Deliverable D-JRP21-WP3.20

Workpackage 3

Responsible Partner: WBVR (NL)

Contributing partners: WBVR (NL)

GENERAL INFORMATION

European Joint Programme full title	**Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards**
European Joint Programme acronym	**One Health EJP**
Funding	**This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.**
Grant Agreement	**Grant agreement n° 773830**
Start Date	**01/01/2018 (BIOPIGEE 01/01/2020)**
Duration	**60 Months (BIOPIGEE 30 Months, requested extension for 6 additional months)**

DOCUMENT MANAGEMENT

JIP/JRP deliverable	D-JRP21-WP3.20 HEV infectivity test adapted for testing of HEVs in biofilm
Project Acronym	JRP21-FBZ3.1-BIOPIGEE
Author	Renate Hakze- van der Honing/ Wim van der Poel (WBVR)
Other contributors	WBVR
Due month of the report	M48
Actual submission month	M48
Type *R: Document, report DEC: Websites, patent filings, videos, etc.; OTHER*	R **Save date**: 20-Dec-21
Dissemination level *PU: Public (default) CO: confidential, only for members of the consortium (including the Commission Services)*	PU
Dissemination *Author's suggestion to inform the following possible interested parties.*	OHEJP WP 1 ☐ OHEJP WP 2 ☐ OHEJP WP 3 ☒ OHEJP WP 4 ☐ OHEJP WP 5 ☐ OHEJP WP 6 ☐ OHEJP WP 7 ☐ Project Management Team ☒ Communication Team ☒ Scientific Steering Board ☒ National Stakeholders/Program Owners Committee ☐ EFSA ☒ ECDC ☒ EEA ☐ EMA ☐ FAO ☐ WHO ☐ OIE ☐ Other international stakeholder(s): …… Social Media: ..... **Other recipient(s): via publication** .....

BIOPIGEE

HEV infectivity test adapted for testing of HEVs in biofilm

Participating country

NL (WBVR)

Background

For detecting HEV in the eviromental samples and in biofilm an easy to use and reliable method needs to be developed. This assay will be used to test the precence of in HEV in biofilms, pig farm environmental samples and in different kinds of HEV positive pig samples.

Objective

The objective was to develop and evaluate a model for testing biofilms/surface microlayers for the precence of HEV.

Methods

Sampling Method
Surfaces in pig pens where biofilms are expected were wiped with electrostatic dust collectors (EDCs). In comparison with a swab the use of cloths (EDCs) increase the surface that can be tested. After collection, the EDCs were tested or stored in a 50 ml tube (Greiner) at -80°C until the procedure was continued. Medium (5 ml) was added to each tube and incubated 1.5 hour while 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. Six days after inoculation, a sample was collected for analyse with real time rtPCR to detect HEV.

RNA isolation and Real time RT-PCR
RNA isolation was carried 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 amplified on the LC480 (Roche diagnostic) machine with the real time rtPCR of Jothikumar et al. (2006) using the TaqMan Fast Virus 1-step Master Mix (applied biosystems).

Results/Conclusion

EDC's spiked with different HEV concentrations were tested (1:10, 1:20, 1:40, 1:80). After culturing, all the samples showed a reduction of 10-12 Ct when tested in the RT-PCR.
The developed sampling method is relatively easy to perform and can be executed in any laboratory without the need for sophisticated equipment. However further validation will be needed.

Future work

For further validation of the test system, we aim to visit pig farms and sample biofilms/surface microlayers and test these for the presence of HEV.

This work of the project BIOPIGEE has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.