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Tracking novel adenovirus in environmental and human clinical samples: no evidence of endemic human adenovirus type 58 circulation in Córdoba city, Argentina

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SUMMARY

In recent years, several types of human adenovirus (HAdV) have arisen from the recombination between two or more previously known HAdV types, but their epidemiology is poorly understood. In this study, we investigated the circulation of HAdV-58, a recently described HAdV isolated from an HIV-positive patient in Córdoba city, Argentina. For this purpose, a 30-month survey was conducted to study the presence of this type of adenovirus in sewage samples collected at the inlet from a wastewater treatment plant in Córdoba city, Argentina. *Complementarily, the virus was sought in stools of HIV-positive patients.* Although HAdVs were detected in human stool samples and in a high percentage of sewage samples, no evidence of HAdV-58 circulation was detected. We suggest that there is no endemic circulation of HAdV-58 in the geographical local area. The trend is that the number of identified HAdVs increases over time. In this context, understanding the current circulating HAdVs may be biologically relevant.

Key words: Circulation, human adenovirus, sewage.

INTRODUCTION

Human adenoviruses (HAdVs) are a group of double-stranded DNA viruses classified within the family Adenoviridae, genus *Mastadenovirus*, and grouped into seven species designated A–G [1, 2]. Based on serological methods such as serum neutralization and haemagglutination inhibition assays besides other biological attributes, 51 serotypes of HAdVs were originally differentiated [3–6]. It has been established that most of them are prevalent throughout the world and produce a number of different clinical syndromes. It has been shown that some types (e.g. 40 and 41) circulate in an endemic form and cause gastroenteritis, or paediatric upper respiratory tract infections in the case of type 5. Other types cause sporadic infection and occasional outbreaks, for example types 8, 19, and 37 which are associated with epidemic keratoconjunctivitis [7–9]. Although different types may have different epidemiological patterns, all are transmitted by direct contact, faecal–oral and waterborne transmission.

More recently, bioinformatics and genomic analysis have complemented serology-based methods for

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typing HAdVs [10, 11]. Using these methods, several other new adenovirus types were described as either sequence-divergent and/or recombinant viruses of previously recognized HAdV types. Thus, the number of HAdV types currently exceeds 60. Some of these new adenovirus types have medical importance because they have been associated with pathology, such as HAdV-52 and HAdV-54 [2, 3]. Nevertheless, the epidemiology and implications in the disease process are unknown for most of the recently identified HAdVs.

One newly discovered type, HAdV-58, was first isolated from a stool sample of a chronic excretory HIV-infected patient in Cordoba city, Argentina [12]. The genome of HAdV-58 was completely sequenced and genetically analysed in an extensive manner in order to understand its genomic organization and evolutionary origin [13]. However, its pattern of circulation in the human population is completely unknown. Therefore, tracking novel HAdV-58 in clinical and environmental samples could be very important to determine if the virus is circulating in the local population.

Wastewater carries pathogens which are excreted in bodily fluids and transported into the urban sewage. Numerous viral human pathogens, other than adenoviruses, have been found in a high frequency in these samples; for example, enterovirus, rotavirus and polyomavirus [14–17]. Epidemiological studies conducted on sewage samples have provided important information about the circulation of a virus in a given community. Moreover, communal sewage may serve for the detection of viral infections of low prevalence or unrecognized clinical importance in a population.

HAdVs, through intestinal infections, have been found in high frequency and concentration in sewage [18–20]. The reason for this is that many types of HAdV, which are widespread, can replicate in the gastrointestinal tract and be excreted in the stool. Thereby, sewage samples may serve to detect an endemic adenovirus type in a given community. For this purpose, molecular methods based on DNA amplification have become reliable for detection of adenovirus in sewage [21]. In this way, in order to find evidence of HAdV-58 circulation in Córdoba city, Argentina, we designed specific primers for HAdV-58 and studied sewage samples through a 2½-year period. We also searched for HAdV-58 in the stools of HIV-positive patients. The aim of this study was to contribute to the knowledge of the epidemiology of the recently discovered HAdV-58.

METHODS

Sewage sample collection

A total of 30 independent untreated sewage samples were collected in a treatment plant which receives urban sewage from Córdoba city in Argentina. Samples were collected at the rate of one per month from February 2009 until July 2011.

Virus concentration

Concentration of specimens was performed using the PEG precipitation method as previously described by Lewis *et al.* [22, 23], and modified by Huang *et al.* [24]. The 1·5–l water samples were concentrated 100-fold to 15 ml by high-speed centrifugation (two steps at room temperature for 1 h) and PEG precipitation (10% PEG 6000, overnight at 4 °C). The method used helped to recover viral particles from environmental samples [25].

