21. Development of an ex-vivo infectivity assay for hepatitis E virus

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Pig products have been identified as the main infection route of Hepatitis E virus (HEV) infection in humans. Biosecurity measurements may be applied to reduce HEV in pig farms. To this date HEV detection relies on RT- PCR because culture methods are lacking and efforts to assess HEV infectivity are hardly made. Our aim was to develop a broadly applicable, feasible and sensitive culture system which can be applied for the detection of infectious HEV in swine faeces, environmental materials and food products.
Fresh liver tissue, obtained from a young HEV negative tested piglet was perfused with collagenase IV. Liver cells were isolated and cultured, and subsequently hepatocytes were selected. As soon as these hepatocytes were growing confluent they were aliquoted and stored in liquid nitrogen until use. Prior to the inoculated of HEV positive samples hepatocytes were taken from the liquid nitrogen and were propagated in 6 wells plates. After an incubation for 1.5 hours the inoculate was removed and the cells were washed prior to adding 3ml of the growth medium. On D0 to D7, culture medium was sampled and tested by real time rtPCR. A week after infection we observed a decreace of the Ct values of 8-10 units in the supernatants, and a decrease of 5-8 units in the cell fractions. Based on the decreasing Ct values it can be concluded that there was virus replication. Further study to confirm our observations by EM or antigen detection, and to reproduce the results with different samples are ongoing.

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