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BRIEF COMMUNICATION

EVALUATION OF ENTEROVIRUS 71 IMMUNE STATUS IN SÃO PAULO STATE, BRAZIL

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SUMMARY

Antibodies to Enterovirus 71 (EV71) were evaluated in São Paulo State during 1999-2005. The titer of neutralizing antibodies against EV71 was determined by microneutralization assay, and a titer of ≥ 1:8 was defined as indicative of protected immunity. Neutralizing antibodies to EV71 were observed in 12.4% (55/442) of sera samples, a low protective rate, suggesting that EV71 infection is uncommon in this region, but that there is a relatively high susceptibility to EV71 related diseases, which is worrying considering the recent Asian outbreaks. Also, a significant location-specific difference in seropositivity was observed. Neutralizing antibodies to EV71 were observed in 8.7% (21/241) of São Paulo metropolitan area sera samples, and 16.9% (34/201) of the sera samples from other municipalities. A high number of Brazilian residents live in country and coastal areas without adequate access to piped water or sanitation. This situation may contribute to the EV71 dissemination in these zones. The analysis of environmental samples could possibly make a valuable contribution to studies on the epidemiology of EV71.

KEYWORDS: Enterovirus 71; Neutralizing antibodies; Immune status.

Enterovirus 71 (EV71) belongs to the enterovirus genus of the *Picornaviridae* family, and its infections are manifested most frequently as a mild exanthema known as hand, foot and mouth disease (HFMD)[5]. Otherwise, among the enteroviruses, (except polioviruses), EV71 infections are the most important type because they are frequently complicated by neurologic diseases, including encephalitis, meningitis and epidemic poliomyelitis-like syndromes, that have generated interest and subsequent public health implications[9].

EV71 has emerged as a significant pathogen with potential to cause large outbreaks. The last 10-13 years have seen a shift in the frequency of reported EV71 associated with encephalitis fatalities, in particular during outbreaks in Malasya in 1997 and Taiwan in 1998[1]. Several smaller scale outbreaks have been also recorded in Western Australia, Korea, Japan and Singapore[1]. During 2006, there occurred multiple reports of large-scale HFMD outbreaks in India, Thailand, Hong Kong, Malaysia, Brunei[1], and, more recently, in China, in 2008[20]. However, EV71 activity is not just confined to South East Asia. During 1983-2005, a total of 270 cases of EV71 were reported in United States, and it appeared among the 15 most common serotypes during the 2000-2005 period[11]. In Brazil, prospective studies showed that EV71 was the cause of 6% of acute poliomyelitis-like disease and 5% of Guillain-Barré syndrome[4,19].

The serum neutralizing antibody response is the major indicator of EV71 infection and protective immunity. In Brazil, few studies have been published on the detection of neutralizing antibodies to EV71; in particular there is concern about its involvement in acute flaccid paralysis (AFP) after poliovirus eradication, which is found concentrated in the city of Belém, in the Northern region of the country[2,7]. As limited information is available about the level of immunity against EV71 in São Paulo State, in the country´s Southeastern region - especially after the Asian outbreaks - serological data from 1999-2005 are presented in this study. Previous Ethics Committee approval was granted by the Adolfo Lutz Institute (Ref.12/05). This was an anonymous unlinked study and informed consent was not required.

Serum samples from patients with suspected enterovirus infections are routinely sent to the Enteric Diseases Laboratory of the Adolfo Lutz Institute, the sole facility responsible for *Enterovirus* surveillance in São Paulo State, in order to conduct viral investigation. The level of immunity against EV71 was investigated in a total of 442 randomly selected sera samples, obtained from a convenient retrospective sampling. Each serum represents one individual, and no consecutive samples were obtained from the same individual in different years.

The individuals were divided in the following age strata (in years): 0-5 (n = 155; 35.1%), preschool and kindergarten children (the mainly group affected by severe EV71 infections); 6-15 (n = 103; 23.3%), school-aged children and those of the maximum age that carries through AFP monitoring; and > 15 (n = 184; 41.6%), adult individuals. In this work, two panels of clinical samples were used. The first panel was of 241 serum samples from eight different municipalities in the São

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Paulo metropolitan area (Diadema, Guarulhos, Itapecerica da Serra, Mogi das Cruzes, Osasco, Santa Isabel, São Bernardo do Campo and São Paulo), and the second panel was composed of 201 serum samples from 35 distinct São Paulo State municipalities, including countryside and coastal areas.

