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Authors: Jorge Mariojouls, Gonzalo Castro, María Belén Pisano, Paula Barbero, Anabella Fantilli, Mariel Borda, Fernando Canna, Gabriela Barbás, Viviana Ré

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Title: Hepatitis A outbreak affecting men who have sex with men (MSM) in central Argentina, occurred in July 2017-April 2018, later than the European outbreak.

Jorge Mariojouls,[a] Gonzalo Castro,[a] María Belén Pisano,[b] Paula Barbero,[c] Anabella Fantilli,[b] Mariel Borda,[a] Fernando Canna,[a] Gabriela Barbás,[a] Viviana Ré,[b]

Affiliations
[a] Laboratorio Central, Ministerio de Salud de la Provincia de Córdoba, Argentina.
[b] Instituto de Virología "Dr. J.M.Vanella", Facultad de Ciencias Médicas, CONICET, Universidad Nacional de Córdoba. Argentina.
[c] Area Epidemiología, Ministerio de Salud de la Provincia de Córdoba, Argentina.

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Highlights

- A Hepatitis A outbreak among young adult MSM occurred in 2017-2018 in Argentina.
- HAV genotype IA, strain VRD 521–2016, was the responsible of the outbreak.
- Reinforce official policy of vaccination in MSM is mandatory.

1.Background

Infection with Hepatitis A virus (HAV) is the most common cause of acute viral hepatitis worldwide [1].

In the past, Argentina was considered to be an area of high endemicity for HAV infection, with most people infected in early childhood [2]. Then, HAV was the main cause of liver transplant, with 70.5 to 173.8 reported cases of hepatitis every 100,000 inhabitants per year during 1995–2004 [3]. Nowadays, HAV has shown intermediate endemicity and notification of clinical cases has decreased drastically since the introduction of the vaccine among children of 12 months, in 2005 [4]. Subsequent studies have demonstrated high persistence of protective

antibodies up to 4 and 7 years after immunization [5,6]. However, as a consequence of the implementation of immunization in children, and the improvement in socio-economic, hygienic and sanitation factors, young adults are becoming increasingly susceptible to HAV infections [7].

The most common vehicles for HAV transmission are: the ingestion of contaminated water and contact with infected people. Sexual transmission, especially in men who have sex with men (MSM), has also been reported [8,9].

During June-2016 - May-2017, several outbreaks of hepatitis A (HA) were recorded in Europe, especially described in MSM [9]. The viruses responsible for these outbreaks belonged to genotype IA and grouped with one of these three strains: VRD_521_2016, RIVM-HAV16-090 and V16-25801. Similarly, in the same period, HA outbreaks affecting primarily MSM were reported in North America (USA, January-2017, strains: VRD_521_2016 and RIVM-HAV16-090) and South America (Chile, November 2016 -October 2017, strain: V16-25801; Brazil August 2017 - January 2018, strains: VRD_521_2016 and RIVM-HAV16-090) [10-12].

On 30 December 2017, the local public health authority of Cordoba province, central region of Argentina, informed an increase in the number of cases of HA, primarily among males. Following this information, epidemiological and virological surveillance of HA was enhanced.

We describe an outbreak that disproportionally affected HAV-unvaccinated young adult men, primarily MSM, occurred later than Europe.

2. Objective

The purpose of this study was to describe, characterize and contextualize epidemiologically the HA outbreak has occurred in Córdoba, central region of Argentina, between july2017 and april2018.

3. STUDY DISEGN.

3.1 Case definition

According to the Argentinean Ministry of Health, the surveillance criteria for acute HA cases are: a) suspected clinical case: compatible clinical features with

elevated liver enzymes (2.5 folds the reference value of 7-55 U/L); b) confirmed case: suspected case plus positive anti-HAV IgM, or a suspected case with proven epidemiological link with a confirmed case (cohabiting, contact or having been exposed to the same source in the case of an outbreak) [8].

