



AKADÉMIAI KIADÓ

Molecular epidemiology and characterization of endemic, epidemic and imported hepatitis A virus (HAV) strains in Hungary (2003–2022)

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RESEARCH ARTICLE



ABSTRACT

Hepatitis A virus (HAV) is one of the most important etiological agents of acute viral hepatitis but comprehensive molecular epidemiological study with chrono-phylogeographical data are not available from Hungary.

Between 2003 and 2022, a total of 8,307 HAV infections were registered officially in Hungary of which 400 (4.8%) HAV IgM antibody-positive serum samples were collected countrywide. HAV genomic RNA was successfully detected in 216/400 (54%) sera by RT-PCR subsequently confirmed by sequencing. The complete nucleotide sequences of VP1 region were determined in 32 representative HAV strains. Based on the sequence analysis, 150 (69.4%) strains were characterized as HAV sub-genotype IA and 66 (30.6%) as sub-genotype IB, respectively. Based on the combined epidemiological and molecular data, epidemic, endemic, and imported HAV strains were also characterized. The first two registered countrywide outbreaks started among men-sex-with men (MSM) in 2011 (sub-genotype IA) and 2021 (sub-genotype IB), the continuously circulating endemic/domestic HAV strain (sub-genotype IA) in East Hungary and the travel-related sub-genotype IB strains from Egypt should be highlighted. All HAV strains are deposited in the HAVNET database (<https://www.rivm.nl/en/havnet>).

In this 20-year-long comprehensive molecular epidemiological study, we report the genetic characterization and geographic distribution of endemic, epidemic and imported HAV strains for the first time in Hungary with continuous co-circulation of sub-genotypes IA and IB HAV strains since 2003. These data provide basic information about the HAV situation in the country in an international context and can promote more effective national public health intervention strategies for the prevention of HAV transmissions and infections.

KEYWORDS

hepatitis A virus, molecular epidemiology, genotype, sub-genotype, Hungary

INTRODUCTION

Hepatitis A virus (HAV) is one of the most important etiological agents of acute viral hepatitis caused by one serotype of hepatitis A virus of the genus *Hepatovirus*, family *Picornaviridae* [1]. HAV has been classified into seven genotypes (I–VII) including four human (I, II, III and VII) groups. Most human strains belong to genotype I, which has been divided into sub-genotypes IA and IB [2] by sequence- and phylogenetic analysis. Sequence-based sub-genotype and genetic variant analysis of HAV is a useful method [3] for the investigation of the geographic distribution of certain endemic HAV strains, timely identification of imported variants and following the (national and international) spread of a potential epidemic strain. The molecular epidemiological studies can also reveal relationships that may remain hidden with classical epidemiological investigations, thus facilitating the identification of the potential human, environmental and food sources of the infections and reducing the possibility of faecal-oral, blood-borne and sexual transmissions of further infections.

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In Hungary, the annual number of registered HAV cases between 2003 and 2022 was between 28 (in the year 2020) and 1,556 (in the year 2014) where the total population number was 9.75 million in 2020. The HAV has variable epidemiological and geographical distribution from region to region within the country. In the North-East part of Hungary (Borsod-Abaúj-Zemplén and Szabolcs-Szatmár counties) HAV infections are thought to be endemic. In this geographic region, HAV infections are continuously present in the population at moderate/high levels and transmitted by classical transmission routes mainly among the young population living with low social and hygienic conditions. In some years, more than 80% of the all reported HAV infections were registered only in these 2 (of the 19) counties in Hungary. The capital Budapest is in a special situation for HAV where ~15–20% of the total population lives in high-density and interactive environments. However, in most areas, especially in the west of the country, the annual number of registered HAV cases is low (very low or zero) and/or fluctuating from time to time.

In three early epidemic case studies, co-circulations of HAV sub-genotype IA and IB were reported from Hungary [4–7]; however, detailed data are not available about the geno- and sub-genotypes distribution of HAV strains over time and about the detailed molecular epidemiology of HAV infections in Hungary.

In this 20-year-long molecular epidemiological study, we report the genetic characterization and geographic distribution of endemic, epidemic and imported HAV strains in Hungary between 2003 and 2022.

MATERIALS AND METHODS

Collection of specimens

Serum samples ($N = 400$) positive for HAV IgM antibodies by ELISA method were collected and selected countrywide from Hungary from acute HAV infections and outbreaks

between 2003 and 2022. Most of the specimens originated from three main areas from the North-East, Central (capital and surroundings) and South-West parts of Hungary.