The titer of neutralizing antibodies against EV71 was determined by microneutralization assay. Sera were diluted two-fold, from 1:8 to 1:1024, in triplicate, and each dilution was incubated for two h at 36 °C with a 100 × 50% cell culture infectious dose of EV71/ BrCr strain (PAHO-WHO). The virus-serum mixtures were added to RD cells (human rhabdomyosarcoma - ATCC-CCL-136), and after a 5-day incubation at 36 °C, the cytopathic effect was assessed by phase contrast microscopy. A titer of ≥ 1:8 was defined as indicative of protected immunity, and data were analyzed by Epi Info version 3.4.3 (CDC, Atlanta, GA, USA).

Overall, 12.4% (55/442; 95% IC 9.0-15.0%) of tested samples had neutralizing antibodies to EV71, and 87.6% (387/442; 95% IC 83.9-90.1%) were negative. A different scenario has been reported in Germany in two distinct studies: (i) a non-age-specific evaluation of antibodies in the years of 1997-2007 with a seroprevalence of 60.3%[5], and (ii) an age-adjusted evaluation of individuals ≥ one year during 2006 with a seroprevalence of 42.8%[15]. In Taiwan, a seroepidemiological study performed in 1997 (pre-epidemic), showed rates of about 60-70% in adults and children older than six years of age[3]; and a seropositive rate of 50-60% in pregnant women in results from 2006 to 2008 (post-epidemic)[13]. In China, EV71 antibodies were detected in 44.2% of healthy children aged between 1-6 in 2005[8].

A significant location-specific difference in seropositivity was observed: 8.7% (21/241; 95% IC 5.1-12.3%) of the São Paulo metropolitan area samples, and 16.9% (34/201; 95% IC 11.8-22.2%) of the samples from other municipalities showed neutralizing antibodies to EV71 (p = 0.014). *Enterovirus* are commonly acquired by fecal-oral contamination, therefore numerous variables may be associated with *Enterovirus* seroprevalence including sources of drinking water, sewage systems (or lack thereof), socioeconomic factors, and behavior relating to hygiene. In fact, several *Enterovirus* infections and viral gastroenteritis outbreaks have been microbiologically linked to contaminated municipal water supplies[6,17], including in Brazil[16].

The metropolitan region of São Paulo has relatively high coverage levels for water supply through house connections (98.4%) and adequate sanitation (81.2%), while a high number of Brazilian residents live in country and coastal areas without adequate access to piped water or sanitation[10]. This situation may contribute to the EV71 dissemination in these zones. Similar data were displayed in an age-specific study of seroprevalence of hepatitis A conducted in central Tunisia, where 21.3% of school children living in the urban areas and 87.7% of those living in rural areas had antibodies to hepatitis A[12]. It is a public health priority to improve water quality and sanitation coverage. Moreover, albeit indirectly, the analysis of environmental samples could possibly make a valuable contribution to studies on the epidemiology of EV71.

No correlation between age groups and seropositivity were observed. There was no significant difference in EV71 immunity in preschool children aged 0-5 years (11.0%; 17/155; 95% CI 6.1-15.9%), schooling children aged 6-15 years (14.6%; 15/103; 95% CI 7.3-20.8%), or adults > 15 years (12.5%; 23/184; 95% CI 7.3-16.7%). A similar picture has been reported in Taiwan and Germany. In Taiwan, during 1996-1997 (the pre-epidemic period), in a serosurvey where ≈12% of the children < five years had antibodies to EV71, this seroprevalence reached ≈50% in children aged 5-12 years[14]. In Germany, during 2006, there was a significant increase in the EV71 seroprevalence from 12% among one to four years-olds to 49% among five to nine years-olds[15]. In another study conducted in Germany, the seroprevalence increased with age, but ranged from 36.6% in preschool children aged 0-6 years, to 67.2% and 75% in those aged 10-15 years and adults, respectively[5].

In Brazil, two studies among children ≤ 15 years of age were conducted in the city of Belém. The first study, conducted in 1998, showed that 59.2% of the children tested positive to EV71 antibodies, although the seropositivity decreased with age from 45.4% in children < three years to 10.9% in children aged 12-15 years[7]. The second study, carried out between 1998 and 2001, presented 57.1% of seropositivity to EV71, but only 20.2% for children < three years[2].

