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Sexual behaviour and poor hygiene are related to recent hepatitis a virus community outbreaks

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ABSTRACT

Objectives: there has been a global increase in the incidence of hepatitis A infection. The aim of this study was to examine the characteristics of the increase in our region and the degree of adherence to the recommended hygienic measures after discharge from hospital.

Methods: demographic, clinical and biochemical variables were collected from patients with acute hepatitis A in our health area. The patients were grouped as follows: January 2010 to December 2016 (historical cohort) and January 2017 to October 2017 (recent cohort). A phylogenetic analysis was also performed in the recent cohort. One month after discharge, bacterial growth was evaluated by a culture of the dominant hand imprint and were compared with a control group.

Results: a total of 110 cases were registered with a median age of 36.3 years (range 3-89) and 77.3 *%* were male. The incidence was 0.82/100,000 inhabitants/year and 22.75/100,000 inhabitants/year in the historical and recent cohorts, respectively. Patients in the recent cohort were more frequently male (52.6 *% vs.* 82.4 *%*, p = 0.008) and younger (51.7 [3-89] *vs.* 33.4 [4-74] years, p < 0.001). In addition, 63.8 *%* of the recent cohort were men who had sex with other men and had unsafe sexual practices (37.5 *%*). Phylogenetic analysis showed a predominance of genotype A and a high frequency of the VRD 521-2016 sequence. A higher growth of enterobacteria was observed in patients with hepatitis A compared to the control group (7.3 *% vs.* 1.2 *%*, p = 0.005), despite specific hygienic measures given at discharge.

Conclusions: a recent outbreak of hepatitis A in our area was related with gender, younger age and sexual practices. Hepatitis A infected subjects showed a poor adherence to hygienic measures. Our data suggests the need for policies that encourage preventive actions, particularly vaccination in this high-risk group.

Keywords: Hepatitis A. Outbreak. Men who have sex with men.

INTRODUCTION

The incidence of hepatitis A virus (HAV) infection in Europe in the last two decades has decreased from 10.0 to 2.5 per 100,000 inhabitants per year, occurring in less than 3.0 per 100,000 inhabitants per year in 21 of the 28 countries of the European Union (1).

In Spain, the recorded incidence in 2015 was 1.27 cases per 100,000 inhabitants. This is in accordance with sources from the National Centre for Epidemiology (2), which is very similar to the incidence in surrounding countries. A vaccine for hepatitis A has been available in Europe since 1991 and has likely influenced the decrease in incidence (3), especially in areas of high endemicity and high-risk groups. In our low endemic area, the current HAV vaccination strategy is opportunistic and vaccination of high-risk groups is voluntary.

However, sometimes there may be peaks in incidence. The main route of transmission of HAV infection is the faecal-oral route, usually via the consumption of contaminated food or water. Transmission from person-to-person, as a result of poor hygiene and risky sexual behaviours, is less frequent worldwide (4). However, the main cause of the increase in incidence in Western countries has been due to transmission via unsafe sexual contact in men who have sex with men (MSM). Sporadic outbreaks have been reported since the 1970s, with a higher incidence occurring between 2008 and 2011 (5,6). The most recent outbreaks between 2016 and 2018 affected the whole of Europe (Table 1), including Spain (7,8).

It is important that patients follow a series of hygiene recommendations due to the transmission mechanism. Together with food, water safety control and vaccination, these are the most effective measures to prevent transmission (4). Of all these measures, regular hand washing with soap and hot water or an antiseptic solution are essential to reduce the risk of oral transmission through faeces, especially during the replication period when there is an increased risk of infection (9). However, the degree of compliance with hygiene measures recommended for infected patients after diagnosis is unknown.

The aims of this study were to examine the characteristics of the affected population in our healthcare area during 2017 by comparing them with a historical cohort. A phylogenetic analysis of the different HAV strains was performed in order to geolocate the origin and to evaluate the compliance with hygiene recommendations after a diagnosis of acute infection via a microbiological study of the patients' hands.

