Adeno-associated Virus 2 Infection in Children with non-A-E Hepatitis

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Introduction

- An outbreak of acute hepatitis of unknown aetiology in children was first reported in Scotland in April 2022[1]. Cases aged <16 years have since been identified in 35 countries[2].
- Adenovirus (HAdV) was identified by PCR in 53.1% of tested cases.
- A spike in HAdV infections in Scotland predominantly affecting children <5 years also directly preceded the outbreak of this unexplained hepatitis

- We hypothesize that HAdV may be directly implicated in the pathology of non-A-E hepatitis in children.
- Here, we report a detailed investigation of 9 early cases and 25 control subjects.

Methods

3 comparison groups were created to test the hypothesis:

Case group	9 of the earliest affected children who presented to the hospital with hepatitis of unknown origin between 14 March and 4 April 2022
Control Group 1	13 age-matched healthy children (10 male, 3 female; age range 3-5 years)
Control Group 2	12 children (8 male, 4 female; age range 1-4 years) with PCR-confirmed HAdV infection and normal transaminases

Metagenomic and target enrichment sequencing, and real-time PCR of all available clinical samples (plasma and liver biopsies) were done for all 3 groups to look for both DNA and RNA viruses.

Results

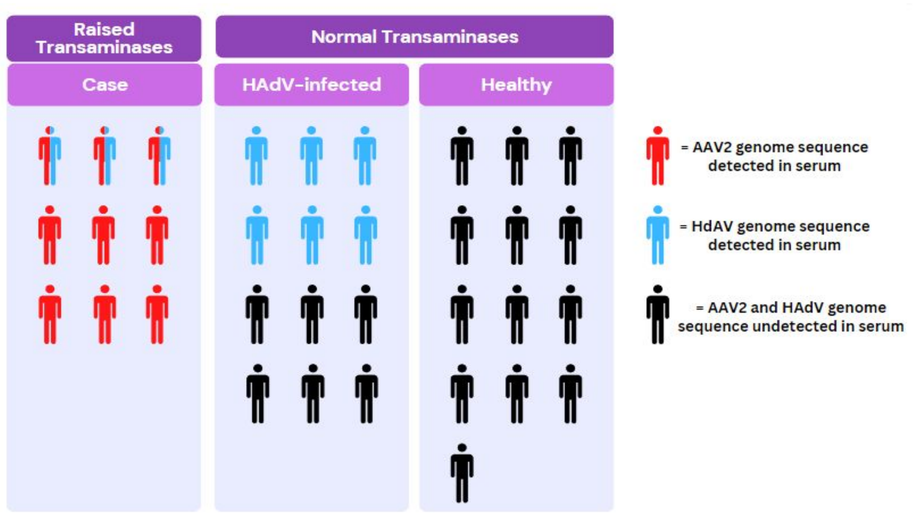

Figure 1. Pictorial diagram of HAdV and AAV-2 genome detected in thee serum of all 3 comparison groups through sequencing

- Fig 1 shows that adeno-associated virus 2 (AAV-2), a parvovirus dependovirus, was detected in 9/9 serum case samples and 4/4 liver case biopsies, but was not detected in control groups.
- HAdV was detected in 3/9 serum case samples and 3/4 liver case biopsies. HAdV was not detected in Control Group 1 (labelled Healthy).

