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Molecular and antigenic characterisation of rotavirus associated with an outbreak of acute infection diarrhea at a nursery in Merida, Yucatan, Mexico.

Original Article

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SUMMARY.

Introducción. Rotavirus (RV), is a major cause of acute infectious diarrhea (AID) in children. The profile genomic and antigenic studies of RV are important to determine the molecular changes that the strains have been presenting presented through the years. The purpose of this study was to carry out the electrophoretic and antigenic characterization of RV strains circulating during an outbreak of AID at a nursery in Merida, Yucatan, Mexico in January, 1996.

Material and methods. Faecal samples were obtained from 29 children and 7 adults and were analysed using a polyacrylamide gel electrophoresis (Page) technique and Elisa with monoclonal antibodies for subgroup, G and P serotypes.

Results. A total of 61.1% of the samples from children and adults were found to be positive for Group A RV. Subgroups I and II were found in a

similar proportion. G1 serotypes were detected in 50%, G2 in 50% and P1A serotypes in 100% of the samples. 50% of the viral RNA electrophoretical patterns, were short patterns and 50% were long patterns. The serotype G2, subgroup I, short patterns and one child who required hospitalization due to acute dehydration.

Discusion. Since the nursery is a closed place the presence of two different strains, indicates that two different strains were circulating at the same time. Short pattern, G2 serotypes and subgrup I were found in 50% of the samples, and this had not been observed in the Yucatán since 1989. Our results suggest that short pattern serotype G2, subgroup I are associated with more serious clinical manifestations. (*Rev Biomed 2001; 12:5-10*)

Key words: Rotavirus, acute infectious diarrhea, serotypes, Yucatan Mexico.

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RESUMEN.**Caracterización molecular y antigénica de rotavirus asociado con un brote de diarrea infecciosa aguda en una guardería en Mérida, Yucatán, México.**

Introducción. El rotavirus (RV), es el ocasiona los casos de diarrea infecciosa aguda (DIA) en niños. Los estudios del perfil genómico y antigénicos de los RV, son importantes, para conocer los cambios moleculares que han presentado las cepas a través de los años. El objetivo de este trabajo fue la caracterización electroforética y antigénica de las cepas de RV que circularon durante un brote de DÍA en una guardería en Mérida, Yucatán, México en Enero de 1996.

Material y método. A las muestras a RV, se les realizó la caracterización por la técnica de electroforesis en gel de poliacrilamida (PAGE) con tinción de nitrato de plata y por un ELISA con anticuerpos monoclonales específicos de subgrupo y de serotipo G y P.

Resultados. Las edades de los niños estuvieron entre 1 mes a 5 años y de los adultos entre 20 a 40 años. El grupo de RV fue el A; el subgrupo I y el II se encontraron en porcentajes iguales. Los serotipos G encontrados fueron el G1, G2 y el serotipo P, fue el P1A. Los patrones electroforéticos cortos y largos se identificaron al 50% respectivamente. El serotipo G2, subgrupo I, patrón corto se encontró en dos adultos y un niño que tuvieron que ser hospitalizados por deshidratación.

Discusión. La presencia de dos cepas diferentes en la guardería, nos indica que pueden estar circulando en un mismo lugar varias cepas a la vez. Nuestros resultados sugieren que cepas de patrón corto, serotipo G2, subgrupo I, se asocian a las infecciones más severas.

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Palabras clave: Rotavirus, diarrea aguda infecciosa, serotipos, Yucatán México.

INTRODUCTION.

Acute infectious diarrhea (AID) is frequently associated with viruses. Rotavirus (RV) has the highest impact due to the severe clinical symptoms (dehydration) in children, the seasonal outbreaks, and approximately one million child fatalities per year worldwide (1-5). Group A RV is the most common and is classified into subgroups according to the antigenic properties of VP6 protein (I, II, non-I, non-II and I + II), the former two being the most frequent ones (6-8). According to the neutralising test for VP7 glycoprotein, 14 different G serotypes have been identified. The predominant ones are G1, G2, G3 and G4. According to VP4 protein protease sensitive, 20 P serotypes have been identified. The P serotypes most commonly detected in human RV are P1A, P1B, P2, P3 and P4 (9-13). In addition to serological assays, sequence comparisons using PCR have been used to define the genotype and its corresponding serotypes (14-17).

