Description of a Widespread Outbreak of Aseptic Meningitis Due to Echovirus 30 in Rio de Janeiro State, Brazil

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Echovirus 30 belongs to the genus *Enterovirus* and is widely associated with aseptic meningitis (AM) outbreaks. In Brazil epidemics due to this serotype were reported in several states but in Rio de Janeiro, before this study, it was only involved in sporadic episodes. We retrospectively collected data from AM notifications charts and enterovirus isolation database from Rio de Janeiro State Health Department (RJSHD) and Enterovirus Reference Laboratory in the year of 2005. An outbreak of AM was detected during March, April and May associated with a high cell culture isolation rate for echovirus 30 (17.4%). Male children with ages varying from 1 to 9 years were more affected. Of the 22 patients with confirmed echovirus 30 disease, clinical information was available in eight; fever, headache and vomiting were the most common manifestations. CSF analysis showed a typical pattern of viral infection with median of cellularity of 100 cells/mm³ and mononuclear cell predominance in 64.7% of the cases. The median of protein and glucose levels of 49 mg/dL and 56.5 mg/dL. The fatality rate was null. Despite its benign course and the lack of treatment options, aseptic meningitis surveillance is crucial for early identification of causative agents of outbreaks, which helps to avoid additional testing and inappropriate use of antimicrobials.

Key-Words: Aseptic meningitis, enterovirus, echoviruses, cerebrospinal fluid.

Aseptic meningitis (AM) is a syndrome characterized by the acute onset of fever and meningism along with cerebrospinal fluid analysis (CSF) typically presenting mononuclear cell predominance and negative bacteriological investigation [1]. Currently, with the widespread use of viral vaccines (mainly polio, mumps and measles), non-polio enterovirus has become the leading cause of infectious AM, being responsible for both sporadic and epidemics cases [2]. The diagnosis of non-polio enterovirus in Brazil is restricted to research centers in which clinical specimens are tested for epidemiological surveillance purposes only through viral culture or PCR.

Echovirus 30 belongs to the genus *Enterovirus* and is associated mainly with cases of AM with an epidemic pattern. This serotype has increasing its incidence and in the last years it is among the most frequently detected enterovirus [3]. In Brazil it has been already reported as the causative agent of outbreaks of AM in several states (Rio Grande do Sul, Paraná, São Paulo, Pernambuco e Pará), affecting more commonly older children and adults and with a low case fatality ratio [4,5].

Between March and May 2005, an outbreak of AM occurred in the metropolitan area of Rio de Janeiro state concurrently with above normal levels of confirmed echovirus 30 isolation from CSF and feces of patients with this syndrome. Clinical, laboratory and epidemiological data are reported in this study.

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Material and Methods

Subjects

In this retrospective study the epidemiological and demographic data of 573 notified cases of AM in 2005 were reviewed and analyzed from the database of the meningitis advisory committee of the Rio de Janeiro State Health Department (RJSHD). AM was considered in all cases with the following criteria [6]: patients of any age with symptoms of acute meningitis (fever, headache, vomiting, nuchal rigidity and irritability or lethargy) along with a CSF profile showing white cell count of ≥ 10 cells/mm³ and negative bacteriological investigation.

Clinical data of the patientsassisted at Instituto Estadual de Infectologia São Sebastião (IEISS), Rio de Janeiro, with confirmed echovirus 30 infection was obtained from the patients' charts and from Enterovirus Reference Laboratory database. Data on age, sex, signs and symptoms, co-morbidities, initial CSF analysis results and clinical outcome at the time of hospital discharge were collected.

CSF Analysis of Clinical Specimens

All patients underwent a lumbar puncture for meningitis evaluation; no patient was submitted to this procedure only for the purpose of collecting samples for the study. The CSF was analyzed for cellular content, protein and glucose levels and bacteriological tests using laboratory routine methods.