METHODS

Study patients

All cases of acute HAV infection treated at the Complejo Hospitalario Universitario de Canarias (CHUC) from January 2010 to October 2017 were registered. Patients were divided into two cohorts: a) 'historical'cohort, comprising cases between January 2010 and December 2016; and b) 'recent' cohort, from January 2017 to October 2017.

Individuals with symptoms compatible with acute infection (abdominal pain, vomiting, diarrhoea, jaundice, fever, weakness, myalgias or arthralgias) and positive for specific IgM antibodies against HAV were diagnosed with acute HAV.

Clinical and epidemiological variables such as sex, age, nationality, educational level (primary, secondary, university), marital status (single, married, stable partner), current job, toxic habits such as alcohol consumption and quantity (> 3 units/day) and drug consumption were recorded in a coded database. This was based on electronic medical records from our hospital and primary care or data obtained via telephonic consultation surveys. Other variables included recent trips (including countries with or without a high endemicity), epidemiological environment (contact with HAV patients), sexual orientation (heterosexual, homosexual, bisexual), risky sexual intercourse, comorbidity quantified by the age-adjusted Charlson comorbidity index (moderately-elevated comorbidity if Charlson index [CI] ≥ 2), days of hospitalisation (number of days at hospital), transplant, death and cause of death. In addition, biochemical and serological variables obtained from the CHUC Central Laboratory at the time of diagnosis were collected including haemograms and data on aspartate aminotransferase [AST], alanine aminotransferase [ALT], gamma-glutamyl transferase [GGT], alkaline phosphatase [AP], total bilirubin, creatinine, international normalised ratio [INR], prothrombin activity [PA], serology B virus [AgHBs], C virus antibodies [anti-HCV], human immunodeficiency virus [HIV], syphilis, cytomegalovirus and Epstein-Barr virus. The diagnosis of severe acute liver failure met the definition provided by the European Association for the Study of the Liver (EASL) (10). The study protocol for this study was approved by the CHUC Ethics and Research Committee and informed consent was obtained from all patients.

Phylogenetic analysis

In order to identify the outbreak, serum samples from patients from the recent cohort were sent to the National Microbiology Centre of the Instituto de Salud Carlos III for analysis. The samples were processed according to the recommendations of the European Centre for Disease Prevention and Control (ECDC). The VP1-2A region (500 NT) was amplified and the maximum-likelihood phylogenetic tree was constructed using 1000 pseudo-repetitions.

Bacterial culture

All patients included and assessed were given instructions at discharge in order to evaluate the degree of adherence to the hygienic measures. Recommendations were based on those developed by the World Health Organization (WHO), such as hand washing with soap and hot water for 30 seconds several times a day.

A dominant palm print was obtained during the follow-up consultation one month after discharge, without prior notice but with the patient's consent. The cultures were grown on two culture media; blood agar and MacConkey agar. Bacterial growth was evaluated, with special emphasis on colonies from the intestinal flora (*Enterobacteriaceae*). Analysis of the cultures was performed blindly in the microbiology laboratory of CHUC by an expert microbiologist with more than 15 years of experience. Growth of more than 10 colonies from the culture was considered as a positive result.

Patients who attended consultations randomly at the Hepatology and Gastroenterology Department during the same period were included as a control cohort. Hygiene recommendations were not provided and the same palm print procedure (as described above) was performed with the patient's consent during the consultation, without prior notice.

Statistical analysis

Descriptive statistical techniques were used such as frequency distributions and percentages for qualitative and statistical central trend variables and dispersion and position for quantitative variables. The results were expressed as absolute values, percentages and means with their standard deviation. Student's t-tests were used for quantitative variables that followed a normal distribution and the Mann-Whitney U test was used for those that did not. Qualitative variables were compared using the χ-square test. Data analysis was performed with the IBM SPSS Statistic 21 software. Differences with a value of $p < 0.05$ were considered as statistically significant.

RESULTS

Patient characteristics of historical and recent cohorts

A total of 110 cases were registered with a median age of 36.3 years (range 3-89), 77.3 % were male. 91 of these cases were (median 33.4 years, range 4-74, 82.4 % male) registered in 2017 (recent cohort), with a cumulative incidence of 22.75 cases per 100,000 inhabitants per year.

