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Rotavirus and coronavirus outbreak: etiology of annual diarrhea in Costa Rican children

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Abstract: In Costa Rica, an annual outbreak of infant diarrheal disease (December and January) was reported since 1976, and rotavirus was incriminated later as the main etiological agent (1976-1981). Apparently the disease has not been systematically studied in Costa Rica after 1981. For that reason the occurrence of the outbreak was retrospectively documented for 1993-1995 and etiology was studied in 48 children treated for diarrhea at the Nacional Children Hospital (capital city of San Jose) during December, 1994 and January, 1995. Rotavirus (33%) and coronavirus (27%) were the main agents. To our knowledge, this is the first time that these viruses are incriminated in an outbreak of diarrhea.

Key words: Diarrhea, diarrheal disease outbreak, rotavirus, coronavirus.

Rotavirus, the main etiological agent of infant diarrhea was described in 1973 (Bishop *et al.* 1973, Flewett *et al.* 1973). Three years later, from December 1976 to January 1977, the first outbreak of diarrheal diseases asociated with this virus was reported in Costa Rica (Hernández *et al.* 1978). Then, other studies showed a profile of diarreal diseases in Costa Rican children with an important annual peak from December to January or February, mainly associated with rotavirus (Mata *et al.* 1983). This annual outbreak of diarrheal disease coincides with the worldwide prevalence of rotavirus (Christensen 1989), which has a seasonal pattern: it is associated with winter or cooler months in warm regions (Christensen 1989).

To our knowledge, viral research of diarrheal disease in Costa Rican children has not been systematically conducted after the 1976-1981 reports. Nevertheless, rotavirus is suspected as the cause of diarrheal outbreaks at the end and beginning of every year since that time.

The aim of this research was the analysis of infectious agents associated with diarrhea in a group of children and a retrospective analysis of the monthly prevalence of diarrheal diseases treated at the Nacional Children Hospital, San José, Costa Rica.

MATERIALS AND METHODS

We studied a group of 48 children with diarrhea, treated at that hospital between December, 1994 and January, 1995. A fecal sample from each was analyzed for parasites by direct microscopy. Smears of fecal suspension were prepared, air dried, methanol fixed, stained by the cold Zield-Neelsen method, and analyzed for the presence of *Cryptosporidium* spp. and

Campylobacter-like organisms. Bacteria were identified by inoculation in agar of MacConkey, SS, and Blaser. Futhermore, rotavirus and coronavirus were studied by Dot-ELISA using a monoclonal antibody against the VP6 of human rotavirus and a polyclonal antibody to calf coronavirus (Jiménez 1990). Additionally, data of clinical symptoms were recorded for each patient.

A retrospective analysis of the prevalence of diarreal disease found at the hospital, was done for the years 1993 - 1995.

RESULTS

The infectious agents were: One case each of *Giardia, Ascaris lubricoides*, and *Campylobacter,* two of *Shigella sonney*, 16 (33%) of rotavirus, and 13 (27%) of coronavirus; seven of these children excreted both viruses simultaneously.

The seriousness of viral diarrheas is exemplified by the finding of 14 cases dehydrated, three associated with rotavirus, six with coronavirus and five with both viruses, compared with 17 cases associated with other agents or without etiologic diagnosis (Table 1). However, that difference was not statistically significant ($p=0.7$); maybe because of the severity of diarrhea: all these cases were hospitalized due to this disease. Moreover, vomit was present in 87% of the cases associated with viruses ($p<0.5$) and 32% of the patients that excreted viruses showed respiratory symptoms ($p<0.5$, Table 1).

The annual profile of diarrheal diseases showed a peak from December to January (Fig. 1).

TABLE 1

Clinical findings in children with diarrhea associated with rotavirus and coronavirus

Clinical signs	Viral diarrheas			The rest of the cases*
	Rotavirus	Coronavirus	Both virus	
Dehydration/ without dehydration	3/6	6/0	5/2	17/8
Days with diarrhea ≤ 3 / >3	6/3	5/1	6/0	19/7
Evacuation/24 hr ≤ 5 / >5	5/4	3/3	5/1	9/12
Vomit/ without vomit	8/1	6/0	6/1	19/4
Respiratory symptoms/ without	3/9	4/5	0/6	7/10

* The rest of the cases includes the cases without etiologic agent identified and those with bacteria or parasites associated with diarrhea. Numbers to the left of virgule refer to left condition in the "clinical signs" colum and viceversa.

