OCTOBER 11-12, 2024 MILAN, ITALY — DeltaCure 3rd International Meeting

P11

Poster for you

Is there a Hepatitis Delta Virus variant in West Africa, not detected by current serological diagnostic tests?

Poussyina Hamouda [1], Athenaïs Gerber [1,2], Bruce Wembulua Shinga [3], Valérian Delagarde [1,2], Adrià Ramirez Mena [3], Ndeye Fatou Ngom [3], Ousseynou Ndiaye [3], Emmanuel Gordien[1,2], Ségolène Brichler [1,2], Gilles Wandeler [3], Moussa Seydi [3,], Frédéric Le Gal [1,2,*]*

(1) Centre national de référence des Hépatites virales B, C et Delta, Laboratoire de Microbiologie clinique, Hôpital Avicenne, Assistance Publique Hôpitaux de Paris, Sorbonne Université, Paris Cité, Bobigny, France. (2) INSERM, U955 équipe 18, Créteil, France (3) Service des Maladies Infectieuses et Tropicales, Centre Hospitalier Universitaire de Fann, Dakar, Sénégal.

Introduction

- **HDV infects around 5% of HBs antigen (HBsAg)-positive patients worldwide**, worsening the prognosis of the liver disease. However, the HDV prevalence is highly variable at regional or country level; in West African countries, HDV prevalence ranges from 2% to 30% [1] (**figure 1**).
- For the **diagnosis of HDV infection,** WHO recommends the detection of anti-HDV antibodies (HDV-Ab) by serological tests in all HBsAg-positive patients. For those with positive HDV-Ab, the virological profile must be completed with the measurement of HDV RNA in order to determine active viral replication (**figure 2**).

Figure 2 **HDV Diagnosis flowchart according to WHO recommendations**

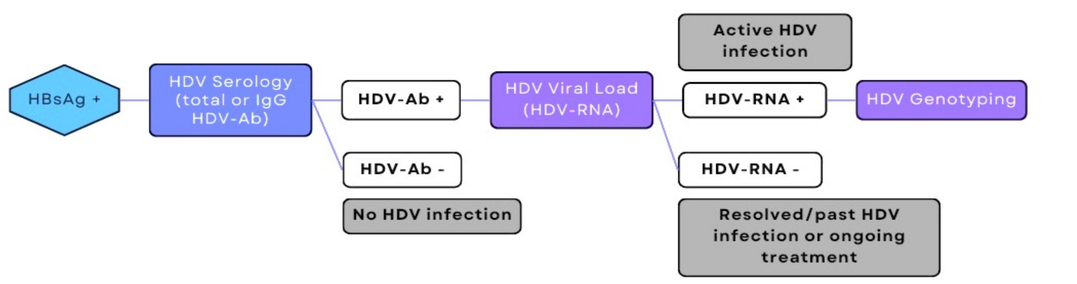
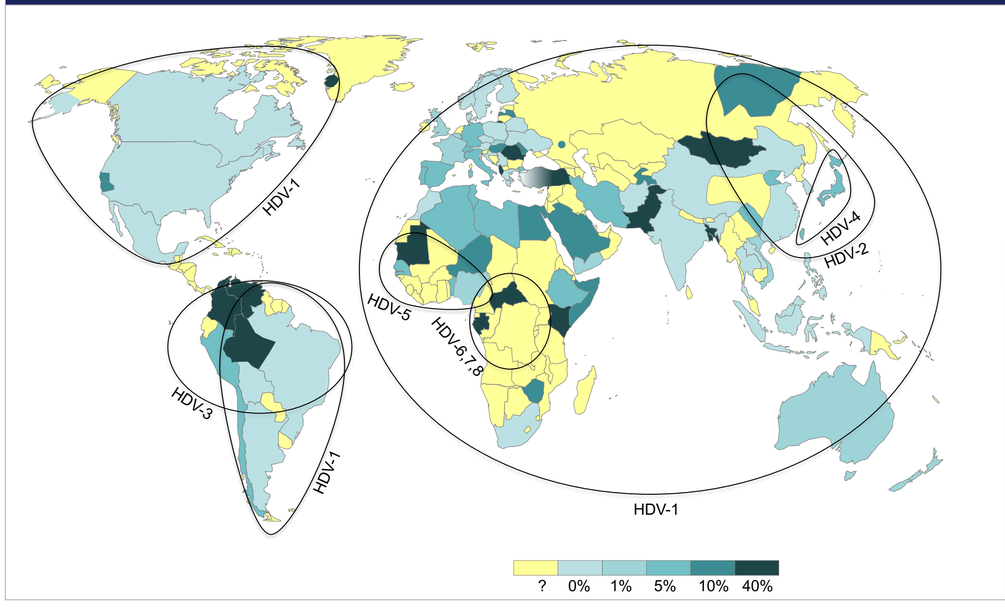