Faecal specimens from HIV-positive patients

A total of 70 faecal samples were collected between 1996 and 1999 from HIV-seropositive individuals (average age 31·8, range 17–55 months). All individuals sampled were in clinical stage C3 of HIV infection, as classified by the Centers for Disease Control (CDC, USA). Samples were collected and stored at −20 °C until processed. Faecal suspensions, 20% in phosphate-buffered saline buffer, were clarified by low-speed centrifugation and supernatant was screened by nested PCR for HAdV detection.

DNA extraction

DNA was extracted from 0·5 ml of concentrate using a phenol–chloroform protocol. Briefly, each sample was resuspended in 0·5 ml extraction buffer [10 mmol/l Tris–HCl (pH 8·0), 5 mmol/l EDTA, 0·1% SDS] and vortexed. After 10 min at room temperature, 1 ml phenol–chloroform was added. After 30 min at 56 °C, the mixture was centrifuged at 12 000 ***g*** for 30 min and the aqueous phase was separated. After overnight incubation of the samples with absolute ethanol at −20 °C, samples were pelleted by centrifugation at 12 000 ***g*** for 30 min. Ethanol was discarded and the precipitated DNA was air-dried and resuspended in 50 ml double-distilled water. All DNA extracts were stored at −20 °C.

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Nested PCR for HAdVs

The region of the hexon gene encoding loops 1 and 2 was amplified by a nested PCR as described previously [10]. The first set of primers included HX5-1 (forward primer): 5′-AAGATGGCCACCCCCTCG ATGATGCCGCAGT-3′, and HX3-1 (reverse primer): 5′-CACTTATGTGGTGGCGTTGCCGGCC GAGAACGG-3′. The reaction was done in a total volume of 20 μl containing 10 × PCR buffer, 400 mM of each deoxynucleoside triphosphate (dATP, dGTP, dCTP, dTTP), 0·2 μM of each primer, 0·6 μl of 50 mM $MgCl_2$ and 1 U *Taq* DNA polymerase (Invitrogen, USA). The cycling parameters consisted of a total of 40 cycles of denaturing at 98 °C for 10 s followed by annealing and extension at 65 °C for 6 min. The second PCR primer set included HX5-3 (forward primer): 5′-CACATCGCCGGACAGGATGCTTCGGAGTA-3′, and HX3-4 (reverse primer): 5′-GTGTTGTGAGCCA TGGGGAAGAAGGTGGC-3′. The reaction was done in a total volume of 20 μl containing 10 × PCR buffer, 200 mM of each deoxynucleoside triphosphate (dATP, dGTP, dCTP, dTTP), 0·2 μM of each primer, 0·6 μl of 50 mM $MgCl_2$ and 1 U *Taq* DNA polymerase (Invitrogen). The cycling parameters consisted of a total of 40 cycles of denaturing at 94 °C for 1 min, annealing at 40 °C for 1 min, and extension at 72 °C for 2 min. After each PCR reaction, a 10 μl aliquot was subjected to electrophoresis on a 1·5% agarose gel containing ethidium bromide, and the bands (2·8 kbp for the first step and 1·8 kbp for the second step) were visualized using a UV transilluminator.

Specific PCR for HAdV-58

Specific primers for HAdV-58 were designed to amplify a region of 512 bp in loop 2 of the hexon gene. The primers used were as follows, H58F (forward primer): 5′-CCTTTGGCGTGGCTGCTATGG-3′, and H58R (reverse primer): 5′-TAGAGTCAACTTGCG ACACCAGCT-3′. The reaction was done in a total volume of 50 μl containing 10 × PCR buffer, 10 μM of each deoxynucleoside triphosphate (dATP, dGTP, dCTP, dTTP), 0·15 μM of each primer, 2 μl of 50 mM $MgCl_2$ and 2·5 U *Taq* DNA polymerase (Invitrogen). The cycling parameters consisted of a total of 35 cycles of denaturing at 94 °C for 30 s, annealing at 47 °C for 30 s, and extension at 72 °C for 1 min. After PCR, 10 μl of each reaction mixture was subjected to electrophoresis on a 1·5% agarose gel containing ethidium bromide, and the band (512 bp) was visualized using a UV transilluminator. The designed set of primers was shown to be able to detect HAdV-58 DNA. We utilized HAdV-58 strains for the optimization of the reaction conditions, and also as positive control in our assay. HAdV-58 PCR specificity was assayed against prototype strains of HAdV-3, HAdV-5, HAdV-29, HAdV-31, HAdV-40 and HAdV-4, whose results were negative. The sensitivities of the sets of primers for HAdV-58 PCR amplification were checked by a limiting dilution and proved to be 10–100 DNA copies/ml.

