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Prevalence of Hepatitis Delta Virus Infection in Galicia: Results of the Universal Implementation of Double Reflex Testing

Study population

All **chronic HBsAg carriers** who visited any hospital within the Galician health service during the period **between January 2023 and December 2024**

Methods and results

Delta double reflex testing:

HBsAg+ **1962**

↓

Anti-VHD + **85 +**

LIAISON® XL MUREX Anti-HDV Assay; Diasorin

↓

HDV viral load **20 +**

Hepatitis Delta Real Time PCR Kit; Vircell

Outcomes

In our setting, the **seroprevalence of HDV in HBsAg carriers is 4,3%.** Despite the low prevalence, a significant proportion of patients have **active infection (23,5%),** which places them at risk of complications. Consequently, they could benefit from **early diagnosis and novel treatments** such as Bulevirtide.

Carracedo, et al.

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Prevalence of hepatitis delta virus infection in Galicia – Results of the universal implementation of double reflex testing

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Authors contributions

RC: Methodology, Data curation, Formal analysis, Project administration, Writing—original draft, Resources, Supervision, Validation, Visualisation, Investigation.

MAC, SC, MT, NC, MJG, PO, LM, JJC, NP, SP, LRO: Data curation, Project administration, Writing - review and editing, Resources, Investigation.

AA: Conceptualization, Methodology, Project administration, Writing - review and editing, Funding acquisition, Resources, Supervision, Validation.

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Conflict of interest statement

RC, NP y SP received honoraria from Roche Diagnostics and research grants from Gilead; AA received honoraria, travel grants or research grants from Gilead, Abbott Diagnostics, and Roche Diagnostics. All other authors have no disclosures.

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Abbreviations

HBsAg: hepatitis B virus surface antigen

PCR: polymerase chain reaction

IQR: interquartile range

HBV: hepatitis B virus

HCV: hepatitis C virus

HDV: hepatitis Delta virus

HIV: human immunodeficiency virus

Abstract

Introduction: Chronic hepatitis caused by the hepatitis Delta virus is the most severe form of viral hepatitis and carries the greatest risk of complications. The real prevalence of this infection remains unknown.

Objectives: This study seeks to determine the prevalence of both the antibodies and the active infection rates of hepatitis Delta in the Autonomous Region of Galicia, Spain.

Materials and Methods: A prospective study was conducted including all patients carrying the hepatitis B surface antigen who attended any hospital within the Galician Health Service between 2023 and 2024. These patients underwent serum testing for antibodies against the hepatitis Delta virus (LIAISON® XL MUREX Anti-HDV Assay; Diasorin Saluggia, Italia); in those testing positive, the viral load was then assessed (Hepatitis Delta Real Time PCR Kit; Vircell, Granada, Spain). The samples with a positive viral load were subsequently genotyped.

Results: A total of 1,962 patients were included, 85 of whom had anti-HDV antibodies, and 20 of these (23.5%) had a detectable viral load. The prevalence obtained was 4.3%

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in carriers of the hepatitis B virus surface antigen, and 0.03 per 1,000 inhabitants. The most common genotype found in active infections was 1D.

Conclusions: The seroprevalence obtained in this study confirms the low circulation of the hepatitis Delta virus in our region. However, a significant proportion of affected patients have an active infection, and early diagnosis is essential to prevent disease progression and the development of complications.

As expected, most of our patients are infected with strains of genotype 1D, which is predominant worldwide, including Europe. However, we also found three strains of genotype 5 in patients from West Africa.

Keywords: Epidemiology. Hepatitis. Hepatitis Delta virus. Chronic infection. Genotype.

Lay summary

The hepatitis Delta virus (HDV) is a rarely seen virus that affects patients with hepatitis B and can cause the most severe form of viral hepatitis. Until recently, there were no effective treatments for this virus, but since 2020, the use of Bulevirtide has been approved, which is the first direct-acting antiviral specifically targeting HDV.

