#### **ORIGINAL ARTICLE**



# Epidemiology of hepatitis B and C virus infection in Central West Argentina

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#### Abstract

Little information is available regarding the prevalence of viral hepatitis in Central West Argentina. This study aims to give new information regarding HBV and HCV prevalence, genotypes, and risk factors in Central West Argentina and the suitability of dried blood spot (DBS) sampling for HBV and HCV screening. Methods: A total of 622 individuals were included; the mean age was  $36.6 \pm 14.3$  years and 55.4% were females. HBV and HCV markers were detected using serological and molecular analysis, and risk factors were evaluated using statistical analysis. Results: Using serum samples, the HBsAg prevalence was 1.8%, the rate of HBV exposure (anti-HBc positivity) was 5.3%, and the rate of HBV immunity was 34.9%. HBV DNA was found in four out of 11 HBsAg<sup>+</sup> samples, and the viruses in three of these samples were classified as genotypes A1, A2 and F2a. Multivariate analysis showed that anti-HBs positivity was associated with the level of schooling and history of HBV vaccination. The anti-HCV prevalence was 2.6%, and HCV RNA was found in 11 samples, seven of which contained viruses of genotypes 1a (n = 2), 1b (n = 3) and 2 (n = 2). The sensitivity of the DBS assay for HBsAg, anti-HBc, and anti-HCV was 100%, 66.6%, and 75%, respectively, and the specificity was above 98% for all markers when compared to serum. Conclusion: A low rate of HBV immunity was observed, demonstrating the importance of HBV vaccination. High HCV prevalence was found, and HCV 1b was closely related to other Argentinian isolates. Finally, the performance of DBS testing in this population needs more optimization to increase its sensitivity and specificity.

#### Introduction

Viral hepatitis has a significant cost in lives, communities and health systems, with an estimated 1.3 million deaths per year due to acute infections and because of the complications of the chronicity of some viruses, such as cirrhosis and hepatocellular carcinoma. Of these deaths, 95% are

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<sup>3</sup> Virology Section, Central Hospital Mendoza, Mendoza, Argentina attributed to hepatitis B or C. Worldwide, 257 million people are considered to have a chronic hepatitis B virus (HBV) and 71 million have a chronic hepatitis C virus (HCV) infection [1, 2].

Argentina has a population of 40,091,359 inhabitants according to the population census of the year 2010. Its population is composed of descendants of two main migratory flows from Spain and Italy in the early 20th century. Mendoza province has a population of 1,738,929 inhabitants and is located geographically in the central west of the Argentine Republic [3].

In Argentina, the prevalence for hepatitis B and C are very different, as are the population groups affected by these infections. HBV endemicity is low (0.1% to 0.5%) among blood donors in transfusion centers, with new cases of infection occurring mainly in the age group of 25 to 64 years, through the sexual route. HCV prevalence varies from 1.5% to 2.5% showing higher prevalence in small rural areas. In the province of Santa Fe, Argentina, the HCV infection rate reached 4.5% in people older than 50 years [4]. In a rural

village of the city of O Brien, province of Buenos Aires, the overall prevalence of HCV infection was 5.6%, with 12.6% of the people over 40 years old, and 23% of the people 60-70 years old testing positive [5].

HBV is classified into eight main genotypes (designated A-H) and two minor and putative genotypes (I and J) [6]. HCV has been classified into eight genotypes where epidemic subtypes – specifically 1a, 1b, 2a, and 3a – are widely distributed worldwide and account for a large proportion of the total of HCV cases, especially in high-income countries [7, 8]. It is important to determine the distribution of HBV and HCV genotypes because of differences in virological and clinical parameters that might interfere with antiviral treatment and prognosis. In Argentina, some studies have documented the presence of HBV genotypes A, C, D, and F [9, 10] and HCV genotypes 1, 2 and 3 [11, 12]; however, few studies have been reported and little is known about the distribution of these genotypes in western Argentina.

To establish preventive measures against these infections, it is important to determine the prevalence of HBV and HCV infections. However, there is a paucity of studies regarding epidemiology of these viruses in Argentina due to difficulty in obtaining serum samples and recruiting volunteers from the general population. Most studies have examined blood donors or specific groups, such as individuals who are coinfected with HIV [13–15].

