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ENTEROVIRUSES ISOLATED FROM PATIENTS WITH ACUTE RESPIRATORY INFECTIONS DURING SEVEN YEARS IN RIO DE JANEIRO (1985-1991)

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SUMMARY

Enteroviruses were investigated in respiratory secretions collected from patients with acute respiratory infections (ARI) over a seven year period (1985-1991), as part of a longitudinal study of ARI aetiology. All the viruses that are most commonly associated with ARI were found in this study. Among the virus isolates, enteroviruses were only less frequent than respiratory syncytial viruses, adenoviruses and influenzaviruses. Forty five enterovirus samples were isolated from patients with either upper respiratory tract infections (URTI) or lower respiratory tract infections (LRTI). From these enterovirus isolates, thirty one samples were identified as poliovirus (n=18) and non polio enterovirus (n=13) by serum neutralization. Poliovirus were identified as type 1 and 2 and all of them were vaccinal strains. From thirteen non polio enterovirus, twelve were identified as echovirus serotypes 1, 2, 7, 11, 19 and 31. The remainder was identified as coxsackievirus B4.

KEYWORDS: Enteroviruses; Nasopharyngeal secretions; Acute respiratory infections

INTRODUCTION

Respiratory viruses are the most common cause of symptomatic human infections. Children have an average of two to seven, and adults one to three acute respiratory infections (ARI) each year[2]. Respiratory syncytial virus (RSV) is the major cause of lower respiratory tract infection in infants and young children worldwide[16]. It is the main cause of bronchiolitis and pneumonia in children under 6 months of age[14].

In the Northern hemisphere parainfluenza viruses are probably the second main cause of lower respiratory tract infections. In the Southern hemisphere adenovirus is an important aetiological agent of respiratory infections in children mainly the serotypes 1, 2 and 7[9,11,12,15] which are more frequent than parainfluenza virus.

Rhinovirus, coronavirus and enterovirus, including coxsackie A and B viruses, echoviruses, polioviruses and enteroviruses 68-71 can also cause upper respiratory tract infections (URTI) and less frequently lower respiratory tract infection (LRTI) in children[2]. The pathogenic role of enteroviruses in respiratory illness has been described in several studies[7,13]. Most of these studies was done in closed communities or during short periods of time. In Brazil, distinct respiratory tract illnesses associated with enterovirus have been reported in some aetiological studies of ARI[15,19,20]. The frequency of this association in ARI seems worthy of emphasis. This paper reports the occurrence of URTI and LRTI related to enterovirus in Rio de Janeiro. It also indicates the clinical features, virological techniques in diagnosis and epidemiological considerations relative to these enterovirus infections. To achieve the objective of this study nasopharyngeal secretions were obtained from children and adults with evidence of ARI over a seven year period.

MATERIALS AND METHODS

Study area and population

Rio de Janeiro, is a State in southeast Brazil whose population is almost all 93% concentrated in the urban areas. The metropolitan area is divided into two zones namely the city (Município do Rio de Janeiro) and the suburbs (Baixada Fluminense). The city of Rio de Janeiro itself has many shanty towns on the mountain side which are crowded with low-socio economic families. The study sites were located mainly in the east city having a high population density. The population target of this study belongs to a lower socio-economic level. Rio de Janeiro has tropical climate, with an average maximum temperature of 26.5^{0}C and minimum 20.5^{0}C. The temperature range is from 12^{0}C to 36^{0}C with an average relative humidity of 81 and approximately 12cm^3 of rain per year.

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Patients group

Clinical specimens were collected from patients attending different Health care units at Rio de Janeiro. The primary health care unit of the School of Public Health (ENSP) sent 1063 specimens (49% of the specimens) from January 1985 to December 1991. From January 1986 to December 1987 and January to December 1989 the specimens were almost exclusively obtained from this unit. The outpatient department of the Hospital Rafael de Paula Souza (HRPS) sent 168 specimens from February to December 1988, the Hospital Universitário Antônio Pedro (HUAP) sent 65 specimens from January to December 1985 and Hospital Geral de Bonsucesso (HGB) sent 20 specimens from March to June 1991. A total of 552 specimens were obtained from emergency wards: 420 from HGB, 72 from Hospital Salgado Filho (HSF), 25 from Hospital Universitário Gama Filho (HUGF), 20 from Hospital do Andaraí (HA) and 15 from Urgências Pediátricas (URPE) during the periods between January to December 1985, May to July 1987, April to August 1988 and January 1990 to December 1991. The inpatients study yielded 40 specimens from HGB. From May to December 1991, 80 specimens were collected from children in a day-care center (Creche Portugal dos Pelegrinos) and 165 specimens were obtained from other health care units. Specimens were collected from patients showing URTI and LRTI. Most commom URTI were colds, pharyngitis and tonsillitis and LRTI were pneumonia, bronchitis and bronchiolitis. Specimens were obtained mostly from children (93%). Specimens collected from adults (7%) were mainly patients' relatives and hospital, health unit and laboratory staff in close contact with patients. Among the specimens obtained from children 43% were under the age of one, and the remainder of the specimens were mainly from children between 1 and 2 years of age.

