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ATYPICAL HUMAN ROTAVIRUSES IN THE G.D.R.

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Summary. — Following the aetiologic agent of diarrhoea in children aged one to three years we found in the stools a large number of rotaviruses by electron microscopy (EM), although ELISA was negative; the sensitivity of ELISA had been confirmed in previous investigations. In 141 children with diarrhoea 10 conventional (group A) and 21 antigenically distinct rotaviruses were detected. The presence of atypical rotaviruses could be confirmed by electrophoretic analysis of their RNA migration profiles. The electropherotype pattern corresponded to the group C rotaviruses reported by others.

Key words: rotavirus group C of men; gastroenteritis; electron microscopy; ELISA; RNA electrophoresis

Introduction

The laboratory diagnosis of human rotaviruses is already well established. Because of the difficulties to cultivate human rotaviruses in cell cultures, their diagnostic is based mainly on immunological methods using antibodies against simian or bovine rotaviruses which have a common group specific antigen located on the inner capsid of the virion. Rotaviruses, morphologically identical, but antigenically unrelated with the conventional rotaviruses of group A, were detected first in 1980 in swine, calves, and later on in other animals by electron microscopy (EM). Since 1982 atypical rotaviruses (pararotavirus) have been found also in children which fell ill with diarrhoea (Rodger *et al.*, 1982; Dimitrov *et al.*, 1983; Nicolas *et al.*, 1983; Espejo *et al.*, 1984; Arista *et al.*, 1985; Dimitrov *et al.*, 1986). Because of the absence of the group A rotavirus group-specific antigen, their detection was impossible by a common immunological test such as ELISA. To date, EM is the main screening method for atypical rotaviruses. Because of the inability to multiply human atypical rotaviruses in cell cultures, the immunological identification and serological grouping is only possible by ELISA or by immunoelectron microscopy using human convalescent sera. Another effective and useful method for detecting and characterizing atypical rotaviruses is the electropherotyping of the viral genomic dsRNA segments in polyacrylamide gel electrophoresis (PAGE) (Espejo *et al.*, 1984; Snodgrass *et al.*, 1984; Arista *et al.*, 1985; Besselaar *et al.*, 1986; Pedley *et al.*, 1986; Sorrentino *et al.*, 1986).

With this method a very clear distinction between the RNA pattern of the rotavirus group A and the atypical rotaviruses could be made; the latter were classified into rotavirus groups B and C (Flewett *et al.*, 1984; Bridger *et al.*, 1986; Nakata *et al.*, 1986; Pedley *et al.*, 1986).

When establishing ELISA for detection of human rotavirus antigens as diagnostic tool in our laboratory we compared its results with EM findings. A nearly complete correspondence between both methods was described (Schumacher *et al.*, 1986). But recently EM investigations seemed to be more efficient as the results of ELISA; atypical rotaviruses were suspected in the G.D.R. causing diarrhoea in children.

Materials and Methods

Specimens. Faecal solutions (10—20 %) from children with diarrhoea were prepared in bidistilled water by shaking for 30 min and ultrasonication (for 30 min). The solutions were clarified by centrifugation at 2500 rev/min for 15 min. The supernatants were investigated in ELISA and by EM; in addition, the supernatants were homogenized with equal volume of Freon 113, centrifuged and RNA was extracted for PAGE.

Electron microscopy. Negative staining was performed without further concentration (2 % potassium phosphotungstate, pH 6.0).

Immunoelectron microscopy (IEM). Clumping method, solid phase immunoelectron microscopy and decoration method (Kjeldsberg, 1986) were used employing SA 11-, NCDV-, and conventional human rotavirus antisera in dilutions 1 : 100 to 1 : 1000. The samples were viewed in electron microscope Tesla BS 500.

ELISA. Microtitre plates were coated with rabbit immune serum against rotavirus SA 11 in dilution 1 : 5000 or with rabbit serum proved to be without antibodies (pre-immune serum) in the same dilution. The samples were given to both coating variants and covered then with peroxidase-conjugated immune serum from rabbit; o-phenylene diamine was added as substrate. The reaction was stopped with 2 mol/l H_2SO_4. The results were evaluated positive, when the ratio of extinction of immune serum to pre-immune serum (P/N) was more than 2 and the extinction of immune serum was more than 100. The method has been described in more detail elsewhere (Schumacher *et al.*, 1986).