Data on risk factors in the recent cohort were obtained for 79.12 % of patients. Forty-six of the males who responded to the survey claimed to be MSM (63.8 %) and 27 (37.5 %) of these claimed to have had sex with another man at a time consistent with the period of infection. Thirty-three (36.7 %) declared close contact (house living, co-worker, etc) with an affected subject and only three (3.3 %) had recently travelled to an endemic area. Contaminated food or water transmission cases were not found. Of the total number of patients, 14 (15.4 %) were HIV-positive and all were male. Five (35.7 %) of these cases confirmed that they had been having sex with another man and 64.3 % had received the hepatitis B vaccine. The median hospital stay was 2 nights (range 0-7 nights).

Regarding the historical cohort, 19 cases were registered (median 51.7 years, range 3-89, 52.6 % men) with a cumulative incidence of 0.475 cases/100,000 inhabitants/year. Risk factor data were collected in 63.2 % of cases. There were no described cases of risk factors related to MSM. A favourable epidemiological environment for infection was found in 25 %, which included direct contact with patients affected by HAV and was the main risk factor described.

After comparing both cohorts, the results obtained are shown in table 2. The incidence in the recent cohort was significantly higher than that in the historical cohort (OR 47.9, 95 % CI; 46.75 to + Inf, $p < 0.001$). Patients in the recent cohort were more frequently male (82.4 *vs.* 52.6 %, $p = 0.008$) and were younger (33.4 [4-74] *vs.* 51.7 [3-81] years, p

< 0.001) than those in the historical cohort. There was also a greater percentage of MSM in the recent cohort (29.7 *vs.* 0 %, p = 0.04) and fewer comorbidities (CI ≥ 2: 11.8 *vs.* 36.8 %, p = 0.009). None of the subjects in either cohort had been vaccinated against HAV.

Phylogenetic analysis

Phylogenetic analysis was performed on 69 samples, one sample was insufficient and amplification failed in eight samples. Only one sample corresponded to the genotype IB and the rest were genotype IA. Two high homology samples had the RIVM-HAV16-090 sequence, 54 the VRD 521-2016 sequence and 3 had neither of these and had previously undescribed sequences (Fig. 1).

Hand culture analysis

Samples where obtained from 52 controls (45.6 years, range 32-76, 73 % male) and 30 patients infected with HAV (35.6 years, range 21-74, 83.3 % male) from the recent cohort who attended follow-up visits. Growth of different bacteria were observed including *Staphylococcus spp, Streptococcus spp, Neisseria spp, Bacillus spp* and *Enterobacteriaceae spp.* All are usually found on the skin and in the upper respiratory tract, except for the Enterobacteriaceae spp, which are mainly found in the gastrointestinal tract.

As shown in Figure 2, a significantly increased growth of enterobacteria was observed in the HAV infected cohort compared to the control group (7.3 % *vs.* 1.2 %, p = 0.005). No differences were observed for the rest of the species between the groups.

DISCUSSION

The results of our study show that the incidence of HAV infection is low in our environment, except during outbreak periods. The epidemiology changes during these outbreaks, in contrast to infections during low incidence periods. There was a predominance among young males, probably through sexual transmission.

The incidence of hepatitis A in our region was low and similar to the rest of western countries and Spain (4,11). In fact, an incidence of less than one case per 100,000 inhabitants per year was registered in our study during the historical cohort period. However, the incidence in our area increased during 2017 to 23 cases per 100,000 inhabitants per year. This was not an isolated obserevation but was seen internationally. Several outbreaks of hepatitis A were registered in numerous countries during 2016, predominantly affecting MSM (2,12). Similarly, a predominance among MSM was observed in our study, with a few additional characteristics such as the affected individuals were generally single individuals with no stable partner who regularly consumed alcohol and drugs. There were no cases of MSM, although the small number of cases included in the historic cohort impeded comparisons regarding sexual risk habits.