To analyze the immunity level, three neutralizing titer ranges were defined: 1:8-1:16 (low level), 1:32-1:128 (medium level), and 1:256-1:1024 (high level). The analysis of the immunity level in relation to the age of the individuals showed that the number of sera with medium neutralizing antibodies levels to EV71 was highest in the 0-5 year age group (4.5%; 7/155), followed by the > 15 (1.6%; 3/185), and the 6-15 (1.0%; 1/103) age groups. The low immunity level to EV71 increased with age from 6.4% (10/155) in children aged 0-5 years to 12.6% (13/103) in children aged 6-15 years, but declined to 10.8% (20/185) in individuals over 15 years. No high neutralizing antibodies levels to EV71 were observed. Similar data was described in Germany, where EV71 seropositive individuals generally showed lower neutralizing antibodies titers when compared to Coxsackie A 16[15]. This same study showed that the number of individuals with high levels of EV71 neutralizing antibodies declined with age, suggesting that the reinfection of the elderly is rare; otherwise, the number of individuals with high levels of neutralizing antibodies should increase with age[15].

The EV71 seropositivity showed a different frequency for each year: 14.3% (3/21) in 1999; 30.0% (24/80) in 2000; 4.0% (2/50) in 2001; 4.1% (3/73) in 2002; 11.0% (11/100) in 2003; 9.2% (10/109) in 2004; and 22.2% (2/9) in 2005. Unfortunately, the number of sera in each year was flimsy, impairing the statistical analysis. In addition, case records of EV71 infection are not sufficiently detailed to look for temporal trends in occurrence of EV71 diseases across the country, and laboratory records of serodiagnosed EV71 are too few to aid interpretation.

The serological data obtained in this study showed a low rate of protective EV71 antibodies in the population of São Paulo, suggesting that EV71 infection is uncommon in this region, and a relatively high susceptibility of the inhabitants to EV71 related diseases. In accordance with the serological data obtained in this study, EV71 isolations in Brazil were very low during the 1999-2005 period[11,18], as well as in previous times[4,19]. It is worth mentioning that our results correspond to 442 randomly selected samples of sera, obtained from a convenient retrospective sampling from the Enteric Diseases Laboratory, and presenting different symptomatologies. Therefore, the number of sera in each age group and/or year may be fairly small, and the study does not represent the prevalence in the general population. It is possible

that a broader sample size would be required to confirm these findings.

The transmission of strains between countries is made easier by globalization, and that would favor an EV71 infection increase in many countries in the near future, as there are no effective steps to prevent its transmission, nor good therapeutic measures to control its deadly complications. The only means of preventing and controlling the spread of EV71 during outbreaks is by public health surveillance. Currently, there is no vaccine available for this viral infection[21]. Several studies have shown that infants of preschool age are at the highest risk of severe EV71 infection[13], and the transmission of this virus is lower in other age groups[14]. The highly pathogenic potential of EV71 - in addition to its easily transmittable nature - are the causes of a very common pediatric disease[21]. These facts together suggest that public health measures should focus on the population most at risk.

Even though the importance of EV71 has already been defined, more studies are required in order to improve the knowledge of several key aspects related to this virus in Brazil.

RESUMO

Avaliação da situação imunitária contra Enterovírus 71 no Estado de São Paulo, Brasil

Anticorpos para Enterovírus 71 (EV71) foram avaliados no Estado de São Paulo durante 1999-2005. O título de anticorpos neutralizantes contra EV71 foi determinado pelo ensaio microneutralização, e um título de ≥ 1:8 foi definido como indicador de imunidade protetora. Anticorpos neutralizantes para EV71 foram observados em 12,4% (55/442) das amostras de soro, uma baixa taxa de proteção, sugerindo que a infecção pelo EV71 é incomum nesta região e que existe alta susceptibilidade a doenças relacionadas ao EV71, o que é preocupante considerando os recentes surtos asiáticos. Ainda, foi observada diferença significativa na soropositividade em relação à localização, onde 8,7% (21/241) e 16,9% (34/201) das amostras provenientes da região metropolitana de São Paulo, e demais municípios, respectivamente, apresentaram anticorpos neutralizantes para EV71. Um grande número de brasileiros vive em áreas rurais e à beira-mar, sem acesso adequado à água encanada ou saneamento. Essa situação pode contribuir para a disseminação de EV71 nessas regiões. A análise de amostras ambientais poderia gerar contribuição valiosa para estudos sobre a epidemiologia da EV71.

AUTHORS' CONTRIBUTIONS

AL and MCSTT conceived and designed the study; AL, AC, DHR and FFC performed the microneutralization assay; AL, AC and DHR analyzed the data; AL wrote the manuscript; RCCC conducted laboratory supervision. All authors contributed to the preparation and revision of the manuscript, read and approved the final version. AL and MCSTT are guarantors of the paper.

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