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Acute hemorrhagic conjunctivitis epidemic in São Paulo State, Brazil, 2011

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ABSTRACT *Acute hemorrhagic conjunctivitis (AHC) infection is highly contagious and can lead to explosive epidemics. In early February 2011, the Center for Epidemiologic Surveillance of the State of São Paulo Health Secretariat (SES-SP) in Brazil received reports of conjunctivitis outbreaks from rural areas of the state that subsequently spread statewide. This report describes that AHC epidemic and its etiologic agent. Data from the Ministry of Health Information System for Notifiable Diseases (SinanNet) and the SES-SP epidemiologic surveillance system for conjunctivitis, developed to detect outbreaks, confirm the etiologic agent, and carry out control measures, were analyzed. Eye (conjunctival swab) samples were taken from patients with clinical presentation of viral conjunctivitis to perform viral laboratory diagnosis. A total of 1 067 981 conjunctivitis cases were reported to the surveillance system for 2011; there was an increase in the number of cases in epidemiologic weeks 6–26 (summer season) versus previous years. Most cases occurred in the metropolitan region of Greater São Paulo. Of 93 collected samples, 57 tested positive for coxsackievirus-A24 (CV-A24), based on virus isolation in tissue-culture cell lines, reverse transcription polymerase chain reaction (RT-PCR), and enterovirus sequencing of RT-PCR. The data analysis showed that the fast-spreading etiologic agent of the AHC epidemic that occurred in the summer of 2011 was CV-A24. The AHC epidemic was due to an enterovirus that occurred sporadically, spread rapidly and with great magnitude, and had substantial socioeconomic impact due to the high level of absenteeism at work and school.*

Key words Conjunctivitis; coxsackievirus infections; epidemics; epidemiological surveillance; Brazil.

Acute hemorrhagic conjunctivitis (AHC) is a highly contagious viral and rapidly progressive disease (1). It is characterized by abrupt onset of red eye, pain, photophobia, eyelid edema, foreign body sensation or irritation, excessive tearing and eye discharge, follicular reaction, and subconjunctival hemorrhage (1, 2). The etiologic agent is an enterovirus (EV) responsible for a wide spectrum of clinical diseases in humans (3). EVs comprise a large genus in the *Picornaviridae* family consisting of more than 100 serotypes delineated into four species (A–D) based mostly on their phylogenetic relationships (4). Serotypes enterovirus 70 (EV-D70, species enterovirus D) and coxsackievirus-A24 (CV-A24, species enterovirus C) are responsible for most cases and outbreaks of AHC (1, 2, 5).

Because it is highly contagious and related to crowding and poor hygiene conditions, AHC is frequently spread through physical contact and often affects 50% or more of residents in communities with low socioeconomic status (6, 7). The incubation period is 12–48 hours and AHC persists 3–7 days (2, 5). Immunity acquired from contracting AHC decreases substantially within seven years of the

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infection. Loss of herd immunity is a contributing factor to the transmission of the disease, which has epidemic potential similar to influenza (8).

The disease is transmitted person-to-person, usually through contact with contaminated hands or through sharing of contaminated personal care items (1, 5). No specific treatment is available for AHC; however, the illness is self-limiting, and severe complications are rare (9, 10).

Although the ocular disease it causes is self-limiting, EV-D70 has been associated with patient neurologic impairment (acute flaccid paralysis, radiculomyelitis and cranial nerve involvement) during outbreaks. This association was demonstrated in India by hundreds of cases of individuals with conjunctivitis who experienced acute flaccid paralysis of the lower limbs (8).

AHC has the potential to become epidemic, mainly in tropical coastal regions (11). Explosive epidemics have emerged in several locations around the world, presenting approximately 10-year-long cyclical variation. Three major AHC pandemics have occurred: 1969–1971, 1980–1981, and 2002–2004. There are millions of cases of AHC worldwide (1, 2, 7, 11–13).

AHC is characterized by conjunctival congestion, vascular dilatation, edema, subconjunctival hemorrhage, and palpebral conjunctival follicular reaction (1, 2).