Results

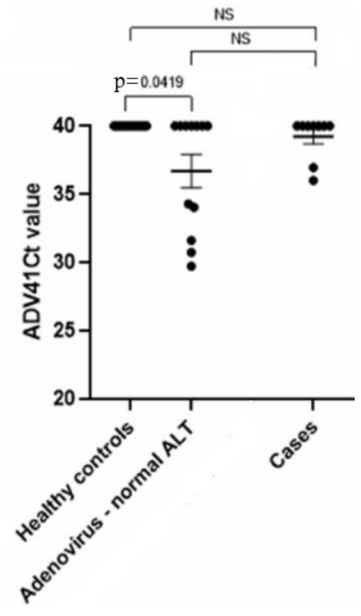

Figure 2. HAdV real-time PCR in cases vs controls

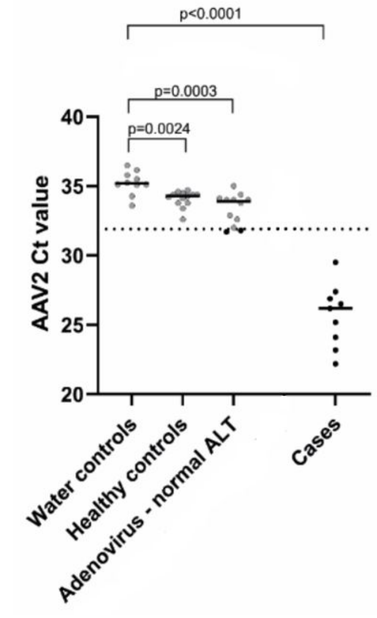

Figure 3. AAV2 real-time PCR in cases vs controls, the estimated detection limit of the assay (Ct=31.9) is shown (dotted line)

- Fig. 2 shows that HAdV Ct values between the Case group and either control groups were not statistically significant according to the Mann-Whitney test.
- Fig. 3 shows that AAV2 Ct values were significantly different between all 3 groups according to the Mann-Whitney test.

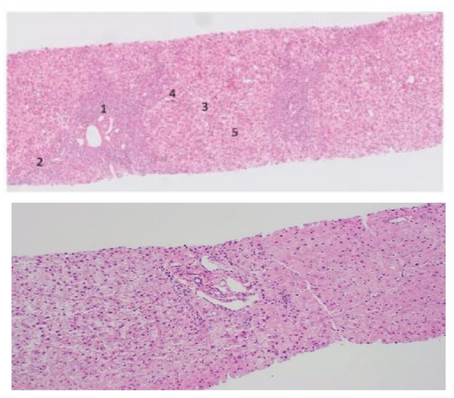

Figure 4. histology of liver biopsies (top) in Scottish children with AAV2 and HAdV-associated hepatitis, and (below) with normal liver tissue[3]

Fig. 4 (top) shows liver parenchyma in children with AAV2 and HAdV-associated hepatitis, present is:

1. Interface hepatitis
2. Feathering (ballooning) change of hepatocytes
3. Apoptotic hepatocytes
4. Mild steatosis present in some zone 2 hepatocytes

Discussion & Conclusion

- AAV2 genome detected independently by sequencing and by real-time PCR was present at significantly higher frequencies (9/9) in cases of paediatric non-A-E hepatitis than controls
- Rather, HAdV was detected at lower levels in the Case group (3/9)
- Dependoparoviruses (AAV2) rely on coinfection with a helper virus for replication, most commonly HAdV or a herpesvirus.
- This suggests the hypothesis of AAV2 co-infection with HAdV as the pathology of non-A-E hepatitis in children, rather than sole HAdV impact as proposed in our initial hypothesis

- Limitation: the presence of AAV2 could be an association with hepatitis and may not represent direct aetiology; rather AAV2 may be a biomarker of recent HAdV infection
- Future work: Larger case-control studies to investigate the role of AAV2 and HAdV in the aetiology of unexplained non-A-E paediatric hepatitis

Acknowledgements & References

We would like to thank Prof. Emma Thomson et al. for conducting the research and Prof. Tan Boon Huan for her kind guidance in crafting this poster.

1. Marsh, K. et al. Investigation into cases of hepatitis of unknown aetiology among young children, Scotland, 1 January 2022 to 12 April 2022. *Euro Surveill* 27, doi:10.2807/1560-7917.ES.2022.27.15.2200318 (2022).
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3. Kothari, Shana & Kalinowski, Michael & Shah, Natasha & Raddawi, Hareth.A Case of Idiopathic Non-Cirrhotic Portal Hypertension in a Patient With a Left Ventricular Assist Device. Journal of Investigative Medicine High Impact Case Reports. (2019).