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Original Article

Molecular characterization of adenovirus circulating in Central and South America during the 2006–2008 period

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Background Human Adenoviruses are recognized pathogens, causing a broad spectrum of diseases. Serotype identification is critical for epidemiological surveillance, detection of new strains and understanding of HAdvs pathogenesis. Little data is available about HAdvs subtypes in Latin America.

Methods In this study, we have molecularly characterized 213 adenoviruses collected from ILI presenting patients, during 2006-08, in Central and South America.

Results Our results indicate that 161(76%) adenoviruses belong to subgroup C, 45 (21%) to subgroup B and 7 (3%) to subtype E4.

Keywords Adenovirus, Latin America, viruses.

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Introduction

Human adenoviruses (HAdv) are well-recognized pathogens, causing a broad spectrum of diseases, including upper and lower respiratory tract infections, gastroenteritis, conjunctivitis, and keratoconjuntivitis, mostly in young children.

Adenoviruses are non-enveloped, double-stranded DNA viruses that cause an estimated 8% of clinically relevant viral disease globally.[1] To date, 51 human adenoviral serotypes have been described on the basis of type-specific antiserum-mediated neutralization of infectivity and grouped in six species or subgroups (A, B, C, D, E, and F), on the basis of hemagglutination inhibition and biochemical criteria.[2] Each species has been associated with different spectra of disease: respiratory infections with HAdvs are usually associated with species B, C, and E whereas species F viruses are restricted to the enteric tract infections.[3,4]

Serotype identification is critical for epidemiological surveillance, detection of new strains and understanding of HAdv pathogenesis. The use of molecular methods for adenovirus diagnosis has significantly increased in the last years, although they have some disadvantages as the homology within species ranges from 50% to 100%, but between subgroups it can go as low as 4%.[5]

Little data are available about HAdvs subtypes circulating in Latin America. There are few studies showing the different proportions of each species and/or subtypes in this region.[6–9] In this study, we have analyzed samples from patients presenting with influenza-like illness (ILI) in Central and South America during the 3-year period 2006–2008 by amplification of the last 300 nucleotides of the hexon gene and comparison to published sequences, to classify adenovirus into species in outpatients with ILI symptoms in this region.

Material and methods

Specimen collection and identification of adenovirus positive samples

In collaboration with 10 Central and South American countries ILI-surveillance networks, 14 106 nasopharyngeal and throat swab were collected from ILI presenting subjects, regardless of age, with an onset of fever (≥38°C) and cough or sore throat during 2006–2008 under the Surveillance for

Respiratory Pathogens Protocol, NMRCD.2002.0019.[10] Adenoviruses were isolated from ILI specimens from: Nicaragua (n = 21), Honduras (n = 19), El Salvador (n = 1), Venezuela (n = 7), Colombia (n = 27), Bolivia (n = 3), Ecuador (n = 17), Peru (n = 267), Paraguay (n = 1), and Argentina (n = 39). Most of the samples came from Peru, thus the number of adenovirus is greater in this country, but the proportion of adenovirus by ILI cases remains low (2·8%). Once collected, swabs were placed in viral transport media and stored at −80°C until transported on dry ice to the NMRCD in Lima, Peru. Virus isolation was carried out by inoculation in Vero cell line for 10 days. Then, viral identification was performed by indirect immunofluorescence using specific anti-sera (D3 Ultra DFA Respiratory Virus Screening & ID Kit; Diagnostic Hybrids, Athens, OH, USA).

DNA extraction and polymerase-chain reaction (PCR)

Viral DNA was extracted from immunofluorescence positive samples using the AmpliTaq Gold® kit (Applied