Molecular methods

HAV IgM-positive serum samples were tested by RT-PCR for HAV. Viral RNA was isolated from sera by TRIzol™ LS Reagent (ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Screening primers newRT/BR5 [5] were used for the detection and characterization of the viral VP1/2A genomic regions which was a common consensus sequencing region recommended by HAVNET, a global network of hepatitis A virus laboratories [3]. In selected cases ($N = 32$) the complete VP1 HAV sequences were also determined. PCR-products were directly sequenced and run on an automated sequencer (310 or 3,500 Genetic Analyzer, Applied Biosystems).

Sequence- and phylogenetic analysis

Phylogenetic analysis based on the VP1/2A nucleotide sequences of Hungarian HAV strains ($N = 216$) was done using the Neighbor-Joining clustering method with a 2-parameter Kimura nucleotide substitution model supported by the online phylogenetic analysis tool of the HAVNET database (<https://www.rivm.nl/en/havnet>). All the 216 HAV strains are deposited into the HAVNET database prospectively and selected strains ($N = 93$) with diverse nucleotide sequences are into the GenBank database under accession numbers AY986357, AY986358, DQ163904, EF190998, KM657825, OL830105-OL830127, and OQ161708-OQ161772.

RESULTS

Between 2003 and 2022, a total of 12,912 viral hepatitis cases including 8,307 HAV infections reported officially in Hungary (Fig. 1). The number of the yearly registered HAV cases decreased by 85% from 2003 to 2011; however, a rapid

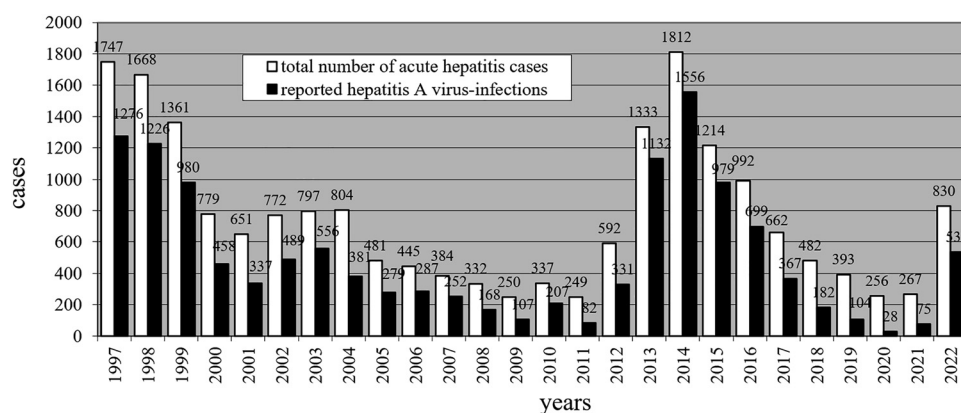
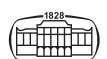


Fig. 1. Yearly distribution of the reported hepatitis A virus (HAV) cases (black column, $N = 13,073$) and all acute viral hepatitis cases (white column, $N = 19,890$; includes the reported acute HAV, HBV, HCV, and HEV infections) in Hungary from 1997 to 2022, respectively (yearly dataset collected from National Public Health Centre, Hungary, <http://www.oek.hu/oek.web>, https://www.antsz.hu/felso_menu/temaink/jarvany)



and significant increase in the cases occurred in 2011/2012 and 2021/2022, respectively (Fig. 1).

A total of 400 (4.8%) HAV IgM-positive specimens of the 8,307 registered HAV cases were tested by RT-PCR within the study period. HAV genomic RNA was successfully detected in 216 (54%) of the 400 specimens by RT-PCR method subsequently confirmed by sequencing. The complete nucleotide sequences of the VP1 region were determined in 32 selected HAV strains (Fig. 2). Based on the VP1/2A sequence analysis 150 (69.4%) strains were characterized as HAV sub-genotype IA and 66 (30.6%) as sub-genotype IB, respectively (Fig. 2). Except for the genotype I, sub-genotype IA and IB strains, other HAV genotypes were not detected in the study period in the country. Sub-genotypes IA and IB strains were continuously co-circulated in Hungary during the study period (data not shown).

Based on the combined epidemiological and sequence data we could characterize the potential HAV strains in registered outbreaks, endemic infections and imported cases in Hungary between 2003 and 2022 (Figs 2 and 3).

