Acta virol. **35** : 408-412, 1991

DETECTION OF TWO ATYPICAL ROTAVIRUSES IN THE PROVINCE OF MISIONES, ARGENTINA

S. N. BASNEC[1], M. O. GIORDANO[1], F. R. BENNUN[1], S. V. NATES[1,*], M. VERGARA[2], A. R. DEPETRIS[1]

[1]Instituto de Virologia "Dr. J. M. Vanella"-Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Estafeta 32-Ciudad Universitaria-5000 Córdoba; and [2]Hospital "Dr. R. Madariaga", Misiones, Argentina

Received November 20, 1990

Summary. - Out of 317 human gastroenteritis cases studied between August 1988-August 1989, two atypical antigenically distinct rotaviruses (pararotaviruses) were detected in faecal samples among 19 rotaviruses shedding children from Misiones province, North-Eastern Argentina. A 1 3/4 year old girl a 3 years old boy, both with vomiting and normal temperature, shed these atypical rotaviruses. Their morphology by electron microscopy was identical to other rotaviruses; they contained 11 double-stranded RNA segments detected by polyacrylamide gel electrophoresis and failed to react with the antibody directed against the rotavirus group specific antigen (Rotazyme II ELISA). The electrophoretic migration of these RNAs (electropherotype) in polyacrylamide gels did not coincide with the typical pattern of distinct size classes observed in most human rotaviruses reported, instead, they appeared to be related to patterns of rotaviruses group C.

Key words: RNA electrophoresis pattern; atypical rotaviruses; human gastroenteritis

Introduction

It is well known that rotaviruses are important causal agents of viral gastroenteritis in children (Kapikian *et al.*, 1980). They have also been described as the aetiological agents of gastroenteritis in many animals (Estes *et al.*, 1983).

Rotaviruses from different mammalian species are morphologically similar and share a common group-specific antigen (Yolken *et al.*, 1978). When analysed by polyacrylamide gel electrophoresis in combination with silver staining (PAGE-SS) their double stranded RNA shows a pattern of 11 segments.

*To whom correspondence should be addresssed

Atypical RNA patterns as detected in PAGE have been reported for some rotaviruses; different terms were proposed, such as pararotavirus (Bohl *et al., 1982),* group B rotavirus (Dimitrov *et al.,* 1983), and atypical rotavirus (Sorrentino *et al.,* 1986), to designate these antigenically and electrophoretically distinct from group a rotaviruses.

In the present report we describe the detection of two human atypical rotaviruses isolated in the Misiones province, Argentina, whose electrophoretic patterns resembled that of group C rotaviruses.

Materials and Methods

Patients and materials. Faecal samples were collected from children admitted with diagnosis of gastroenteritis to "Dr. R. Madariaga" Hospital in Misiones, Argentina, as part of the Annual Collaborative National Survey of Aetiological Agents in Acute Diarrhoea in Children (Argentinian Secrectary of Sience and Technology-SECYT). One of the samples in question was obtained from a 1 3/4 year old girl, admitted on November 1st, 1988 with diarrhoea, normal temperature and vomiting. Diarrhoea four days during the hospitalizion period, with an evacuation frequency of 5-6 times a day. The faecal sample was taken on the first day of admission. The second case was a male child aged 3, admitted on October 5th, 1988. This child had begun with diarrhoea three days before admission, with 6-7 evacuations per day, normal temperature, and vomiting.

*RNA analysis. S*tools were stored at −20°C until they were tested. For polyacrylamide gel electrophoresis 20 % faecal suspendions were homogenized in 0.02 mol/l TRIS-HCl, pH 7.2, and clarified by centrifugation. About 200 μl of supernatants were mixed with equal volume of extraction buffer (500 mmol/l LiCl, 10 mmol/l ethylennediaminetetraacetic disodium salt-EDTA, and 1 % sodium dodecyl sulphate SDS), and 400 μl of phenol-chloroform 1:1. After incubation for 10 min at 56°C, the materials were centrifuged at 16 000 xg 30 min and 200 μl of the aqueous phase were added to 1 ml of ethanol for RNA precipitation (overnight at -20°C). The pellets obtained after centrifugation (16 000 xg, 10 min) were mixed with 15 μl of sample buffer (62 mmol/l TRIS-HCl pH 6.8, 5 % 2-mercaptoethanol, 3 % SDS, 0.01 % bromphenol blue and 30 % glycerol), and loaded on top of 10 % polyacrylamide gel according to Laemmli (1970). Gel running was performed during 18 hr at 15 mA. Gels were stained by the silver technique (Herring *et al.,* 1982).

