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ACUTE GASTROENTERITIS ASSOCIATED WITH COXSACKIE B-6 VIRUS IN INFANTS AND CHILDREN, IN SÃO PAULO, BRAZIL

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SUMMARY

An investigation into the role of enteroviruses in the etiology of infantile diarrhea was carried out among 126 patients attending a Pediatric Clinic in São Paulo, Brazil. A control group of 70 matched children without diarrhea was included in this study. Enteroviruses were isolated from 11.1% of the diarrhea cases while the control group yielded 2.9% of enteroviruses. Predominance of Coxsackie B-6 virus (64.3% of the isolates) was found, followed by Polio 1 (21.4%) and Coxsackie B-3 (14.3%) viruses.

INTRODUCTION

In spite of the relatively high frequency of acute gastroenteritis particularly in infants and young children, a large proportion of these episodes is bound to remain etiologically unexplained.

In previous papers, MURAHOVSCHI & MOURA [9] and MURAHOVSCHI et al. [10] reported the results of the laboratory diagnosis in the study of a group of 126 infants and children aged one month to three years, exhibiting acute gastroenteritis (Summer diarrhea). Out of the 126 cases, 14 (11.1%), or 14.9% of the 87 severe hospitalized cases, were stool-positive for enteroviruses.

The present paper complements the two previous ones, reporting the identification of the viral agents isolated.

MATERIALS AND METHODS

The material studied was collected from January through April, 1963.

Faecal specimens or rectal swabs from the 126 diarrheal and the 70 non-diarrheal subjects were transferred immediately upon collection to screw-capped vials containing 10 ml of a medium composed of Hanks balanced salt solution and antibiotics (penicillin, 500 units/ml; streptomycin, 500 μg/ml; and mycostatin, 200 units/ml) and kept frozen at -70°C prior to further processing. One-tenth milliliter of each specimen was inoculated into each of three tissue culture tubes of the following cell strains: human amnion (AV_3), BHK and HEp-2. Eagle's minimum essential medium plus 10% newborn calf serum was employed as tissue culture medium through-

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out this investigation. Cell cultures were followed daily for the appearance of cytopathic effect for a minimum of 15, usually 21 days. Non-inoculated culture tubes of each cell strain employed were carried along as controls in every experiment. Tubes showing viral effects (CPE) were harvested and passaged at least twice. Cytopathic agents were identified by neutralizing 100 TCD_{50} of virus against the following inactivated (56°C for 1 hr) standard hyperimmune enterovirus sera: Polio 1-3, Coxsackie A 1-14, 17 and 20, Coxsackie B 1-6, ECHO 1-3, 5-19 and REO 1. Neutralization tests were carried out as follows: four-tenths of a milliliter of mixtures of equal virus and serum volumes were incubated at 37°C for 2 hours, and 0.2 ml of the mixture inoculated into three tubes each of AV_3 or Hep-2 cell strains. Cultures were observed for 7 days and final readings taken 48 hours after the corresponding virus controls showed complete cellular degeneration.

RESULTS

Cytopathic agents were isolated in 14 out of the 126 specimens from infants or children with diarrhea, and only in the human amnion (AV_3) cell line cultures, cytopathic effects became evident after an observation period varying from 5 to 8 days after inoculation. Cytopathic effects were not observed in any of the other cell strains inoculated; upon isolation, however, subcultures could be successfully carried out in either AV_3 or Hep-2 cells.

The identification of the 14 isolates can be summarized as follows:

Isolate	Patients	Number of cases	Percent
Poliovirus 1	IF 112 IF 133 IF 201	3	21.4
Coxsackie B_3	IF 4 IF 145	2	14.3
Coxsackie B_6	IF 11 IF 40 IF 104 IF 106 IF 108 IF 124 IF 127 IF 129 IF 135	9	64.3
Total		14	100.0

In the control groups, two agents were isolated: one typed as Coxsackie virus B_6, and another one which could not be neutralized by any of the sera tested.

DISCUSSION

Acute gastroenteritis and diarrhea are a prominent cause of morbidity among persons of all ages throughout the world, an in many countries, as in Brazil, they stand as a significant cause of infant mortality.

The data published in the pertinent literature generally conform to the textbook notion that diarrhea and gastroenteritis epidemics in infants and children are most commonly caused by ECHO viruses. The role of Coxsackie viruses in these syndromes, however, seems to be rather unsettled: for instance, whereas MELNICK & WENNER[8] and PARROT[11] recognize only Coxsackie A viru-

ses (types 18, 20, 21, 22 and 24 in particular, according to the former Author) as associated to infantile diarrhea, KLEIN et al. [6] point Coxsackie B viruses types 3 and 4 as possible agents of this disease, exempting Group A Coxsackie agents. On the other hand, KIBRICK [5] states that infantile gastroenteritis and diarrhea are mostly associated with ECHO viruses and that "Coxsackie A viruses have also been implicated as a cause of this syndrome but their role is less certain". Several Authors (CANDEIAS et al. [2], MARIE et al. [7], POHNJAPELTO [13] and TRAVASSOS et al. [15]) report the association of Coxsackie A viruses to infantile episodes of gastroenteritis and diarrhea: PRENZEL & LENNARTZ [14], in Germany, and PELON et al. [12] in Costa Rica, on the other hand, reported the association of Coxsackie viruses B 2 and B 4, and B 5, respectively. Notwithstanding, the former Authors did not consider their isolates (Coxsackie B 2 and B 4) as necessarily implicated, from a clinical standpoint. BEHBEHANI & WENNER [1] report the simultaneous isolation of Coxsackie virus A 17, A 20 and B 2, ECHO 2 and 16, in a sample of 38 diarrheal infants. FELICI & ARCHETTI [4], during an epidemic of acute gastroenteritis in children, in Milan, have also isolated Coxsackie B-3 along with ECHO 9.

In the sample presently studied, comprising 126 diarrheal infants and children aged one month to three years, the results of the final typings in the 14 cases found to harbor enteroviruses have been quite unexpected, as 9 (64.3%) out of them were Coxsackie B-6, followed by 3 (21.4%) Poliovirus and 2 (14.3%) Coxsackie B-3. A Coxsackie B-6 virus was also to be identified in one isolate from the control group.

Nevertheless, it should be stressed that there are no solid grounds for accepting a definite etiologic correlation between the viruses isolated and the clinical manifestations observed. The simultaneous isolation of poliovirus and Coxsackie B-3 virus, along with a Coxsackie B-6 in the control group and the evidentiation of enteropathogenic bacteria in 5 out of the 9 cases positive for Coxsackie B-6, must also be taken into consideration.

On the other hand, according to data already available (CARVALHO [3]), in this particular area prime-infection of the population with enteroviruses does take place exactly in the age-range involved in the present study, suggesting that the prevalence of Coxsackie B-6 might simply reflect an outbreak of this particular agent in the community investigated.

RESUMO

Gastrenterite aguda associada ao vírus Coxsackie B-6 em lactentes e crianças, em São Paulo, Brasil.

Pesquisou-se a participação de enterovírus na etiologia da diarréia infantil, em 126 pacientes de uma Clínica Pediátrica de São Paulo, Brasil. Um grupo-controle de 70 crianças sem diarréia foi incluido neste estudo. Isolaram-se enterovírus em 11,1% dos casos de diarréia, enquanto no grupo-controle a porcentagem correspondente foi de 2,9%. Observou-se predominância do vírus Coxsackie B-6 (64,3% dos isolamentos), seguido pelo vírus Pólio-1 (21,4%) e pelo vírus Coxsackie B-3 (14,3%).

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