Complete nucleotide sequence analysis of the VP1 genomic region of Echoviruses 6 isolated from sewage in Greece revealed 98% similarity with Echoviruses 6 that were characterized from an aseptic meningitis outbreak 1 year later

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Abstract

The molecular characterization of two enterovirus strains (LR51A5 and LR61G3) isolated from the sewage treatment plant unit in Larissa, Greece, in May and June 2006 and the investigation of their relationship with enteroviruses of the same serotype isolated in Greece in 2001 and 2007 were performed by complete VP1 sequence analysis of the isolates. The close phylogenetic relationship and the high nucleotide similarity (98%) led to the conclusion that the virus isolated from sewage in 2006 was associated with that isolated from an aseptic meningitis outbreak 1 year later. Bootscan analysis of the VP1 genomic region revealed that intraserotypic multi-recombination events might have been involved in the evolutionary past history of the LR51A5 and LR61G3 isolates.

Keywords: Echovirus 6, intraspecies recombination, molecular analysis, VP1

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Enteroviruses are important causative agents of potentially fatal human diseases, such as acute haemorrhagic conjunctivitis, aseptic meningo-encephalitis and acute flaccid paralysis [1]. Enteroviruses are transmitted by the faecal-oral route, are excreted in human faeces and can be found in natural and man-made aquatic environmental samples. Environmental surveillance constitutes a great tool for estimating the extent and the duration of poliovirus circulation in a population. In India polioviruses were detected in environmental samples 3 months before poliomyelitis cases [2]. Although a lot of studies have been reported concerning polioviruses in environmental samples, little attention has been paid to the molecular epidemiology of non-polio enteroviruses isolated from sewage or other environmental samples and their relationship with circulating enteroviruses in humans during the same time period [3].

In the present study two Echoviruses 6 (E6) (LR51A5 and LR61G3) were isolated in May and June 2006 from the sewage treatment plant unit of Larissa city, Thessaly, Greece. During the summer of 2007 E6 was associated with an aseptic meningitis outbreak [4]. Complete VP1 sequence of the two isolates (LR51A5 and LR61G3) was obtained and nucleotide and amino acid sequence analysis was conducted. In order to investigate the relationship with other E6 isolates phylogenetic analysis of a part of VP1 was performed.

The sewage samples were concentrated by the two-phase separation method proposed by WHO [5] and the viruses were isolated in Rd cells. Viral RNA extraction and its reverse transcription into cDNA was performed as has been previously described [6]. In order to obtain the full nucleotide sequence of the VP1 genomic region, PCR with three different primer sets was performed: CL1653 (1653- CAT CAACCTACATCCCAATAACC-1676) and CR2648 (2626-GAAGTTTTCCACTGACGATTCC-2648) (present study); 292 and 222 [7] and EUG3a, EUG3b, EUG3c, and EUC2, EUC2a, EUC2b [8].

Phylogenetic and molecular analysis was conducted using MEGA version 4 software. For the phylogenetic analysis of the LR51A5 and LR61G3 isolates, partial sequences of the E6 prototype strain, such as those of strains isolated in 1979–2006 in Europe and Asia, were used. In order to investigate the phylogenetic relationships of LR51A5 and LR61G3 with other Greek isolates, partial sequences of the echoviruses 6 isolated in Greece from aseptic meningitis outbreaks in 2001 (Ver, Paran, Tsikan and Kras) and in 2007 (ECV6/51.07/2007/GRC and ECV6/82.07/2007/GRC) were used. The phylogenetic analysis was based on a neighbour-joining (NJ) algorithm and the reliability of the trees was determined by bootstrap analysis with 100 pseudoreplicate data sets. Finally, for the detection of recombination events in VP1, SimPlot and bootscan analysis between the aligned sequences were created with the aid of SimPlot

software (version 3.5.1), with a window size of 140nt for full VP1 analysis.

Based on the criterion for molecular typing of enteroviruses in VP1, sequencing of the VP1 genomic region revealed that LR51A5 and LR61G3 belong to the E6 serotype, as LR51A5 and LR61G3 showed 77% (>75%) nucleotide and 93% (>88%) amino acid identity with the respective region of the E6 reference strain D' Amori [9]. The two isolates revealed between them 99–100% nucleotide similarity, leading to the assumption that it was the same virus that circulated for at least 2 months in the city of Larissa.