A total of 6 officially registered HAV outbreaks (namely Transdanubia 2006; Úny 2006; Budapest 2007/2008; MSM 2011/2014; Mohács 2017, and MSM 2021/2022) were confirmed that has been associated with sub-genotypes IB ($N = 4$) and IA ($N = 2$) strains (Fig. 2). Outbreaks MSM 2011/2014 (sub-genotype IA; between years 2011 and 2014) and MSM 2021/2022 (sub-genotype IB; years 2021/2022 and beyond) were the first two registered epidemics in Hungary where the main initial source and the mode of HAV transmission were confirmed as sexual (men-sex-with men, MSM). Both epidemics are associated with cases of countrywide infections. The MSM 2011/2014 HAV IA strain (the prototype strain is HUN-A357, KM657825; Fig. 2) has the closest (99.05%) nucleotide identity to strain HAV/Terrassa07/Catalonia/Spain (HQ401245) using GenBank Blastn search. The MSM 2021/2022 HAV IB strain (the prototype strain is HUN-A394, DQ161772; Fig. 2) has the closest (97.81% and 96.46%) nucleotide identity to strain HAV/Egy/BI-11/2015/Egypt (KX228694) [8] and the cluster of IB strains from New York, USA in years 2016–2019 [9]. On the other hand, we could find only one HAV strain (HUN-A378 from July 2017; OL830121) in this study, that had 100% nucleotide identity to one (VRD_521_2016) of the so-called three European sub-genotype IA HAV epidemic strains (i) RIVM-HAV16-090 (EuroPride from Sept 2016, The Netherlands); (ii) VRD_521_2016 (from Dec 2016 UK/Spain) and (iii) V16-25801 (LT796556, from Aug 2016, Germany) circulating among MSM in many European and other countries [10, 11]. While the sexual orientation is not known in this 40-year-old man (HUN-A378) the infection is thought to be imported (from Romania) (Fig. 2).

Only sub-genotype IA HAV strains were identified in East Hungary. Identical or genetically highly similar sub-genotype IA strain (named lineage “a”) was found to be continuously circulating in North-East Hungary as the potential domestically endemic HAV variant in the country (Fig. 3). Sub-genotype IA lineage “a” HAV strains were also detected in Veszprém county (West Hungary) (Fig. 3),

where it was proved the domestic travel origin (family visit) of the infections from North Hungary using classic epidemiological methods.

Based on the patient’s medical history, a total of 10 cases were confirmed as travel-related imported HAV infections caused by sub-genotype IA (from Romania $N = 3$; Brazil $N = 1$ and Austria $N = 1$) and sub-genotype IB (from Egypt $N = 3$, Spain $N = 1$ and Romania $N = 1$) HAV strains (Fig. 2). Eight of these 10 imported infections had unique nucleotide sequences which were not present in any HAV strains detected in Hungary during the study period (Fig. 2). The strains imported from Egypt [HUN-A239 (OQ161739), HUN-A244 (OQ161742) and HUN-A250 (OQ161748)] have 98.35–98.96% nucleotide identities to sub-genotype IB HAV strains circulating in Egypt as the closest match in GenBank database using Blastn searches.

DISCUSSION

In this two-decades-long comprehensive molecular epidemiological study, we report the genetic characterization and geographic distribution of endemic, epidemic and imported HAV strains for the first time in Hungary between the years 2003 and 2022. During the study period, HAV genotype I, sub-genotypes IA and IB strains were co-circulated in 70%/30% proportion in the study population year after year since the year of 2003. No genotypes other than genotype I HAV strains were identified and low number of HAV cases reported during the SARS-CoV-2 pandemic years 2020/2021.

Both sub-genotype IA and IB strains were associated with outbreaks, imported cases and endemic infections in Hungary. On the other hand, interestingly, only sub-genotype IA strains (fundamentally lineage “a”) were identified in the East part of the country confirming the potential endemic presence of this genetic variant and also the regional difference of the circulating HAV strains in Hungary. Sub-genotype IB strains were identified in (South)-West and central (and the capital of) Hungary.