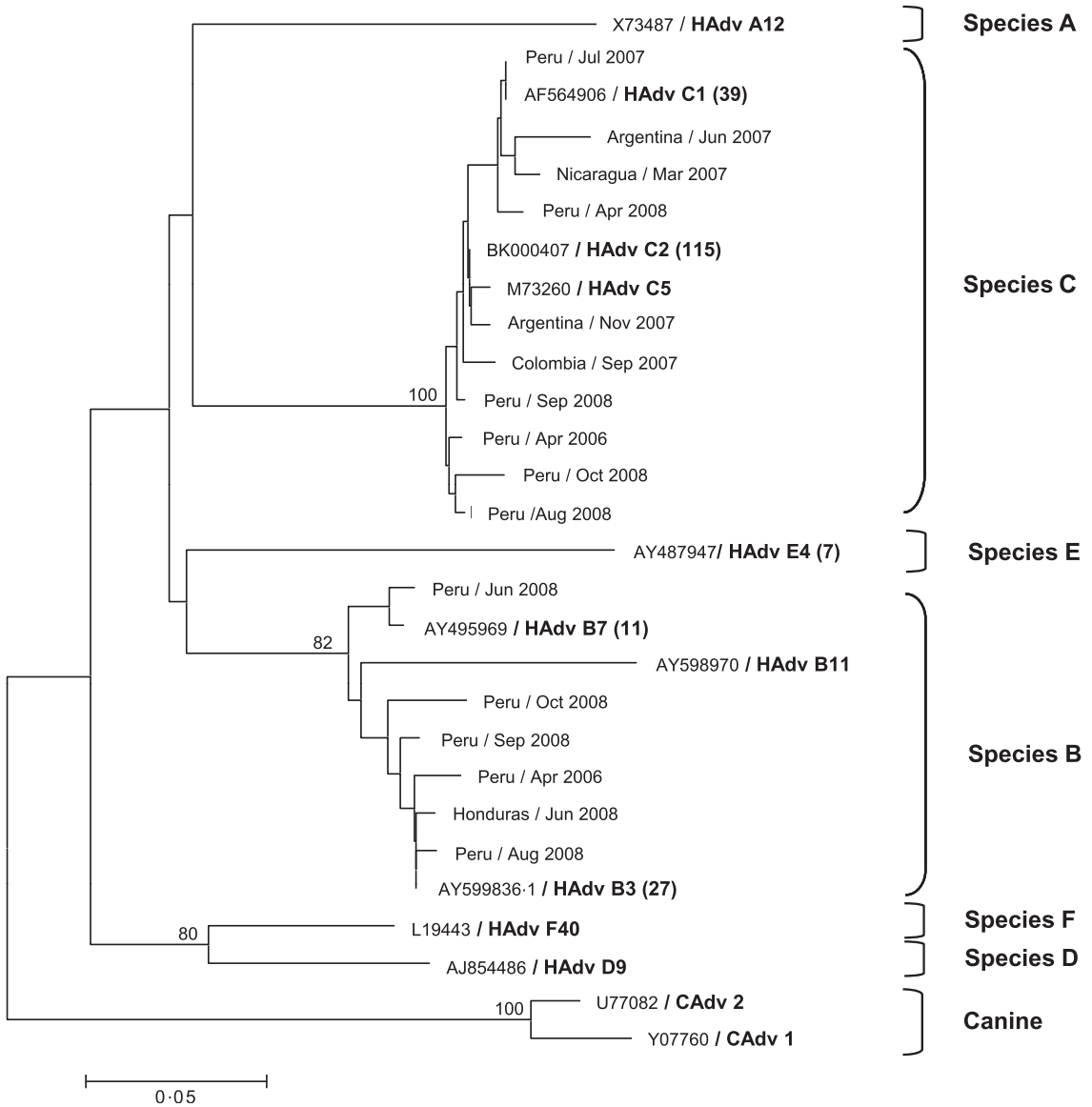

Figure 1. Adenovirus–phylogenetic tree. The last 300 nucleotides of the adenovirus *hexon* gene were amplified, sequenced, and compared with published sequences from GenBank. We have labeled the samples according to the following format: 'Country of collection/Month–Year of collection.' The comparison sequences are complete genome sequences from GenBank, these are presented in the following format: 'Accession Number/Serotype in Bold' (Number of isolated virus sharing the same sequence). Nucleotide sequences were aligned by using clustal x. Phylogenetic analyses were performed using the Kimura two-parameter model as a model of nucleotide substitution and using the neighbor-joining method to reconstruct phylogenetic trees (mega version 2.1). The samples are grouped into species. Canine adenoviruses were used as out-group sequences for comparison.

