Journal of Infection and Public Health 16 (2023) 1462–1470

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: www.elsevier.com/locate/jiph

Hepatitis A virus subgenotypes in Latvia, 2008–2021

Oksana Savicka [a,b,*,1], Diana Dusacka [a], Reinis Zeltmatis [a], Sergejs Nikisins [a], Inga Azina [c,d], Ludmila Ivancenko [c], Ieva Tolmane [c,e], Baiba Rozentale [c,d]

[a] Riga East Clinical University Hospital, Laboratory "Latvian Centre of Infectious Diseases", National Microbiology Reference Laboratory, Riga, Latvia
[b] Riga Stradins University, Department of Infectology, Latvia
[c] Riga East Clinical University Hospital, hospital "Latvian Centre of Infectious Diseases", Riga, Latvia
[d] Riga Stradins University, Department of Public Health and Epidemiology, Latvia
[e] University of Latvia, Department of Medicine, Latvia

ARTICLE INFO

Article history:
Received 15 December 2022
Received in revised form 21 June 2023
Accepted 17 July 2023

Keywords:
Hepatitis A virus (HAV)
HAV genotypes
Men who have sex with men (MSM)
Persons who inject drugs (PWID)
Outbreak
Phylogenetic analysis

ABSTRACT

Background: In Latvia outbreaks of the HAV were observed between 2008 and early 2010 and again in 2017–2018. However, the risks of introducing and spreading infection still exist, as the virus spreads easily when personal hygiene is not followed.
Methods: To determine the spread of HAV subgenotypes in the territory of Latvia the VP1/P2A genomic region of HAV was amplified and sequenced for 259 case serum samples. The study carried out a molecular biological investigation and molecular epidemiological investigation. Demographic data (sex, age), disease data (hepatitis symptoms, hospitalization, vaccination) and epidemiology data (part of the outbreak, possible source of infection, recent travel) were collected. Based on the obtained sequences, the phylogenetic tree was built and analyzed for the homology and belonging to different isolated HAV clusters from other countries.
Results: From the obtained data, it was concluded that HAV subgenotype IA had 13 clusters and 12 sporadic cases, HAV subgenotype IB had eight clusters and 11 sporadic cases, HAV subgenotype IIIA had one cluster and nine sporadic cases. It was found that the sources of infection among the investigated cases were different, they were mostly associated with contact with a patient with HAV, travel, as well as between persons who inject drugs and men who have sex with men, and the prevalence of HAV similar sequences was observed in different years. It was concluded that patients with HAV subgenotype IA had the longest hospitalization duration and averaged 9.3 days, while patients with subgenotype IB - 7.3 days, subgenotype IIIA - 7.7 days. Analyzing the data on vaccination, it was found that mostly all were not vaccinated or had an unknown vaccination status.
Conclusions: All of this has led to the conclusion that the application of molecular biological methods of the HAV and a careful analysis of epidemiological data can help to better understand the ways of spreading the infection, investigate local outbreaks, detect cases of imported infection and track the recirculation of the virus.

1. Introduction

HAV infection, which is a major cause of acute hepatitis, poses an important public health problem worldwide [1–3]. Hepatitis is a major health problem in both developing and developed countries. An estimated 1.4 million clinical cases of hepatitis A occur worldwide every year [4].

* Corresponding author at: Riga East Clinical University Hospital, Laboratory "Latvian Centre of Infectious Diseases", National Microbiology Reference Laboratory, Riga, Latvia.
E-mail address: oksana_savicka@inbox.lv (O. Savicka).
[1] ORCID: 0000-0003-0047-3644

https://doi.org/10.1016/j.jiph.2023.07.012

The manifestation can be asymptomatic or symptomatic, ranging between mild and fulminant hepatitis, which is rare [5,6].

The virus can spread through the faecal-oral route, ingestion of contaminated food and water or direct contact with an infected person [7,8]. Lack of safe water, as well as poor sanitation and hygiene are risk factors for HAV infection. Epidemics can be prolonged and cause substantial economic loss [5,9]. The particles of HAV are stable and resistant to room temperature and low pH conditions that favor its transmission via the fecal-oral route. Before symptomatic disease, the incubation period is usually approximately 28 days with viremia and viral elimination in feces during the first weeks after infection explaining the high probability of contamination in individuals who practice oral-anal sex and persons who injects drugs [1,10]. However, fecal shedding of HAV can last for months after resolution of symptoms and such patients could be a source of further spreading of HAV [11,12].

HAV belongs to the family *Picornaviridae* and the genus *Hepatovirus*. HAV contains a 7.5 kb genome encoded by a positive-sense, single-stranded RNA. HAV has six genotypes (I-VI); genotypes I-III are infectious to humans [5,10]. Genotypes I and III are each divided into subgenotypes A and B. The nucleotide variation between isolates of different genotypes is ~15 %, and variation between subgenotypes ranges between 7 % and 7.5 % in the VP1 capsid protein-P2A protease junction [5,13–17].

Hepatitis A occurs sporadically and also as outbreaks. Molecular detection and typing of the VP1/P2A genomic region of HAV is used for genotype detection and outbreak investigations [18,19].

In Latvia, hepatitis A is a notifiable disease, with all cases reported to the Centre for Disease Prevention and Control of Latvia (CDC) [20]. Since the last HAV outbreak in Latvia, which occurred in 2008–2009 and resulted in 5107 cases, the number of hepatitis A cases has remained steady at around 10 cases per year, all of which have been sporadic and without epidemiological links to each other. However, in 2017, an increase in cases was observed, followed by continued case reporting up to 2019.

In the present study, we characterized the HAV strains involved in the acute hepatitis A cases identified in Latvia in 2008 – 2021 during outbreaks as also sporadic cases by sequencing and a phylogenetic analysis. HAV samples were typed to determine whether these cases were linked to one another, to risk groups or connected other to outbreaks. We describe our investigations, including genotype sequence characteristics of the cases and phylogenetic analyses, and summarise the available surveillance data of HAV in Latvia from 2008 to 2021.

2. Materials and methods

2.1. Study setting

Riga East clinical university hospital, Infectology Centre of Latvia is the main institution in the field of infectious diseases and houses Latvia's National microbiology reference laboratory (NMRL). The NMRL is Latvia's primary centre of detection, confirmation and molecular typing of HAV. Clinicians should notify probable and confirmed cases, and laboratories are required to report positive HAV results to the CDC. Laboratory diagnosis is based on at least one of the following three laboratory criteria according to the 2018 EU case definition for acute hepatitis A: (i) detection of hepatitis A virus nucleic acid in serum or stool, (ii) hepatitis A virus-specific antibody response or (iii) detection of hepatitis A virus antigen in stool [21]. Serological tests for anti-HAV IgM are the most common and a mainstay in the diagnosis. When the CDC receives notification reports from clinicians or laboratories, all cases of hepatitis A are investigated by the in-house epidemiologists, who contact the HAV patient and collect information about hospitalisation, vaccination, hepatitis symptoms, and travel during the incubation period.

In Latvia from 2008 to 2021 5726 hepatitis A cases were notified [22]. Serum samples were obtained from 259 individuals with clinical manifestations of acute hepatitis A for HAV typing, with a mean age of 33.7 years (range, 3 – 77 years).