The genome of group A RV consists of four groups of segments (1-4, 5-6, 7-9 and 10-11) (18-21). Changes in those groups are common, the most remarkable being in the mobility of the segments 10 and 11 in group A RV, which is responsible for the different patterns, (long, short and supershort). The long ones are the most frequent and are associated with G1, G3, G4 serotypes and subgroup II. Short patterns are correlated with G2 serotype and subgroup I (22-25). The combination of subgroup II, G1, G3 serotypes and long electrophotypes are the most prevalent worldwide (10,12,24,26).

In Yucatan (Southeast Mexico), epidemiology of RV infection has been variable. From 1985 to 1989, it remained constant throughout the years with a discrete increase in the autumn. Between 1990 and 1993 cases only occurred from October to December, since 1994 an increase was observed in the number of diarrhea episodes during the first months of the years (Data to be published). The G and P serotypes circulating

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during those periods have been G1, G3 and G4 and P1A, P1B and P2. Long RNA electrophoretic patterns have predominated in appearance. It should be noted that in 1989, an outbreak occurred associated with the strain having G2 serotype, short electrophoretic pattern and subgroups I, in which the clinical symptoms of the patients were so severe that most of the patients required hospitalization (1,11,12).

Molecular epidemiology based on the genomic profile of RNA (by Page) and the antigenic characterisation of RV strains isolated during outbreaks is a key tool to clarify what is happening within RV strains. Studies on genomic and antigenic of RV have been important to identify molecular changes in the strains throughout the years. In this study, we report the molecular and antigenic characterisation of RV isolates during an outbreak at a nursery in Merida, Yucatan, Mexico.

MATERIALS AND METHODS.

A prospective and descriptive study was carried out at a nursery where an AID outbreak lasting less than a month occurred in January, 1996. The nursery is located in the city of Merida, Yucatan, Southeastern region of Mexico, where mean annual temperature is 26°C and mean annual rainfall is 951 mm³.

Fecal specimens were collected from each child who presented a diarrhea episode. The infant's mother was interviewed to determine the number of evacuations their consistency (liquid, pasty or formed) and the presence or absence of vomiting and fever. Samples were also taken from nursery staff who presented a diarrhea episode, in order to determine the dissemination of infection.

RNA patterns analysis was done using Page with 10% running gels with 3% stacking gels, staining was done with silver nitrate (1,27).

Immunoassays were performed using monoclonal antibodies (Mab) specific to serotypes and subgroups, using (NUNC-Immunolon II of Denmark plates). Mab dilution used was 1/1000.

(The monoclonals were donated by Dr. Koki Taniguchi). The Mabs used were as follows: for subgroup I (S2-37); subgroup II (YO-5); for G serotypes: G1 (ku4), G2 (S2-2G10), G3 (YO-1E2), G4 (ST-2G7); for P: P1A (YO-2C2), (YO-1S3), P1B (S2-2F2) (9). A clinical sample was considered positive if the optical density 450 value was twice as high as the negative control. Wa, DS-1, SA11, ST3 strains were used as positive control.

RESULTS.

The nursery staff consisted of 10 adults, who took care of 70 children. During the outbreak, which lasted one month, 36 clinical samples (29 from children and 7 from adults) were collected. Bacteriological and parasitological studies were carried out on each one of the samples obtaining negative results.

