- **HEV is highly resistant to inactivation by alcohols and commercially available alcohol-based disinfectants**
- **Ethanol disrupts the quasi-envelope of HEV while leaving the naked virion intact**
- **Phosphoric acid is an important factor rendering anti-HEV activity**

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Hepatitis E virus is highly resistant to alcohol-based disinfectants

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1 Introduction

The Hepatitis E virus (HEV) is the most common cause of acute viral hepatitis worldwide and mainly transmitted via the fecal-oral route or consumption of contaminated food products. Due to the lack of efficient cell culture systems for the propagation of HEV, limited data regarding HEV sensitivity to chemical disinfectants are available. Consequently, preventive and evidence-based hygienic guidelines on HEV disinfection are lacking.

2 Aim

In this study we evaluated different principal components of hand disinfectants as well as commercial hand disinfectants for their virucidal activity against HEV using a recently described high titer cell culture HEV model.

3 Method

We used a robust HEV genotype 3 cell culture model which allows quantification of viral infection of quasi-enveloped and naked HEV particles. For HEV genotype 1 infections the primary isolate Sar55 in a faecal suspension was applied. Standardized quantitative suspension tests using end point dilution and large-volume-plating were performed for the determination of virucidal activity of alcohols (1-propanol, 2- propanol, ethanol), WHO disinfectant formulations and five different commercial hand disinfectants against HEV. Iodixanol gradients were conducted to elucidate the influence of ethanol on quasi-enveloped viral particles

5 Conclusions

Different alcohols and alcohol-based hand disinfectants were insufficient to eliminate HEV infectivity with the exception of one commercially ethanol-based product which including phosphoric acid. These findings have strong implications for the efficient prevention measures to reduce viral transmission in clinical practice

6 Acknowledgements

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4 Results

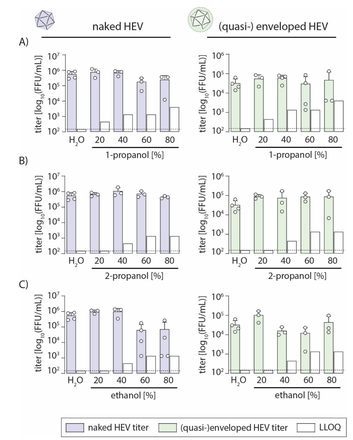

Figure 1. Suspension test of alcohols against HEV gt3
Cell-culture derived naked (left) and quasi-enveloped virus particles (right) were used in a standard suspension test to evaluate the virucidal activity of 1-propanol (A), 2-propanol (B) and ethanol (C) at different concentrations. Sterile water was used as negative control. Coloured bars represent infectious titer, white bars represent the lower-limit of quantification (LLOQ), the dotted line represents the detection limit of the assay (n=3-4, means ± STD).

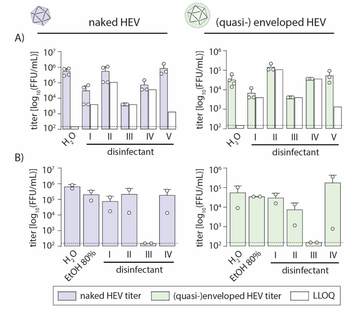

Figure 4. Suspension test of commercially available hand disinfectants
Different commercial hand disinfectants were tested in the standard suspension test with end point dilution (A) or large-volume plating assay (B) using cell-culture derived naked (left) and quasi-enveloped virus particles (right). Sterile water was used as negative control. Coloured bars represent infectious titer, white bars the lower-limit of quantification (LLOQ) and the dotted line the detection limit of the assay (n=2-4, means ± STD).).

formulations	main ingredients	changes to general formulation
VP-2069	*ethanol (50-60%); 1-propanol (9-11%)*	***left out*: butandiol-1,3; propylene glycol;**
VP-2070	*ethanol (50-60%)*	***left out*: 1-propanol;**
VP-2071	*ethanol (50-60%); 1-propanol (9-11%)*	**no changes**
VP-2072	*ethanol (50-60%); 1-propanol (9-11%)*	**Left out: phosphoric acid;**

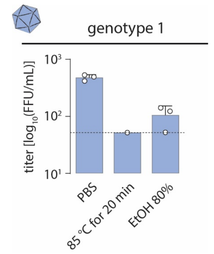

Figure 2. Large-volume plating assay of ethanol against HEV gt1
HEV primary isolate gt1 strain Sar-55 stool suspension was used in a large-volume plating assay to evaluate the virucidal activity of ethanol. Sterile PBS served as negative control; heat inactivation for 20 min at 85°C was used for total inactivation. Coloured bars represent infectious titer, the dotted line represents the detection limit of the assay (n=3, means ± STD).

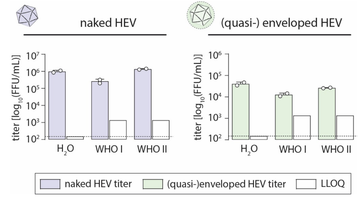

Figure 3. Virucidal activity of modified WHO formulations against HEV
Cell-culture derived naked (left) and quasi-enveloped virus particles (right) was used in a standard suspension test (end point dilution) to evaluate the virucidal activity of modified WHO formulations. Sterile water was used as negative control (n=2, means ± STD).

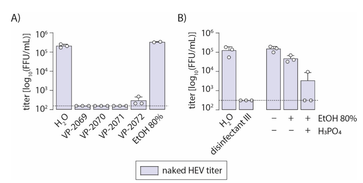

Figure 5. Large-volume plating assay of different formulations of disinfectant III
Different formulations (VP, for specific see table below) of disinfectant III were tested in a large-volume plating assay using cell-culture derived naked virus (A). Sterile water and ethanol (EtOH 80% final concentration) were used as controls. To evaluate the synergistic effect of ethanol and phosphoric acid in disinfectant III, both parts were tested separately or in combination (B). Coloured bars represent infectious titer, the dotted line represents the detection limit of the assay (n=3, means ± STD).

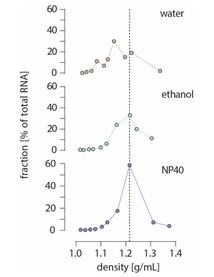

Figure 6. Ethanol disrupts the quasi-envelope structure of HEV
Cell-culture derived quasi-enveloped virus particles were treated in the quantitative suspension test for 30 seconds with addition of ethanol (80% final concentration). After 30 seconds of incubation, the mixture was diluted with medium and subjected to an iodixanol gradient centrifugation. Ten fractions were harvested from the bottom and HEV RNA content was determined for each fraction.

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