3.2 Enhanced surveillance.

Due to the increase of HA cases in the notification system of Argentina in calendar week 51/2017, we (arbitrarily) considered a possible outbreak beginning in the calendar week 29/2017 (starting 30 July), when the first case of HA in an adult MSM was recognised (Figure 1). In this context samples of serum and / or stool from 32 HA cases were retrospectively studied.

3.3 Samples, Viral extraction and molecular analysis

Nucleic acid isolation, reverse transcription Nested PCR (RT-Nested PCR) and sequencing were conducted as described elsewhere using unified protocols published in the HAV-Net, in all samples (serum or stool) of cases with positive IgM anti-HAV notified since calendar week 29 [13]. Positive samples were sequenced using an automatized sequencer (3500xL Genetic Analyzer - Applied Biosystems). Sequences obtained were entered into the HAV-Net database, and were used to perform phylogenetic analysis. A rooted maximum likelihood phylogenetic consensus tree for sequences of 360 nucleotide (nt) long fragments in the VP1/2A junction region was inferred using MEGA version 6 software [14].

4. Results

Until 20 April 2018, 32 cases of HA were notified in Cordoba province since July 2017 (week 29). Of these, 28 were male, and 4 were female (Table 1). The main characteristics of these patients are shown in Table 1.

Sexual orientation was known for 27 cases (23 men, 4 woman); 16 (69.6%) identified themselves as MSM and 50% (8/16) of them were infected with human immunodeficiency virus (HIV). Median age of the MSM cases was 31.9 years (range: 21–55 years); all of them lived in Cordoba city (second most populated city of Argentina). In terms of disease severity, among patients for

which clinical information was available (n= 23), jaundice occurred in 90% of the cases (n= 21) and severe hepatic failure in 26% of them (n=6). Three cases, had a travel history outside Argentina to Peru, Chile, and Ecuador, during the assumed period of infection. Most of the adults involved in the outbreak were unvaccinated, except one MSM who was vaccinated with one dose of a monovalent hepatitis A vaccine 15 years before disease onset. Two cases were unvaccinated children living in a different geographical area, 86 km away from Córdoba city.

For comparison, in the same period July 2014 – April 2015, July 2015 – April 2016, and July 2016-April 2017, 3, 7 and 5 cases of HA were reported, respectively. Among these, none were identified as MSM.

All samples of this study were subjected to RT-Nested-PCR, 14 of them had a positive result and were sequenced. A good quality sequence could be obtained for 11 samples. Phylogenetic analysis of them showed one cluster of MSM-related HAV strains from Cordoba, Argentina (Figure 2), which grouped with the strain VDR521-2016, associated with outbreaks in MSM in Europe between June 2016 and May 2017 [9]. Two sequences obtained from patients who were not MSM (CbaARGFDAYO Case Nov/2017: Female patient, 6 years old, not vaccinated, inhabitant at 86Km from Córdoba city, and CbaARG_15 Case March/2018: Female adult patient reporting travel to Ecuador) grouped within strain V16-25801 group, together with Chilean MSM-associated sequences (Figure 2).

The sequence CbaARG_25, corresponded to a non-vaccinated male adult, not associated with MSM, reported in April 2018, grouped separately from the outbreak sequences, with the reference sequence X83202 (from Italy, 1990's decade) and with the strain CbaARG_31_2016, amplified from a case of HA occurred at the beginning of 2016.

5. Discussion

Here we report a hepatitis A outbreak among MSM in the central region of Argentina, started in mid-2017. All sequences amplified were phylogenetically closely related, indicating the circulation of the same strain belonging to

genotype IA (VDR- 521-2016) in MSM and suggesting a common source of infection.

Currently, as both sanitary and socioeconomic conditions have been improved in some areas of the world, the age of infection has shifted to older groups, and thus the number of symptomatic cases has increased. In this sense, in a similar way to was happened in other parts of the world, in this study cases occurred in patients of average age of around 30 years.

Previous studies conducted in our region, demonstrated above 40% susceptibility to HAV infection, both in healthy adult individuals and in immunocompromised HIV infected patients, especially in that age range [7,15]. On the other hand, we evidenced the presence of HAV RNA in sewage and river samples during the same period in which susceptible individuals were found. These findings demonstrate the potential risk of infection for these individuals in our area [7,16].