As in other countries, AHC epidemics in Brazil occur in the summer season (7, 14). The first notification of an AHC outbreak from EV-D70 in Brazil occurred in 1981 in the State of Amapá—the first entry of this disease agent in the country and in South America (15). In 1984, a major AHC epidemic from EV-D70 occurred in several other states in Brazil, including São Paulo (15). In 2003, a national outbreak of AHC caused by CV-A24 occurred in Brazil; it was considered the worst outbreak of conjunctivitis in 20 years, nationwide and in São Paulo State (1, 11, 13). After this major AHC epidemic, the Center for Epidemiologic Surveillance (CVE) of the State of São Paulo Health Secretariat (SES-SP) organized a surveillance system for conjunctivitis.

Acute hemorrhagic conjunctivitis epidemic in São Paulo state, Brazil, 2011

In early February 2011, the CVE/SES-SP began receiving reports of conjunctivitis outbreaks, mainly from penal institutions. The first reported outbreak occurred in a prison in the city of Flórida Paulista in the Western region of São Paulo State, and eventually spread statewide. The aim of the study reported here was to 1) describe the epidemiologic features and 2) identify the etiologic agent of this 2011 AHC epidemic in São Paulo State, Brazil. The study was retrospective and descriptive and was conducted with convenience (clinical) samples and secondary data from the conjunctivitis epidemiologic surveillance system of the SES-SP. The data are routinely collected by the public health services.

Data analysis

Conjunctivitis is not a reportable disease in Brazil, but notification of outbreaks is mandatory. For outbreaks, data are entered in the Brazilian Ministry of Health Information System for Notifiable Diseases (SinanNet). SinanNet is designed to collect, transmit, and disseminate data generated from epidemiologic surveillance at three levels of government (municipal, state, and federal), through a computer network, to provide evidence for research studies and inputs for reportable disease data analysis.

In São Paulo State, the epidemiologic surveillance system for conjunctivitis was designed to detect outbreaks, confirm the etiologic agent, and carry out control measures. State public health professionals from the regional Epidemiologic Surveillance Groups (*Grupos de Vigilância Epidemiológica*, GVEs) have been trained to carry out clinical diagnosis of conjunctivitis and epidemiologic surveillance as well as sample collection for etiologic diagnosis. These professionals then train the municipality health professionals.

To help ensure early detection of outbreaks, data from individual conjunctivitis cases are collected at the local level and forwarded to the GVE. At the end of every epidemiologic week ("epi week"), data from all municipalities that monitor the disease[3] in each GVE coverage area are analyzed, consolidated, and sent electronically to the São Paulo State epidemiologic surveillance system. Epidemiologic surveillance for conjunctivitis is not implemented in all municipalities because it is not a mandatory municipal activity. Municipalities that do not conduct regular surveillance for conjunctivitis only collect data on individual cases when there is an outbreak (i.e., sporadic cases of conjunctivitis are not monitored). When a conjunctivitis outbreak is detected, patient eye (conjunctival swab) samples are collected to identify the etiologic agent.

Monitoring the data allows for observation of any sudden increase in the number of cases, which can be a warning sign of probable occurrence of an outbreak in a particular city or region that will require field investigation.

Virus isolation and identification

From February to June 2011, conjunctival swabs were obtained through convenience sampling of persons with a clinical presentation suggestive of AHC. Once an outbreak was identified, health workers were instructed to collect conjunctival swabs from the next set of conjunctivitis cases that presented at their clinics within 48 hours of symptom onset. A total of 93 samples were sent to the Enteric Diseases Laboratory at the Adolfo Lutz Institute, the regional reference health center for São Paulo State for conjunctivitis viral surveillance, to conduct the diagnostic evaluation.

All swabs were inoculated in two types of tissue-culture cell lines—human rhabdomyosarcoma (RD, ATCC CCL-136), and human lung diploid cells (MRC-5, ATCC CCL-171)—and observed daily for cytopathic effects (CPEs) for at least two weeks (16). Samples with CPEs, in any type of tissue culture, were considered positive for the disease, and reverse transcription polymerase chain reaction (RT-PCR) and viral protein 1 (VP1) partial sequencing identified their serotypes.