2.2. HAV serological tests

The presence of anti-HAV IgM antibodies were tested by enzyme immunoassay (EIA) using AxSYM HAVA-M 2.0 (Abbott Diagnostics, Germany) (n = 100), ETI-HA-IgMK-PLUS (DiaSorin, Italy) (n = 1), Architect HAVAb-IgM, (Abbott Diagnostics, Germany) (n = 99), Cobas Anti-HAV IgM (Roche Diagnostic, Germany) (n = 59) according to the manufacturer's instructions.

2.3. RNA extraction, nested polymerase chain reaction (PCR), sequencing and sequencing analysis

The protocol for molecular detection and typing of the VP1/P2A genomic region of HAV, National Institute for Public Health and the Environment, the Netherlands [23] was used for genotyping by sequencing 460 nt.

Automated RNA extraction from serum samples was performed on NucliSens easyMaq, Biomerieux instrument (bioMerieux, Durham, North Carolina, United States (US)), amplification on GeneAmp 9700 thermal cycler (Applied Biosystems, Waltham, Massachusetts, US), and sequencing on Applied BioSystems 3130xl genetic analyser (Applied Biosystems).

All acquired HAV sequences were submitted to the HAVNET database and compared with reference sequences, including three outbreak strains widely circulating among MSM (VRD_521_2016, RIVM-HAV16–090 and V16–25801) and reference strains for genotypes (IA, IB, IIIA) obtained from GenBank database (X75215, M14707, FJ227135).

A phylogenetic tree was inferred by using the Maximum Likelihood method based on the Tamura-Nei model with bootstrap analysis (1000 replicates). All positions containing gaps and missing data were eliminated. The phylogenetic tree was generated by MEGA (6.0) software [24].

2.4. Epidemiological data

Epidemiological data including age, sex, hepatitis-related symptoms, information about hospitalisation, vaccination against HAV, possible source of infection (contact with an HAV case, MSM, PWID, food-borne or unknown), recent travel and suspected country of origin of the virus for typed HAV samples was collected.

2.5. Statistical Analyses

Descriptive statistical methods were used to characterize the patients. For quantitative data, the arithmetic mean (Mean, M) with the standard deviation (Standard Deviation, SD) of the dispersion indicators and the median (Median, Me) was evaluated.

The results were evaluated with an α-error of 5 %, thus, if the p-value obtained in the results was less than 0.05, the null hypothesis was rejected and the test result was recognized as statistically significant.

If statistical data did not correspond to normal distribution, non-parametric tests such as Mann-Whitney U Test and Kruskal-Wallis Test were applied.

Correlation analysis, Spearman's rank correlation coefficient was used to determine the relationship between the variables.

Data processing was performed using the computer program IBM SPSS Statistics, Version 27.0.

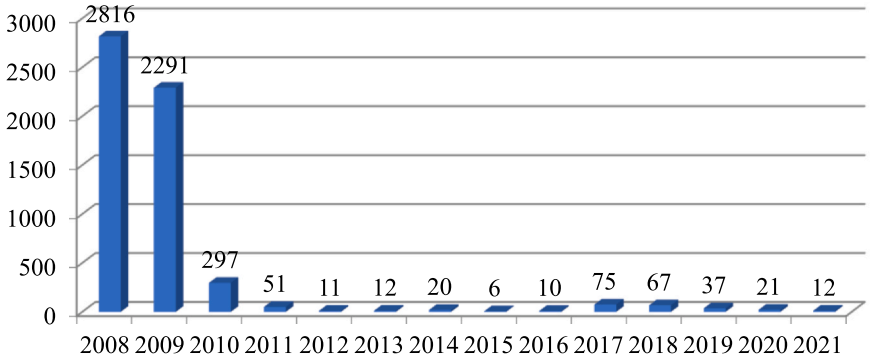

Fig. 1. Notified hepatitis A cases in 2008–2021 in Latvia.

3. Results

3.1. Notified hepatitis A cases in 2008–2021 in Latvia

Fig. 1. shows reported 5726 hepatitis A cases from 2008 to 2021 in Latvia. Evaluating the distribution of diagnosed cases by year, it can be established that the number of new cases is fluctuating, however, there is a tendency to increase the number of cases during outbreaks (2008 – 2009, 2017–2018).

3.2. Subgenotypes of hepatitis A cases in Latvia

Serum samples from cases (259/5726; 4.5 %) were sequenced to determine HAV subgenotypes. There is no conflict of interest to declare 1 shows the amount of the sequenced 259 HAV samples. The majority were HAV subgenotype IA - 72.0 % (n = 187), subgenotype IB - 23.0 % (n = 59), subgenotype IIIA - 5.0 % (n = 13). In 2017 most of the cases were subgenotype IA (n = 55) and they belonged to one of three HAV subgenotype IA outbreak strains in Europe among MSM during the 2016/2017 (VRD_521_2016, n = 30; RIVM-HAV16–090, n = 7; V16–25801, n = 2), however in 2018 some HAV strains associated with HAV outbreak among MSM also were detected. Other clusters and sporadic cases of HAV subgenotype IA infections were also detected. (Table 1).

3.3. Demographics

Fig. 2 shows age and sex distribution among the 259 sequenced HAV cases. The mean age of patients at diagnosis was 33.7 years (SD ± 14.9 years). The youngest patient was 3 years old, while the oldest patient was 77 years old. The median age was 32 years. 41 (16 %) of this group of patients were children and overwhelmingly more - 218 (84 %) were adults. Comparing the age of patients at the time of diagnosis, the age of men and women differs statistically significantly (p = 0.049).

3.4. Data on hepatitis symptoms, hospitalization, vaccination

All 259 patients had symptoms of hepatitis. The total number of hospitalized patients was 242/259 (93.4 %), non-hospitalized patients 17/259 (6,6 %).

Among hospitalized patients, adults were 205/242 (84.7 %) and children were 37/242 (15.3 %). The average age of the hospitalized patients was 33.7 years (SD ± 14.9 years), with a median of 32 years, the gender distribution was uneven: men – 54.1 % (n = 131), women – 45.8.1 % (n = 111). Among hospitalized patients, HAV subgenotype IA was 178, subgenotype IB - 52, subgenotype IIIA - 12.

Comparing the gender of hospitalized patients and HAV subgenotype, there is no statistically significant association (p = 0.790), but comparing the age distribution of hospitalized patients with HAV subgenotypes is statistically significantly different (p = 0.08).

Among non-hospitalized patients, adults were 14/17 (82.4 %) and children were 3/17 (17.6 %). The mean age of non-hospitalized patients was 33.4 years (SD ± 16.0), with a median of 34 years, the gender distribution was even: men – 52.9 % (n = 9), women – 47.1 % (n = 8). Among non-hospitalized patients, HAV subgenotype IA was 9, subgenotype IB - 7, subgenotype IIIA - 1.

When comparing non-hospitalized patients' gender and HAV subgenotype, there is no statistically significant difference (p = 0.758), as well as the age distribution by HAV subgenotype is not statistically significantly different (p = 0.837).

The number of analyzed patients who were hospitalized in REUH inpatient LIC from 2012 to 2021 amounted to 97/259 (37.5 %), for an average of 6.9 days (SD ± 4.5 days) from the date of illness, with a median of 6 days. The duration of hospitalization for the examined group was on average 8.7 days (SD ± 7.8 days), with a median of 7.0. The minimum number of days was – 1, the maximum number of hospitalized days was 73 days.