A total of 19 (65%) children and 3 (43%) adults (61.1% of overall samples) were positive to RV. The age of the infected patients ranged from 1 month to 5 years old in children and 20 to 40 years old in adults. Clinical manifestations were acute and the average number of diarrhea episodes was 17 evacuations within 48 hr. The average body temperature was 37°C and vomiting occurred in all patients. However, 2 adults and 1 child required hospital attention due to acute dehydration and fever (38°C). Only group A RV was detected. Subgroups I and II were found in similar proportions within samples. The G serotypes detected were G1 (50%), G2 (50%) and P serotypes detected were P1A (100%). The RNA electrophoretic short and long patterns were found in equal proportion (50%) (table 1). G2P1A setotype was found in the samples of the adults.

DISCUSSION.

An outbreak of acute infections diarrhea in a nursery school was described in this study. RV was the only pathogenic found in 65% of the children's faecal samples and in 43% of the adults.

Table 1
Serotypes, subgroups and electrophoretic pattern of RV detected during an outbreak at a nursery in Merida, Yucatan, Mexico.

Number of samples	Serotypes	Subgroup	Electrophoretic pattern
11 (50%)	G1P1A	II	Long
11 (50%)	G2P1A	I	Short

Two different strains of RV were circulating during this outbreak, since two electrophoretic patterns were observed by the Page technique. They were short pattern and long pattern, suggesting the presence of two different sources of infection in the nursery. This differs from the facts normally reported by other authors since RV outbreaks which occur in closed areas, like the nursery, are usually caused by one strain like that reported in 1994 by Castro L (11).

In the nursery, the children are separated into different areas, according to their age. Each group has its own rooms, bathrooms, play areas and, above all, its own personnel, with the aim of preventing the spreading of possible infection. This diarrhea outbreak caused by RV spread through the nursery rapidly. The precursors of the infection were a person who prepared the food and a baby. This could be corroborated with initial date of the infection.

The age of the presentation of the infection varied between 1 month and 5 years in the children and between 20 and 40 years in the adults. The clinical symptoms were more serious among the adults since 66% of the required hospital attention due to acute dehydration, which suggests that the strains undergo some change in their virulence, the strains with serotypes G2P1A are unusual because G2 is frequently associated with P1B (30).

14 G serotypes and 20 P serotypes have been identified; G1-4 and P1A, P1B being the most important among humans. Despite the possibility of there being a combination of both serotypes, epidemiological studies show that the G1, G3 and G4 serotypes are most frequently associated with P1A serotype and the G2 with the P1B. In this

study, the antigenic characteristic of the strains demonstrated the presence of both G1 and G2 serotypes combined with the long electrophoretic pattern, subgroup II and with the short electrophoretic pattern subgroup I respectively. As for the relation with P serotype, both G1 and G2 serotypes were found with a single P1A serotype. Therefore this change was considered to be important in the presentation of the serious clinical manifestations.

This AID outbreak caused by the G2 serotype of RV is the second to occur in Merida in 10 years. It should be noted that when this serotype is present, the clinical symptoms are more serious. Based on published studies, we know that natural infection by RV with the G1, G3, G4 serotypes, gives, as a result a heterotypic response to any of the serotypes. This doesn't occur for G2 since it has a low frequency of circulation which is why there is a considerable number of people susceptible to this serotype and who, on being infected, can present very serious clinical symptoms.

Since environmental factors in 1989 and 1996 did not have any variation in relation to rainfall, humidity or temperature, we considered that they did not play an important role in the appearance of the outbreak associated with RV having short RNA pattern in the area. We consider that this could be appearing in cyclical and natural way.

The risk of infection among children in nurseries is due to characteristics such as the age and the state of immune response. The environment and the lack of knowledge among staff about infection prevention and control are also important.

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The latter is influenced by personal hygiene practices of the people taking care of children, as well as environmental sanitary conditions, food preparation, physical space and quality of the nursery (28).

The increase of nurseries has caused a significant impact on epidemiology of diseases and health cost to society, increasing the risk of infections, not only in children, but also in staff who take care of children and the potential spread of infections outside this environments (23,29).

Therefore, it should be considered important to establish epidemiological surveillance systems with training courses directed to staff of different assistance centres, in order to prevent the potential RV dissemination.

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