Specimens

Nasopharyngeal secretions (NPS) were collected from these patients showing ARI within 7 days of the disease onset. Seventy eigth percent of the specimens were taken in the first four days of illness. NPS were transported on ice (4^{0}C) to the laboratory within two hours after collection. One aliquot of about 0.5ml of NPS was taken for virus isolation, diluted in virus transport medium (VTM), after that NPS was processed for immunofluorescence. We identified RSV, parainfluenza, influenza and adenovirus utilizing specific anti-sera as described[15]. Some specimens were also processed for viral antigens detection by enzyme immunoassay[17,18].

Virus isolation and identification

HEp-2, MRC-5 and MDCK cells were used for virus isolation. Isolates in HEp-2 and MRC-5 cells were identified by using immunofluorescence (RSV, adenovirus and Herpes simplex), neutralization assays (adenovirus), and acid-lability[5] (rhinovirus and enterovirus). Isolates obtained in MDCK cells were identified by using hemadsorption inhibition (parainfluenza) and hemagglutination inhibition (influenza). Isolates identified as enterovirus were stocked at liquid nitrogen or frozen at -70^{0}C until they were reinoculated in HEp-2 cells and those that could not be recovered in this cell line were inoculated in RD cell. These were identified as poliovirus and non polio enterovirus by serum neutralization, using specific anti poliovirus 1,2 and 3 hyperimmune equine sera (Centers for Disease Control-CDC-Atlanta-USA). Non polio enterovirus were identified by serum neutralization test, using anti-sera for echovirus and group A and B coxsackievirus serotyping (Central Public Health Laboratory-CPHL-Londres-UK). Poliovirus were characterized intratypically by molecular hybridization and the results were confirmed by polymerase chain reaction, using probes and primers specifically designed to recognize vaccine-related poliovirus sequences (Sabin 1, 2 and 3), Brazillian wild poliovirus types 1 and 3 and a group probe for enterovirus detection [3,21].

RESULTS

During a seven year period 2153 specimens were received and virus were detected both by isolation and/or direct methods in 548

TABLE 1
Viral isolation and identification from ARI samples during the period from 1985 to 1991

Year	Samples			Identification**						
	received	virus positive*	Isolates	RSV	Adeno	PF3	Flu	Herpes	Rhino	Entero
1985	362	108	68	17(49)	8(8)	0(0)	17(25)	2	8	14
1986	172	41	27	5(10)	8(12)	0(1)	4(8)	1	1	7
1987	289	65	45	18(36)	9(11)	0(0)	6(6)	0	4	8
1988	248	74	69	45(49)	8(8)	0(3)	8(8)	0	0	6
1989	139	7	3	0(1)	1(1)	0(1)	0(2)	0	0	2
1990	475	142	102	65(99)	22(22)	0(0)	13(19)	0	0	1
1991	468	111	65	18(55)	26(28)	0(1)	12(18)	1	0	7
Total	2153	548	379	168(299)	82(90)	2(6)	60(86)	4	13	45

*Positive by virus isolation and/or rapid diagnosis (immunofluorescence directly on cells in NPS)
**RSV(Respiratory syncytial virus), Adeno(adenovirus), PF3 (Parainfluenza type 3), Flu (Influenzavirus), Herpes (Herpes simplex), Rhino (rhinovirus) and Entero(Enterovirus).

