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# Spread of hepatitis E virus from pig slurry to the water environment

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**Objectives:** Spread of pig slurry as an organic fertilizer is commonly used in Danish agriculture. The slurry is spread untreated so pathogens able to survive in slurry tanks will be widely distributed in the environment. The objective of this study was to examine if hepatitis E virus (HEV), which is known to be excreted in faeces from pigs, will be transported through the soil and into the drainage system of a field due to precipitation or will be retained in the soil matrix. Water from the drainage system is not treated before it is discharged into larger water reservoirs (lake, fjords, streams), and hence could present a risk for virus transmission to wildlife and shellfish. We tested the presence of HEV in water drained from a test field where slurry from a Danish pig farm had been applied and in mussels from different regions in Denmark with fields in close proximity.

**Methods:** Slurry from a Danish pig farm was spread on a tile-drained field of loamy soil. Water that arrived at the drainage system located 1 m below surface was collected over a time period of 4 month. Samples were collected on a weekly basis and when water flow in the drainage system exceeded a certain threshold (an event). In addition, samples of water collected from wells located along the field and groundwater. Archived mussels from different regions in Denmark were included in the study. Virus was concentrated from water using Poly Ethylene Glycol precipitation and virus from the digestive tissue of the mussels was extracted using proteinase K treatment. Subsequently, viral genomic RNA, from both water and mussels, was purified using the NucliSENS miniMAG system and detection and quantification of HEV and mengovirus (used as process control) were performed by real time RT-PCR.

**Results:** Water samples representing a total of 14 events were tested. HEV was detected in the first event following spread of pig slurry. In agreement with this, the weekly sample of this period also tested positive. HEV was not found in any of the subsequent water samples. Of the 70 blue mussel samples, that mainly originated from fjords, none tested positive.

**Conclusion:** HEV is regarded as a zoonotic virus with pigs as the primary reservoir. The pathway to humans and other mammals is unclear. Here we show that under Danish conditions, spread of pig slurry can cause viral contamination of water reservoirs, making HEV accessible to the population and wildlife. This indicates a possible route of HEV transmission from pigs to other reservoirs. We also show that retention in soil matrix is at a minimum as HEV was detected at first rainfall after application of pig slurry. The viability of the viruses found in this study is still unclear since HEV cannot be cultivated in cells. We did not find any

HEV positive mussel samples indicating that the release of HEV from fields is not a concern for shellfish production.