Deliverable
Workpackage

Responsible Partner: WBVR
Contributing partners: WBVR

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BIOPIGEE

Method for testing persistence of infectious HEV in surface microlayers
D-JRP21-WP3.5

Objective

The objective of this subtask will be to further adapt and implement the developed HEV infectivity assay for testing of microlayer surfaces /biofilms for assessing the inactivation of infectious HEV.

Participating countries

Netherlands (WBVR)

Background

As soon as the most suitable infectivity assay has been selected, this assay will be used to test different kinds of HEV positive pig samples and pig farm environmental samples. For the eviromental samples an easy to use and reliable method need to be developed.

Methods

Sampling Method

Surfaces in pig stabels were sweped with sterilized electrostatic cloths. In comparising with a swab the use of cloths increases the surface that can be tested. After collection, the swipes were stored in a 50 ml tube (Greiner) at -80°C. 5ml of medium was added to each tube and incubated 1.5 hour rolling at room temperature. After testing using HEV PCR, supernatants obtained from HEV positive cloths were used to perform cell culture propagation as described below.

Cell culture

A fresh liver, obtained from a young piglet was perfused with Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) (Gibco) until most of the blood was flushed away. After cutting the liver in small pieces, it 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 5 minutes 1200rpm and the pellet was washed ones with DMEM/F12 (Gibco). The liver cells were 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 growing confluent during a couple of days, these were used for infection experiments or can be fozen in liquid nitrogen for later use.

Primarly hepatocytes were seeded in growth medium on a 6 wells plate format coated with Collagen I. When an almost confluent monolayer of the hepatocytes was observed, the cells were inoculated with 1ml test sample. After an incubation of 6 hours the inoculate was removed and the cells were washed with DMEM/F12 prior to adding 2ml of the maintenence medium containing DMEM/F12 with 2 % Fetal bovine serum (FBS), 1 % anti/anti (Gibco) and 40ul/ml suppl-B (Gibco). The medium was refreshed for about 50% each 2nd or 3rd days depending on the day of inoculation. 6 days afther inoculation a sample was collected for analyse with real time rtPCR to detect HEV.

RNA isolation and Real time RT-PCR
RNA isolation was carry out using the directzol kit (Zymo Research), 100µl sample was added to 300µl Trizol LS and performed according to manual. The RNA was elueted in 50µl elution buffer. The HEV RNA was amlified on the LC480 (Roche diagnostic) machine with the real time rtPCR of Jothikumar (2006) using the TaqMan Fast Virus 1-step Master Mix (applied biosystems).

Results/ Conclusions
The developed method is relatively easy to perform and can be executed in any laboratory without the need for sophisticated equipment. However it still needs further validation.

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