EV-positive cultures were collected and stored at –70° C for ribonucleic acid (RNA) isolation. Specimens were frozen and thawed three times and clarified by centrifugation at 14 000 x g for 10 minutes at 4° C. Viral RNA was isolated from the 200 µL of infected cell culture supernatant using QIAamp Viral RNA Mini Kit (Qiagen, Valencia, California, United States) according to the procedure recommended by the manufacturer. The RT-PCR for CVA-24 and EV-D70 was performed according to the protocol previously described (17).

RT-PCR products were purified with the PureLink® purification kit (Invitrogen, Carlsbad, California, United States) according to the manufacturer's

[3] Collection of data on conjunctivitis is only conducted by municipalities that consider it necessary to monitor the disease.

recommendations. Forward and reverse reactions were carried out with primers S3 and AS3 respectively, using the BigDye® Terminator Kit v3.1 (Applied Biosystems, Foster City, California, United States). Dye-labeled products were run on an automated sequence analyzer (ABI Prism® 3130 Genetic Analyzer, Applied Biosystems). All sequencing chromatograms obtained were edited manually to obtain contiguous (contig) sequences, using Sequencher 4.7 software (Gene Codes Corporation, Ann Arbor, Michigan, United States). Molecular typing based on VP1 sequences was performed using online Enterovirus Genotyping Tool version 0.1 (http://www.rivm.nl/mpf/enterovirus/typingtool).

Ethics

The secondary data analyzed in this study were obtained from the Sanitary Ophthalmology Center at the CVE/SES-SP and are available as public information. In addition, all data were analyzed anonymously. During the epidemic, clinical samples were collected from patients receiving local public health services. This activity was considered a public health response to the AHC epidemic and thus did not require review by the Institutional Review Board, according to Brazil's Resolution 466 (12 December 2012) on research involving human beings (18).

Case data

From February to June 2011, there was a substantial increase in the number of cases reported to the surveillance system during epi weeks 6–26 (summer season) (1 067 981 cases for 2011) (Figure 1). The largest proportion of cases occurred among individuals 15 years old and older (64.5%) and school-age children (29.3%).

AHC cases were reported in almost all regions of São Paulo State, with an overall incidence rate of 2 568 per 100 000 inhabitants.

Most reported cases occurred in the metropolitan region of Greater São Paulo, 48% of which occurred in the City of São Paulo. The map in Figure 2 shows the incidence rate (per 100 000 inhabitants) of conjunctivitis in various municipalities of São Paulo State during the epidemic of 2011. The highest incidence rates (> 20 000 per 100 000 inhabitants) were observed in 12 municipalities in the Central and Western regions of São Paulo State.

During the epidemic, 93 conjunctival swabs were collected from patients with clinical presentations suggesting AHC, and analyzed for virus isolation. Most of them had reported a three-day period between onset of symptoms and first medical assistance. According to the laboratory results, 57 (61.3%) (n = 93) virus isolates causing CPEs in tissue-culture cells were obtained. All virus isolates were identified as CV-A24 by RT-PCR and confirmed by VP1 partial sequencing. The causative agent was identified before the epidemic peak.

After the implementation of the conjunctivitis epidemiologic surveillance system in São Paulo State, an increase was noted[4] in the number of both notified cases and conjunctival outbreaks (both larger than expected for 2011). The data indicated the presence of an AHC epidemic, and the etiologic agent was identified as fast-spreading CV-A24.[5]