Outbreaks associated with MSM have been described since the 1980s to the present day (5,6). Phylogenetic analysis of the virus is a useful tool for epidemiological studies of these outbreaks, because it detects traceability and evaluates the relationship that exists between different regions and the present type of hepatitis A virus transmission (13). In October 2016, the Netherlands reported at least 17 cases of hepatitis A among the one million people that attended the *Europride conference* in Amsterdam, of which 11 corresponded to an identical IA genotype, RIVM-HAV16-090 (Dutch strain) (14). As described by Freidl et al., they found an identical match with a strain described in an outbreak among MSM in Taiwan in May 2016 when comparing the sequence of this strain in different databases (HAVNET and GenBank) (15). However, 2 cases were due to genotype IA and strain VRD-521-2016 (British strain), which was re-identified in December 2016 in the United Kingdom during another outbreak of the disease (16). This sequence had not previously been identified in the United Kingdom, but phylogenetic analysis showed links to sequences from travellers returning from Central and South America. Interestingly, up to 14 cases were documented of patients who had travelled to Spain, 3 had of which travelled to the Canary Islands. The Dutch strain RIVM-HAV16-090, mostly found among MSM in London, was identified in 13 subjects. Therefore, the same strains were observed in our recent cohort outbreak and 90 % of the samples were similar to the British strain (VRD 521-2016) and only two to the

Dutch strain (RIVM-HAV16-090). This observation is in agreement with the predominance of the British strain in other outbreaks in our country, such as that reported in Barcelona (Table 3).

New outbreaks were reported in Spain during 2017 (Table 3) and in 21 other European countries. The viruses involved shared genotypes with outbreaks in the Netherlands and the United Kingdom, where there was also a predominance of MSM. In our cohort, the phylogenetic analysis of the samples confirmed the predominance of the British strain, which was also the most frequently noted in Barcelona and Lombardy (Italy). The vast majority of the strains involved in these outbreaks were related to transmission among MSM. Although the description of an outbreak in the Lazio region (Italy) reported up to 6 % of strains that were neither the British nor the Dutch strain and were related to cases of food poisoning (17). These data suggest that HAV infection during outbreaks in the western countries are related to the route of sexual transmission and in particular to specific strains. Therefore, it is preventable with appropriate measures, such as vaccination.

Tenerife is a common tourist destination. However, we did not collect cases of infection among foreigners in our study. The most likely reason is that the development time for symptoms is longer than the average stay of tourists. Other explanations could be that the northern area (the orogin of our cohort) does not receive as many tourists as other parts of the island, or the subject were treated in private hospitals outside the public domain.

Data obtained in recent years and in particular from 2016 until the end of 2017, reveal the high risk of transmission among MSM (18). The sexual contact route and poor adherence to hygienic measures to prevent transmission, especially hand hygiene, perpetuates the transmission of the virus in the population during the outbreak. Our data highlight a poor compliance with hygiene recommendations in this population, as growth of enterobacteria was found in the hand culture of 7.3 % of patients infected with HAV, despite the hygiene recommendations given at discharge. In contrast, a statistically significant poorer hygiene was noted when compared to the control group, in which growth was observed in just 1.2 % of the cultures. Therefore, just as vaccination is fundamental, the awareness of the patient-at-risk is considered also indispensable. Thus, highlighting the importance of education about hand hygiene measures, sexual education and basic knowledge of the disease, which includes the abstinence from risky sexual relationships.

Although there are no published data available, the health resource burden resulting from an outbreak is high considering the length of hospital stay, tests, complications, transplants and deaths, although infrequent. In our cohort, up to two-thirds of the patients were hospitalised, i.e in the hospital for more than 24 hours, either in the emergency room or on admission. Similar data have been reported during different outbreaks, although one important limitation of our study is the selection bias that may have influenced our high rate of hospitalisation. As only those who came to the hospital on an emergency basis were recorded, the most symptomatic and serious cases were the ones reported.