RNA analysis. Electrophoresis was performed in 8 % polyacrylamide slab gels in the Laemmli system (Laemmli, 1970).

Extraction of viral RNA. The Freon treated supernatants (400 µl) were incubated with 0.1 mg/ml thermitase (Schreier *et al.*, 1988) and 1 % sodium dodecyl sulphate (SDS) in 50 mmol/l Tris/HCl, pH 7.4 for 1 hr at 37 °C. The RNA was extracted with Phenol saturated with Tris/HCl buffer and precipitated with ethanol at −20 °C. The RNA was resuspended in sample buffer (62 mmol/l Tris/HCl, pH 6.8, 2 % SDS, 5 % mercaptoethanol, 10 % glycerol, 0.001 % bromophenol blue) prior to analysis. Electrophoresis was performed for 3 hr at 20 mA. Finally, the gel was stained overnight with ethidium bromide (2 µg/ml) and photographed in the UV light.

Results

In 1987 children aged 1—3 years with acute diarrhoea were investigated for rotaviruses in the frame of a comprehensive study. The study was restricted to a small town of the western part of the G.D.R. The same faecal specimen of each child was checked both by ELISA and EM. Altogether faecal samples from 141 children were investigated. Rotaviruses have been found in 31 cases. In 120 samples the same results were found with both methods, 110 cases were negative, and 10 cases were rotavirus-positive. But in additional 21 cases rotavirus particles were seen by electron micro-

Table 1. Investigation of specimens from children with diarrhoea

Test	Diagnosis	Specimen number
ELISA negat, EM negat	No rotavirus	110
ELISA posit, EM posit	Rotavirus group A	10
ELISA negat, EM posit	Atypical rotavirus	21
Total		141

scopy despite of negative ELISA results (Table 1). In the majority of these specimens many rotavirus particles were seen, most of them with a single shell (Figs. 1—2). These samples were investigated by IEM. No reaction with antibodies against group A human rotaviruses, SA 11 or NCDV could be observed (Figs. 3—4) in the solid phase immunoelectron microscopy, by clumping or by decoration methods.

The electropherotypes of these presumably antigenically distinct viruses were determined in PAGE. The migration profiles of the 11 RNA segments were visible in 12 samples; they were identical with the migration pattern of the RNA of human atypical rotaviruses. Some examples are shown in Fig. 5, in comparison with the migration profiles of the RNA segments of the reference strain SA 11 and the two group A human rotaviruses with long or short migration patterns, respectively. In the other 9 EM-positive but ELISA negative specimens the quantity of material submitted for RNA-PAGE was not sufficient to determine the electropherotype pattern.

Discussion

In the G.D.R. usual methods for detection of human rotaviruses are counter immunoelectrophoresis, ELISA and EM. The immunological methods employ polyclonal antibodies against the simian rotavirus SA 11 because of requiring special conditions for cultivation of human rotaviruses in cell cultures. The sensitivity and specificity of the ELISA used in our laboratory has been proved in many comparative tests and a nearly complete correspondence of the results with those of EM could be shown.

In 1987 in a study on aetiology of diarrhoea in children in the age group of 1—3 years unexpected differences between EM and ELISA were found. As already described in other countries, the presence of antigenically distinct rotaviruses was supposed, because the viruses were detectable neither by ELISA nor by immunoelectron microscopic methods.

By 21 samples the viral RNA migration profile was investigated in PAGE; atypical rotaviruses could be verified in 12 cases. The RNA pattern strongly resembled the profiles described in previous reports with atypical human rotaviruses (Pereira *et al.*, 1983; Espejo *et al.*, 1984; Arista *et al.*, 1985; Besselaar *et al.*, 1986; Bridger *et al.*, 1986; Dimitrov *et al.*, 1986; Sorremtino *et al.*,

1986) belonging to group C (Flewett *et al.*, 1984; Bridger *et al.*, 1986). The rotavirus group B strains causing epidemic outbreaks of acute gastroenteritis in adults in China exhibited a different migration pattern (Tao *et al.*, 1984; Wang *et al.*, 1985; Nakata, 1986; Dai *et al.*, 1987). In the remaining 9 cases an electrophoretic analysis was not successful, but very likely all 21 viruses detected with EM can be classified as atypical rotaviruses.