Enzyme-linked immunosorbent assay (ELISA) and electron microscopy (EM). Samples with atypical RNA patterns, all the positive RNA-PAGE samples and 10 % of the negative ones were tested by ELISA (Rotazyme II-Abbott) according to the manufacture instructions, and viewed by electron microscopy (for details see Muchinik *et al.,* 1981).

Results

Out of 317 human faecal samples obtained from patients with gastroenteritis, residents of Misiones, Argentina, investigated between August 1988-August 1989, 19 were positive for rotavirus when tested by PAGE-SS. The RNA analysis of the electrophoretic patterns of two of them were different from the electropherotypes "long" and "short" of group A rotavirus detected until now in our laboratory (Fig. 1).

When comparing the RNA profiles we observed differences in groups II and III. One of the segments belonging to group III migrated within the range of group II, and the group III remained with two bands. The extension of the elec-

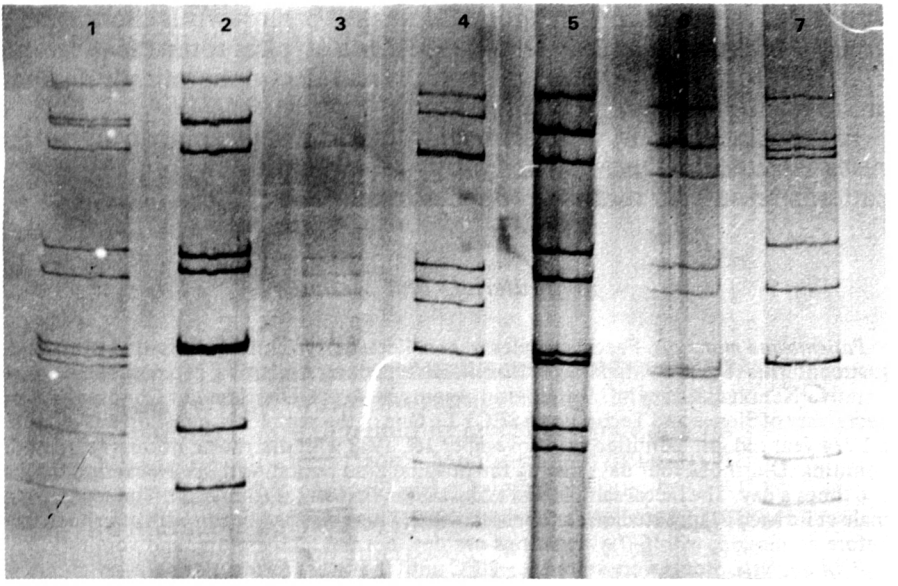

Fig. 1
Comparison of profiles of the 11 rotavirus genome segments after electrophoresis in 10 % polyacrylamide gels and silver staining
Lanes 1 and 2: Rotavituses type "long".
Lanes 3 and 4: Atypical rotaviruses described.
Lanes 5 and 6: Rotaviruses type "short".
Lane 7: Similar rotavirus SA 11 genomic RNA included as control.

trophoretical migration of the samples resembled that of "long" electropherotype (Fig. 1, lanes 3-4). Segments 3 and 4 (group I) and segments 8 and 9 (group III) showed co-migration in both cases.

Viewing by EM showed the characteristic morphology of rotavirus particles, but the results of the ELISA test for group specific antigen were negative in both samples.

Discusion

During the last four years we analysed 2154 faecal samples from different provinces of Argentina. We found a prevalence range of rotavirus infection between 5 and 15 %, detecting among the samples studied 14 distinct electrophoretic patterns, 3 "short" and 11 "long" (in preparation). Among the 317 samples analysed from Misiones province during the last year, in two we detected an atypical electropherotype pattern, as compared with those currently observed among other human isolates. To date, different authors

reported the presence of atypical rotavirus in man (Nicholas *et al.*, 1983) and in animals (Bohl *et al.*, 1986). According to Flewett *et al.* (1985) the majority of these atypical rotaviruses represent a distinct group classified as group C. However, recently atypical human rotaviral strains were described in other countries, which were not included in this group (Hung *et al.*, 1984; Besselaar *et al.*, 1986).

Ushijima *et al.* (1989) have reported four human group C rotaviruses with two distinct electrophoretic patterns. One of them is strikingly similar to the pattern of the two strains isolated in our laboratory. In addition, Nicholas *et al.* (1983), Rodger *et al.* (1982), and Dimitrov *et al.* (1983) also have reported very similar atypical rotavirus patterns. Like us, all these workers found in these electrophoretic patterns a segment belonging to group III that migrated within the range of group II, remaining the group III with two bands. On the other hand, they are different from the antigenicaly distinct virus isolated by Tao *et al.* (1984).