Identification of antibodies against HAV in the population aged below 40 years is not recommended and screening is more cost-effective in people over 50 years of age, due to the high prevalence of positive serology in this population (19). HAV vaccines have been available in Europe for three decades (9), achieving seroconversion in more than 95 % of subjects (20,21). The WHO does not recommend universal vaccination in countries with high endemicity, although it is recommended in developing countries with intermediate endemicity where a high proportion of the adult population is susceptible to HAV infection. Meanwhile, selective vaccination of high-risk groups is recommended in countries with low or very low endemicity, such as our health care area (22). However, European countries such as Greece (23) and specific regions such as Catalonia (Spain) (24) have systematically introduced vaccination against HAV. Selective vaccination of high-risk groups for HAV is cost-effective for high-risk patients, as reported in studies from two decades ago (25). Recent outbreaks involve a high prevalence of HIV patients. For example, none of the patients were vaccinated in our cohort, despite the effectiveness of the vaccine in the prevention of HAV infection in this population (26). In contrast, at least 64 % of HIV patients were vaccinated against HBV. This highlights the low risk awareness of HAV infection in this population and also among physicians.

Recent studies from Puglia in 2009 (27) and the United States in 2015 (28) have also shown that universal vaccination significantly reduces the incidence of acute hepatitis during the post-vaccination period. In contrast, an analysis performed after the introduction of a universal vaccination in Catalonia in the 1990s showed a significant reduction in the number of cases in children. However, there was an increase in the number of cases involving immigrants and MSM (29). The latter can be explained because these high-risk groups were not included in the universal vaccination plan, which was applied to the paediatric population. This highlights the need for opportunistic vaccination in the adult population.

In conclusion, recent outbreak of hepatitis A in our area were highly related with gender, younger age and sexual practices. Hepatitis A infected subjects showed a poor adherence to hygienic measures. Therefore, improved vaccination and awareness campaigns should be implemented to avoid the spread of new outbreaks in the future.

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Table 1. Characteristics of outbreaks published at the European level (2016-2018)

Outbreak	**United Kingdom (16)**	**Netherlands (14)**	**Germany (30)**	**Lombardy (31)**	**Israel (8)**	**Rome (17)**	**North France (32)**
Sample size, n	52	17	38	244	19	481	49
Period of inclusion	Jul/16-Jan/17	Jul/16-Feb/17	Nov/16-Jan/17	Jan/17-May/17	Dec/16-Jun/17	Jan/16-Mar/17	Feb/17-Jul/17
Average age (range)	36	33 (26-52)	31 (11-52)	33 (18-76)	31 (22-41)	33 (1-84)	36 (31-49)
Foreigners, n (%)	NA	11	NA	NA	NA	NA	NA
Men, n (%)	34 (65.4)	17(100)	37 (97)	230 (94)	18 (95)	449 (93,3)	48 (98)
MSM, n (%)	43	17	30 (79)	133 (55)	17 (90)	NA	32 (65)
Risk contact, n (%)	9	NA	NA	(63)	NA	NA	NA
Contact with affected, n (%)	NA	NA	6 (16)	(18)	NA	NA	9 (18)
Travel, n (%)	19[a]	2[d]	6[b]	NA	5 (26)	NA	13 (27)
ALT (UI/L)	NA	NA	NA	2368 (47-8914)	NA	NA	NA
Total bilirubin (mg/dL)	NA	NA	NA	6.6 (0.4-18)	NA	NA	NA
Hospitalisation, n (%)	NA	5 (29)	8 (21)	180 (74)	19 (100)	NA	34 (69)
HIV, n (%)	NA	NA	NA	46 (19)	1 (5)	NA	11 (23)
Genotype							
IA, n (%)	37 (100)	13	12 (100)	183 (93)	14	129 (99)	38 (100)
IB, n (%)	0	0	0	14 (7)	1	1 (< 1)	0
Other, n (%)	0	0	0	0	0	0	0
Phylogenetic analysis							
British strain, n (%)	24 (65)	2 (11)	2 (17)	117 (59)	6 (40)	112 (86)	24 (63)

Dutch strain, n (%)	13 (35)	11 (89)	3 (25	79 (40)	8 (53)	3 (< 1)	13 (34)
German strain, n (%)	0	0	7 (58)[c]	1 (1)	0	0	1 (3)
Other, n (%)	0	0	0	0	1 (7)	17 (13)	0

NA: Non-available data. [a]Fourteen travelled to Spain and two to Germany. [b]Two travelled to Spain. The rest went to Austria, Greece, Malta and Taiwan (1 each). [c]Sequence from Germany was isolated in 2013 in Italy during a European food-related outbreak (30). [d]Two patients reported that they visited Barcelona and one reported a visit to Argentina.