Argentina follows the World Health Organization (WHO) recommendations about HAV vaccination [17], which includes free HAV risk-based vaccination for MSM (although the scope of coverage is unknown) and no-free vaccination for travellers to endemic areas [8, 18]. Additionally, the official notification system does not register sexual orientation data in our country. Thus, the magnitude of sexually transmitted HAV is likely underestimated. The incorporation of detailed questionnaires to capture sexual history of the patients would be very useful to establish epidemiological links between cases. In this sense, some limitations of our study were: a)- the relatively low number of samples analysed, together with the low number of variables provided by the epidemiological records, which did not allow statistical analyses to compare course of infection, time of hospitalization, morbidity of infection, comorbidities, among HIV(+) vs HIV(-) MSM patients, b)- the impossibility of amplify the RNA genome in all IgM (+) samples, probably due to a bad sample conservation for molecular assays, or low viral load by the time of sampling.

Molecular characterization helped to confirm the outbreak and demonstrated that laboratory results, when combined with reliable epidemiologic data, can be effective in understanding transmission networks. Although all MSM-related sequences grouped with sequences from EU outbreaks suggesting imported cases, none of the patients had reported travelling to Europe during the

incubation period, so establishing the infection link is very difficult. However, this does not rule out that some MSM traveller with subclinical HA or a case not officially notified may have been the link between the outbreaks.

During this study, 4 cases of HA not associated to MSM were reported; their sequences were phylogenetically distinct from the MSM sequences. Two cases were non-vaccinated adult women, who travelled to high endemic areas in South America. The lack of immunization in adults makes these patients a reservoir for the virus (HAV-infected person can be viremic up to 6 weeks through their clinical course), since it is excreted in stool for up to 2 weeks prior to becoming symptomatic, being able to generate new cases or a new outbreak situation. The other 2 cases that were not related to the outbreak belonged to Peruvian unvaccinated children, living in an area 86km away from the city of Córdoba. The immigration of individuals without vaccination coverage is a matter to be addressed when trying to control HAV in our region.

Many questions arise from this study: why three strains of HAV caused outbreaks in MSM around the world? Where did the outbreak originate? Which is the evolutionary history of the strains? How did these strains move around the world? Why did only one of the strains cause the outbreak of hepatitis A among MSM in our region? Phylodynamic studies could answer some of these questions, and should be made in the near future.

The improved surveillance of HAV will detect new possible outbreaks in specific populations (as MSM), evaluate the impact of the post-outbreak intervention, and will allow to obtain a better understanding of the HAV transmission in our area.

Finally, based on: (a) the recent HAV outbreaks among MSM reported worldwide, (b) the fact that MSM usually become infected at later ages, when the severity of the disease is more evident, (c) the low proportion of immune individuals for HAV observed among young adult individuals, and (d) the access to free hepatitis A vaccination in our country, we strongly recommend to improve communication strategies to raise awareness among health personnel and the general population to increase vaccination. This will help to mitigate viral dissemination in our population and to prevent new outbreaks of HAV in MSM.

Authors' contributions
VR and GB conceived the study. PB and JM obtained the blood samples and recorded epidemiological data. JM and MB performed serological test. JM and FC maintained the clinical and epidemiological database. GC, AF, and MBP performed all the molecular testing and sequencing. VR, JM and MBP wrote the initial manuscript. All the authors reviewed, commented and approved the manuscript.

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FIGURE 1. Hepatitis A cases occurred during the outbreak from April 2017 to July 2018, Argentina (n = 32). Each case is located in the week of onset of illness. Sex, sexual orientation, HIV-coinfection and travel history is indicated.