Figure 1 **HDV prevalence and species worldwide**



- This pipeline is challenged by the **high variability of HDV**, with 8 different species and several subtypes for each species. In West Africa, HDV-1 and HDV-5 are predominant.
- According to current knowledge, commercial serological tests detect all HDV species with good sensitivity and specificity. Pangenotypic molecular diagnostic tests are also available, even if some other kits have more questionable performances, depending on suppliers and species [2,3] .

Figure 3 **Map of Senegal**

- Last year, a study described the presence of HDV RNA in several **Gambian patients** with negative HDV-Ab [4]. Although these results suggested false-negative serological results due to a possible HDV variant, a contamination during amplification could not be formally excluded.
- At the same period, we conducted an epidemiological study of HBV and HDV in **Senegal** (SEN-B), a neighboring country of the Gambia (**figure 3**).
- **Therefore, we aimed to investigate the hypothesis of a possible HDV variant in the SEN-B cohort.**

Material and methods

COHORT

- **SEN-B enrolled 914 HBsAg-positive patients**, diagnosed between 2019 and 2023 and followed at Dakar Fann University Hospital (Senegal).
- The median age was 32 years, and 487 (53%) were male (table 1).
- **HDV-Ab were found in 13 / 914 individuals (1.4%)**, using the Liaison®XL Murex Anti-HDV assay (Diasorin). HDV-RNA extraction was realized with the m2000sp automatic device (Abbott Diagnostics) from 500µL of plasma, allowing a high sensitivity of detection combined with RT-qPCR with EurobioPlex EBX-071® kit (Eurobio). **More than 60% replicate HDV** and species **HDV-1, 5 and 7** were identified [5].

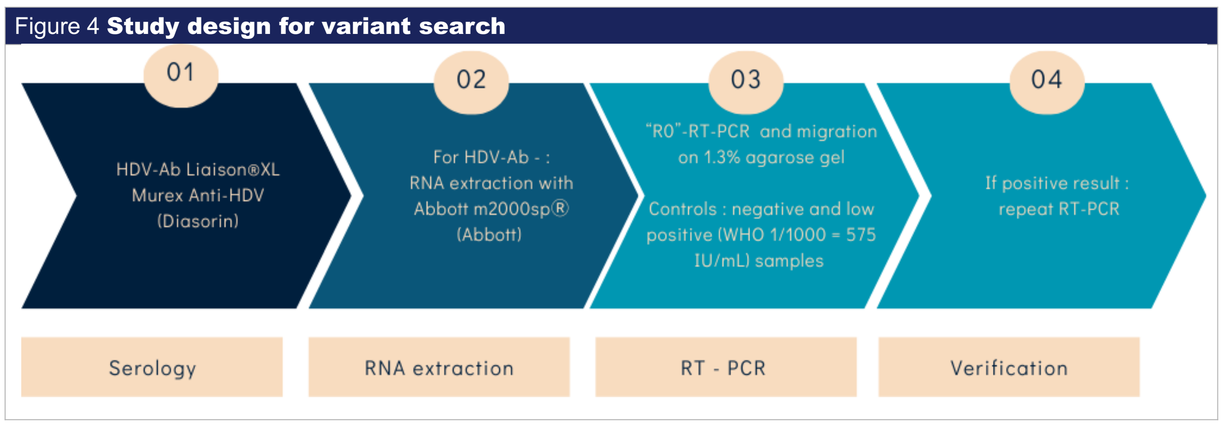
Table 1 **Characteristics of the study participants by HDV-Ab status**