Table 2. Comparison of historical and recent cohorts

	Historical cohort (n = 19)	**Recent cohort (n = 91)**	**p value**
Age, X ± SD	51.7 ± 24.8	33.2 ± 13.5	< 0.001
Male, n (%)	10 (52.6)	75 (82.4)	0.005
Country of birth, n (%)			
- Spain			
- Brazil	18 (95)	86 (95)	-
- Venezuela	0	1 (1)	-
- Argentina	1 (5)	3 (3)	-
	0	1 (1)	-
MSM, n (%)	0[2]	46 (63.4)[1]	0.000
Risky sexual contact, n (%)	0[2]	27 (37.5)[1]	0.040
Contact with affected individuals, n (%)	3 (25)[2]	33 (36.7)[1]	0.868
Travel to endemic area, n (%)	2 (16.7)[2]	3 (3.3)[1]	0.147
Previous HAV vaccination, n (%)	0	0	
HIV, n (%)	0	14 (15.4)	0.079
Charlson Index ≥ 2, n (%)	7 (36.8)	11 (11.8)	0.009
Severe acute liver failure, n (%)	0	2 (2.2)	0.514
Death, n (%)	0	1 (1.1)	0.646
Other sexually-transmitted infections[3]			
- Syphilis, n (%)	1 (5)	13 (14.3)	-
- Gonorrhea, n (%)	0	6 (6.6)	-
- Chlamydia, n (%)	0	9 (9.9)	-
Incidence (per 100,000 inhabitants/year)	0.475	22.75	< 0.001

X: mean; SD: standard deviation. [1]Available information for the historical cohort (n = 72, 79,1 %). [2]Available information for the recent cohort (n = 12, 63.2 %). [3]Sexually-transmitted infection in the last 3 years before HAV infection.

Table 3. Characteristics of outbreaks in Spain (2016-2018)

Outbreak	**Tenerife**	**Barcelona (33)**	**Málaga (34)**	**Oviedo (35)**	**Cantabria (36)**
Sample size, n	91	46	51	108	148
Period of inclusion	Jan/17-Oct/17	Jan/17-Jun/17	Jan/16-Dec/16	Nov/16-Dec/17	Jun/16-Sept/18
Average age (range)	33.2 (4-74)	33.5 (28-50)	36 (25-45)	39.5 (RIQ 15)	33 (24.6-42)
Foreigners, n (%)	5 (5)	19 (41)	18	7 (6.5)	6 (3.4)
Men, n (%)	75 (82.4)	44 (96)	46 (90)	103 (95.4)	125 (84.5)
MSM, n (%)	46 (63.9)	31 (67)	26 (62)	63 (63.9)	31 (73.8)
Risky sexual contact, n (%)	27 (37.5)	25 (54)	23 (45)	NA	28 (48.3)
Contact with affected, n (%)	33 (36.7)	12 (26)	3 (8)	4 (3.8)	28 (48.3)
Travel, n (%)	15 (16.5)	NA	0	3 (2.8)	1 (0.7)
Severe acute liver failure, n(%)	2 (2.2)	NA	0	18 (16.2)	0
ALT (UI/L)	2626.7 ± 1762.4	3572 (2426-4665)	1550 ± 1350	3126 ± 2196)	2.587.0 (1.721-3.951)
Prothrombin activity	NA	63.3 (45-71)	67 (56-84)	66 ± 25	71.5 (15.7)
Total bilirubin (mg/dL)	6.9 ± 3.2	5.7 (4.2-8.2)	6.8 ± 3.94	8 (4.75)	5.6 (3.0)
Hospitalisation, n (%)	70 (76.9)	18 (39)	26 (51)	NA	58 (44.3)
Liver transplant	1 (1.1)	0	0	0	0
Sexually transmitted infections, n (%)	12 (13.2)	12 (26)	12 (44)	22 (20.4)	31 (20.9)
Toxic habits, n (%)	39 (42.9)	13 (28)	NA	NA	NA
HIV, n (%)	14 (15.4)	1 (2.7)	8 (19)	8 (7.4)	15 (10.2)
Genotype					
IA, n (%)	60 (98)	37 (100)	NA	NA	NA
IB, n (%)	1 (2)	0	NA	NA	NA
Other, n (%)	0	0	NA	NA	NA
Phylogenetic analysis					
British strain, n (%)	54 (90)	29 (78)	NA	NA	NA
Dutch strain, n (%)	2 (3)	4 (11)	NA	NA	NA
German strain, n (%)	0	2 (5.5)	NA	NA	NA
Other, n (%)	3 (5)	2 (5.5)	NA	NA	NA