This study, in which all hospitals in the Galician Health Service have participated, aims to determine the prevalence of hepatitis Delta in our region. To this end, during 2023 and 2024, all HBsAg carriers who visited any hospital in Galicia were tested to determine whether they were infected with HDV. Based on the data obtained, we estimate that the prevalence in our setting is 0.03 per 1,000 inhabitants, which is low. However, a significant proportion of affected patients have active infection and are at risk of serious complications in the short to medium term. It is therefore essential to diagnose them early to prevent the disease from progressing and to enable them to benefit from treatment against HDV.

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Introduction

The hepatitis Delta virus (HDV), discovered in the 1970s by Rizzeto *et al*., is a defective RNA virus that requires the presence of the hepatitis B virus surface antigen (HBsAg) to complete its life cycle in human hepatocytes (1). This virus is primarily transmitted parenterally and can affect all age groups, although it is more common in intravenous drug users and other at-risk groups such as people living with HIV, men who have sex with men, and immigrants from endemic areas. It is distributed worldwide, albeit unevenly, with endemic areas having the highest prevalence: Mongolia, Vietnam, Pakistan, Japan, Taiwan, the Middle East, the Amazon basin, West Africa, the Mediterranean basin, Eastern Europe, Greenland, and the Pacific islands of Kiribati and Nauru (1–3). There are eight described genotypes that are distributed heterogeneously. Genotype 1, which is present worldwide, is predominant in Europe and North America. Genotype 3 is found mainly in South America, genotypes 2 and 4 in Asia, and genotypes 5 to 8 in Africa (1,2).

HDV infection can occur in two ways: simultaneously with the hepatitis B virus (HBV) in a naive patient (coinfection) or in a patient who is a chronic carrier of HBsAg (superinfection). Coinfection usually presents as acute hepatitis that resolves spontaneously in 90–95% of immunocompetent adults. By contrast, in cases of superinfection, the chronic hepatitis rate is between 70% and 90%. Chronic hepatitis due to HDV is the most severe form of viral hepatitis and carries the highest risk of complications (1,2,4). Treatment based on pegylated interferon alpha has been the only treatment available for the last 40 years despite its limited efficacy until the development of Bulevirtide, an inhibitor of viral entry into the hepatocyte which has become the first direct-acting antiviral drug against HDV to be marketed (2).

In industrialized countries, the introduction of the HBV vaccine has successfully reduced the prevalence of HDV infection in native populations. However, in recent years an increase in the number of cases of hepatitis Delta has been observed due to

immigration from endemic areas such as Eastern Europe and West Africa (2,4).

The real prevalence of HDV infection is still unknown due to the highly heterogeneous nature of studies conducted thus far, the lack of standardized screening methods, and the scarcity of resources for diagnosing this infection in many endemic areas (1,5). In recent years, three large meta-analyses have been published, and these have estimated the seroprevalence of HDV in chronic HBsAg carriers worldwide to be between 4.5% and 13.02% (12–72 million people) (6–8). In Spain, the latest seroprevalence study of vaccine-preventable diseases estimated a prevalence of 7.7% in this same group (9). However, the national prevalence remains uncertain because available studies provide partial data that may not reflect the true status of this infection in our country.

The main clinical practice guidelines for the management of hepatitis B in Europe and Spain recommend screening for hepatitis D in all chronic HBsAg carriers at least once in their lifetime (10–12). On the one hand, this strategy helps to determine the real prevalence of the infection and local epidemiology, which is essential for establishing the most appropriate HDV micro-elimination strategies for each population in order to achieve the World Health Organisation's goal of eliminating viral hepatitis by 2030. On the other hand, it also allows for earlier therapeutic interventions and thus minimizes complications arising from the disease (1,2).

The objective of this study is to determine the real prevalence of HDV infection in our setting.

Methods

A cross-sectional, observational study with prospective data collection on chronic HDV infection was conducted in the Autonomous Region of Galicia (Spain), which, according to the Galician Institute of Statistics, has a population of 2,705,833 inhabitants (https://www.ige.gal/igebdt/datos-basicos).

