Fecal virome in decompensated liver cirrhosis patients

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INTRODUCTION

The gut virome is the viral part of the gut microbiome and is composed of **viruses infecting eukaryotic cells** (e.g. **human** and **plants**) and **viruses infecting bacteria (phages)**. Over the past decade, these **phages** have gained interest as mediators of human health and disease because of their interactions with the gut bacteria and possible effect on the immune system. In this study, the gut virome is characterized in patients with decompensated liver cirrhosis, a disease in which the gut microbiome contributes to disease progression but where the role of the gut virome is yet to be elucidated.

METHODS

MUCOSA-PREDICT cohort
- 93 decompensated liver cirrhosis patients
- 309 fecal samples*

NetoVIR protocol
- viral enrichment
  - centrifugation
  - filtration
  - nuclease treatment
- DNA & RNA extraction
- random amplification
- library preparation
- Illumina sequencing

Bioinformatic processing
- read trimming
- per sample *de novo* assembly (> 1 kb)
- cross-sample clustering (95% ID – 85% cov)
- viral identification (n = 41,284)**
- abundance & normalisation (70% coverage)

* 292 samples remained after normalisation

** **eukaryotic viruses**: protein homology (DIAMOND) **or** nucleotide homology (BLASTn) with CheckV completeness estimate
phages: Virsorter2 signal with CheckV completeness estimate **or** *Inoviridae* protein homology (DIAMOND)

RESULTS

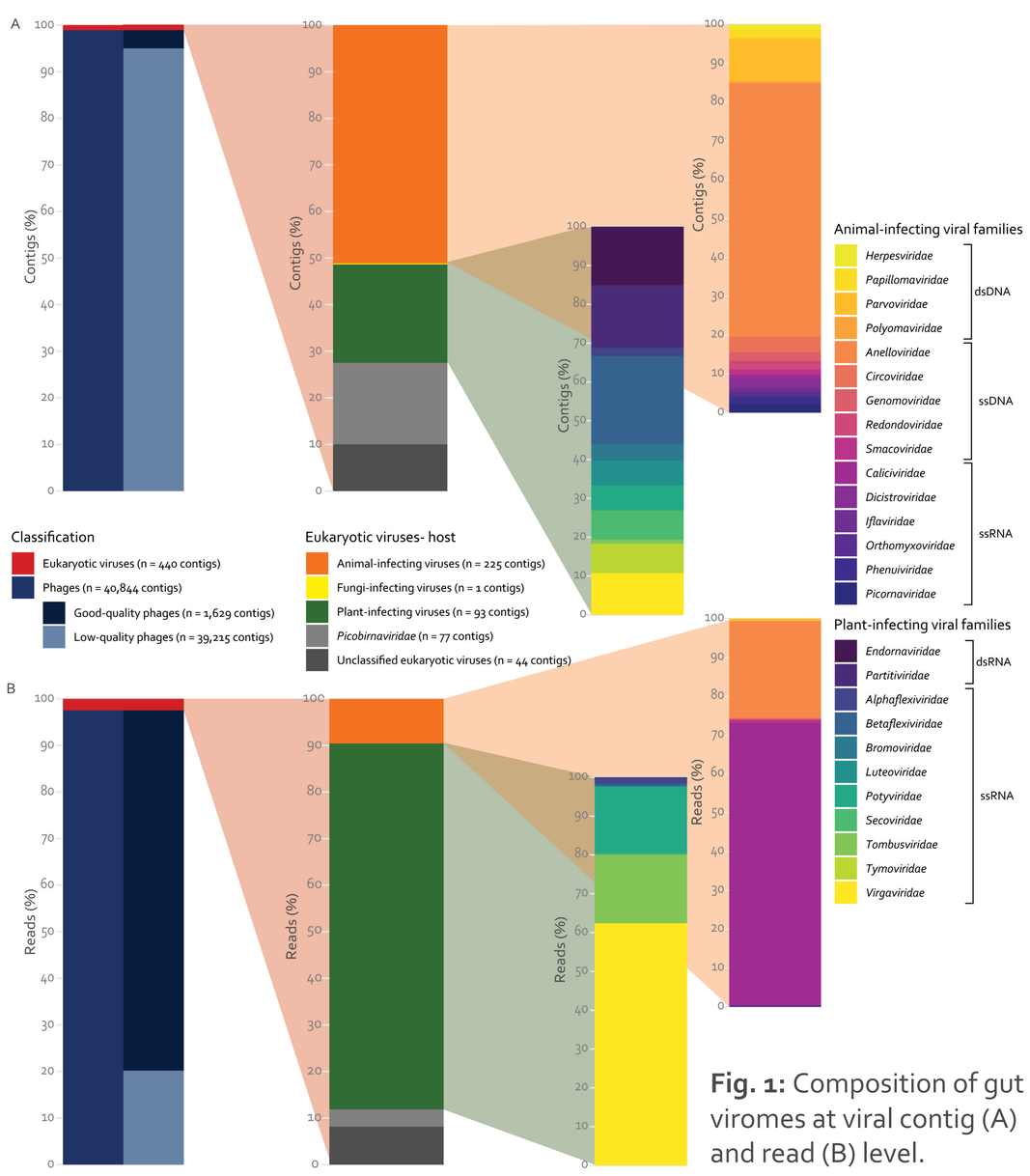

Fig. 1: Composition of gut viromes at viral contig (A) and read (B) level.