TABLE 2
Clinical and epidemiological data of patients from which poliovirus were isolated

Year	Serotype	Age	Health care unit*/Syndrome**	Date of specimen collection	Date of antipolio vaccination campaign	
					1st round	2nd round
1985	P1	3m	ENSP/URTI	20/06	15/06	17/08
	P1	4m	ENSP/URTI	03/09		
	P1	3m	ENSP/URTI	01/10		
1986	P1	1y7m	ENSP/LRTI	03/02	14/06	16/08
	P1	2m	ENSP/LRTI	07/04		
1987	P1	2m	ENSP/LRTI	28/05	23/05	15/08
	P1	1y3m	ENSP/LRTI	30/09		
1988	P1	1y6m	HRPS/URTI	29/02	21/05	13/08
	P1	2y2m	HRPS/URTI	21/03		
	P2	5m	HRPS/LRTI	27/04		
	P1	2y	HRPS/LRTI	04/10		
	P2	2y	HRPS/URTI	04/10		
	P1	4y	HRPS/URTI	04/10		
1989	P2	6m	ENSP/LRTI	14/06	10/06	12/08
1991	P1	1m	HGB/LRTI	20/06	15/06	31/08
	P1	3m	HGB/URTI	24/06		
	P1	1y	HGB/LRTI	27/06		
	P1	3m	ENSP/LRTI	30/07		

*ENSP (Escola Nacional de Saúde Pública), HRPS (Hospital Raphael de Paula Souza), HGB (Hospital Geral de Bonsucesso)
**URTI and LRTI - upper and lower respiratory tract infection

clinical specimens. Among them, 379 virus were isolated and identified as respiratory syncytial virus, adenovirus, influenzavirus, enterovirus, rhinovirus, herpesvirus and parainfluenzavirus type 3 (Table 1).

Enterovirus were isolated from forty five NPS. Forty two strains were isolated from children with ages between 0 and 5 years old, twenty one presenting LRTI and the other twenty one showing URTI. Three samples were isolated from adults presenting URTI. In this study, we did not obtain mixed enterovirus isolates either with different enterovirus serotypes, or with other respiratory viruses.

From 29 isolates obtained in MRC-5 cells, 16 behaved as enterovirus and 13 as rhinovirus when tested by acid lability. Among twenty nine isolates obtained in HEp-2, 21 were recovered in the same cell line and one was recovered only after passage in RD. Among the sixteen isolates in MRC-5, three were recovered in HEp-2 and 6 were recovered only after passage in RD. Thus, from the forty five enterovirus samples originally isolated, just thirty one were recovered in HEp-2 (n=24) and RD cells (n=7). Eighteen samples were identified as poliovirus and thirteen as non polio enterovirus by serum neutralization. All poliovirus were isolated and identified in HEp-2 cells. The poliovirus identified as being serotype 1, hybridized positively with specific probe for Sabin 1 and those identified as serotype 2, with the specific probe for Sabin

TABLE 3
Non polio enterovirus isolated from ARI patients

Year	Non poliovirus serotypes	Age	Health unit*	Syndrome**	Date of specimen collection
1985	E31	5m	ENSP	LRTI	21/03
	E11	2y 3m	HGB	LRTI	13/05
	E11	23y	IOC	URTI	15/05
	E19	27y	IOC	URTI	20/05
	E2	4y	ENSP	URTI	08/08
	E11	2y 8m	ENSP	LRTI	22/08
1986	E1	1y	ENSP	LRTI	31/01
	E11	5y	ENSP	LRTI	18/04
	Cox B4	2m	ENSP	URTI	30/07
1987	E7	1m	URPE	LRTI	01/07
	E11	6m	ENSP	URTI	30/10
	E2	1y 4m	ENSP	URTI	20/11
	E31	3y 2m	ENSP	URTI	21/09

*ENSP (Escola Nacional de Saúde Pública), HGB (Hospital Geral de Bonsucesso), IOC (Instituto Oswaldo Cruz) and URPE (Urgências Pediátricas)
**URTI and LRTI - upper and lower respiratory tract infection

TABLE 4
Predominant clinical features presented by patients infected with non polio enterovirus

Clinical features*		Patients		
		Echovirus	Coxsakievirus	subtotal
URTI	cold (rhinorrhea, cough)	2	0	2
	pharyngitis-tonsilitis	4	1	5
LRTI	bronchitis	4	0	4
	pneumonia	2	0	2
Total		12	1	13