NA: Non-available data.

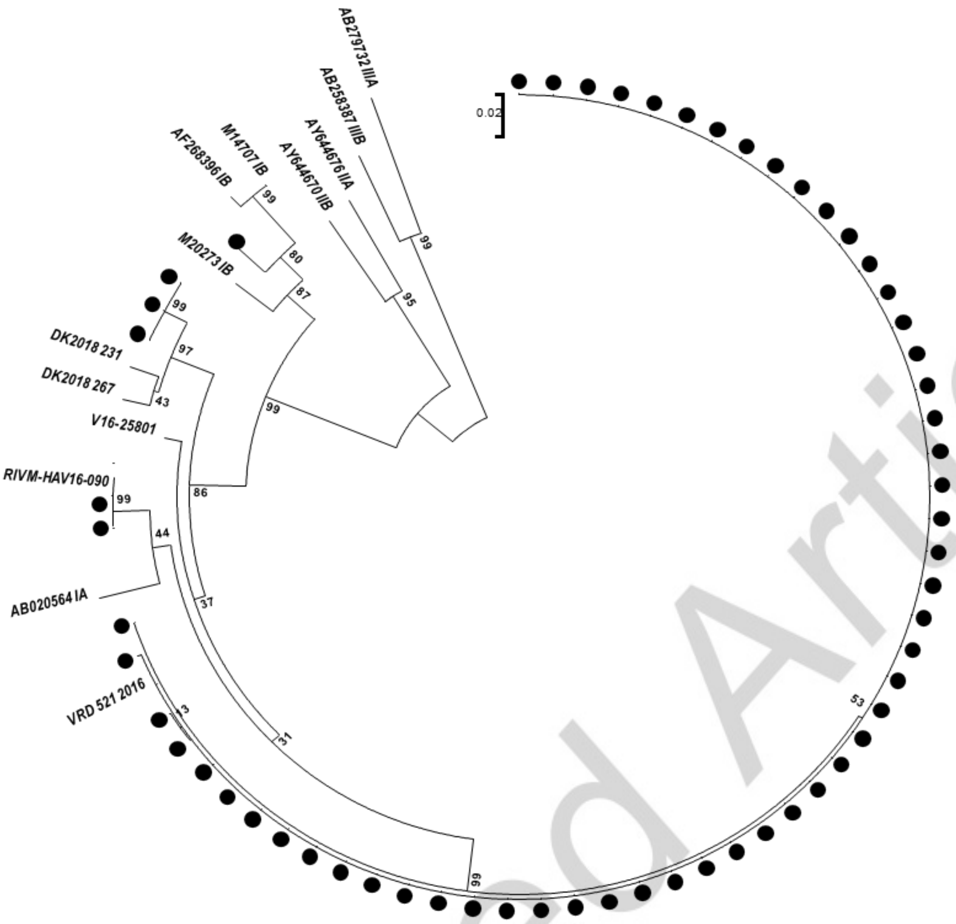

Fig. 1. Phylogenetic tree VP1-2A (Maximun-Likelihood 1000 pseudorepetitions). The VRD 521-2016 sequence was the most frequent in our recent cohort, followed by the RIVM-HAV16-090 sequence.

Fig. 2. Growth rate (%) of different bacterial groups in culture media after dominant hand impregnation. A significantly greater growth of Enterobacteriaceae spp was found in subjects affected by hepatitis A virus (HAV) infection (n = 30, black bars) than in control cases (n = 52, white bars).