In recent years, the use of dried blood spot (DBS) samples for HBV and HCV diagnosis in different populations has been investigated [16–19]. DBS collection does not require trained health professional to perform venipuncture, does not require centrifugation for serum preparation and does not require that samples be kept cold during transport. Although DBS samples have not yet been used for epidemiological studies of hepatitis in Argentina, their use could potentially facilitate diagnosis and the performance of epidemiological studies.

In this study, we estimate the prevalence rates of HBV and HCV in different populations of Mendoza province, Argentina, to identify risk factors, to identify the most prevalent genotypes of HBV and HCV, and to evaluate the suitability of DBS sampling for HBV and HCV screening in this population.

### **Patients and methods**

#### Patients

This cross-sectional study included 622 patients recruited in three different counties of Mendoza Province in western Argentina. Mendoza is the fourth largest province of Argentina, with 18 departments and more than 900,000 inhabitants in the Mendoza metropolitan area, and the province is an important wine-producing region.

All participants in the study were over 18 years old and gave written informed consent to participate. The protocol of this study was approved by the Ethics and Research Committee of the Central Hospital of Mendoza, Argentina. Individuals were recruited from three departments of the province:

- Junin: A total of 252 individuals were recruited in the department of Junín during a campaign for hepatitis testing and counselling from October 20 to 24, 2014. This department is 60 kilometres from the city of Mendoza, has an area of 263 km<sup>2</sup>, and has 35,045 inhabitants. All of the subjects lived in small rural town and were farmers, state employees, or merchants.
- San Martin: A total of 205 individuals were recruited in San Martín department from December 10 to 13, 2014 before they attended a music festival. San Martin is located 45 kilometers from the city of Mendoza, and has an area of 1,504 km<sup>2</sup> and 108,448 inhabitants. Individuals from different provinces of Argentina come to the festival, but only residents of at San Martin were included in the study.
- Mendoza Capital: A total of 165 individuals were admitted to the public health service of Central Hospital of Mendoza from October to December 2014. Mendoza Capital has an area of 54 km<sup>2</sup> and 110,993 inhabitants.

Respondents were assured about confidentiality, that their participation was voluntary, and that they had the right to withdraw from the study at any time. All participants were given a verbal explanation of the objectives and methodology of the research and were included in the study after obtaining signed informed consent. All individuals who tested positive were sent to public health units to have access to treatment.

#### **Blood and DBS sampling and processing**

A blood sample (5 mL) was collected from each participant by venipuncture, using a Vacutainer device. The sample was allowed to clot to separate the serum for analysis and was stored at -20 °C until analysis.

DBS samples were obtained by spotting 3–5 drops (approximately 75  $\mu$ L) of whole blood onto Whatman filter paper (Whatman no. 903, G&E Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) until a 12-mm pre-cut circular paper disk was completely filled. DBS samples were air-dried at room temperature for 4 h, placed in individual sealable plastic bags containing silica desiccant sachets, and stored at -20 °C.

The blood was then eluted from the paper and processed as described previously [16]. For anti-HCV, a 3-mm disc was punched from each DBS sample and placed into a microtube containing 300 microliters of phosphate-buffered saline (PBS) with 0.5% bovine serum albumin (BSA) at 4–8 °C for 18–24 h. For HBsAg and anti-HBc detection in DBS samples, a 6-mm disc was eluted in 700  $\mu$ l of 0.5% PBS/BSA at 4–8 °C for 18–24 h.

#### HBV and HCV testing in serum and DBS samples

Serum samples were tested for serological markers of HBV (HBsAg, anti-HBc, anti-HBs) and anti-HCV using commercial enzyme immunoassay (ELISA) kits (Diasorin, Pomezia, Italy) according to the manufacturer's guidelines. The same ELISA kits were used for both serum and DBS samples.

DBS samples were tested for anti-HCV, HBsAg and anti-HBc. Anti-HCV was detected in DBS samples using a fivefold increased sample volume (100  $\mu$ l), and the sample diluent was decreased to 100 microlitres for commercial EIA (Murex HCV Ab, Diasorin). The cutoff was calculated according to the supplier's instructions [17].

HBsAg and anti-HBc were detected in DBS samples as described previously, but the sample volume and cutoff value for the assays were modified as follows: HBsAg, 150  $\mu$ L of eluate and reactive samples with optical density OD  $\geq$  0.115; anti-HBc total, 100  $\mu$ L of eluate and reactive samples with OD  $\leq$  0.261 [16].