Study population. All adult patients who were HBsAg carriers and who attended any Galician Health Service hospital between 2023 and 2024 as part of routine clinical

practice to confirm the suspected diagnosis or to monitor a known disease were included in the study. Subsequently, clinical and epidemiological data (sex, age, country of origin, transmission route, hepatitis C virus (HCV) and human immunodeficiency virus (HIV) coinfections) were collected anonymously from patients who met the inclusion criteria.

Serological and molecular study. All patients who tested positive for HBsAg underwent a determination of total HDV-specific antibodies in the same serum sample (double reflex testing) using a chemiluminescent immunoassay (LIAISON® XL MUREX Anti-HDV Assay; Diasorin Saluggia, Italy). In those with a positive test, a quantitative real-time reverse transcription PCR (Hepatitis Delta Real Time PCR Kit; Vircell, Granada, Spain) was performed, after total nucleic acid extraction (EZ1® DSP Virus Kit; Qiagen, Hilden, Germany), to determine viral load and thus to differentiate past infection (undetectable viral load) from active infection (detectable viral load). This PCR technique has a limit of detection of 23 IU/mL (13). Subsequently, samples with detectable viral load, regardless of its value, were genotyped using a massive sequencing strategy based on amplicons. To this end, a specific PCR was performed with two pairs of overlapping primers that amplified the entire viral genome (14). These amplicons were then processed using *Illumina* technology and the NextSeq 1000 system. The resulting sequences were assembled using CLC-Genomics-Workbench software and the reference sequences of the eight genotypes obtained from the hepatitis Delta virus database (HDVdb; http://hdvdb.bio.wzw.tum.de/).

Statistical analysis: To evaluate the distribution of the "age" category, the Kolmogorov-Smirnov test was applied, with a result of $p < 0.05$, and hence the median and interquartile range (IQR) were chosen to be used to analyse patient ages. Furthermore, the Chi-square test was used to compare categorical variables, considering that differences are statistically significant when the value of $p < 0.05$. Comparative analyses between subgroups were included for seroprevalence by geographic origin, sex, and healthcare area. These analyses could not be performed for the prevalence of active infection due to insufficient statistical power because of the small sample size. Multivariate models were also not applied since the study focused on HDV prevalence, with few positive cases (n = 85), few coinfections, and limited information on

transmission routes. Also, insufficient clinical variables were collected to make this type of analysis possible. Statistical analysis of the data was performed using IBM SPSS Statistics software (version 30.0).

This study has been approved by the Santiago-Lugo Research Ethics Committee (CEIm code: 2023/109). The project was conducted in compliance with the 1964 World Medical Association Declaration of Helsinki, Royal Decree 1090/2015, December 24th, on clinical trials, and the Convention on Human Rights and Biomedicine, signed in Oviedo on April 4th, 1997.

Results

A total of 1,962 HBsAg carriers were included in the study, of whom 85 had antibodies against HDV, representing a seroprevalence of 4.3% in HBsAg carriers and 0.03 per 1,000 inhabitants. Of the 85 patients with antibodies against HDV, 20 (23.5%) had a detectable viral load and thus had an active HDV infection. No statistically significant differences in antibody prevalence were found between native and immigrant patients or between health areas. However, significant differences were indeed found between the prevalence in men and women, with a higher occurrence observed in the male population.

Patients with HDV antibodies were predominantly men (66/85), of Spanish origin (54/85), and had a mean age of 56 years (IQR: 23-86). The transmission route was unknown for 62 patients. In cases where the transmission route was known, parenteral transmission was the most common (18/23). Cases of sexual (2/23) and vertical (3/23) transmission were also found. Regarding coinfections, 25.9% (22/85) were infected with HIV and 32.9% (28/85) with HCV.

In active infections, the mean age was 51 years (IQR: 25-65), and 80% (16/20) were men. The results, as broken down by health area, are set out in Table 1.