Fig. 3 shows the duration of hospitalization. The number of patients with subgenotype HAV IA was 69.1 % (n = 67), with subgenotype HAV IB 24.7 % (n = 24), with subgenotype HAV IIIA 6.2 % (n = 6). The average number of hospitalized patients with HAV subgenotype IA was 9.3. days, (minimum day was 1-day, maximum day

Table 1
Distribution of hepatitis A virus subgenotypes, Latvia, 2008–2021 (n = 259).

	Number of cases per year											Total	
	2008	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	n	%
HAV sequenced samples	100	1	2	5	4	6	59	52	18	11	1	**259**	**100**
IA (total)	95	1	2	4	2	2	55	20	4	2	0	**187**	**72**
IA (VRD_521_2016)*	-	-	-	-	-	-	27	3	-	-	-	**30**	**16**
IA (RIVM-HAV16–090)*	-	-	-	-	-	-	5	2	-	-	-	**7**	**3.7**
IA (V16–25801)*	-	-	-	-	-	-	2	0	-	-	-	**2**	**1.1**
IA (other cases)	-	-	-	-	-	-	21	15	-	-	-	**-**	**-**
IB	1	0	0	1	0	1	3	29	14	9	1	**59**	**23**
IIIA	4	0	0	0	2	3	1	3	0	0	0	**13**	**5**

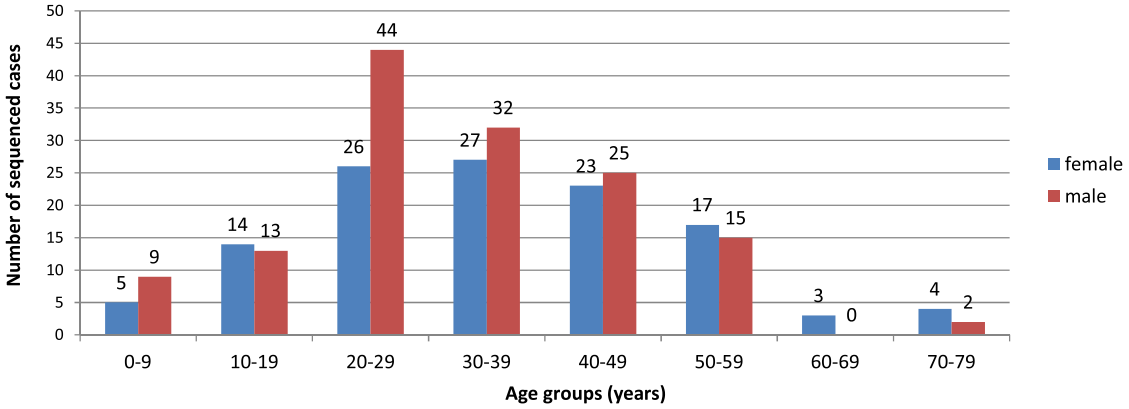

Fig. 2. Age and sex distribution of sequenced hepatitis A cases, Latvia, 2008–2021 (n = 259).

– 73 days), with subgenotype IB was 7.3. days (minimum day was 2 days, maximum days – 15 days), with subgenotype IIIA 7.7. days (the minimum day was day 1, the maximum day was day 12).

The vaccination data of the study group showed that the majority of patients were not vaccinated against HAV - 89.6 % (n = 232), 10.0 % (n = 26) did not know their vaccination status and in one case - 0.4 % (n = 1), when the patient was in contact with an HAV patient and was vaccinated with a single dose of HAV vaccine, but still got sick.

3.5. Epidemiological data of hepatitis A cases

Data on possible source of infection showed that only 27.4 % (n = 71) had a known source of infection and 72.6 % (n = 188) had an unknown source of infection. Of the identified sources of infection, one case was associated with fruit from Azerbaijan, one case was associated with fruit from Uzbekistan, four cases were among individuals who self-identified as MSM and isolated HAV RNA nucleotide sequences belong to cluster RIVM-016–90, seven cases were related to each other from the outbreak in 2008, 23 cases were PWID, 35 cases were determined to be in contact with a HAV patient.

Data on travel history during HAV incubation and suspected country of origin of the virus showed that 81.5 % (n = 211) were local cases and 18.5 % (n = 48) were linked to other countries: Austria (n = 1), Bulgaria (n = 1), Philippines (n = 1), France (n = 1), Greece (n = 1), Estonia (n = 1), Italy (n = 1), Nepal (n = 1), Netherlands (n = 1), Nigeria (n = 1), Sri Lanka (n = 1), Turkmenistan (n = 1), Ukraine (n = 1), Great Britain (n = 2), Morocco (n = 2), Tajikistan (n = 2), Spain (n = 3), Egypt (n = 4), India (n = 4), Kazakhstan (n = 4), Russia (n = 4), Uzbekistan (n = 4), Germany (n = 6).

3.6. Phylogenetic analysis

3.6.1. Subgenotype HAV IA

Fig. 4 shows subgenotype HAV IA results. 187 HAV sequences fall into 13 clusters and 12 sporadic cases with no identified epidemiological association. The average age of patients with HAV subgenotype IA was 35.5 years, men were 103, women – 84, adults – 165, children – 22.

The first cluster consists of 96/187 (51.3 %) identical sequences, 95 cases were reported in 2008 and one in 2012. 23/96 cases were associated with PWID from the 2008 HAV outbreak, 6/96 were associated with the 2008 outbreak, 67/96 were of unknown source. The case from 2012 was linked to travel in the Great Britain, the other cases were local.

The cluster named VRD_521_2016, which was associated with the HAV outbreak in EU/EEA countries, consists of 30/187 (16 %) identical sequences, 27 cases were registered in 2017, 3 - in 2018. In 11/30 cases, the source of infection was determined - contact with HAV patients, in 19/30 - the source of infection is unknown, but two cases were associated with travel to Germany, one to Spain and Austria, the remaining cases were local.

The cluster named RIVM-HAV16–090, which was associated with the HAV outbreak in EU/EEA countries, consists of 7/187 (3.7 %) identical sequences, 5 cases were registered in 2017, 2 - in 2018. In 1/7 cases, the source of infection was determined - contact with an HAV patient, 4/7 had identified themselves as MSM, 2 / 7 - the source of infection is unknown, and two cases were associated with travel to Germany, one to Spain, France and Russia, the other cases were local.

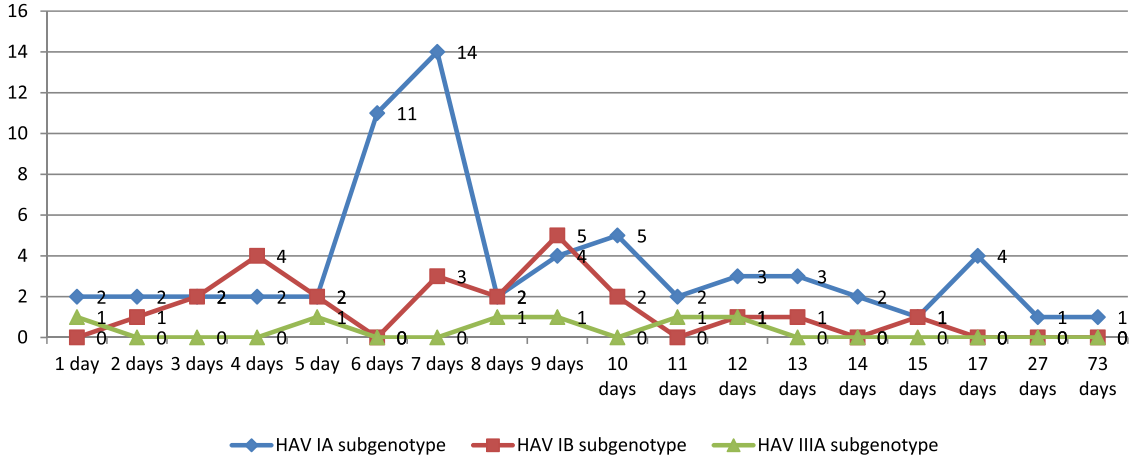

Fig. 3. Duration of hospitalized patients with HAV subgenotypes IA, IB, IIIA (n = 97).