The RNA profile shown also resembles the electropherotypic pattern described by Sorrentino *et al.* (1986) which was isolated from a near geographical area in Argentina. It would be interesting to know the antigenic relationship among different strains isolated in the same area, to determine their role as human diarrhoea aetiological agents.

Studies are in progress to analyse a higher number of samples to determine the prevalence of these agents, its seasonal distribution and the severity of the cases as compared with those of convention rotavirus. These would allow the evaluation of their clinical and epidemiological significance. Since the group C rotavirus cannot be detected by the commercially available ELISA test, one should be cautious in interpreting ELISA negative result in children with gastroenteritis. So, the prevalence and real role of these viruses in the aetiology of gastroenteritis will remain unknown if electrophoresis of the samples is not undertaken. This must be evaluated on proposed vaccine development programmes.

Acknowledgements. This research was partially supported by a grant (11 E 025/88) of the Secretaria de Ciencia y Técnica de la República Argentina (SECYT).

References

Besselaar, T. G., Rosenblatt, A., and Kidd, A. H. (1986): Atypical rotavirus from South African neonates. *Arch. Virol.* **87**, 327–330.

Bohl, E. H., Saif, L. J., Theil, K. W., Agens, A. G., and Cross, R. F. (1982): Porcine pararotavirus: detection, differentiation from rotavirus and pathogenesis in gnotobiotic pigs. *J. clin. Microbiol.* **15**, 312–319.

Dimitrov, D. H., Estes, M. K., Rangelova, S. M., Shindarov, L. M., Melnick, J. L., and Graham, D. Y. (1983): Detection of antigenically distinct rotaviruses from infants. *Infect. Immun,* **41**, 523–526.

Estes, M. K., Plamer, E. L., and Obijesky, J. F. (1983): Current Topics in *Microbiol. Immunol.* **105**, 123–184.

Flewet, T. H., Beards, G. M., Sanders, R. C., and Hall, C. J. (1984): New diarrhoea virus of man. In *"Abstracts Sixth Inter. Congr. Virology"* Sendai, Japan, Sept. 1-7, p. 212.

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.

Hung, T., Chen, G., Wang, C., Yao, H., Fang, Z., Chao, T., Chou, Z., Ye, W., Chang, X., Den, S., Liong, X., and Chang, W. (1984): Waterborne outbreak of rotavirus diarrhoea in adults in China caused by a novel rotavirus. *Lancet* **I**, 1139-1142.

Kapikian, A. Z., Wyatt, R. G., Greenberg, H. B., Kalica, A. R., Kim, H. W., Brandt, C. D., Rodriguez, W. J., Parrot, R. H., and Chanock, R. M. (1980): Approaches to immunization to infants and young children against gastroenteritis due to rotaviruses. *Rev. Infect. Dis.* **2**, 459-469.

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

Muchinik, G., Cristein, S., and Platza, A. (1981): Rotavirus infection in children hospitalized for diarrhoea in Argentina. *Ann. Trop. Paediatr.* **1**, 167-173

Nicholas, J. C., Cohen, J., Fortier, B., Lourenco, M. H., and Bricout, F. (1983): Isolation of human pararotavirus. *Virology* **124**, 181-184.

Rodger, S. M., Bishop, R. F., and Holmes, I. H. (1982): Detection of a rotavirus like agent associated to diarrhoea in an infant. *J. clin. Microbiol.* **16**, 724-726.

Sorrentino, A., scodeller, E. A., Bellinzoni, R., Muchinik, G., and La Torre, J. L. (1986): Detection of an atypical rotavirus associated with diarrhoea in Chaco, Argentina. *Trans. Roy. Soc. Trop. Med. Hyg.* **80**, 120-122.

Tao, H., Changan, W., Zhaoying, F., Zinyi, C., Xuejian, C., Xiaoquang, L., Guangmu, C., Henli, Y., Tungxin, C., Weiwe, Y., Shuansen, D., and Weicheng, C. (1984): *Lancet* May 26, 1139-1142.

Ushijima, H., Honma, H., Mukoyama, A., Shinozaki, T., Fujita, Y., Kobayashi, M., Ohseto, M., Morikawa, S., and Kitamura, T. (1989): Detection of group C rotaviruses in Tokyo. *J. med. Virol.* **27**, 299-303.

Yolken, R. G., Barbor, B., Wyatt, R. G., Kalika, A. R., Kapikian, A. Z., and Chanock, R. M. (1978): Enzyme-linked immunosorbent assay for identification of rotaviruses from animal species. *Science* **201**, 259-262.