Of the samples with a detectable viral load, a total of 17 could be sequenced. The remaining three samples could not be analysed because the sequences obtained were of poor quality, this likely due to the viral load being too low. 82.3% (14/17) of the sequenced strains belonged to genotype 1D. The countries of origin of these patients

were Spain (11/14) and Romania (3/14). The other three samples analysed were classified as genotypes 5A (1/3) and 5B (2/3), and all corresponded to individuals from West African countries. The patient with genotype 5A was a 60-year-old male coinfected with HIV from Guinea-Bissau. The other two patients were also male, aged 25 and 35, from Mali and Senegal, respectively, and neither of them had any coinfections. In none of these cases was the transmission route known with certainty.

Discussion

The real prevalence of HDV remains unknown, despite it being the cause of the most severe chronic viral hepatitis and carrying a higher risk of complications such as cirrhosis, hepatic decompensation, or hepatocellular carcinoma (1).

In recent years, it has been observed that in developed countries similar to Spain, HDV seroprevalence in the native population is declining due to universal HBV vaccination and, indirectly, due also to improvements in prevention measures against parenterally transmitted viruses (15,16). Previous studies conducted in our region confirm this trend when comparing data from before and after the incorporation of the HBV vaccine into the national vaccination schedule (17–19). The present study confirms that the prevalence in our region remains low, less than 5%. However, no significant differences were found compared to data published in recent years (17,20,21). This fact could be explained by the trend currently being witnessed throughout Europe: the aging of native patients, many of whom were infected in the 1980s and 1990s, and the arrival of new infected patients from endemic areas, such as Eastern Europe and West Africa (15,16). Such a hypothesis would explain why the prevalence has remained stable in recent years rather than continuing a downward trend.

Regarding other studies similar to ours conducted in Spain, it can be seen that the estimated prevalence in all of these is similar to that obtained for our population, despite the specific demographic differences in each area that can affect, among other factors, migratory flows (22–24). Considering the results obtained in all these studies conducted in recent years in very different geographical areas of Spain, it could be inferred that the prevalence of HDV in our country among HBsAg carriers is currently

between 4% and 6%, lower than the estimate in the last national seroprevalence study (9). However, a national multicentre study would be necessary to confirm this hypothesis.

As expected, in our setting, the majority of patients with active HDV infection are infected with genotype 1 strains, which is the most prevalent genotype worldwide and also in Europe (1,2). Regarding those patients from endemic areas, we found that individuals from Eastern Europe, specifically Romania, were also classified as genotype 1. This same finding has been reported in other studies in countries similar to ours, such as Italy, which included Romanian patients, and which serves to confirm that the predominant genotype in Europe is genotype 1 (25). On the other hand, we had three patients from West Africa who were infected with strains belonging to genotype 5. This genotype, like genotypes 6 to 8, is usually found in Africa or in patients originating from this continent, so our finding here is in line with expectations (1,2).

Incorporating HDV screening into routine testing as part of a one-step diagnostic approach (double reflex testing) has proved to be a beneficial strategy, in that it allows for expanded diagnostic coverage of this virus (22). Although the prevalence of HDV infection is low in our setting, a significant proportion of those affected have a detectable viral load (23.5%), which may be even higher since there may be an underestimation due to cases with viral loads below the limit of detection of the PCR technique. Patients with active infection would benefit greatly from the new treatments available to prevent disease progression and associated complications, which not only impact the patient positively but also the healthcare system in general due to the long-term costs involved. At this point, microbiological diagnosis is essential, since the clinical presentation of HDV and HBV infection can easily be confused with HBV monoinfection (1). Furthermore, understanding the local epidemiology is essential to implement the best programs for eliminating this hepatitis. Such an approach is not possible without implementing HDV screening in routine clinical practice, as recommended by most hepatitis B management guidelines (10,12,26). In short, double reflex testing is a strategy that has proved cost-effective in low-prevalence settings where new, specific HDV treatments are also available, as is the case in our population (22).