Samples found to be negative in the preliminary screening were considered seronegative. Samples that initially tested borderline or positive were retested using a commercial ELISA to confirm the results. Indeterminate samples were excluded from the analysis.

#### **Molecular analysis**

Anti-HCV-positive serum samples were tested by real-time PCR (Abbott HCV, Abbott, USA), which has a dynamic range of linear quantification of 20 to  $1.7 \times 10^8$  IU/mL. HBsAg-positive serum samples were tested by real-time PCR (Cobas TaqMan HBV Test, Roche, USA), which has a dynamic range of linear quantification of 29 to  $1.1 \times 10^8$  IU/mL.

RNA and DNA were extracted from serum samples using a commercial kit (High Pure Viral Nucleic Acid Kit, Roche Diagnostics, Germany) following the manufacturer's instructions. Qualitative detection of HCV RNA in serum was performed using an RT semi-nested PCR for amplification of the NS5B region of HCV using primers described by Sandres-Sauné et al. [20] and the conditions described previously [21]. For amplification of HBV DNA in serum samples, DNA was subjected to a PCR using oligonucleotides to amplify the partial polymerase/surface genes of HBV [22] and conditions described previously [23]. Sequence analysis was performed using PCR products that were purified using a QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany). Direct nucleotide sequencing reaction was done in both directions using a Big Dye Terminator Kit (version 3.1, Applied Biosystems, Foster City, CA, USA) with the same oligonucleotides used in PCR for HBV or HCV. Sequencing reactions were analyzed on an ABI3730 automated sequencer (Applied Biosystems). The sequencing protocol was performed as described by Otto et al. [24].

The nucleotide sequence was analyzed using MEGA 7.0 software [25]. Phylogenetic trees were constructed by the neighbor-joining and maximum composite likelihood methods with a dataset composed of 65 reference sequences from different HBV subgenotypes available in the GenBank database. The reliability of the phylogenetic tree was assessed by bootstrap test (1000 replicates). HCV phylogenetic analysis included 71 reference sequences representative of HCV genotypes 1 to 7 available in the GenBank database (referred to in the phylogenetic tree by subtype, followed by the GenBank accession number).

#### Data collection and analysis

Demographic and behavior factors related to hepatitis were obtained from each participant using a questionnaire and entered into an SPSS data sheet along with the virological results. The prevalence was calculated for HBV and HCV markers in the studied population. Descriptive statistics were generated for the data, and the chi-squared test for independence or for trend were used to compare categorical variables. A *p*-value < 0.05 was considered statistically significant. All calculations were performed using the Statistical Package for the Social Sciences (SPSS for Windows, release 20.0; SPSS, Chicago, IL, USA).

## Results

#### **Population characteristics**

A total of 622 individuals were included in this study, with the largest number recruited in Junin, followed by San Martin and Mendoza. The overall mean age was 36.6  $\pm$  14.3 years, and the majority were female (55.4%). The socio-demographic characteristics of the 622 individuals included in this study are shown in Table 1. All variable frequencies differed significantly according to subject group, except for the number of people in the home, history of hemodialysis, and previous history of blood transfusion.

Table 1 Main characteristics of the population studied in Mendoza Province, Argentina

