

RESEARCH NOTE

Outbreak of Acute Haemorrhagic Conjunctivitis in Cuba

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Acute haemorrhagic conjunctivitis (AHC) is a highly contagious eye disease, which appeared for the first time in Africa in 1969. The causative agent was identified by R Kono et al. (1972 *Lancet* 1: 1191-1194) as a new type of Picornavirus: Enterovirus type 70 (E70). Other outbreak occurred in 1970 in Singapore and probably in Java Island was caused by another new human pathogen that was identified as a variant of Coxsackievirus A24 (CA24v) (M Yin-Murphy 1972 *Southeast Asian J Trop Med Public Health* 33: 303-309). Since then, both agents have caused outbreaks in different parts of the world, sometimes with a pandemic behaviour. AHC was unknown in Cuba until 1981 when E70 affected more than 800,000 persons (P Más et al. 1985 *Rev Cub Hig Epid* 23: 384-390, 1986 *Rev Cub Hig Epid* 24: 396-398). Later, the CA24v was recognized as the causative agent of a new epidemic occurred in the summer of 1986 (P Más et al. 1987 *Bol Epid INHEM* 8: 1-2, MM Comellas 1989 *Rev Cub Hig Epidemiol* 27: 71-79). E70 re-emerged in 1989 (MM Comellas et al. 1992 *Rev Cub Med Trop* 44: 228-229) and the last epidemic of AHC in Cuba was due to CA24v in 1993.

In July and until December of 1997, Clinical Cuban Centers reported a sudden increase of the number of cases of AHC (137,136 reported cases) (Dirección Nacional de Estadísticas del Ministerio de Salud Pública de Cuba 1997 *Informe Anual*). In order to determine the causative agent of this outbreak we received 53 conjunctival swabs and 150 paired sera patients with clinical diagnosis of AHC. We apply two Reverse Transcription-Polymerase Chain Reaction (RT-PCR) protocols to four conjunctival specimens: the RT-nested PCR protocol designed by I Casas et al. (1995 *J Virol Methods* 53: 25-36) which use general primers that recognize the 5' non-translated region (5'NTR) of all enteroviruses serotypes which have been sequenced (including CA24v and E70); simultaneously, we apply other RT-PCR protocol, this time designed by HA Rotbart et al. (1992 *J Clin Mic* 30: 160-165) employing general primers that also promotes the amplification of 5'NTR but exclude to CA24v. The 50% of samples were positive with the Casas' primers and the 100% were negative with Rotbart' primers, which suggested the presence of CA24v. Infections caused by enteroviruses are often clinically indistinguishable. However in AHC syndrome, only two agents are involucrate: CA24 and E70. With the combined use of these generic primers we have rapidly determined which virus was possibly related with the aetiology of this outbreak.

To corroborate this finding, all conjunctival specimens were inoculated in monolayers of laryngeal carcinoma cell line (HEp-2) at 37°C and fibroblastic diploid embryonic human cells (PHuE-1) at 33°C to favour the growth of CA24v and E70, respectively. Fifty-six point six percent of cases showed a typical cytopathic effect of Enterovirus and it was an evident more sensitivity of HEp-2 cells (96.6% of viral isolation). All strains were identified as CA24v by Neutralization test, using type specific antiserum against CA24v reference' strain, produced in our laboratory.

We also determine the presence of neutralizing antibodies against CA24v and E70 reference' strains in the 150 paired sera. Neither serum showed neutralizing titre against E70. Although, we found a 40.4% of seropositivity (cut-off titer 1:10) to CA24v reference' strain. The geometric mean titre of the first serum was 1:2,7, meantime in the second serum this titer was 1:45.6 which is expected to the ocular infection produced by CA24v (KH Lin et al. 1994 *Kao Hsiung I Hsueh Ko Hsueh Tsa Chih* 10: 606-612).

The mechanism and route of transmission of AHC is essentially by direct contact with the ocular infected secretions and only few authors reports the role of the enteric route as an important mechanism of transmission. In order to know this role

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we inoculated in cell culture of HEP-2 at 37°C, a 10% suspension of nine faecal samples of nine patients with AHC. We have detected the presence of CA24v in 100% of cases. Two of these patients continuously collected specimens of faeces during three months and we detected the virus during six weeks, as a typical enterovirus shows. These results could explain the role of the enteric route in the epidemiology and pathogenesis of AHC due to CA24v. We know about the high mutation rate

in this agent (K Miyamura et al. 1990 *Arch Virol* 114: 37-51). Possibly, in the “enteric nature”, the CA24v find the opportunity to mutate and these accumulative nucleotide changes leads to new intratypic variants that promote the next outbreak. This event or the possibility of the importation of a new antigenic variant could explain the origin of the Cuban outbreak of AHC in 1997. The molecular characterization of Cubans isolates of AHC is in progress.