. *Journal of General Virology* **35**, 385–386.

Voller, A., Bartlett, A. & Bidwell, D. (1978). Enzyme immunoassays with special reference to ELISA techniques. *Journal of Clinical Pathology* **31**, 507–520.

Wyatt, R. G., Kalica, A. R., Mebus, C. A., Kim, H. W., London, W. T., Chanock, R. M. & Kapikian, A. Z. (1981). Reovirus-like agents (rotaviruses) associated with diarrheal illness in animals and man. *Perspectives in Virology* **10**, 121–145.

Yolken, R. H., Barbour, B., Wyatt, R. G., Kalica, A. R., Kapikian, A. Z. & Chanock, R. M. (1978). Enzyme-linked immunosorbent assay for identification of rotaviruses from different animal species. *Science* **201**, 259–262.

Yolken, R. H., Kim, H. W., Clem, T., Wyatt, R. G., Kalica, A. R., Chanock, R. M. & Kapikian, A. Z. (1977). Enzyme-linked immunosorbent assay (ELISA) for the detection of human reovirus-like agent of infantile gastroenteritis. *Lancet* ii, 263–267.

Zissis, G. & Lambert, J. P. (1978). Different serotypes of human rotaviruses. *Lancet* i, 38–39.

Zissis, G. & Lambert, J. P. (1980). Enzyme-linked immunosorbent assays adapted for serotyping of human rotavirus strains. *Journal of Clinical Microbiology* **11**, 1–5.

EXPLANATION OF PLATES

Plate 1

RNA electrophoretic patterns of bovine, simian and human rotaviruses. Channel A, bovine rotavirus, strain UK; channel B, simian rotavirus, strain SA11 and channel C, human rotavirus, subgroup 2. Electrophoresis in 3·5–10% polyacrylamide gradient, 25 mA, 15 h.

Plate 2

RNA electrophoretic patterns of human rotavirus from São Paulo. Channels A–J, random ELISA-positive faecal samples; 20 μl of extracted RNA from each sample. Electrophoresis in 7·5% acrylamide, 25 mA, 15 h.