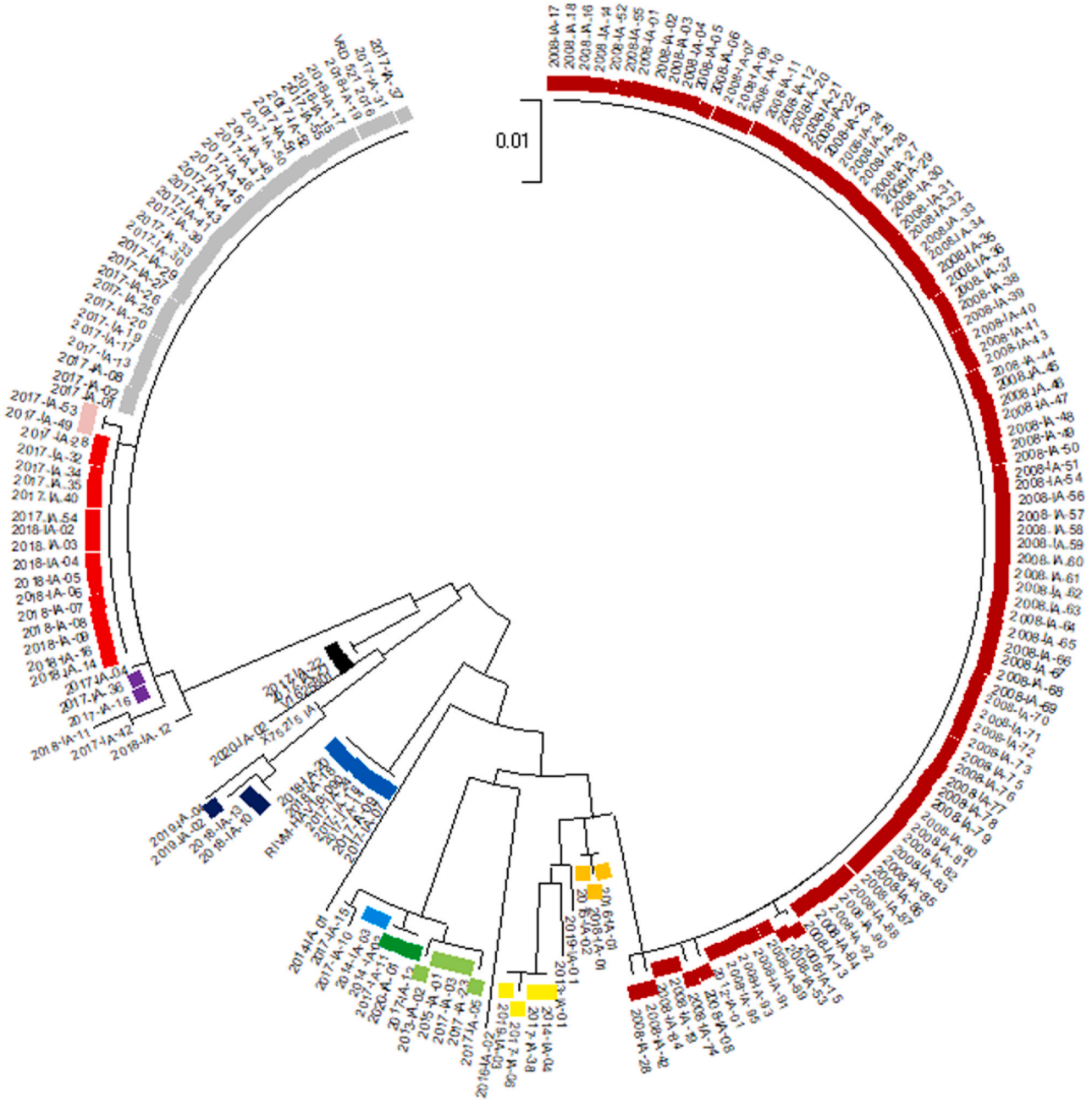

Fig. 4. Maximum likelihood phylogenetic tree of the hepatitis A virus VP1/2A genomic region sequences from hepatitis A cases subgenotype IA (n = 187).

A cluster named V16–25801, which was associated with an outbreak of HAV in EU/EEA countries, consists of 2/187 (1.1 %) identical sequences, cases were reported in 2017. The source of infection is unknown, but the cases were linked to travel to Germany and Estonia.

The second cluster consists of 16/187 (8.5 %) identical sequences, 6 cases were reported in 2017 and 10 cases in 2018. In 3/16 cases, the source of infection was determined - contact with an HAV patient, for the rest the source of infection is unknown. One case was linked to travel to Ukraine and one to the Netherlands, the other cases were local.

The third cluster consists of 3/187 (1.6 %) identical sequences, one case was reported in 2015, one in 2016 and one in 2018. The source of infection is unknown, but one case was linked to travel to Bulgaria and one to Greece.

The fourth cluster consists of 4/187 (2.1 %) identical sequences, one case was reported in 2014, two in 2017, one in 2019. The source of infection is unknown, but two cases were linked to travel to Russia and Uzbekistan.

The fifth cluster consists of 5/187 (2.7 %) identical sequences, one case was reported in 2013, one in 2015, three in 2017. The source of infection is unknown, but one case was linked to travel to Russia, one to Italy, the rest were local.

The sixth cluster consists of 3/187 (1.6 %) identical sequences, two cases were reported in 2017, one in 2020. The source of infection is unknown, but one case was linked to travel to Spain, the others were local.

The seventh cluster consists of 2/187 (1.1 %) identical sequences, two cases were reported in 2014. In one case, the source of infection was fruit that was brought from Uzbekistan, in the second case, the source of infection is unknown. The cases were local.