EUKARYOTIC VIROME

Only 440 viral contigs (1.1%; **Fig. 1A**) represent eukaryotic viruses, making up 2.5% of all viral reads (**Fig. 1B**). Most eukaryotic viral contigs belong to the ***Anelloviridae*** viral family (147 contigs; **Fig. 1A**), a family of viruses with a small circular ssDNA genome, considered to be widely prevalent and not (yet) associated with disease. In one sample, up to 18 different ***Anelloviridae*** species are observed (**Fig. 2B**), whereas most samples do not contain any eukaryotic viruses. ***Peper mild mottle virus*** (*Virgaviridae* family) is the most prevalent eukaryotic viral species (28%), in contrast to the majority of eukaryotic viral species which are sample-specific (**Fig. 2A**). **Eukaryotic viruses** generally represent only a small fraction of the viral reads (IQR: 0 – 0.19%). Only a limited number of samples show high relative abundances of **plant-infecting viral families** (**Fig. 2C**), along with one sample being dominated by an **animal-infecting virus**, a *Norwalk virus* (*Caliciviridae* family), a known cause of gastrointestinal disease.

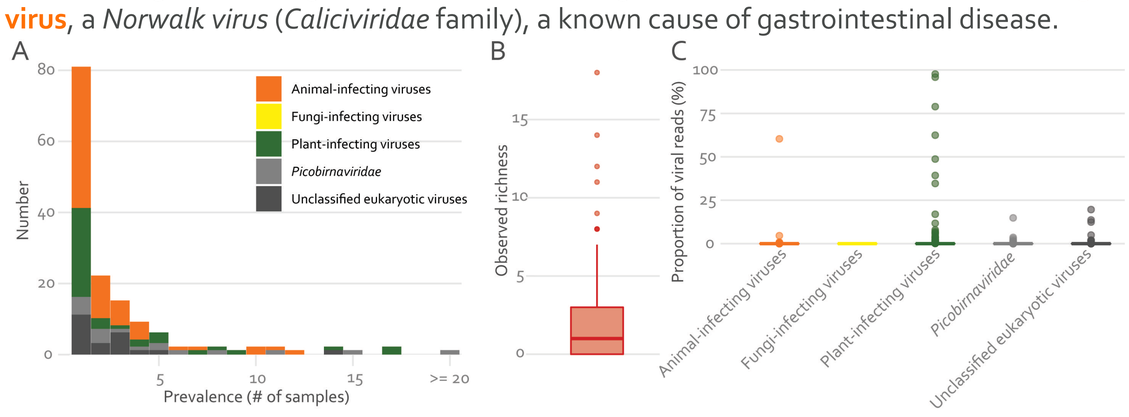

Fig. 2: Prevalence (A), diversity (B) and relative abundance (C) of eukaryotic viral species.

PHAGEOME

For phages, taxonomical classifications are often unavailable, hence analyses at species level are not possible. To avoid the bias that comes with including multiple fragments from the same phage genome in the downstream analysis, only **good-quality phages** representing more than 50% of a phage genome are included. Although this is only a small fraction of the phage contigs (4.0%; **Fig. 1A**), they dominate the phageomes (79.4% phage reads; **Fig. 1B**) and viromes in general (IQR: 66.9 – 95.5% viral reads; **Fig. 3C**). A median of 17 **good-quality phages** are observed per sample (**Fig. 3B**). Although the majority of **good-quality phages** remains specific to a single sample, a larger fraction of contigs is shared in more than 10 subjects (67/1,629 (4.1%) **good-quality phages** vs. 10/440 (2.3%) **eukaryotic viral species**; **Fig. 3A**). The most prevalent **good-quality phage**, a *Skunavirus* (*Siphoviridae* family), is present in 94 samples (32%).

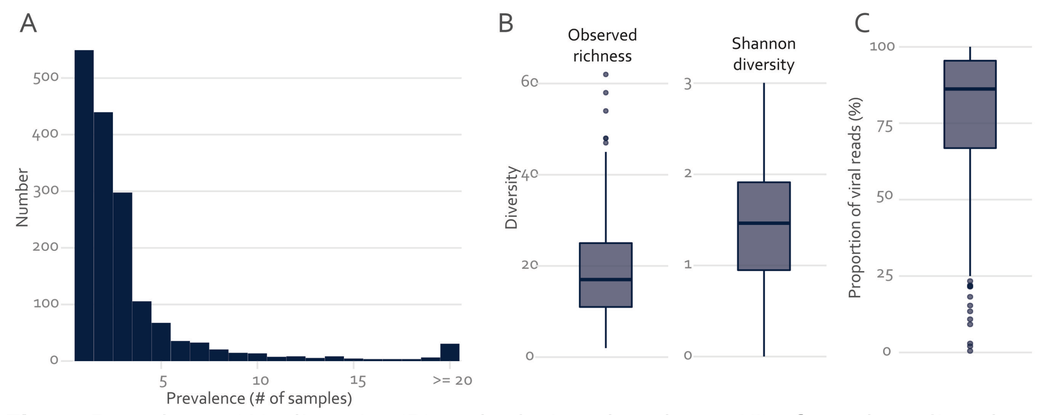

Fig. 3: Prevalence (A), diversity (B) and relative abundance (C) of good-quality phages.

CONCLUSION

In general, the gut **phageome** is not only more abundant, but also more diverse than the **eukaryotic virome**. The general low-abundance, sparsity and diversity of **eukaryotic viruses** in the human gut virome makes them unsuitable candidates for biomarkers. However, the presence of **human-infecting viruses** in certain individuals might influence disease. On the other hand, highly prevalent and abundant **phages** might be valuable biomarkers for disease. Identification of such biomarkers by associating the virome data with clinical metadata and other –omics are ongoing, along with predicting the bacterial hosts of these **phages**. The potential role of **phages** on progression of chronic liver disease is currently still unclear, but this study will gain new insights into the pathophysiology of decompensated liver cirrhosis. This can potentially result in new applications for diagnosis, therapy and/or monitoring of decompensated liver cirrhosis.

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