Two waves of infections were associated with a significantly increased number of countrywide HAV cases identified within the previous 20 years. Interestingly, the initial source and transmission mode of these infections in both events were sexual, confirming a novel, but fundamental epidemiological impact of the sexually transmitted HAV infections/outbreaks in the country for the first time. Both events started to spread among MSM population in the capital Budapest causing prolonged transmissions of HAV but it caused by two different HAV sub-genotypes, IA (started at the end of the year 2011) [7, 12, 13] and IB (started at the end of year 2021) (<https://www.ecdc.europa.eu/en/news-events/spread-hepatitis-virus-strains-genotype-ib-several-eu-countries-and-united-kingdom>). Because of the low level of HAV immunity (high susceptibility) in the study population under age of 40 [7] and the high risk of faecal-oral HAV transmission during sexual practices among MSM people in Europe [10, 11, 14, 15] novel national and international public health strategies [16, 17],



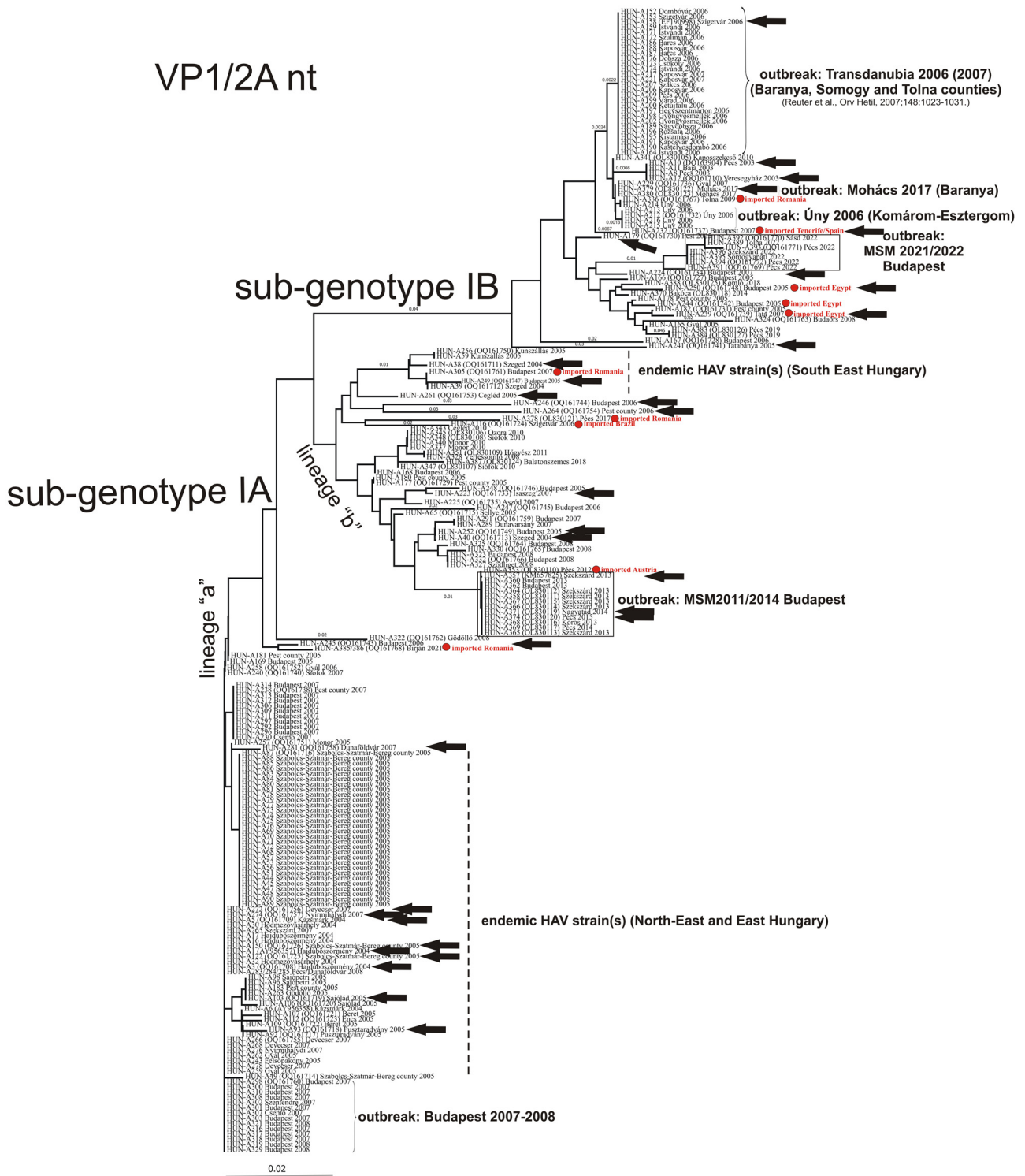
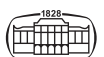


