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Workpackage 3

Responsible Partner: WBVR (NL)

GENERAL INFORMATION

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BIOPIGEE

HEV infectivity assay available

Objective

The objective was to develop and optimise a culture system for hepatitis E virus using primary pig liver cells and ultrathin pig liver slices (Task JRP21-WP3-T3: Study of HEV stability in relation to disinfection approaches)

Participating countries

NL (WBVR)

Methods

To be used in an HEV infectivity assay, fresh liver samples obtained from a young piglet were perfused with Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) (DMEM/F12) (Gibco) until most of the blood is flushed out. After cutting the liver in small pieces, the tissue was inoculated with and incubated in 0,1% collagenase IV in DMEM/F12 for 1 hour at 37 °C. Liver cells were collected and detached from each other using a 70µm cell strainer. The cell suspension was centrifuged for 5 minutes at 1200rpm and the pellet was washed ones with DMEM/F12 (Gibco). The liver cells awere cultured in growth medium containing DMEM/F12 with 10 % Fetal bovine serum (FBS), 1 % anti/anti (Gibco) and 40ul/ml suppl-B (Gibco) in a T150 culture flask coated with Collagen I. The flasks were incubated with 12ml 100ug/ml Collagen 1 in 0.02M HAc, After two hours these were washed with PBS and dried for 4 hours or used directly. The next day the cells were washed stringently to get rid of cells other than hepatocytes. As soon as the cells had been grown confluent during a couple of days, these were used for infection experiments or stored / frozen in liquid nitrogen for later use.

One day before infection the hepatocytes were seeded into 6 wells plates in growth medium. The next day, normally an almost confluent monolayer could be observed. The hepatocytes were inoculated with 200µl test sample. After an incubation of 1.5 hours the inoculate was removed and the cells were washed with DMEM/F12 prior to adding 2ml of the growth medium. The medium was refreshed for about 50% each 2nd or 3rd days depending on the day of inoculation, after removing of the inoculate at the start of the experiment and before refreshing the medium a sample was collected to be analysed using real time rtPCR.

Table 1: Characteristics of the inoculum

Origin	Land	Species	Year	Reference
HEV infected hepatocytes	Netherlands	Pig	2005	W.v/d Poel, WBVR

Results/Conclusion

HEV replication could be demonstrated using harvested hepatocytes in primary culture. During a period of seven days, cells and supernatants were tested using RT-PCR. Ct-values were plotted and decreases of CT values from a range of 27-34 to a range of 19-25 could be observed in both cells and supernatants. Thus HEV replication could be confirmed.

Future work

For further validation of the test system, the hepatocyte cell system will be infected with additional rt-PCR positive samples of different origin.

Table 2: Rt-PCR positive samples of different origin

Origin	Country	Species	Year	Reference
Sausage	France	Pig	2011	A. Berto, 2013
Faeces	Netherlands	Pig	2019l	W. van der Poel, WBVR
Liver Gt3	Netherlands	Pig	2007	W. van der Poel, WBVR
Liver Gt4	Belgium	Pig	2008	R. Hakze-van der Honing,2011
Sausage	Netherlands	Pig	2018	B. Hogema, Sanquin

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