Variable	Total $(n = 622)$	San Martin $(n = 205)$	Junin (n = 252)	Mendoza ( $n = 165$ )	<i>p</i> -value
Age, years, mean $\pm$ SD	36.6 ± 14.3	29.6 ±11.0	$40.5 \pm 14.2$	39.6 ± 14.9	<0.0001
Gender					
Female	345 (55.5%)	60 (17.4%)	176 (51.0%)	109 (31.6%)	< 0.0001
Male	277 (44.5%)	145 (52.3%)	76 (27.4%)	56 (20.2%)	
Marital status, married	229 (36.8%)	18 (7.9%)	143 (62.4%)	68 (29.7%)	< 0.0001
Level of education					< 0.0001
Illiterate	71 (11.4%)	07 (9.8%)	36 (50.7%)	28 (39.4%)	
Primary education	255 (41.0%)	103 (40.4%)	91 (35.7%)	61 (23.9%)	
Secondary education	226 (36.3%)	90 (39.8%)	79 (34.9%)	57 (25.2%)	
College	70 (11.2%)	5 (7.1%)	46 (65.7%)	19 (27.1%)	
Number of people at home (mean $\pm$ SD)	$3.1 \pm 3.9$	$3.0 \pm 6.0$	$3.0 \pm 2.0$	$3.0 \pm 2.0$	>0.99
Number of rooms at home (mean $\pm$ SD)	$2.4 \pm 1.2$	$3.0 \pm 1.0$	$2.0 \pm 1.0$	NA	< 0.0001
History of blood transfusion	75 (12.0%)	18 (24.0%)	37 (49.3%)	20 (26.6%)	0.15
Previous HBV vaccination	229 (36.8%)	28 (12.2%)	90 (39.3%)	111 (48.5%)	< 0.0001
History of haemodialysis	03 (0.5%)	01 (33.3%)	00 (0.0%)	02 (66.7%)	0.21
Toothbrush sharing	34 (5.5%)	11 (32.3%)	03 (8.8%)	20 (58.8%)	< 0.0001
Tattoo history	211 (33.9%)	141 (66.8%)	32 (15.2%)	38 (18.0%)	< 0.0001
Use of illicit narcotic substances	179 (28.8%)	152 (84.9%)	11 (6.1%)	16 (8.9%)	< 0.0001
Sharing of nail pliers	447 (71.8%)	155 (34.7%)	201 (44.9%)	91 (20.3%)	< 0.0001
Depilation	153 (24.6%)	43 (18.9%)	81 (52.9%)	29 (18.9%)	0.001
History of intravenous medicine	209 (33.6%)	NA	139 (66.5%)	70 (33.5%)	0.01

NA: not applicable

# HBV serological profile in serum samples and risk factors

Table 2 shows that 11 out of 622 (1.8%) individuals were HBsAg positive. The rate of past HBV infection (anti-HBs and anti-HBc positivity) was 3.5% (22/622), and the rate of HBV exposure (anti-HBc positivity) was 5.3% (33/622). Out of 217 HBV-immune individuals, 195 had been vaccinated (only anti-HBs detected). The highest prevalence of HBsAg

(3.0%), anti-HBc (9.1%), and anti-HBs (37.6%) was found in a hospital-based survey in the city of Mendoza.

HBV DNA was found in four out of 11 HBsAg-positive serum samples using real-time PCR, and the mean viral load  $\pm$  SD was 2.7  $\pm$  0.9 log IU/ml. Three samples (with a mean viral load of 3.1  $\pm$  0.5 log IU/ml) could be amplified by PCR and sequenced. They were further classified as genotypes A1 (sample collected in the city of Mendoza), A2 (Junin) and F2a (San Martin).

Marker	Total (n = 622)	San Martin (n = 205) n (%)	Junin (n = 252)	Mendoza (n = $165$ )	<i>p</i> -value
HBsAg					
Positive	11 (1.8%)	01 (9.1%)	05 (45.4%)	05 (45.4%)	0.172
Negative	611 (98.2%)	204 (33.4%)	247 (40.4%)	160 (26.2%)	
Anti-HBc					
Positive	33 (5.3%)	09 (27.3%)	09 (27.3%)	15 (45.4%)	0.038
Negative	589 (94.7%)	196 (33.3%)	243 (41.2%)	150 (25.4%)	
Anti-HBs					
Positive	217 (34.9%)	65 (29.9%)	90 (41.5%)	62 (28.6%)	0.469
Negative	405 (65.1%)	140 (34.6%)	162 (40.0%)	103 (25.4%)	
Anti-HCV					
Positive	16 (2.6%)	01 (6.3%)	05 (31.3%)	10 (62.5%)	0.003
Negative	606 (97.4%)	204 (33.7%)	247 (40.7%)	155 (25.6%)	

**Table 2**Hepatitis B and Cmarkers in Mendoza Province,Argentina

Phylogenetic analysis of the HBV sequences showed that HBV/A1 and A2 were related to Brazilian and European sequences, respectively, while HBV/F2 was related to strains from Brazil and Venezuela (Fig. 1).

Statistically significant differences in anti-HBc antibody prevalence were observed between subject groups, with the highest prevalence found in Mendoza County (Table 2). The demographic and behavioral factors that were statistically significantly associated with anti-HBs positivity in univariate analysis were age, level of education, and history of HBV vaccination, with all remaining significant in multivariate analysis except for age (Table 3).