The high number of atypical rotaviruses in our study is surprising as atypical rotaviruses two times exceeded the conventional rotaviruses (21 : 10). With the wide use of ELISA as a common method for rotavirus antigen detection the significance of atypical rotaviruses may be underestimated. In our laboratory as routine diagnostic for children with diarrhoea from hospitals in Berlin only ELISA is being performed. In few exceptional cases the samples were checked by EM. At such occasions, in 1986 and 1987 rotavirus was found in faeces of 3 children from Berlin by EM only. An electrophoretic analysis has not been done or was unsuccessful. Thus, confirmation of atypical rotavirus was not made in these cases. But it can be taken as a hint, that atypical rotaviruses may cause diarrhoea presumably also in other regions of G.D.R. not only in the restricted area. In healthy children we did not find any rotaviruses; but the number of investigated samples was small. Further investigations are necessary to confirm the clinical and epidemiological importance of atypical rotaviruses. It would be a great progress to overcome the restricted multiplication of atypical rotaviruses; there is an urgent need for establishing a new diagnostic procedure to detect pararotaviruses in addition to EM and PAGE.

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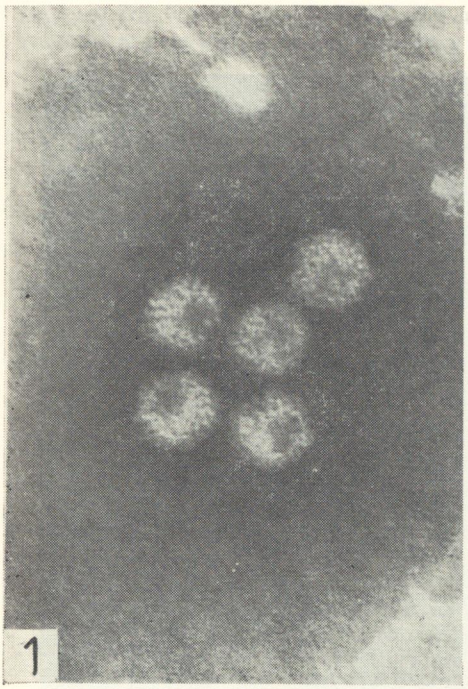