This study has not only provided insights into the current prevalence of HDV in our setting, confirming its low circulation, but has also been a turning point in the diagnosis of HDV infection in Galicia, in that it has facilitated the implementation of one-step double reflex testing as part of routine clinical practice in all microbiology laboratories of the Galician Health Service. Furthermore, this is the first prospective study at a national level that involves all hospitals in an autonomous region, which constitutes a significant milestone in HDV epidemiological surveillance. It is also worth noting that not only antibody prevalence but also active infection prevalence was analysed. Therefore, the results reported here provide a representative picture of how this virus behaves in our population, which will allow us to establish the most appropriate strategies for eliminating this hepatitis.

Key points

KEY POINTS

- Chronic hepatitis Delta is the most severe form of viral hepatitis and carries the greatest risk of complications.
- The real prevalence of the hepatitis Delta infection remains unknown.
- Bulevirtide is the first direct-acting antiviral drug against HDV being approved in Europe.
- The seroprevalence of HDV in chronic HBsAg carriers in Galicia is 4.3% and almost a quarter of those affected have an active infection.
- The implementation of the double reflex testing of VHD in microbiology laboratories has been shown to facilitate earlier diagnosis, thus enabling more efficient management of the infection.
- This study provides real data on the prevalence of VHD in an entire autonomous region for the first time in Spain and has enabled the integration of double reflex testing in all Microbiology Departments of the Galician Health Service.

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Table 1. HDV infection prevalence results and analysis of the main epidemiological data in the seven health areas belonging to the Galician Health Service.

	Coruña-Cee	**Ferrol**	**Lugo- A Mariña-Monforte de Lemos**	**Ourense-Verín-O Barco de Valdeorras**	**Pontevedra-O Salnés**	**Santiago-Barbanza**	**Vigo**	**TOTAL**
Total HBsAg carriers, n	408	138	157	181	290	413	375	**1962**
Anti-HDV prevalence, n (%)	25 (6,1)	8 (5,8)	4 (2,5)	9 (5)	11 (3,8)	15 (3,6)	13 (3,5)	**85 (4,3)**
Median age, years (IQR)	54 (37-86)	59 (43-65)	49,5 (47-60)	58 (35-72)	58 (46-65)	58 (25-78)	53 (27-79)	**56 (23-86)**
Sex, n (%)								
Man	19 (76)	8 (100)	3 (75)	8 (88,9)	9 (81,8)	10 (66,7)	9 (69,2)	**66 (77,6)**
Woman	6 (24)	0	1 (25)	1 (11,1)	2 (18,2)	5 (33,3)	4 (30,8)	**19 (22,4)**
Origin, n (%)								
Native	19 (76)	7 (87,5)	1 (25)	6 (66,7)	5 (45,5)	10 (66,7)	7 (53,8)	**54 (63,5)**
Immigrant	6 (24)	1 (12,5)	3 (75)	3 (33,3)	6 (54,5)	5 (33,3)	6 (46,2)	**31 (36,5)**
Active infection prevalence, n (%)	7 (28)	2 (25)	3 (75)	3 (33,3)	0	4 (26,7)	1 (7,7)	**20 (23,5)**
Median age, years (IQR)	51 (40-65)	54 (43-54)	51 (48-60)	37 (35-55)	0	59,5 (25-65)	32	**51 (25-65)**
Sex, n (%)								
Man	5 (71,4)	2 (100)	2 (66,7)	3 (100)	0	4 (100)	0	**16 (80)**
Woman	2 (28,6)	0	1 (33,3)	0	0	0	1 (100)	**4 (20)**
Origin, n (%)								
Native	5 (71,4)	2 (100)	1 (33,3)	1 (33,3)	0	3 (75)	0	**12 (60)**
Immigrant	2 (28,6)	0	2 (66,7)	2 (66,7)	0	1 (25)	1 (100)	**8 (40)**

Anti-HDV: total antibodies against hepatitis Delta virus; HBsAg: hepatitis B surface antigen; IQR: interquartile range