

Low Prevalence of Hepatitis E Viral RNA in Retail Pig Livers, Pig Blood Curds, Pig Intestines, Lambs and Oysters in Hong Kong

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Background

- Hepatitis E virus (HEV) is increasingly recognized as a cause of foodborne hepatitis.
- Phylogenetic analysis reveals close genetic relatedness of HEV between isolates from clinical cases and infected pigs, suggesting zoonosis as a probable source of human HEV infections.

Aim

- To evaluate the prevalence of HEV in high-risk food items known to harbor the virus in Hong Kong.

Methods

- Five high-risk food items were investigated: **pig livers, pig blood curds, pig intestines, lambs and oysters.**
- All samples were purchased from local retail points between April 2014 and March 2015, inclusively, on a biweekly basis.
- Samples (2 g for oysters and 250 mg for other food types) were homogenized in TRIzol reagent using Precellys Minilys and viral RNA was extracted and purified using QIAamp Viral RNA Mini kit.
- Viral RNA detection was performed using a widely used and broadly reactive quantitative reverse-transcription—polymerase chain reaction (RT-qPCR) assay targeting open reading frame 3 (Jothikumar et al 2006).
- All samples were spiked with an exogenous RNA (TATAA Biocenter) during extraction. A subset of samples were randomly selected for spike RNA detection according to acceptance sampling standard MIL-STD-105.
- Prevalence was determined and 95% confidence level was estimated using binomial exact calculation.

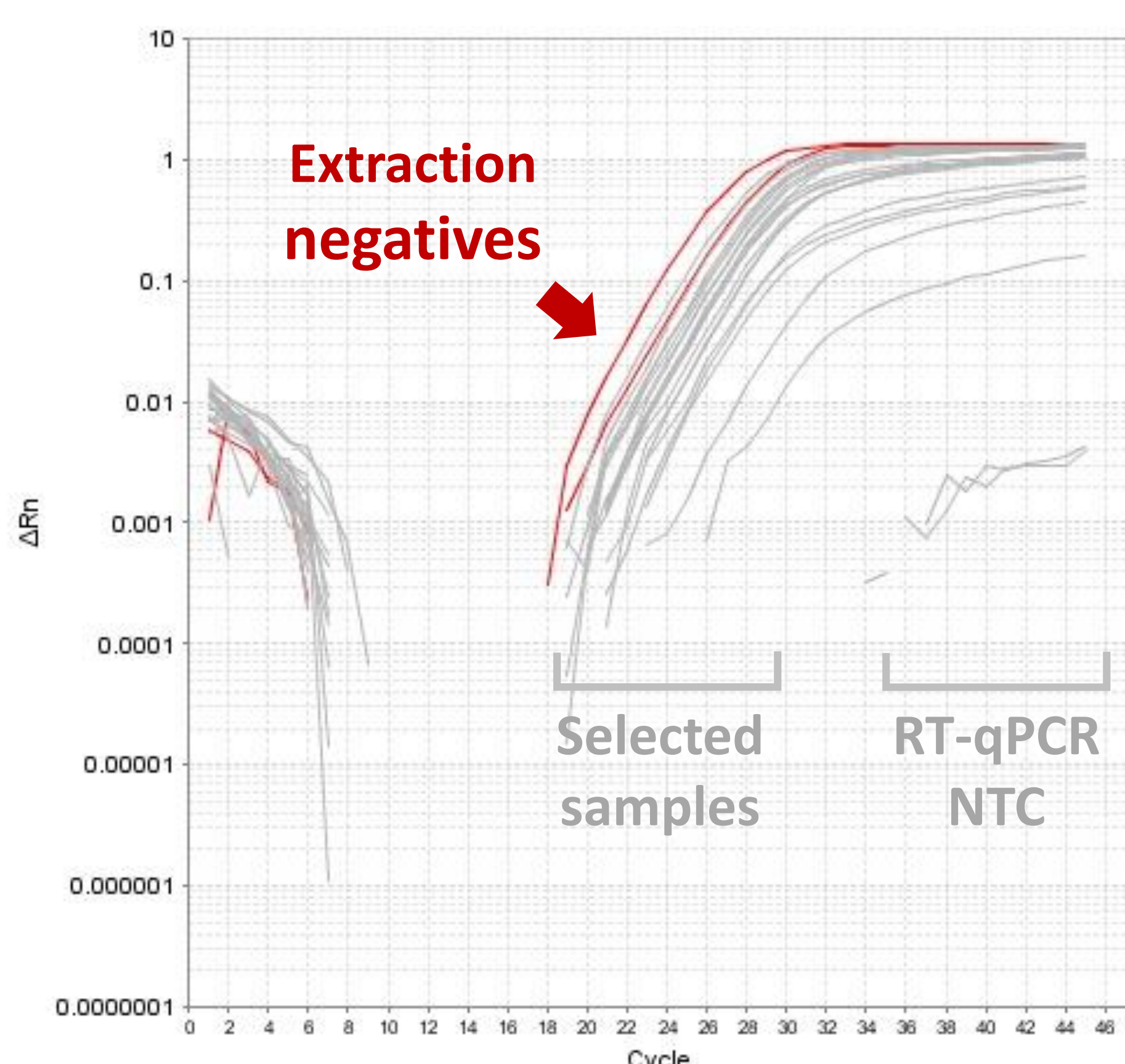


Figure 1. Representative amplification plot of spike RNA detection using RT-qPCR assay (master mix: TaqMan® Fast Virus 1-Step Master Mix [Thermo Fisher]; probe and primers [TATAA Biocenter]) according to manufacturers' instructions. Shown are 20 extracted pig liver RNA samples from multiple extraction batches together with extraction negative and RT-qPCR negative controls.

Results

- A total of 239 pig livers, 120 pig blood curds, 120 pig intestines, 120 lambs, and 239 oysters were sampled and tested for HEV RNA.
- Spike RNA was robustly detected in almost all (98.5%) selected cases, showing only minimal-to-mild RNA loss in extraction and/or RT-qPCR inhibition (**Figure 1**).
- HEV RNA was detected in decreasing positive rate in pig livers (1.7%), pig intestines (0.8%) and oysters (0.4%). Pig blood curds and lambs were HEV negative (**Table 1**).
- HEV RNA positive samples were collected in May, June, July and November 2014.
- Median RT-qPCR cycle threshold value of HEV RNA positive samples was 38.0 (interquartile range, 32.5–42.0).

Table 1. HEV RNA positive rate in 5 food items.

	No. Tested	No. HEV +ve	Prevalence, % (95% CI)
Pig livers	239	4	1.7 (0.5–4.2)
Pig intestines	120	1	0.8 (0.0–4.6)
Oysters	239	1	0.4 (0.0–2.3)
Pig blood curds	120	0	0.0 (0.0–3.0)
Lambs	120	0	0.0 (0.0–3.0)

Conclusion

- HEV RNA was detectable at low prevalence in retail pig livers, pig intestines and oysters. Considering all these food items are popularly consumed in our society, precautionary measures should be taken during purchase, storage, preparation, and cooking of these high-risk food items to minimize HEV infections.

Ongoing Work

- Phylogenetic analysis is underway to delineate genetic relatedness of HEV detected in different food items and local clinical cases of HEV infections.

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