RESULTS

HAdV DNA was detected in 86·7% (26/30) [95% bootstrap confidence interval (CI) 72–96] of the sewage samples tested. Samples testing positive were then assayed for specific HAdV-58 DNA detection. All samples were negative for HAdV-58 DNA (Table1). In stools of HIV-positive patients, HAdV was detected in 8·6% (6/70) (95% bootstrap CI 2–15) and all were negative for HAdV-58 DNA. Five out of six HAdVs detected in HIV-positive patients were typed by sequencing the loop 2 region. Two samples were found to be HAdV-41, another two were HAdV-5, and the remaining one was HAdV-27 (data not shown).

DISCUSSION

A 30-month survey (February 2009 to July 2011) was conducted to investigate for the presence of HAdVs, and specifically HAdV-58, in sewage samples collected at the inlet from a wastewater treatment plant in Córdoba city, Argentina. The reason for this study was that there are no previous studies of the circulation of this virus in the human population. Results disclosed no evidence of HAdV-58, although a high percentage of the samples were shown to contain HAdVs. Positive results obtained for HAdVs in this study showed that PEG concentration procedure as described in the Methods section is useful for obtaining the viral nucleic acids. The highly prevalent detection of HAdV in sewage was consistent with results obtained in other parts of the world where these viruses are a constant contaminant of sewers carrying human waste [26, 27]. On the other hand, the lack of detection of HAdV-58 in sewage samples could strongly indicate that this virus has no endemic circulation pattern in the region. This assertion is also supported by the failure to detect specific antibodies

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Table 1. *Monthly PCR results for human adenovirus (HAdV) and HAdV-58 in sewage*

Sample	PCR results	
	HAdVs	HAdV-58
2009		
February	+	–
March	–	–
April	+	–
May	+	–
June	+	–
July	–	–
August	+	–
September	+	–
October	+	–
November	+	–
December	+	–
2010		
January	+	–
February	+	–
March	+	–
April	+	–
May	+	–
June	–	–
July	+	–
August	+	–
September	+	–
October	+	–
November	–	–
December	+	–
2011		
January	+	–
February	+	–
March	+	–
April	+	–
May	+	–
June	+	–
July	+	–

against HAdV-58 in the local population in accord with a previous study [12]. Moreover, the present study covered a 2½-year period of surveillance, sufficient time to find a microorganism which has an endemic circulation pattern in a region. Therefore, if HAdV-58 is not endemic in the local area, its initial isolation could link the virus to another epidemiological pattern, i.e. the virus could circulate in a very limited portion of the population.

HAdV-58 was found and originally isolated from a chronically infected male in Córdoba city and it was found in our laboratory that this patient excreted the virus for at least 3 months. Since this man had AIDS, we also looked for HAdV-58 in individuals infected with AIDS, but it was not found. Recently, a novel human adenovirus, HAdV-65, was isolated from faeces of children with acute gastroenteritis in Bangladesh [28]. Corresponding genes of HAdV-65 were related to a hexon gene of HAdV-10, a penton base gene of HAdV-37 and HAdV-58, and a fibre gene of HAdV-9. This caused us to suspect that HAdV-65 could be the consequence of a co-infection with different HAdV strains, including HAdV-58. Therefore, the epidemiology of HAdV-58 deserves further researches.

The studies in sewage have been shown to be useful to find strains of HAdV in a geographical area. In a study conducted on sewage samples in Canada, the authors discovered the circulation of two genome variant strains of HAdV-3, i.e. Ad3a16 and Ad3a18, which were reported to be circulating with high virulence in South Korea and Japan [27].

In recent years, molecular methods based on DNA amplification allowed the identification of new types of HAdV. Many of these were shown to be the product or contain traces of homologous recombination of previously known adenovirus types. In relation to this, it is well known that natural recombination is a very common event among circulating adenovirus and that it is a force that drives the evolution of this virus. Therefore, the trend is that the number of HAdVs identified increases over time. In this context, understanding the current circulating field strains may provide the information to determine if the emergent recombinant HAdV is biologically relevant. Therefore, once the magnitude of the circulation of a new adenovirus strain is known, a series of studies that associate virus infection and human pathologies may be performed.

Adenovirus vectors are being tested for use in gene therapy, cancer therapy and vaccines, which further highlights the importance of understanding the circulation of all adenovirus types in human populations. In this study we found no evidence for HAdV-58 circulation in Córdoba city. This perspective underlines the need to perform more investigations on the circulation of the HAdV strains recently discovered.

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DECLARATION OF INTEREST

None.

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