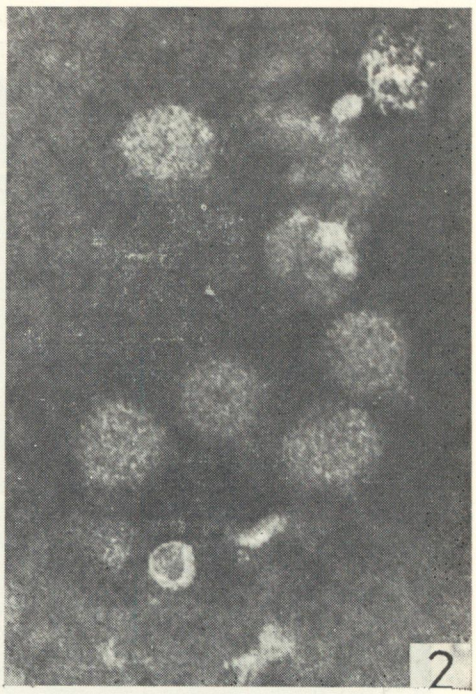

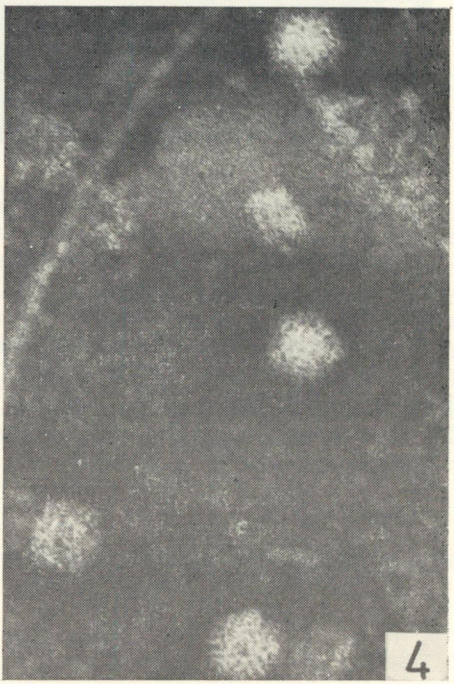

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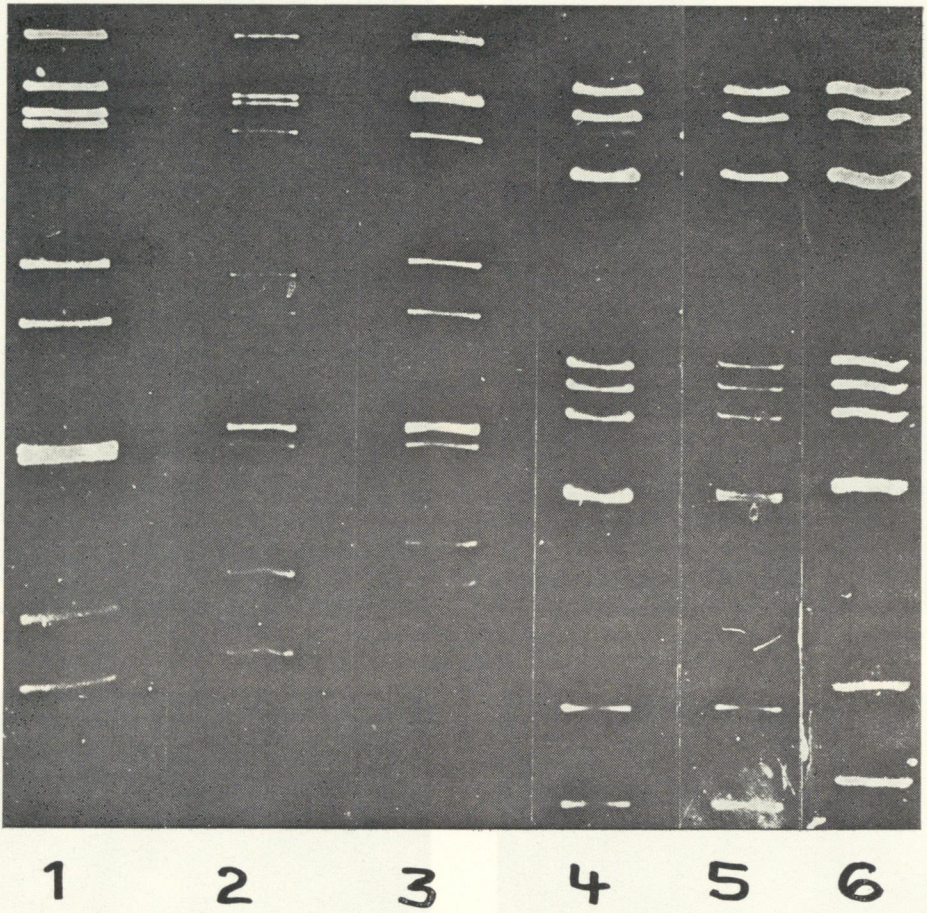

Fig. 5.

Figs. 1—2. Atypical rotavirus particles; (1) single shelled; (2) double shelled. 180 000 ×

Figs. 3—4. Immunoelectron microscopy, clumping method; (3) immune complex of conventional human rotavirus with antibody against human rotavirus strain Wa; (4) atypical rotavirus did not react with the same antibody. 180 000 ×

Fig. 5. Polyacrylamide gel electrophoresis: electrophoretic migration profile of RNA segments of simian rotavirus SA 11 (lane 1), conventional human rotavirus with long pattern (lane 2), with short pattern (lane 3), and of 3 atypical human rotaviruses (lanes 4—6).