Characteristics	HDV-Ab negative **n = 901**	HDV-Ab positive n = 13	p-value
Age (years)	32 (26 - 41)	36 (27 - 40)	0.50
Male sex	479 (53.2)	8 (61.5)	0.50
HDV-RNA +	0	8 (61.5)	-
HDV species	-	HDV-1 (n=1), HDV-5 (n=6) and HDV-7 (n=1)	-

STUDY FLOWCHART

- **We evaluated the presence of HDV RNA in the samples of the remaining 901 HDV-Ab negative individuals**. We performed the conventional "*R0*"-RT-PCR as previously developed in our lab, using pan-genotypic primers defined in the most conserved region of the HDV genome, able to detect all known HDV-species. The sensitivity of our assay is around 20 IU/mL, according to the WHO-HDV standard.
- In addition, in each amplification series, we included both a negative control and a low positive control to confirm the efficacy, the sensitivity as well as the absence of contamination over the amplification process (figure 4).

Figure 4 **Study design for variant search**



Results and discussion

- **No HDV-RNA positive sample in the 901 HDV-Ab negative samples**
- **There is no evidence that HDV variants, undetectable by current serological assays, circulate in this part of Africa**
- **Our results strengthen our position in favor of a systematic screening of HDV infection with a double reflex testing strategy:**
  - **I. serological HDV-Ab screening in all HBV-positive people**
  - **II. followed by the quantification of HDV-RNA by RT-PCR to determine HDV replication among those with positive HDV-Ab**

References

1. Stockdale AJ, Chaponda M, Beloukas A, Phillips RO, Matthews PC, Papadimitropoulos A, et al. Prevalence of hepatitis D virus infection in sub-Saharan Africa: a systematic review and meta-analysis. The Lancet Global Health. 2017 Oct 1;5(10):e992–1003
2. Rocco C, Bonavolta R, Vallefuoco L, Braschi U, Sorrentino R, Terracciano D, Portella G (2019) Comparison of anti-hepatitis D virus (HDV) ETI-AB-DELTAK-2 assay and the novel LIAISON® XL MUREX anti-HDV assay in the diagnosis of HDV infection. Diagn Microbiol Infect Dis.
3. Brichler S, Le Gal F, Neri-Pinto F, Mansour W, Roulot D, Laperche S, Gordien E (2014). Serological and Molecular Diagnosis of Hepatitis Delta Virus Infection: Results of a French National Quality Control Study. Journal of Clinical Microbiology
4. Chemin I., Ceesay A. H. ,Pujol F., Now G., Dubois A., Heintz S., Vo Quang E., Shimakawa Y., Thursz M., Zoulim F., Ingiliz P., Urban S., Testoni B., Lemoine M., (2023). HDV prevalence and genotypes in the Gambia: presence of active infection despite serological negativity. International DeltaCure meeting, poster 20
5. Shinga Wembulua B., LeGal F., Ndiaye O., Sandrine Pandi M., Messan Akotia H., Sidick Badiane A., Hamouda P., Larboulette M.H., Tine J., Ndiaye K., Béguelin C., Ngom N.F., Wandeler G., Seydi M., and Ramírez Mena A. (2024) Hepatitis delta and liver disease among people living with hepatitis B with or without HIV co-infection in Senegal: a cross sectional study. (submission in progress)

Contact
frederic.legal@aphp.fr

HDV reference centre Avicenne Hospital — Assistance Publique Hôpitaux de Paris — SEN-B — Swiss National Science Foundation — SMIT Service des Maladies Infectieuses et Tropicales CHNU Fann-Dakar — CTA OPALS Centre de Traitement Ambulatoire — CRCF — Université de Berne — Inselgruppe