Fig. 2. Phylogenetic analysis based on the VP1/2A nucleotide sequences of sub-genotypes 1A and IB hepatitis A viruses ($N = 216$, deposited in the HAVNET database) between 2003 and 2022 from Hungary is inferred using the Neighbor-Joining clustering method with a 2-parameter Kimura nucleotide substitution model supported by the online phylogenetic analysis tool of HAVNET database (<https://www.rivm.nl/en/havnet>). HAV strains are labelled as strain name (HUN-A...), GenBank accession number, the name of the settlement of the infection, and year of infection. HAV strains associated with reported outbreaks (braces), potential endemic strains (dashed line), imported strains (red circle) and MSM outbreaks (black rectangle) are shown. The country of origin of the imported HAV cases is also indicated. HAV strains with complete VP1 sequences ($N = 32$) are indicated with black arrows



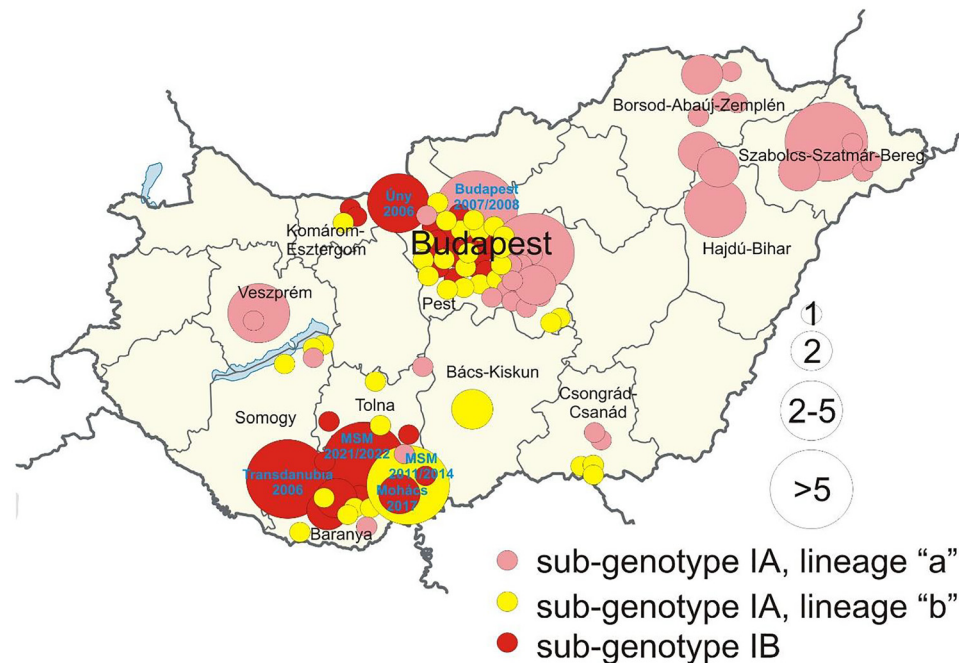


Fig. 3. Geographic distribution of hepatitis A virus cumulative cases confirmed by RT-PCR and sequencing methods according to the patient's place of residence between 2003 and 2022 in Hungary. The dot map shows the sub-genotypes IA and IB strains by colour coding. The size of the circle refers to the number of confirmed cases. Sub-genotype IA lineage "a" (pink colour) represents the potential endemic/domestic HAV lineage in North-East and East Hungary (see also the phylogenetic tree in Fig. 2). The name of the registered epidemics ($N = 6$) is marked by blue letters. The name of the counties ($N = 11$) and the capital Budapest with available HAV-positive specimens are indicated. MSM = men sex with men

education and effective vaccination campaigns should be implemented especially among MSM population for prevention of significant excess of HAV infections and outbreaks caused by different locally and internationally spread HAV strains in Europe including Hungary.

This study has some limitations: The HAV infections are underestimated because only the symptomatic individuals who sought health care were reported. Only ~5% of the registered HAV cases were investigated by molecular methods and some geographic regions (counties) are over- (Southwest Hungary), and some others are under- or not represented in this study.

In summary, continuous co-circulation of sub-genotypes IA and IB HAV strains were identified in Hungary between 2003 and 2022. Using the combined epidemiological and sequence data the potential HAV strains in outbreaks and imported cases of endemic/domestic infections have been characterized. These data can promote more effective national epidemiological preventive work for the prevention of HAV infections and provide basic information about the genetic diversity of HAV strain in Hungary in the international context.

Conflict of interest: No conflict of interest was present.

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