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Table 1. Adenoviral subgroups in circulation

Country	HAdv B	HAdv C	HAdv E	Total	% of adenovirus isolates/ILI cases
El Salvador	0	1	0	1	0·38
Honduras	1	11	0	12	5·28
Nicaragua	2	9	0	11	2·23
Venezuela	3	3	0	6	1·72
Colombia	3	6	0	9	4·29
Ecuador	2	7	0	9	1·81
Peru	32	114	7	153	2·84
Paraguay	0	1	0	1	0·43
Argentina	2	9	0	11	6·43
Total	45	161	7	213	

Number of adenovirus from each subgroup (B, C, and E) determined by molecular analysis and sequencing by country of collection. The last column presents the percentage of adenovirus isolations among the total ILI cases by country.
ILI, influenza-like illness; HAdv, human adenoviruses.

Biosystems, Foster City, CA, USA) and tested by PCR. An amplicon of a moderately conserved region of the adenovirus Hexon gene (amino acid 900–971) was amplified with the following specific primers: Adeno 3 (5′-CCTTTGG CGCATCCCATTCT-3′) and Adeno 4 (5′-GCGCTTGTCA TAGGTGCCCA-3′).[11]

Sequencing and phylogenetic analysis

For direct sequencing of viral nucleic acids from clinical specimens, genes fragments were amplified and sequenced with the use of Big Dye terminator cycle sequencing kit (version 3.1; Applied Biosystems, Ann Arbor, MI, USA) on a Genetic Analyser system (version 3130xL; Applied Biosystems).

Gene sequences were assembled aligned and edited using sequencer and bioedit (version 7.0.0; Isis Pharmaceuticals, Inc., Dublin, Ireland) software. Phylogenetic trees were generated with clustal x version 2.0.1 and mega version 3.1 software.[12]

Results and discussion

Influenza-like illnesses surveillance programs help to monitor the progression and distributions of many viruses and may be used to alert early changes in strains causing differences in infectivity or severity of infections and possible outbreaks.

Molecular typing provides a rapid identification of almost any serotype belonging to the Adv species A, B, C, D, E, and F. Many studies about the phylogenetic evolution of adenovirus exist focusing mainly on the *hexon* gene, with different results depending on the region chosen. We chose the last 300 nucleotides of this gene, which is a moderately conserved region common to all HAdvs subgroups.

A total of 402 HAdvs were isolated from the ILI-surveillance-network. 213 adenoviral samples were selected trying to include all listed countries; amplified and sequenced, subgroups and subtypes were inferred by comparison of sequenced samples with published ones from GenBank. Table 1 shows the analysis of subgroups B, C, and E by country (no other subgroups where detected), and we can see that during the 2006–2008 period, there were mostly two adenoviral species in circulation: C (76%) and B (21%). Subgroup E was only seen in Peru after August 2008 and represents 3% of the total HAdvs found. This distribution agrees with recently published studies[4,13,14] but differs from previous studies showing that the predominant species circulating in Latin America was B.[7,13,15,16]

Some studies have reported that adenoviral outbreaks are seasonal, during a 3-year period we did not observe any seasonality pattern on adenoviral infectivity by subgroups or subtypes.

Finally, Figure 1 shows the phylogenetic tree of all sequenced HAdvs, where the most represented subtype is HAdv C2, followed by HAdv C1 and then by B species, subtypes 7 and 3.

As this is passive surveillance study, the collected data does not allow the analysis of incidence rates or disease burden, it is a descriptive view of respiratory specimens from symptomatic cases. We are currently planning additional studies to address these issues.

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Conflict of interest statement

We declare that we have no conflict of interest.

Disclaimers

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

The study protocol was approved by the Naval Medical Research Center Institutional Review Board (Protocols NMRCD.2002.0019) in compliance with all applicable Federal regulations governing the protection of human subjects.

Disclosure

None of the authors has a financial or personal conflict of interest related to this study. The corresponding author had full access to all data in the study and final responsibility for the decision to submit this publication.

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