Virus Isolation and Identification

All specimens (CSF and Fecal extracts from patients investigating AM) were inoculated in volumes of 0.2 mL onto cell cultures for the purpose of virus isolation. Continuous cell lineages RD (human rhabdomyosarcoma) and HEp2 (human laryngeal carcinoma) were selected for its ability to support most of Enterovirus serotypes replication [7]. The tubes were maintained at 36ºC for seven days, submitted to a

blind passage and kept at 36ºC for an additional seven days. Cell cultures demonstrating cytopatic effect (CPE) were kept at –20ºC until further identification.

Specimens showing Enterovirus characteristic CPE were submitted to RNA extraction using Trizol LS ® (Life Technologies), according to the manufacturer protocol. Specific Enterovirus group-RT-Polymerase Chain Reaction was performed using a primer pair (R – 222 and F – 292) that amplifies a 357 bp fragment inside the gene codifying for the main capsid protein, VP1, as described by Oberste et al.[8]. After 30 cycles of denaturation at 95ºC / 2'', annealing at 42ºC / 4'' and extension at 60ºC / 3'', in a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems), the products were analyzed by electrophoresis in a 1% agarose gel containing ethidium bromide (0.5 mg/mL), using *50 bp ladder* (Invitrogen), as molecular weight marker and visualized in a transiluminator (UV light). Specific products were gel purified using the *QIAquick® Gel Extraction*" (Qiagen) kit and then quantified by comparison, in 1% agarose gel, with *Low DNA Mass Ladder* (Invitrogen) molecular mass marker.

Cycle-Sequencing reactions were performed using the ABI BigDye Terminator Cycle Sequencing Ready Reaction (PE Applied-Biosystems) in a GeneAmp® thermocycler, with cycles of 1'' at 95ºC, 3'' at 42ºC and 3' at 60ºC. Sequence products obtained using both primers (222 and 292) in individual reactions, were purified by Isopropyl alcohol precipitation and analyzed using the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Enterovirus serotypes were obtained comparing the sequences with those located at GenBank using the Program Blast [9].

Endemic Diagram Construction and Statistical Analysis

All data from patients' charts and from the RJSHD were inserted and analyzed by using the SPSS statistics software, version 13.0 for windows (SPSS, Inc., Chicago, IL.). Descriptive statistic tools (mean, median and standard deviation) were performed. The annual incidence of aseptic meningitis was assessed using as denominator the population data taken from 2007 estimation by Brazilian Institute for Geography and Statistics (IBGE).

For construction of AM endemic diagram in Rio de Janeiro, notified cases of AM were analyzed between the years of 2000 to 2004. The mean and the standard deviation of the frequency distribution of cases for each month of the year were obtained and then it was added to each month mean its SD multiplied by 1.96 (z of a normal distribution with 95% sensitivity) in order to construct the upper limit of the diagram which represented the epidemic threshold.

Ethics

This study was projected according Brazilian laws about research involving human beings and was approved by the Ethical Committee of Evandro Chagas Clinical Research Institute, FIOCRUZ, Rio de Janeiro.

Results

Demographic and Epidemiological Data

A total of 573 cases of aseptic meningitis were notified during 2005 in Rio de Janeiro state; the incidence was of 3.71 cases/100,000 inhabitants. Data about age and gender distributions are shown in Table 1. The majority of cases occurred in children between one and nine years old; in this age group male represented 60.7% of the cases whilst female 39.3%. Above this age group gender predominance was not observed.

Cases of AM occurred throughout the studied period concentrating on Rio de Janeiro metropolitan region. Frequency distribution of AM cases shows an epidemic period between the months of March and May (Figure 1). The initial period of epidemic started in March with 99 cases (expected 36 cases), the height occurred in April with 122 cases (expected 38) and the termination in May with 58 cases (expected 39). In Brazil this period corresponds to the end of summer and the beginning of fall.

Enterovirus Isolation and Identification

During the year of 2005, 171 CSF and feces specimens from AM cases were analyzed for enterovirus, the number of specimens sent for virus isolation varied in this period from none to 85 per month; of the 48 (28.0% of total specimens sent) non-polio enterovirus isolated, echovirus 30 was identified in 17 CSF and 5 feces (45.8% of positive samples). The identification of this agent occurred exclusively in the epidemic period and its mean isolation rate was of 17.4% (22/126). Non-sequenced non-polio enterovirus was detected out of the outbreak period in the other 26 (54.2%) CSF samples (Figure 2).