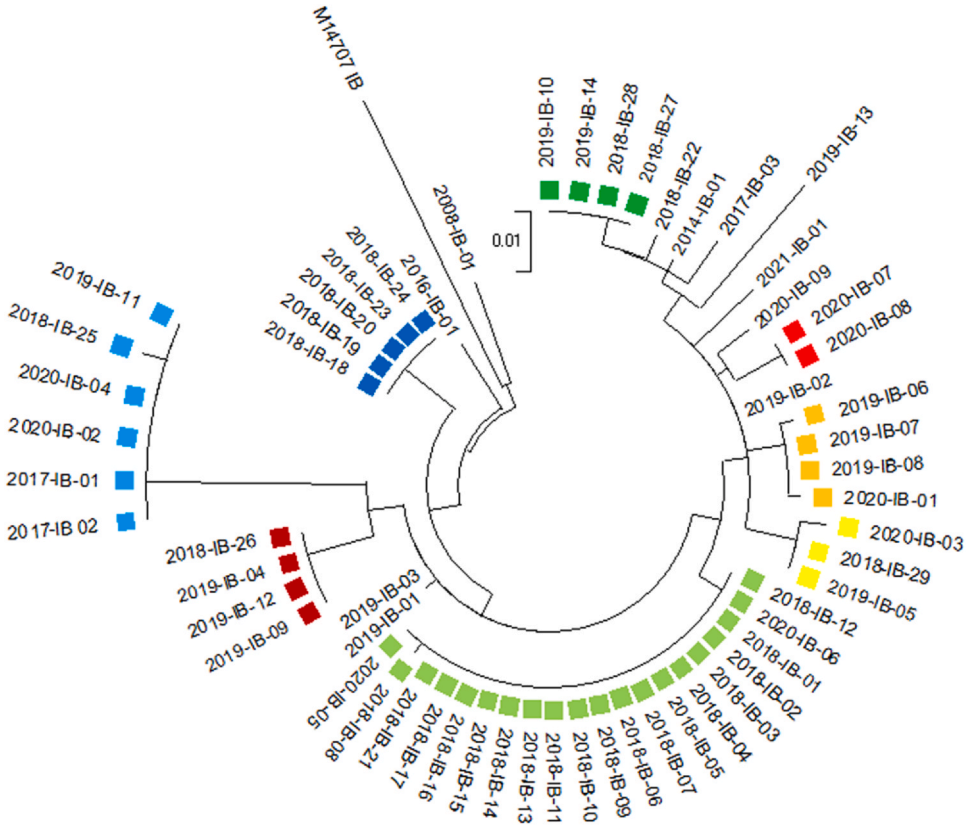

Fig. 5. Maximum likelihood phylogenetic tree of the hepatitis A virus VP1/2A genomic region sequences from hepatitis A cases subgenotype IB (n = 59).

The eighth cluster consists of 2/187 (1.1 %) identical sequences, two cases were reported in 2017. The source of infection is unknown, the cases were linked to travel to Uzbekistan and Kazakhstan.

The ninth cluster consists of 3/187 (1.6 %) identical sequences, two cases were reported in 2018, one in 2019. The source of infection is unknown, but one case was linked to travel to Morocco, the others were local.

The tenth cluster consists of 2/187 (1.1 %) identical sequences, the cases were reported in 2017, the source of infection is unknown and the cases were local.

Sporadic cases accounted for 12/187 (6.4 %) sequences. Cases were registered: one in 2013, one in 2014, one in 2016, four in 2017, two in 2018, two in 2019, one in 2020. In 11 cases, the source of infection is unknown and one had contact with an HAV patient. Two cases were associated with travel to Kazakhstan, one to Germany, Uzbekistan, Morocco, the Philippines. The rest were local.

Reference sequence IA X75215 for subgenotype HAV IA from the GeneBank database and VRD_521_2016, RIVM-HAV16–090, V16–25801 from HAVNET database have been included for comparison. The European strains circulating among men who have sex with men are marked in coloured bars: VRD_521_2016 (gray bars), RIVM-HAV16–090 (blue bars), V16–25801 (black bars). Subgenotype HAV IA clusters are marked in coloured bars: HAV IA cluster 1 (dark red bars), HAV IA cluster 2 (red bars), HAV IA cluster 3 (orange bars), HAV IA cluster 4 (yellow bars), HAV IA cluster 5 (light green bars), HAV IA cluster 6 (green bars), HAV IA cluster 7 (light blue bars), HAV IA cluster 8 (purple bars), HAV IA cluster 9 (dark blue bars), HAV IA cluster 10 (pink bars).

3.6.2. Subgenotype HAV IB

Fig. 5 shows subgenotype HAV IB results. 59 HAV sequences fall into eight clusters and eleven sporadic cases with no identified epidemiological association. The average age of patients with HAV subgenotype IB was 27.8 years, men were 30, women - 29, adults - 38, children - 21.

The first cluster consists of 4/59 (6.8 %) identical sequences, two cases were reported in 2018 and two in 2019, the source of infection is unknown and the cases were local.

The second cluster consists of 2/59 (3.4 %) identical sequences, two cases were reported in 2020, the source of infection is unknown and the cases were local.

The third cluster consists of 4/59 (6.8 %) identical sequences, three cases were reported in 2019, one case in 2020, the source of infection is unknown and the cases were local.

The fourth cluster consists of 3/59 (5.1 %) identical sequences, one case was reported in 2018, one in 2019, one in 2020, the source of infection is unknown, but two cases are local and one case was associated with traveling to Egypt.

The fifth cluster consists of 20/59 (33.9 %) identical sequences, 18 cases were registered in 2018 and two in 2020. In 2018, 16/18 cases were related to HAV infection among relatives in one family, where the first recorded case was in a two-year-old child and the suspected source of infection was food from Egypt. Of the two registered cases in 2020, one was linked to travel to Egypt. In 16/20 cases, the source of infection was determined - contact with an HAV patient, 4/16 - the source of infection is unknown.

The sixth cluster consists of 4/59 (6.8 %) identical sequences, one case was reported in 2018, three in 2019, the source of infection is unknown and the cases were local.

The seventh cluster consists of 6/59 (10.2 %) identical sequences, two cases were reported in 2017, one in 2018, one in 2019, two in 2020, the source of infection is unknown and the cases were local.

The eighth cluster consists of 5/59 (8.5 %) identical sequences, all reported in 2018. The possible source of infection in four cases was linked to contact with an HAV patient, all cases were local.