HCV serological profile in serum samples and risk factors

In this study, the overall anti-HCV prevalence was 2.6%, and 11 of the subjects had detectable HCV RNA in their serum (mean viral load,  $4.4 \pm 1.7 \log \text{UI/mL}$ ). Among the 16 anti-HCV-positive individuals, the mean age was 49 years. Most of them were males (62.5%), and had a primary education (56.2%). The most frequently reported risk factors were a history of tattooing (25%) and drug consumption (37.5%). However, only the subject group was found to be statistically relevant in multivariate analysis (Table 2).

HCV RNA was amplified using semi-nested PCR, and seven samples were reactive and could be genotyped. Phylogenetic analysis of these sequences with a dataset containing reference sequences produced a maximum-likelihood tree in which three samples were classified as subtype 1a and

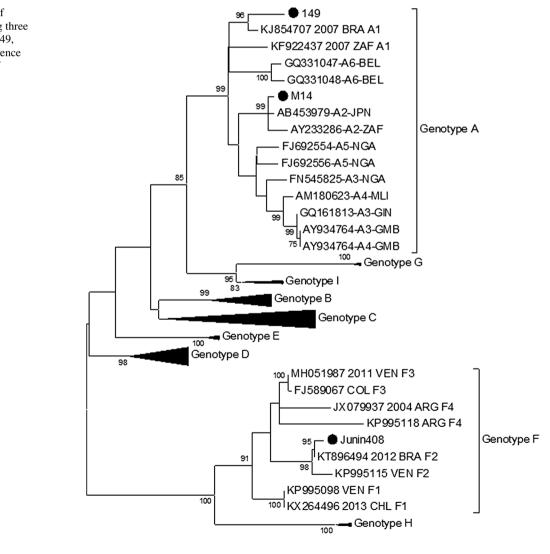


Fig. 1 Phylogenetic tree of HBV sequences, including three isolates from this study (149, M14, Junin 408) and reference sequences from nine HBV genotypes

0.02

Table 3Bivariate andmultivariate analysis ofdemographic and behaviourfactors associated with anti-HBsreactivity in Mendoza Province,Argentina

Variable*	Anti-HBs		Bivariate	Multivariate analysis	P-value
	Positive (n $= 217$ )	Negative (n $= 405$ )	analysis <i>P</i> -value	OR (95% CI)	
Age					
$\leq$ 30 years	105	159	0.028	0.825 (0.528 - 1.289)	0.397
> 30 years	112	246			
Level of education*					
Illiterate	12	58	0.000	1.553 (1.214 – 1.987)	0.000
Primary education	75	180			
Secondary education	85	141			
College	45	25			
Self-reported HBV vacci	nation*				
Yes	120	109	0.000	4.073 (2.684 - 6.181)	0.000
No	68	255			

\*Totals do not add up to 622 due to missing values

formed a monophyletic group closely related to other strains from Argentina and Latin America. Two strains grouped with the HCV-1b reference sequences and were more closely related to Brazilian isolates, and two isolates grouped with HCV-2c and clustered with European strains (Fig. 2).

# Evaluation of DBS sampling for detection of HBV and HCV markers

The sensitivity of HBsAg, anti-HBc, and anti-HCV detection in DBS samples was 100%, 66.6%, and 75%, respectively and the specificity was above 98% for all markers when compared to serum samples. Overall, the kappa index indicated a good agreement between results obtained with serum and DBS samples for all markers (Table 4). Agreement values determined by the kappa statistic indicated the sensitivity and specificity of the assays. In this context, the assays demonstrated good agreement with the serum results, although some differences in sensitivity were observed, depending on the marker used. HBV DNA did not interfere with the sensitivity of HBsAg detection, since no false negative results were obtained with DBS samples. The anti-HBc sensitivity also varied according to sampling location with values of 86.6%, 44.4% and 66.6% for Mendoza, Junin and San Martin, respectively.

The sensitivity of anti-HCV detection DBS in samples varied according to the site of recruitment, with a detection rate of 100% in Mendoza County, 40% in Junin, and 0% in San Martin. Among the 12 positive concordant anti-HCV results in serum and DBS, 10 serum samples were positive for HCV RNA (mean viral load  $\pm$  SD, 4.7  $\pm$  1.4 log IU/ml). Among the four false negative results for anti-HCV in DBS samples, only one sample had HCV RNA in serum (1.4 log IU/ml). A higher anti-HCV sensitivity value was obtained when anti-HCV/HCV-RNA-positive serum samples were

considered truly reactive. In this situation, the overall anti-HCV sensitivity for DBS samples was 90%.