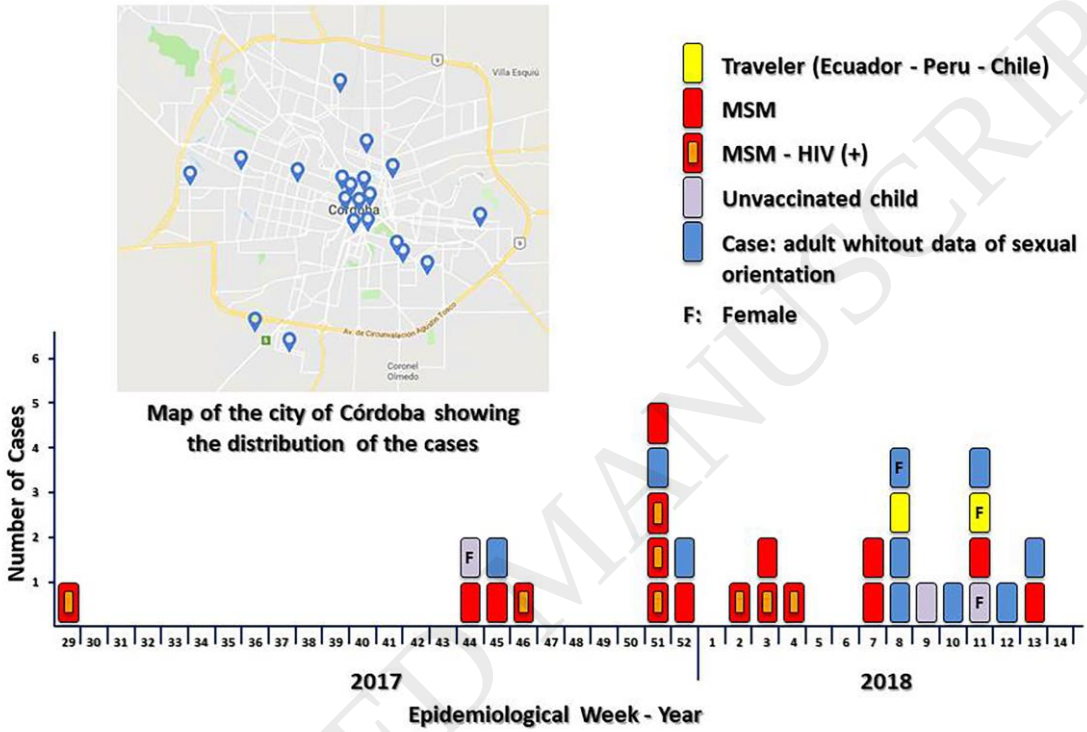

FIGURE 2. Phylogenetic analysis (Maximum Likelihood) of the VP1/2A genomic region (360nt) of viral strains from hepatitis A cases in Argentina, 2017- 2018. Red circles: sequences from MSM outbreak from Argentina 2017-2018. Yellow circles: other Argentinean sequences from the same period of the outbreak, of probable endemic circulation. Blue diamonds: European outbreak strains. Samples CbaARG_10, 11, 13 and 16 are from HIV+ patients.