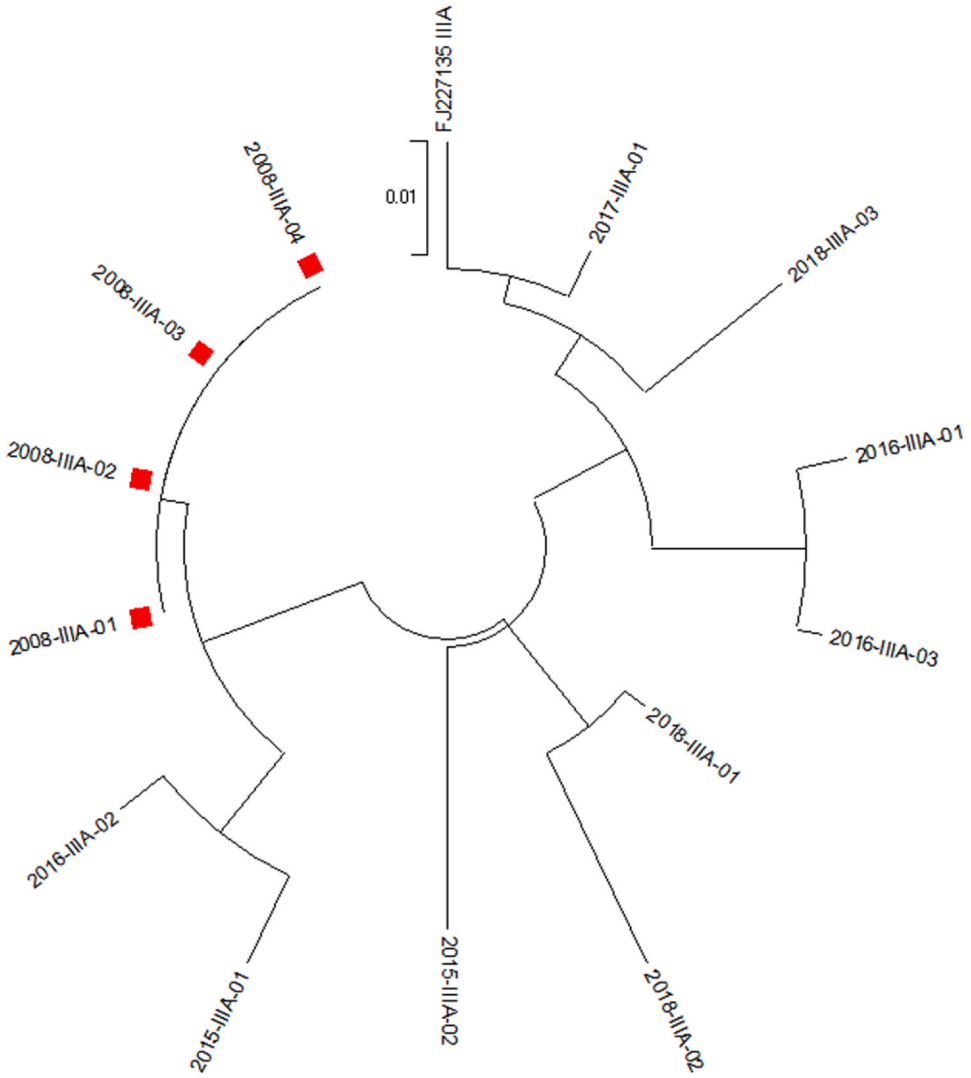

Fig. 6. Maximum likelihood phylogenetic tree of the hepatitis A virus VP1/2A genomic region sequences from hepatitis A cases subgenotype IIIA (n = 13).

Sporadic cases accounted for 11/59 (18.6 %) sequences. One case was registered in 2008, 2014, 2016, 2017, 2018, 2020, 2021, four in 2019. Possible sources of infection are unknown. Two cases were associated with travel to Egypt, one to Nigeria, one to Sri Lanka.

Reference sequence IB M14707 for subgenotype HAV IB from the GeneBank database has been included for comparison. Subgenotype HAV IB clusters are marked in coloured bars: HAV IB cluster 1 (dark red bars), HAV IB cluster 2 (red bars), HAV IB cluster 3 (orange bars), HAV IB cluster 4 (yellow bars), HAV IB cluster 5 (light green bars), HAV IB cluster 6 (green bars), HAV IB cluster 7 (light blue bars), HAV IB cluster 8 (blue bars).

3.6.3. *Subgenotype HAV IIIA*

Fig. 6 shows subgenotype HAV IIIA results. 13 HAV sequences fall into one cluster and nine sporadic cases with no identified epidemiological association. The average age of patients with HAV subgenotype IIIA was 34.5 years, 7 men, 6 women, 12 adults, 1 child.

The first cluster consists of 4/13 (30.7 %) identical sequences, the cases were registered in 2008, the source of infection is unknown and the cases were local. It is possible that this HAV subgenotype was imported to Latvia, as homology with HAV sequences with the country-of-origin Pakistan was found.

The first branch consists of 2/13 (15.4 %) similar sequences, cases were registered in 2017–2018 and were associated with travel in India. One case was associated with an Indian citizen whose country of residence was Latvia, in both cases the source of infection is unknown.

The second branch consists of 2/13 (15.4 %) similar sequences, the cases were registered in 2016 and were associated in one case with travel in Turkmenistan and the source of infection is unknown, and in the second case with the consumption of unwashed fruits brought from Uzbekistan.

The third branch consists of 2/13 (15.4 %) similar sequences, cases were registered in 2018 and were associated with travel in India, and one case was associated with an Indian citizen whose country of residence was Latvia, in both cases the source of infection is not known.

The fourth branch consists of 2/13 (15.4 %) similar sequences, cases were reported in 2015–2016 and were associated with travel in Tajikistan, both cases with an unknown source of infection.

The fifth branch consists of 1/13 (7.7 %) sequences, the case was recorded in 2015 and was linked to travel in Kazakhstan, the source of infection is unknown.

Reference sequence IIIA FJ227135 for subgenotype HAV IIIA from the GeneBank database has been included for comparison. Subgenotype HAV IIIA cluster 1 is marked in red bar.

4. Discussion

Reporting of HAV is mandatory and the surveillance system is at national level in all EU Member States, Iceland and Norway, except for the United Kingdom, which has organized surveillance differently [25].

From 1990–2008, laboratory diagnosis of HAV in Latvia was based on detection of Anti-HAV IgM in blood serum and detection of HAV Ag in feces by EIA method. With the development of technology, since 2008, the Sanger sequencing method has been applied, with the help of which molecular epidemiology was started, and also gave the opportunity to determine not only the circulating genotypes of HAV, but also to build a phylogenetic tree based on the nucleotide sequence to track HAV clusters.

In 2008–2009, an outbreak of HAV was registered in Latvia (5107 cases) and initially the spread of infection was associated with PWID and then spread widely to the rest of the population, continuing to be registered in 2009 [26]. The 2008 outbreak can be attributed to the large number of susceptible individuals caused by rapidly declining population immunity to hepatitis A. Control measures implemented included: contact tracing of cases; vaccination recommendations for contacts of cases; quarantine and medical observation of cases; public health education through mass media and specific prevention recommendations for food handlers, schools and the general public. During HAV outbreak in 2008–2009 vaccination against hepatitis A has not been provided free of charge, vaccination has been recommended to risk groups and contacts. As a result, a significant increase in the number of people vaccinated against hepatitis A has been observed since September 2008 corresponding to the spread of the epidemic. [27]. As a result, sporadic cases of acute viral hepatitis A were reported in Latvia every year until 2017, and most cases were associated with travel to endemic regions. The hepatitis A vaccine is not included in the national immunization calendar in Latvia. Vaccination is only recommended for citizens who travel to countries or areas where HAV is endemic or have with low or intermediate levels of infection.

In late 2016 and early 2017, outbreaks of HAV were reported in EU/EEA countries with a common source of infection, mostly among MSM [28,29], and a trend of increasing HAV cases was observed in 2017 in Latvia with similar HAV sequences from EU/EEA outbreaks, as well as sporadic cases linked to travel or contact with an HAV patient.

Analyzing the molecular epidemiological data on the spread of HAV from 2008 to 2021, it can be established that three subgenotypes of HAV have been found in Latvia - IA, IB, IIIA. The majority of HAV cases were of subgenotype IA (72 %), which is also the most common genotype worldwide [19]. The remaining number of cases were with HAV subgenotype IB (23 %) and HAV subgenotype IIIA was found (5 %).

The patients included in study were hospitalized for an average of 6.9 sick days. A study from Sri Lanka reported an average of 7.8 days of hospitalization [30], while a study from South Korea reported an average of 5.3 days of hospitalization [31].

The average number of hospitalized patients with subgenotype HAV IA was 9.3 days, with subgenotype IB – 7.3 days, and with HAV IIIA – 7.7 days. One study reported 7.4 days for subgenotype IA and 7.8 days for subgenotype IIIA [30]. There are no significant differences between hospitalization days and HAV subgenotypes.