Clinical Aspects

Of the 22 patients with confirmed echovirus 30 disease, clinical data was available in eight and CSF analysis results were in all seventeen CSF samples. There was a male predominance (54.5%) with age varying from 2 to 36 years old (median of 4.5 years). The most common manifestations were headache, 100.0% (8/8); fever, 87.5% (7/8); vomiting, 62.5% (5/8); and nuchal rigidity, 52.5% (5/8). CSF analysis demonstrated pleocytosis with median of 100 leukocytes/mm³ (32 to 325 leukocytes/mm³) with mononuclear cell predominance in 64.7% (11/17). Protein level was slightly elevated with a median of 49 mg/dL (28 to 84 mg/dL) and a median glucose level of 56.5 mg/dL (43 to 92 mg/dL). The case fatality rate was null.

Discussion

Our study reports the first echovirus 30 related outbreak in Rio de Janeiro state, which occurred in the year of 2005, showing its clinical, laboratory and epidemiological aspects. This outbreak started in March with progression until end of April and return to endemic levels in June.

Aseptic meningitis is a syndrome with a broad list of possible etiological agents, and it is greatly underreported in

Table 1. Age and gender distribution of notified cases of aseptic meningitis in the state of Rio de Janeiro in the year of 2005.

Age groups	Male (%)	Female(%)	n(%)
< 1	28 (71.8)	11 (28.2)	39 (6.8)
1 - 4	102 (65.4)	54 (34.6)	156 (27.2)
5 - 9	131 (66.5)	66 (33.5)	197 (34.3)
Subtotal	**261 (66.6)**	**131 (33.4)**	**392 (68.3)**
10 - 14	30 (46.9)	34 (53.1)	64 (11.1)
15 - 49	56 (50.4)	55 (49.6)	111 (19.3)
>= 50	1 (20.0)	4 (80.0)	5 (0.9)
IGN	-	1 (100.0)	1 (0.4)
Total	348 (60,7)	225 (39.3)	573 (100.0)

IGN - Ignored.

Figure 1. Endemic diagram for aseptic meningitis for Rio de Janeiro state (lines) and case distribution per month in 2005 (bars) with an outbreak detected between March and May, Rio de Janeiro state, 2005.

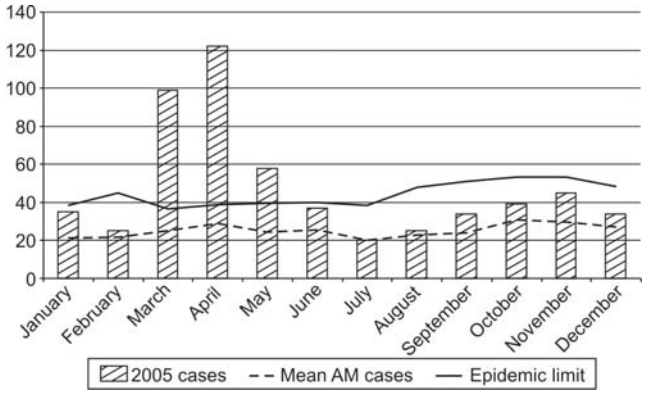
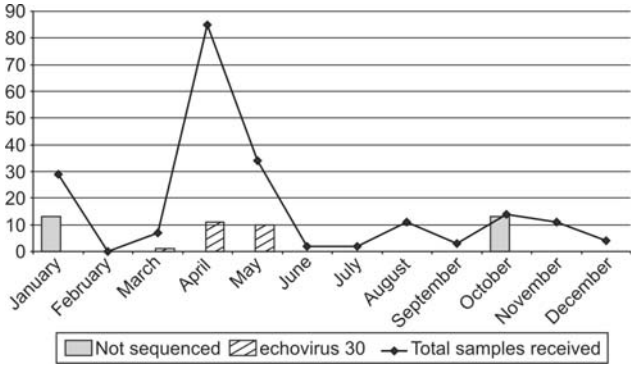


Figure 2. Total specimens analyzed and non-polio enterovirus isolation and identification during 2005, Rio de Janeiro.