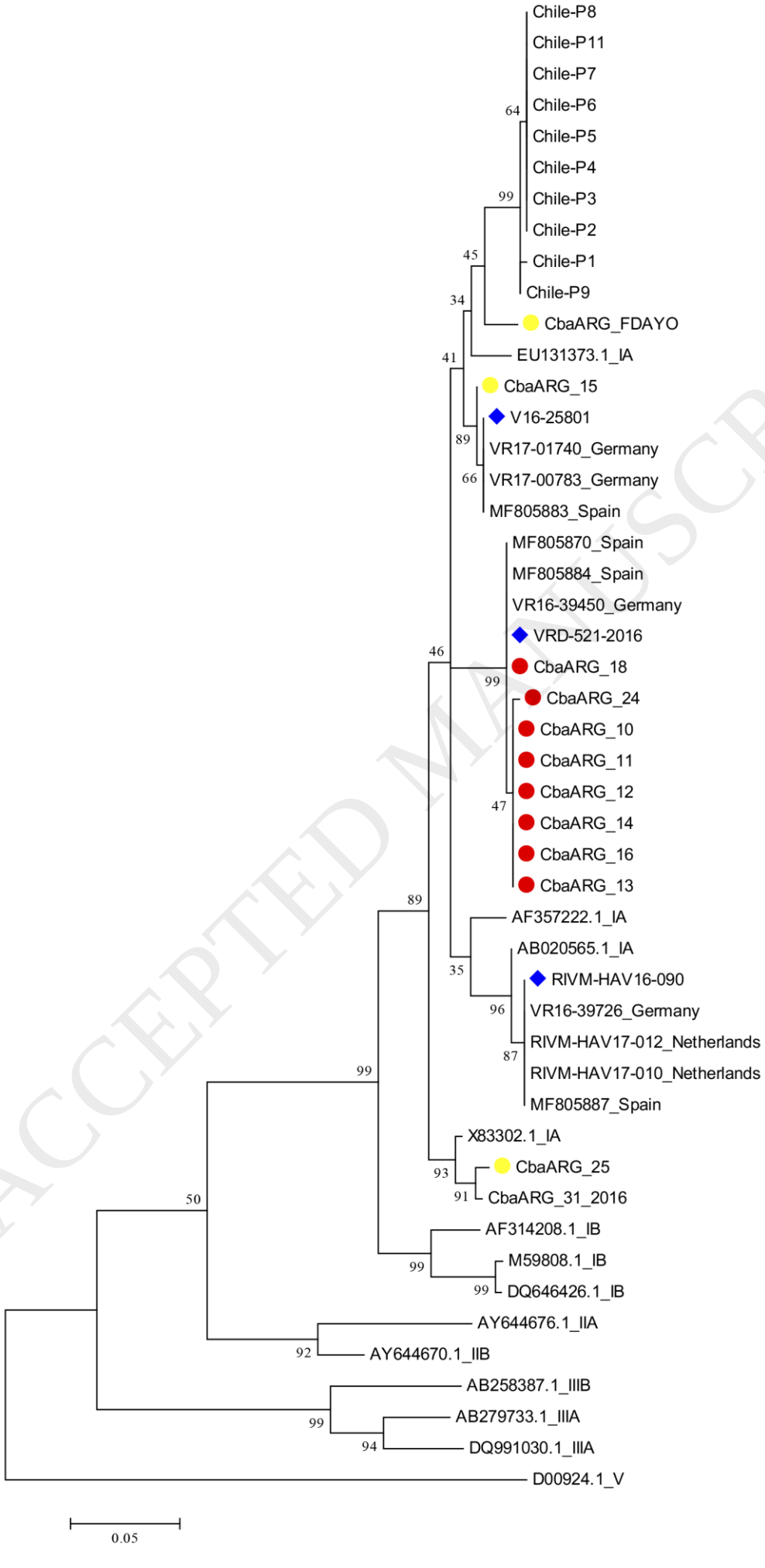

Table 1. Characteristics of notified acute hepatitis A cases in Cordoba, Argentina. July 2017-April 2018

Characteristics	Total (n=32)[a]
Demographic Characteristics	
Age, median (range) of adults patients[b]	31.9 (21-55)
Male patients, number	28
Female, number	4
Clinical -epidemiological characteristics and risk factors	
MSM/men, ratio	16/23
Travel abroad 2 months before symptom onset, number[c]	3
Contact with infected individuals, number [d]	3
Severe hepatic failure[e]	6
Sexually transmitted co-infections[f]	
HIV, number	8
HBV, number	1
HCV, number	0
Vaccination	
Patients vaccinated, number[g]	1

HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus, MSM: men who have sex with men; NA: not applicable.
[a] Sexual orientation recorded among 27 patients.
[b] Two patients were unvaccinated Peruvian children (one male five years and one female six years old) inhabitants of a small town 86Km away from the city of Cordoba.
[c] Three patients (two females and one male) travelled abroad, to Chile, Ecuador and Peru, respectively.
[d] Three patients reported have had close contact with somebody with known acute hepatitis A.
[e] Data available among 23 patients.
[f] Information missing for some patients.
[g] One MSM case that was vaccinated with one dose of a monovalent hepatitis A vaccine 15 years before disease onset