*URTI and LRTI - upper and lower respiratory tract infection

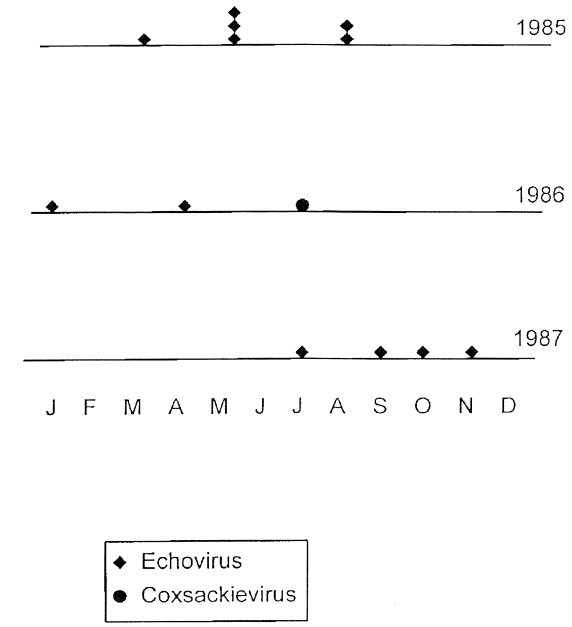

Fig. 1 - Isolation of non polio enterovirus from 1985 to 1987

2. None of the poliovirus isolates reacted with the specific probe for Sabin 3, or with the probes for wild poliovirus strains. All isolates were also tested by PCR, using specific primes confirming both poliovirus serotypes found, and their vaccinal character. Clinical and epidemiological data of patients from which poliovirus were isolated are showed in Table 2. Since 1988, all enterovirus isolates were identified as poliovirus. Poliovirus type 1 was more frequently isolated than poliovirus type 2 (15/18 isolates, Table 2).

From the thirteen non polio enterovirus, one was identified as coxsackievirus B4 and twelve were identified as echovirus serotypes 1, 2, 7, 11, 19 and 31 by serum neutralization test (Table 3). Non polio enterovirus were most frequent among the children living in the shanty town (outpatients). Only two isolates were obtained from children attending emergency wards. Both non polio enterovirus obtained from adults were isolated from laboratory staff. Table 4 shows the predominant clinical features showed by patients shedding non polio enterovirus. LRTI was the most common diagnosis among children and URTI was more frequent among adults. Figure 1 shows the non polio enterovirus occurrence during three consecutive years (1985-1987). No clear seasonality could be noticed.

DISCUSSION

Our main objective was to investigate the occurrence of enterovirus related to URTI and LRTI in Rio de Janeiro. Enteroviruses frequently have been isolated from the throat in person with respiratory infection and these studies have supported the role of enterovirus in ARI[7,13]. The results obtained here strongly support the aetiologic role in UTRI and LRTI, particularly when the virus is isolated from nasopharyngeal secretions. Enterovirus were fourth among the most frequently detected virus associated with ARI. This result is in agreement with the ones obtained in Rio de Janeiro during the previous four years[15]. In the first two years of this study in Rio de Janeiro (1980-1981), enteroviruses were second only to adenoviruses in frequency of isolation[20]. This huge difference in data is due to technic and material utilized in those studies. SUTMOLLER et al.[20] obtained their results by virus isolation in cell culture through oropharynx swabs (OS). From 1982 NPS rather than OS were used for viral isolation and for antigens detection by immunofluorescence or enzyme immunoassay directly from the secretions[15,19].

HEp-2 cells are largely used for respiratory tract virus isolation, because they are very sensitive to the respiratory syncytial virus and adenovirus isolation. It is also able to detect herpes and some enterovirus[6]. When using this cell line in order to recover stocked enteroviruses we obtained higher percentual when original isolates were from the same cell line (72%) and lower when they have been isolated before in MRC-5 (19%). These results were expected because MRC-5 cell as well as other diploid cell lines is very sensitive to isolate a greater number of enterovirus, mainly coxsackievirus and echovirus[6,8]. RD cell was used to recover viruses which were not recovered in HEp-2 because MRC-5 cell was no more available.

Non polio enterovirus were isolated in 1985, 1986 and 1987 (Table 3) due to the fact that we used MRC-5 cells only during these years. The lack of MRC-5 cells during subsequent years justifies the fact that we only have obtained rhinovirus and non polio enterovirus isolation until 1987.

In our study, non polio enterovirus were most frequent among children with benign and moderate ARI attending outpatient departments. The most severe cases were of 2 children in emergency wards. These results of location versus clinical presentation were also found in our previously ARI study[15].