Brazil; in our setting difficulties in performing etiological diagnosis in clinical practice lie in the high costs of testing and to the technical expertise required. Nevertheless, several studies that investigated the etiologies of AM in different regions of the world in the last 30 years found that non-polio enterovirus were responsible for the majority of cases varying in frequency from 52% to 92% [10-12].

In our study, the gender and age distribution of the AM cases showed a similar pattern described in other studies about central nervous system disease caused by enterovirus, with a male predominance and affecting children from 1 to 9 years old. The reason for male predominance is not known, but it is hypothesized that this gender has a higher degree of exposition to the risk factors involved in transmission [13].

AM cases in temperate climate areas are reported to have a seasonal distribution with peak of incidence in the summer, probably associated with the period of school vacancies and recreational activities [14]. In tropical and subtropical areas, cases of AM have a uniform distribution along the year as shown in the endemic diagram constructed in our study. In a previous study, dos Santos et al. [4] showed a similar pattern, but there was a tendency for cases to occur in periods of higher temperatures.

Echovirus 30 has an epidemic pattern of occurrence, and AM is its most commonly reported syndrome [3]. This serotype was responsible, in previous years, for outbreaks of AM in several Brazilian states (Paraná, Rio Grande do Sul, Pernambuco e Pará), always with benign clinical course and outcome [4]. In other regions of the world, echovirus 30 is already the most frequently isolated serotype from CSF of patients with AM [15, 16] and surveillance data from United States indicate that echovirus 30 has caused large outbreaks, intercalated by periods of quiescence [3]. Molecular epidemiology studies performed during outbreaks evidenced five different genotypes of echovirus 30, which is the proposed explanation for this epidemiological behavior [17,18].

In Rio de Janeiro state the circulation of echovirus 30 in association with sporadic cases of AM has been reported since 2001 [4]. In 2005 between the months of March and May an increase was observed in the AM notified cases above the epidemic level for that period. Concurrently echovirus 30 was isolated from clinical specimens sent to the Enterovirus Laboratory in a proportion far beyond (17.4%) that considered normal for sporadic cases, which is less than 10%. This high rate of isolation was similar to that described in an echovirus

30 associated outbreak in Taiwan in 2001 [18]. These facts confirm the occurrence of an outbreak caused by echovirus 30 for the first time in the state.

Clinically we observed typical meningitis syndrome with abrupt onset of fever and headache as the most common manifestations. The case fatality rate was null among the patients with confirmed echovirus 30 disease, which shows the benign course of the disease. Nevertheless, prospective neurological evaluation of these cases would be necessary to exclude long term neurological sequels.

Surveillance data presented in this study represents all notified cases, included those probably not caused by enterovirus, since other causative agents are not uncommon in our country, such as *Leptospira sp*., dengue virus, *Bartonella sp*. and Herpes simplex virus [2,11,19]. Epidemiological data about the etiological agents responsible for AM in Rio de Janeiro are largely unknown, so the real extension of enterovirus involvement in cases of AM in our setting is an unresolved question. Other important aspect that we were not able to describe in this outbreak was the mode of disease transmission; enteroviruses are acquired in the great majority of cases by fecal-oral contamination, which could be related to contact with a common source, like food and water, or by person to person contact [20]. Both mechanisms could have been responsible for the propagation of cases in our study, however methodological limitations imposed by the retrospective design did not permit this analysis.

Echovirus 30 is associated with outbreaks of aseptic meningitis with a benign outcome; an increase in this serotype frequency in Brazil is expected manifesting with large outbreaks of AM between periods of quiescence. Intensifying and improving of AM surveillance is crucial for early identification of causative agents of outbreaks, which helps to avoid additional testing and the unnecessary use of antimicrobials. More studies are necessary to establish other causative agents of AM in Brazil and to elucidate their mechanisms of transmission.

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