The main limitation of the study was to understand the possible source of infection due to the long incubation period of HAV infection. Our data show that only 27.4 % had an identified source of infection and 72.6 % had an unknown source of infection. Comparing the data obtained in our study on the sources of infection, it can be concluded that similar data are also available in other studies in different parts of the world. Among our data, the most frequently reported source of infection was related to households or close contact with an infected person, similarly reported in a study from the USA [32]. However, our other potential sources of infection include contact with a risk group, e.g., MSM, travel to HAV-endemic countries, and PWID. Contaminates food and water are an infrequent source of infection.

Other possible sources are among PWID, which have been studied in Scandinavia and North America [33–35] and travel to countries where HAV is endemic [36]. Previous studies have shown that travel to countries with high or moderate HAV endemicity is a risk factor for residents of countries with low HAV endemicity. Travel remains a major risk factor for HAV infection in the EU/EEA. In participating European countries, 27.8 % of reported hepatitis A cases were travel-related [37]. The results of our study showed that 18.5 % of HAV cases during the incubation period were also travel related and 81.5 % were local cases.

Also, some of our cases were associated with self-identified MSM, which has also been implicated as a source of HAV infection in studies from the Netherlands and England [38,39]. In our study, MSM cases belong to the isolated HAV RNA nucleotide sequence cluster RIVM-016–90, and our study lacks information on sexual behavior because only four patients self-identified as MSM, but these cases were also travel-related.

In our study, in two cases, the source of HAV infection was linked to the consumption of unwashed fruits brought from Azerbaijan and Uzbekistan. Although other studies have suggested that contaminated food and water are a rare source of infection, although they have been linked to outbreaks [10].

Despite the fact that the largest number of cases was local and with an unknown source of infection, there was a trend in the distribution of the identified HAV sequences in different years, which may indicate local circulation of the virus.

5. Conclusion

In conclusion, we report the first detailed comprehensive molecular epidemiological study of the hepatitis A virus in Latvia. This study highlights the genetic diversity of HAV circulating in the country. The combination of diagnostic methods, molecular biology methods and epidemiological data allows public health to identify clusters, establish links with other outbreaks and compare Latvian strains with other strains. This approach helps to understand the epidemiological process of viral hepatitis A. In our study we have not HAV molecular divergences among sources of infection. All cases which were epidemiologically linked to each other were confirmed by sequencing.

Funding

This research was carried out in accordance with the Declaration of Helsinki, as revised in 2013 [40]. Ethics Committees of Riga Stradins University (Reference: 4/08.09.2018), Riga East University Hospital (Reference: ZD/08–06/01–19/210) and Centre for Disease Prevention and Control of Latvia (Reference:6.1–3/8) approved the study. HAV cases were not directly involved in this study. Only data extracted from notifiable disease surveillance systems were used. All identifiable personal information was removed for privacy protection and therefore no informed consent was required.

Declaration of Competing Interest

There is no conflict of interest to declare.

References

[1] Vaughan G, Goncalves Rossi LM, Forbi JC, de Paula VS, Purdy MA, Xia G, Khudyakov YE. Hepatitis A virus: Host interactions, molecular epidemiology and