Respiratory illnesses caused by enterovirus follow the typical enterovirus season pattern of summer and early fall[7,13]. In this study, no seasonality related to enterovirus could be observed.

The enterovirus types isolated from the respiratory secretions (Table 3) are in agreement with the literature, which associates non polio enterovirus, mainly echovirus and coxsackievirus, to URTI and LRTI in children and adults[7,13]. In a former study concerning to ARI virus etiology in Rio de Janeiro during 1980 to 1981[20] we observed the circulation of echovirus serotype 6, 7, 9, 11, 17, 18 and 21 and coxsackievirus A7 and B4. From 1982 to 1985[15], echovirus serotype 1, 2, 19, and 31, as well as coxsackievirus B5 were detected. Echoviruses were 92% of the non polio enterovirus isolated from 1985 to 1987. We isolated echovirus serotype 11 along these three years, and this was the most predominant among non polio enterovirus in the year 1985. Our results are according with previous findings, where echovirus serotype 11 are the most frequent enterovirus associated with respiratory infections[10].

Coxsackievirus A are difficult to isolate in cell culture and certain serotypes (A1, A19, A22) are only isolated in newborn mice[5]. Since we did not use this animal model for this purpose, our study was limited to those enteroviruses which were isolated in the cell system described (HEp-2 and MRC-5).

Poliovirus identification showed similar result to the one obtained by NASCIMENTO et al.[15]. In that study about viral etiology of ARI between 1982-1985 in Rio de Janeiro, there were 8 poliovirus type 1 isolated, and only 2 virus were serotyped as type 2. The predominant isolation of serotypes 1 and 2 has also occurred in studies from stool samples[1,4]. All poliovirus isolates were vaccinal strains. Thus, despite the fact that wild poliovirus circulated in Brazil until 1989, and it was detected in Rio de Janeiro until 1988, we did not isolate any wild poliovirus from NPS.

In this study we did not use either probe or primer specific for wild poliovirus serotype 2 because this virus not have been detected in Brazil since 1985, according to Health Ministry and World Health Organization. The primers and probes utilized in our study were highly specific for the Brazilian wild poliovirus, and thus they may not identify imported wild type poliovirus.

The recovery of vaccinal poliovirus from children showing ARI can be linked with routine vaccination programs (systematic vaccination at 2,4 and 6 month of age), or associated with the mass vaccination campaign (twice a year, children between 0 and 5 year old), taking place in Brazil (Table 2). Even though we do not have accurate information about the vaccinal state of each child analysed in this study, we could observe that all the children probably received at least one dose of vaccine, except one child who was one month old when tested. This observation is based on the time when the material was collected, the child's age and on the period when the annual vaccination occurred.

The recovery of vaccinal poliovirus from this one month old child suggested that either she had had contact with other vaccinated children, or that she participated in the Vaccination Campaign that took place five days before specimen collection. The other poliovirus strains were isolated from secretions collected between the 5th and 57th day after the vaccinations campaigns, apart from five samples that were collected before the campaign's date. These five children may have recently participated in the routine vaccination program, or they have been in contact with children who had recently received periodic vaccination.

The isolation of vaccinal poliovirus from respiratory secretions of children with ARI is partly related to the large utilization of OPV in Brazil, during the poliomyelitis control and eradication programs, and also because while campaigns were taking place, there was a consequent increasing of the vaccine strains in circulation.

RESUMO

Enterovirus isolados de pacientes com infecção respiratória aguda durante sete anos no Rio de Janeiro

Os enterovírus foram investigados em secreções respiratórias coletadas de pacientes com infecção respiratória aguda (IRA), durante um período de sete anos (1985-1991), dentro de um estudo longitudinal da etiologia das IRAs. Neste estudo foram encontrados todos os vírus que são mais comumente associados com IRA. Entre os vírus isolados, os enterovírus foram apenas menos frequentes que vírus respiratório sincicial, adenovírus e influenzavírus. Quarenta e cinco amostras de enterovirus foram isoladas de pacientes com infecção do trato respiratório superior ou inferior. Entre estes enterovírus isolados, trinta e uma amostras foram identificadas como poliovírus (n=18) e enterovírus não polio (n=13) pelo teste de soroneutralização. As amostras de poliovirus isoladas foram classificadas como amostras vacinais do tipo 1 e 2. Dos treze enterovírus não polio, doze foram identificados como echovírus sorotipos 1, 2, 7, 11, 19 e 31 e apenas um foi identificado como coxsackievírus B4.

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