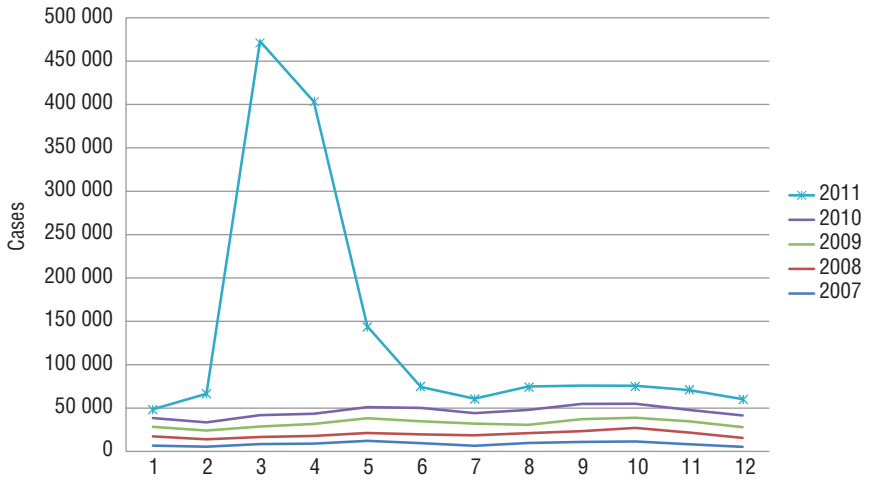
History of CV-A24 in Brazil

In Brazil, CV-A24 was first described as the causative agent of an AHC outbreak in 1987. Sixteen years after that outbreak, in 2003, CV-A24 reemerged as the cause of the AHC epidemic in various Brazilian states, including São Paulo State (1, 12). The next outbreak in São Paulo State attributed to CV-A24 occurred eight years later (2011). AHC epidemics caused by EV occur sporadically, spread rapidly, reaching a large magnitude, and have a significant socioeconomic impact (1, 10). Because of the extremely contagious nature of the disease, the infection spreads rapidly among young adults and results in lost workdays, reducing local productivity, and increasing the use of medical services (9). As no effective treatment has been established for AHC, its epidemiologic aspects must be closely monitored to prevent or control future outbreaks (9, 10).

Limitations

The high level of sub-notification for AHC (disease notification at the municipal level) in São Paulo State was a study limitation (the municipal teams had difficulties updating their notification systems). This problem affected the Brazilian information system for outbreaks (SinanNet), where data on major epidemics must be recorded. Because the municipal teams could not register all of their cases, the Sanitary Ophthalmology Center of the CVE/SES-SP (São Paulo State Health Secretariat) had to send recommendations to the municipalities, and help them update their system.

FIGURE 1. Number of conjunctivitis cases reported to the epidemiologic surveillance system, São Paulo State, Brazil, 2007–2011[a]



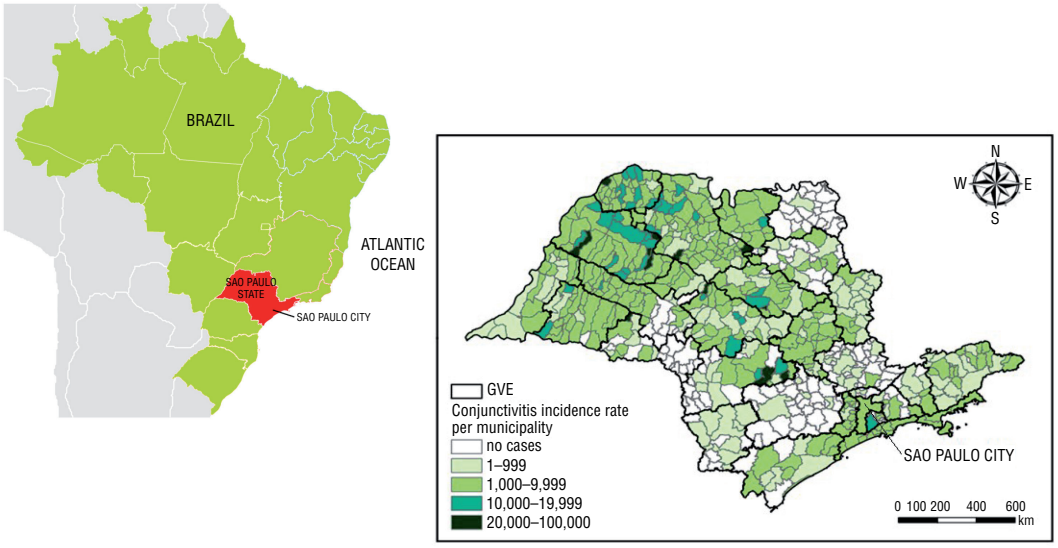
Source: Sanitary Ophthalmology Center, Center for Epidemiologic Surveillance, State of São Paulo Health Secretariat, and the Brazilian Ministry of Health Information System for Notifiable Diseases (SinanNet).