#### Discussion

The prevalence of viral hepatitis has been investigated in Argentina [4, 5, 9–15], but less is known about Central West Argentina, where most of the available data are from blood bank studies. In the present study, we determined the prevalence of HBV and HCV in individuals from Central West Argentina and found differences in HBV and HCV prevalence according to subject group.

The overall HBsAg prevalence was 1.8%, which is higher than that reported for blood donors from Misiones (0.74%) [26] and Mendoza County (0.35%) [13] but lower than that found for men who have sex with men (MSM) (24%) [27]. When HBsAg prevalence was analyzed according to subject group, low prevalence was observed in San Martin (0.48%) compared to Junin (1.98%) and Mendoza (3.03%), which could reflect different levels of HBV exposure and the importance of specific preventive measures in these settings.

Concerning HBV genotypes, this study confirmed the circulation of genotypes A1, A2 and F2a in Central West Argentina. These genotypes are also circulating in the neighboring regions of Misiones and Cordoba [9, 26, 28]. Phylogenetic analysis revealed that the HBV/A1 strain clustered in the Asia-American clade, close to Brazilian sequences. HBV/A2 showed genetic relatedness to viral strains from Brazil and Europe, while HBV/F2 was related to strains from Brazil and Venezuela. Subgenotypes A1, A2 and F are most prevalent in Asian-African, European and Amerindian populations, respectively. The circulation of these subgenotypes in Latin American countries such as Argentina and Brazil

Fig. 2 Phylogenetic tree of HCV sequences, including seven isolates from this study (starting with "ARG") and reference sequences from seven HCV genotypes

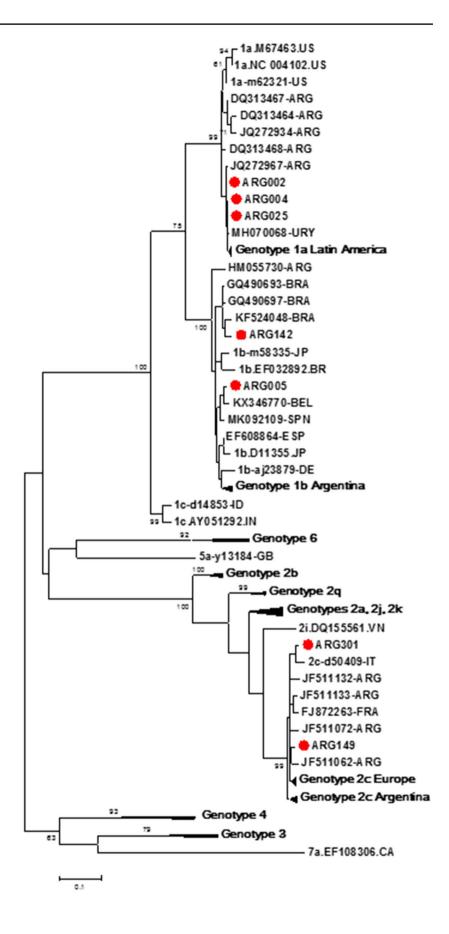


Table 4 Quality parameters of
HBsAg, anti-HBc, and anti-
HCV detection in DBS samples
from Mendoza Province,
Argentina

Variable	HBsAg	Anti-HBc	Anti-HCV
Sensitivity, % (IC)	100.0 (71.5 - 100.0)	66.6 (48.2 - 82.0)	75.00 (47.6 - 92.7)
Specificity, % (IC)	98.8 (97.6 - 99.5)	99.8 (99.1-100.0)	99.8 (99.1-100.0)
Positive predictive value, % (IC)	61.1 (35.7-82.7)	95.6 (78.1 – 99.8)	92.3 (63.9-99.8)
Negative predictive value, % (IC)	100.0 (99.4-100.0)	98.2 (96.7-99.1)	99.3 (98.3-99.8)
Kappa statistic, n (IC)	0.753 (0.571-0.953)	0.776 (0.65 -0.901)	0.824 (0.669-0.978)
False positive (n)	07	01	01
False negative (n)	00	11	04
True positive (n)	11	22	12
True negative (n)	604	588	605

may be related to the presence of these three ethnic groups in their populations [29, 30].