For the molecular epidemiology study, a phylogenetic tree was initially inferred with a 358nt segment of the VP1 genomic region (from positions 2446 to 3307 within the genome), as it spanned most of the available sequences of E6 Greek and other isolates in GenBank. As shown in Fig. 1, the environmental Greek isolates cluster with the echoviruses 6, ECV6/51.07/2007/GRC and ECV6/82.07/2007/GRC, isolated in the 2007 aseptic meningitis outbreak in Greece. Comparison of the nucleotide sequences of LR51A5 and LR61G3 with that of aseptic meningitis revealed 98% similarity. The high nucleotide similarity led to the conclusion that the LR51A5 and LR61G3 isolated from sewage in 2006, shared the same genotype with the echoviruses 6 isolated from aseptic meningitis cases in summer 2007. LR51A5 and LR61G3 did not cluster with the echoviruses 6 of the 2001 outbreak. The nucleotide sequence diversity between the LR51A5 and LR61G3 sequences and those of the 2001 Greek isolates was 18%.

SimPlot and Bootscan analysis of the VP1 region of the isolate LR51A5 is presented in Fig. 2. Four distinct regions are presented where the LR51A5 isolate has high nucleotide homology with different E6 strains. As presented in bootscan analysis, in the 327nt of the 5′ end of VP1 the strain with the highest identity (85%) is 25781_Tumen_2006 (EF397646), isolated in Russia in 2006. Passing to the 328–430nt region there is a change of identity, where the closest strain is 87CF36eu with 89% nucleotide similarity. In the 431–620nt region the strain with the highest similarity (89%) is the 25465_Tambov_05 (EF397644), isolated also in Russia in 2005, and 240nt downstream in the 3′ end of VP1 the strain with the highest similarity (84%) is 25781_Tumen_2006. In phylogenetic trees (data not shown) of these four parts of the VP1 genomic region, the different clustering of LR51A5 and LR61G3 was observed. These results imply that intraspecies recombination events had occurred in the specific genomic region in the past evolutionary history of the E6 strains. Recombination is a known phenomenon for polio and non-polio enteroviruses and is frequently located in the non-capsid genomic region [10–13]. While interspecies recombination is rare within the VP2-VP1 capsid region

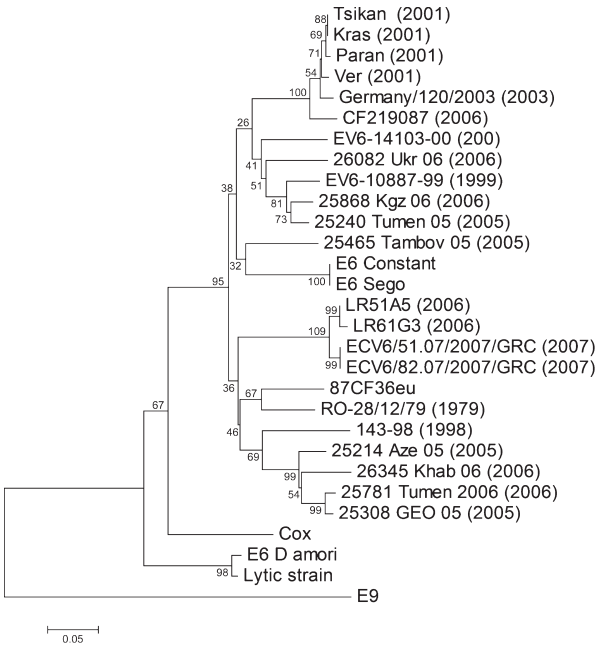

FIG. 1. Phylogenetic tree of 358nt (2446–3307nt) of a region of VP1 of LR51A5 and LR61G3 and other E6 strains: D' Amori (AY302558), Lytic strain (U16283), Cox (AF081322), Sego (DQ345772), Constant (DQ345771), 25214_Aze_05 (EF397658), 26345_Khab_06 (EF397654), 26082_Ukr_06 (EF397650), 25308_GEO_05 (EF397643), 25214_Tumen_05 (EF397641), 25781_Tumen_2006 (EF397646), 25465_Tambov_05 (EF397644), 25868_Kgz_06 (EF397647), 143–98 (AB268168), RO-28/12/79 (AJ279162), Germany/120/2003 (AY956571), CF219087-06 (AM711095), EV6-14103-00 (AY896761), EV6-10887-99 (AY896760), 87CF36eu (AJ241437), Ver (AY697453), Paran (AY697448), Tsikan (AY697451), Kras (AY697456), ECV6/51.07/2007/GRC (FM878835), and ECV6/82.07/2007/GRC (FM878836). The year of isolation of each strain is presented in parentheses. The construction of the phylogenetic tree was based on a neighbour-joining (NJ) algorithm. Support for specific tree topologies was estimated by bootstrap analysis with 100 pseudoreplicate datasets.

[14,15], intratypic recombinants are produced 100 times more often than intertypic [16], although a few intraserotypic recombination events in the capsid region have been reported for non-polio enteroviruses [17].