[a] The numbers in the x-axis correspond to the months of the year.

[4] The authors had analyzed the data on conjunctival outbreaks from 2007–2010, all non-epidemic years.

[5] From 2010 to 2012 (summer season), AHC outbreaks caused by CV-A24 had reemerged in China, Egypt, and France, and were associated with cyclic peaks (1, 19–21).

FIGURE 2. Incidence rate of conjunctivitis (per 100 000 inhabitants), by municipality of residence, epidemiologic weeks 6–26, São Paulo State, Brazil, 2011[a]



Source: Sanitary Ophthalmology Center, Center for Epidemiologic Surveillance, State of São Paulo Health Secretariat, and the Brazilian Ministry of Health Information System for Notifiable Diseases (SinanNet).

[a] GVE: regional Epidemiologic Surveillance Group (*Grupo de Vigilância Epidemiológica*).

Recommendations

Health professionals working for the state epidemiologic surveillance system should receive continual training so that they will be able to identify and carry out the diagnostic, therapeutic, and educational measures required to control epidemics. Teams working in municipal and regional epidemiologic surveillance must continue to identify and investigate the AHC epidemic and carry out control activities promptly.

These study results could be used to build evidence on the transmission of conjunctivitis in tropical areas. The nature of the etiologic agent (CV-A24), along with human and climatic variables, should be closely monitored, indicating the importance of continued surveillance of EV serotypes circulating among conjunctivitis cases.

Conclusions

Epidemiologic features of the data obtained in this study confirmed the occurrence of an AHC epidemic in São Paulo State during the summer of 2011. The etiologic agent for the epidemic was identified as CV-A24. The authors recommend implementation of continuous control measures, additional research, and the development and use of specific response guidelines, as well as constant vigilance for imminent epidemics of this disease.

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Conflicts of interest. None.

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RESUMEN

Epidemia de conjuntivitis hemorrágica aguda en el estado de São Paulo, Brasil, 2011

La conjuntivitis hemorrágica aguda es sumamente contagiosa y puede provocar epidemias fulminantes. A principios de febrero del 2011, el Centro para la Vigilancia Epidemiológica de la Secretaría Estatal de Salud de São Paulo (SES-SP), en el Brasil, recibió informes de brotes de conjuntivitis en zonas rurales del estado que posteriormente se difundieron por todo el territorio estatal. En este informe se describe esa epidemia de conjuntivitis hemorrágica aguda y su agente causal. Se analizaron datos del Sistema de Información para las Enfermedades de Notificación Obligatoria del Ministerio de Salud (SinanNet) y del sistema de vigilancia epidemiológica de la SES-SP para la conjuntivitis, que fue creado para detectar brotes, confirmar el agente causal y adoptar medidas de control. Se obtuvieron hisopados conjuntivales de pacientes con un cuadro clínico de conjuntivitis vírica para hacer el diagnóstico vírico en el laboratorio. En el 2011 se notificaron al sistema de vigilancia 1 067 981 casos de conjuntivitis; el número de casos observados de la semana epidemiológica 6 a la 26 (estación del verano) fue mayor que en años anteriores. La mayoría de los casos se produjeron en la zona metropolitana del gran São Paulo. De las 93 muestras obtenidas, 57 resultaron positivas para el virus Coxsackie tipo A24 (vC-A24), según los resultados del aislamiento vírico en líneas celulares obtenidas por histocultivo, la reacción en cadena de la polimerasa con retrotranscriptasa y la secuenciación enterovírica del producto de esta. El análisis de los datos reveló que el agente causal, de rápida propagación, de la epidemia de conjuntivitis hemorrágica aguda que tuvo lugar en el verano del 2011 fue el vC-A24. La epidemia se debió a un enterovirus que apareció de forma esporádica, tuvo una diseminación rápida y de gran magnitud y repercutió de manera importante en la esfera socioeconómica debido al gran ausentismo laboral y escolar que ocasionó.

Palabras clave Conjuntivitis; infecciones por coxsackievirus; epidemias; vigilancia epidemiológica; Brasil.