evolution. Infect Genet Evol 2014;21:227–43. https://doi.org/10.1016/j.meegid.2013.10.023
[2] Shin E, Kim JS, Oh KH, Oh SS, Kwon M, Kim S, Park J, Kwak HS, Chung GT, Kim CJ, et al. A waterborne outbreak involving hepatitis A virus genotype IA at a residential facility in the Republic of Korea in 2015. J Clin Virol 2017;94:63–6. https://doi.org/10.1016/j.jcv.2017.07.006
[3] Quiñones B, Lee BG, Martinsky TJ, Yambao JC, Haje PK, Schena M. Sensitive genotyping of foodborne-associated human noroviruses and hepatitis A virus using an array-based platform. Sensors (Basel) 2017;17:1–16⟨https://doi.org/10.3390/s17092157⟩.
[4] World Health Organization. WHO/CDS/CSR/EDC/2014.Hepatitis A. Department of Communicable Disease Surveillance and Response. Available at: ⟨http://www.who.int7csr/disease/hepatitis/HepatitisA-whocdscsredc2014-pdf⟩, accessed on April 30, 2018.
[5] Walker BW. Hepatitis A infection: on alert for outbreaks. Nursing 2018;48:66–9.
[6] Lemon SM, Ott JJ, Van Damme P, Shouval D. Type A viral hepatitis: a summary and update on the molecular virology, epidemiology, pathogenesis and prevention. J Hepatol 2017;68:167–84. https://doi.org/10.1016/j.jhep.2017.08.034
[7] Lin KY, Chen GJ, Lee YL, Huang YC, Cheng A, Sun HY, Chang SY, Liu CE, Hung CC. Hepatitis A virus infection and hepatitis A vaccination in human immunodeficiency virus-positive patients: a review. World J Gastroenterol 2017;23:3589–606. https://doi.org/10.3748/wjg.v23.i20.3589
[8] de Almeida Ribeiro CR, Amado LA, Tourinho RS, Pinto Lima LR, Melgaço JG, de Almeida AJ, Bastos LS, Lewis-Ximenez LL, de Paula VS. Accuracy of rapid test for diagnosis of hepatitis A with different infection rate settings and with predictive modeling. Future Microbiol 2019;14:247–58. https://doi.org/10.2217/fmb-2018-0211
[9] World Health Organization (WHO): Hepatitis A. ⟨https://www.who.int/news-room/fact-sheets/detail/hepatitis-a⟩; 2022 [accessed 22 June 2022].
[10] Nainan OV, Xia G, Vaughan G, Margolis HS. Diagnosis of Hepatitis A Virus Infection: a Molecular Approach. Clin Microbiol Rev 2006;19:63–79.
[11] Yotsuyanagi H, et al. Prolonged fecal excretion of hepatitis A virus in adult patients with hepatitis A as determined by polymerase chain reaction. Hepatology 1996;24(1):10–3. doi: 10.1053/jhep.1996.v24.pm0008707246.
[12] Fujiwara K, et al. Frequent detection of hepatitis A viral RNA in serum during the early convalescent phase of acute hepatitis A. Hepatology 1997;26(6):1634–9. https://doi.org/10.1053/jhep.1997.v26.pm0009398009⟨https://doi.org/10.1128/CMR.19.1.63-79.2006⟩.
[13] Ishii K, Kiyohara T, Yoshizaki S, Kawabata K, Kanayama A, Yahata Y, Takahashi T, Kinoshita H, Saitou T, Sunagawa T, et al. Epidemiological and genetic analysis of a 2014 outbreak of hepatitis A in Japan. Vaccine 2015;33:6029–36. https://doi.org/10.1016/j.vaccine.2015.04.061
[14] Kanda T, Nakamoto S, Wu S, Nakamura M, Jiang X, Haga Y, Sasaki R, Yokosuka O. Direct-acting Antivirals and Host-targeting agents against the hepatitis A virus. J Clin Transl Hepatol 2015;3:205–10. https://doi.org/10.14218/JCTH.2015.00016
[15] McKnight KL, Lemon SM. Hepatitis A virus genome organization and replication strategy. Cold Spring Harb Perspect Med 2018;8:1–18. http:/doi.org/10.1101/cshperspect.a033480.
[16] Blanco Fernández MD, Torres C, Riviello-López G, Poma HR, Rajal VB, Nates S, Cisterna DM, Campos RH, Mbayed VA. Analysis of the circulation of hepatitis A virus in Argentina since vaccine introduction. Clin Microbiol Infect 2012;18:E548–51. https://doi.org/10.1111/1469-0691.12034
[17] Barde PV, Shukla MK, Pathak R, Kori BK, Bharti PK. Circulation of hepatitis A genotype IIIA virus in paediatric patients in central India. Indian J Med Res 2014;139:940–4. PMCID: PMC4165008, PMID: 25109730.
[18] Bruni R, Taffon S, Equestre M, Chionne P, Madonna E, Rizzo C, et al. Key role of sequencing to trace hepatitis a viruses circulating in Italy during a large multicountry European foodborne outbreak in 2013. PLoS One 2016;11(2):e0149642. https://doi.org/10.1371/journal.pone.0149642
[19] Holmberg SD. Hepatitis a epidemiology goes global. Clin Infect Dis 2012;54(6):782–3. https://doi.org/10.1093/cid/cir945
[20] Latvijas Vēstnesis. Epidemioloģiskās drošības likums. [Epidemiological Safety Act]. Riga: Saeima; 11 Dec 1997. Latvian. Available from: ⟨https://likumi.lv/ta/id/52951-epidemiologiskas-drosibas-likums⟩.
[21] European Commission (EC). Commission Implementing Decision (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions C/2018/3868. Official Journal of the European Union. 6.7.2018. L 1, ⟨http://data.europa⟩. eu/eli/dec_impl/2018/945/jo; 2018 [accessed 6 July 2018].
[22] Centre for Disease Prevention and Control (CDC) of Latvia. Epidemiological bulletin of Infectious diseases in Latvia 2008–2021. Riga: CDC of Latvia. ⟨https://www.spkc.gov.lv/sites/spkc/files/pielikumi⟩.
[23] National Institute for Public Health and the Environment (RIVM). HAVNET. Bilthoven: RIVM, ⟨https://www.rivm.nl/en/havnet/⟩; 2019 [accessed 16 Dec 2019].
[24] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 2013;30(12):2725–9. https://doi.org/10.1093/molbev/mst197
[25] European Centre for Disease Prevention and Control (ECDC). Surveillance Atlas of Infectious Diseases. 2008-2020. Hepatitis A. Stockholm: ECDC; 2020⟨https://www.ecdc.europa.eu/en/hepatitis-a/surveillance/atlas⟩.
[26] Perevoscikovs J, Lucenko I, Magone S, Brila A, Curikova J, Vennema H. Community-wide outbreak of hepatitis A in Latvia in 2008 - an update. Eur Surveill Jan 22 2009;14(3):19092.
[27] European Centre for Disease Prevention and Control (ECDC). Technical meeting on hepatitis A outbreak response. ⟨https://www.ecdc.europa.eu/en/publications-data/technical-meeting-hepatitis-outbreak-response/⟩, 2008 [accessed November 2008].
[28] European Centre for Disease Prevention and Control (ECDC). Epidemiological update: Hepatitis A outbreak in the EU/EEA mostly affecting men who have sex with men. Stockholm: ECDC,; 2018⟨https://www.ecdc.europa.eu/en/news-events/epidemiological-update-hepatitis-outbreak-eueea-mostly-affecting-men-who-have-sex-men-2/⟩ [accessed 12 September 2018].
[29] Ndumb P, Freidl G, Williams C, Mardh O, Varela C, et al. Hepatitis A outbreak disproportionately affecting men who have sex with men (MSM) in the European Union and European Economic Area, June 2016 to May 2017. Eur Surveill 2018;23(33):1700641. https://doi.org/10.2807/1560-7917.ES.2018.23.33.1700641
[30] Niroshana J, Kiyohara T, Agampodi SB, Samaraweera PK, Kulasooriya GK, Ranasinghe JC, Semage SN, Yoshizaki S, Wakita T, Ishii K. Clinical features and transmission pattern of Hepatitis A: an experience from a Hepatitis A outbreak caused by two cocirculating genotypes in Sri Lanka. Am J Trop Med Hyg 2016;95(4):908–14. https://doi.org/10.4269/ajtmh.16-0221
[31] Hyun J, Seo Y, An H, Yim S, Seo M, Kim H, Kim C, Kim J, Keum B, Kim Y, joon H, Lee H, Um S, Kim C, Ryu H. Optimal time for repeating the IgM anti-hepatitis A virus antibody test in acute hepatitis A patients with a negative initial test. Korean J Hepatol 2012;18(1):56–62. https://doi.org/10.3350/kjhep.2012.18.1.56
[32] Staes CJ, Schlenker TL, Risk I, Cannon KG, Harris H, Pavia AT, Shapiro CN, Bell BP. Sources of infection among persons with acute hepatitis A and no identified risk factors during a sustained community-wide outbreak. Pediatrics 2000;106(4):E54. https://doi.org/10.1542/peds.106.4.e54
[33] Harkess J, Gildon B, Istre GR. Outbreaks of hepatitis A among illicit drug users, Oklahoma, 1984–87. Am J Public Health 1989;79(4):463–6. https://doi.org/10.2105/ajph.79.4.463
[34] Grinde B, Stene-Johansen K, Sharma B, Hoel T, Jensenius M, Skaug K. Characterisation of an epidemic of hepatitis A virus involving intravenous drug abusers—infection by needle sharing? J Med Virol 1997;53(1):69–75. https://doi.org/10.1002/(sici)1096-9071(199709)53:1<69::aid-jmv12>3.0.co;2-s
[35] Leino T, Leinikki P, Hyypia T, Ristola M, Suni J, Sutinen J, Holopainen A, Haikala O, Valle M, Rostila T. Hepatitis A outbreak amongst intravenous amphetamine abusers in Finland. Scand J Infect Dis 1997;29(3):213–6. https://doi.org/10.3109/00365549709019029
[36] Steffen R, Kane MA, Shapiro CN, Billo N, Schoellhorn KJ, van Damme P. Epidemiology and prevention of hepatitis A in travelers. JAMA 1994;21(272(11)):885–9.
[37] Beaute J, Westrell T, Schmid D, Muller L, Epstein J, Kontio M, Couturier E, Faber M, Mellou K, Borg ML, Friesema I, Vold L, Severi E. Travel associated hepatitis A in Europe, 2009 to 2015. Eur Surveill 2018;31(23(22)). https://doi.org/10.2807/1560-7917.ES.2018.23.22.1700583
[38] Bruisten SM, Steenbergen JE, Pijl AS, Niesters HG, Doornum GJ, Coutinho RA. Molecular epidemiology of hepatitis A virus in Amsterdam, The Netherlands. J Med Virol 2001;63(2):88–95.
[39] Sundkvist T, Aitken C, Duckworth G, Jeffries D. Outbreak of acute hepatitis A among homosexual men in East London. Scand J Infect Dis 1997;29(3):211–2. https://doi.org/10.3109/00365549709019028
[40] World Medical Association (WMA). WMA Declaration of Helsinki– ethical principles for medical research involving human subjects. Ferney-Voltaire: WMA; 2018⟨https://www.wma.net/policies-post/wma-declaration-ofhelsinki-ethical-principles-for-medical-research-involvinghuman-subjects/⟩ [accessed 06 July 2022].