A high frequency of HBV exposure (anti-HBc positivity) was found in a hospital setting (10%) compared to other settings (4.59% in San Martin and 3.57% in Junin) and was statistically significant. Previous studies of blood donors revealed a low prevalence of HBV exposure in the Central West region (2.0%) [13] compared to the present study, which could reflect subject group.

In the present study, more than half (65.1%) of the subjects were not immune to HBV, suggesting the importance of universal vaccination in the general population. Anti-HBs positivity was associated with the level of schooling and previous history of HBV vaccination. Individuals with a high level of education had a higher probability of being immune to HBV, and similar results have been reported for beauty professionals in Brazil [31].

A few studies have been conducted to determine the rate of HBV immunization in the Argentinian population. Segura et al. [27] showed that only 7% of MSM reported HBV vaccination, demonstrating low coverage of HBV vaccination in this group. In Argentina, HBV vaccination has been obligatory for health care workers since 1992 and is recommended for high-risk groups. HBV vaccination was incorporated into the National Immunization Calendar for newborns since November 2000, with the first dose recommended in the first 12 hours of life, and vaccine coverage in 2015 was about 94%. In 2012, the National Program for the Control of Immune Preventable Diseases launched the universal vaccination of adults older than 20 years old in an effort to control and eliminate infectious diseases. Despite the introduction of these measures, we observed a low prevalence of HBV immunity.

An overall anti-HCV prevalence of 2.6% was observed in individuals from Central West Argentina, which is higher than that reported in a previous study of a Mendoza blood bank (0.5%) [13]. However, anti-HCV prevalence was high in a hospital setting Mendoza County (6.1%) compared to Junin (2%) and San Martin (0.5%), as was also found in a tertiary hospital in Buenos Aires (5.2%) [32]. In Brazil, differences in HCV prevalence were also found according to population characteristics and location [29, 33, 34], which reflects different levels of exposure in these groups and the importance of adopting specific measures to control infection according to risk of exposure.

The prevalent HCV genotypes were genotypes 1 and 2, which were also found previously in Mar del Plata [11] and Cordoba [12]. HCV-2 isolates clustered with 2c sequences from Italy and other European countries [35]. The genetic relatedness among these isolates could be due to large amount of European migration to Argentina or possible family travel to and from ancestral lands. Isolates of HCV1b were closely related to other strains from Argentina, demonstrating the endemic circulation of some strains, while HCV1a strains were more closely related to Brazilian isolates.

The suitability of DBS samples as an alternative to serum sample for detecting HBV and HCV markers was investigated. The best performance was observed with HBsAg, as was found in previous studies [16, 36], and it was not dependent on HBV DNA reactivity. We found a low sensitivity for detection of anti-HBc as was found previously in HIV-infected individuals [37]; however, information on HIV status was not available in the current study. Low sensitivity of HCV detection in DBS samples was observed when compared to studies on HCV-monoinfected and HCV/HIVcoinfected subjects [17, 38]. These differences could reflect differences in HCV prevalence and HCV RNA status, since high sensitivity was observed in a high-HCV-prevalence setting (100% in Mendoza County), indicating that this assay could be useful for detecting active HCV cases that should be treated. The overall anti-HCV sensitivity increased to 90% when anti-HCV/HCV-RNA-positive serum samples were considered truly reactive.

#### Conclusions

This study gives new information regarding the prevalence of HBV and HCV in the Central West Region of Argentina. Different HBV and HCV prevalence was found according to

subject group, showing the importance of different measures for preventing these infections. A large number of individuals were not immune to HBV, which could reflect low vaccination coverage in this population. High HCV prevalence was found in this study, and HCV 1b was closely related to other Argentinian isolates, showing the circulation of endemic isolates in this country. Finally, the performance of DBS testing needs more optimization to increase its sensitivity and specificity. The results of this study could be considered a pilot evaluation of DBS performance in this population.

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#### **Compliance with ethical standards**

**Conflict of interest** The authors disclose no actual or potential conflict of interest, including any financial, personal or other relationships with people or organisations, within two years of the beginning of this study that could inappropriately influence the study.

**Ethical approval** This study was approved by the institutional review board of the Central Hospital of Mendoza.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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