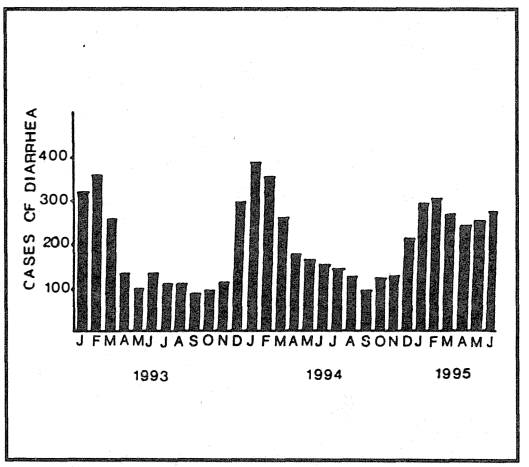

Fig 1. Monthly prevalence of diarrhea in children treated at the National Children Hospital, San José, Costa Rica: there is an annual outbreak from December to the first months of each year.

DISCUSSION

Data suggest that the annual outbreak of diarrhea is strongly associated with viral agents, at least with rotavirus and coronavirus. The former was incriminated previously in that outbreak (Hernández *et al.* 1977, 1980, Mata *et al.* 1977, 1983, Simhon *et al.* 1979, Odio *et al.* 1980). This is the first time that coronavirus has been related to that seasonal peak of diarrhea. It is possible that coronavirus might have been missed in previous research done in Costa Rica, because samples observed under electron microscopy were clarified using genesolv, which could have destroyed this phospholipid enveloped virus. Additionally, many samples were analyzed only by ELISA using polyclonal antibodies to rotavirus. Nevertheless, in an electron microscopy

research of viral diarrheas in calves, where the samples were not clarified with genesolv, both rotavirus and coronavirus were found with similar prevalence (Hernández *et al.* 1987). The data presented herein concerning coronavirus were obtained with a Dot-ELISA using polyclonal antibodies against a bovine strain, suggesting cross-reactivity between coronavirus strains from humans and calves. Nevertheless, there may be different antigenic strains of human enteric coronaviruses, because antigenic relatedness of the enteric human coronavirus with other strains from respiratory infections has been described (Germa *et al.* 1984); conversely, Mortesen *et al.* (1985) did not find that cross reactivity.

The relationship between respiratory symptoms and excretion of viruses (rotavirus and/or coronavirus) in our patients was not statistically significant (p=0.08). However, coronavirus is related with respiratory symptoms and with sporadic cases of diarrhea in children (Christiensen 1989). Likewise, rotavirus has been incriminated with respiratory infections that precede the intestinal symptoms (Zheng *et al.* 1991). Hence, the possible involvement of respiratory tract in coronavirus and rotavirus infections could explain the rapid spred of diarrheal disease in hospitalized children, as indeed occurs during the annual outbreak (Odio *et al.* 1980).

To our knowledge this is the first seasonal outbreak of child diarrhea simultaneously associated with coronavirus and rotavirus.

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RESUMEN

En Costa Rica, entre 1976 y 1981 se documentó la ocurrencia de un brote anual (diciembre y enero) de diarrea en niños, incriminando a rotavirus como el agente más importante. Pero hasta donde sabemos, la enfermedad no ha sido sistemáticamente investigada en Costa Rica después de 1981. Por esa razón, se investigó la ocurrencia de tal brote retrospectivamente para los años 1993 a 1995 y se estudió la etiología en 48 niños con diarrea atendidos en el Hospital Nacional de Niños entre diciembre, 1994 y enero, 1995. Rotavirus (33%) y coronavirus (27%) fueron los principales agentes encontrados. Hasta donde sabemos, esta es la primera vez que esos dos virus son incriminados conjuntamente en la causalidad de un brote de diarrea.

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