Environmental surveillance was successfully used to demonstrate wild and vaccine-derived poliovirus transmission in a community [18,19] and has been also used as a prevention tool in India as polioviruses have been detected in environmental samples 3 months before paralytic poliomyelitis cases [2]. Existing research information on environmental samples has revealed further non-polio enterovirus species [8,20,21], but to our knowledge there is no correlation of these isolates with those from clinical cases. In the present study, for the first time an echovirus 6 was detected in sewage

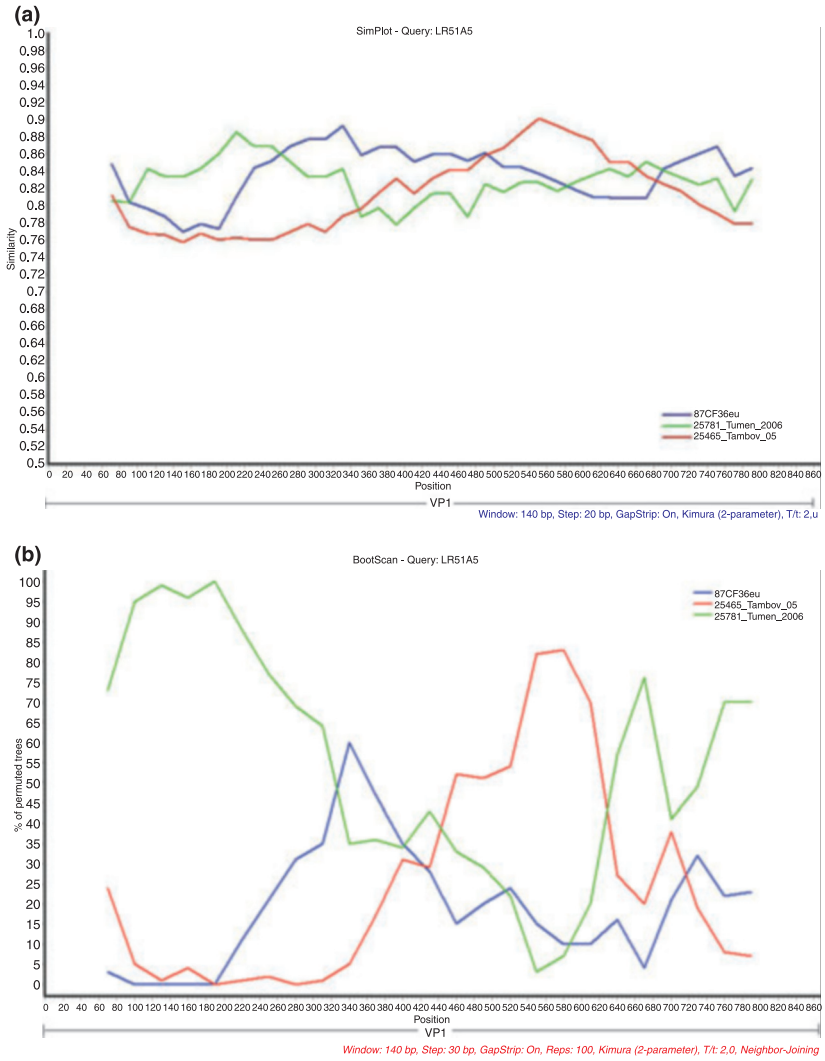

FIG. 2. (a) SimPlot and (b) Bootscan analysis of the VP1 region of the LR51A5 isolate, created by SimPlot software (version 3.5.1), with a window size of 140nt.

1 year before the molecular characterization of viruses from aseptic meningitis cases.

For epidemiological studies of enteroviruses, nucleotide sequences of a part of the VP1 genomic region are usually used. Our findings support that complete VP1 nucleotide sequence analysis is a prerequisite for the revelation of serial intratypic recombination events in VP1 that may play a major role in enterovirus evolution, and that molecular epidemiological studies in only a part of this region may lead to erroneous conclusions.

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Transparency Declaration

The authors declare that they have no conflicting or dual interest.

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Genetic diversity of noroviruses from outbreaks, sporadic cases and wastewater in Luxembourg 2008–2009

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Abstract

The genetic diversity of norovirus strains obtained from gastroenteritis outbreaks, sporadic case surveillance and wastewater plants was compared in Luxembourg from October 2008 until June 2009. Except for GI.6 and GIV.1 strains detected exclusively in wastewater, all other genotypes were also found in human samples. Of the nine NoV genotypes detected, only three (GII.4, GIIb/II.3 and GIIc/II.12) were associated with institutional outbreaks. The majority of sequences from all sources belonged to genotype GII.4, including two potentially new subvariants. Strains collected in the context of outbreaks may significantly under-represent the overall genetic diversity of NoVs circulating in a country.

Keywords: Genetic diversity, Luxembourg, norovirus, nursing homes, sporadic cases, surveillance, wastewater

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