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Contributing partners: IZSAM/ISS/IZSLER (IT), WBVR (NL)

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BIOPIGEE

Produce report on evidence gathered by field studies

Participating countries

UK (APHA), IZSAM/IZSLER (IT), WBVR (NL)

Background

Salmonella and hepatitis E virus (HEV) are zoonotic pathogens which are usually subclinical in pigs. Salmonellosis can cause gastrointestinal diseases in humans with over 90 thousand confirmed cases and 156 deaths in the EU in 2017. *Salmonella* is an important zoonotic pathogen in pigs, that can cause clinical disease. Many sow herds and finishing herds are infected with *Salmonella*, and therefore pose a threat for the contamination of pork and pork products, and ultimately consumers. Human infection with HEV is an increasing public health concern across Europe. The virus is carried asymptomatically by pigs, and can be passed to humans via contaminated pork products if pigs are slaughtered while actively infected. The virus is believed to be widespread in EU pig herds, but little is currently known about the primary sources of infection for pigs or the ability of the virus to persist in the environment. This, coupled with a current lack of suitable vaccine, means that trying to prevent infection in pigs is unlikely to be an effective control option. Instead, the best option may be to employ herd management strategies that increase the chance that, once infected, pigs naturally overcome infection before slaughter. However, in order to advise on what these management strategies should entail, a better understanding of HEV epidemiology within typical pig production systems is first needed, in addition to an understanding of how cleaning and disinfection could aid in preventing the spread of both *Salmonella* and HEV between groups or batches of pigs.

A previous study demonstrated that porcine reproductive and respiratory syndrome virus (PRRSv) and HEV co-infection results in a longer latent period and a longer duration of HEV faecal shedding under experimental conditions. It is unclear whether this association is replicated in commercial farming conditions.

This report will focus on trials carried out in four institutes (WVBR, IZSAM and IZSLER, APHA) across three European countries (NL, IT and UK) which aim to improve our knowledge of HEV (and *Salmonella*) epidemiology and potential control measures.

Aims and Objectives

The aim of the Netherlands' longitudinal field study was to estimate the transmission rate of HEV between consecutive batches of fattening pigs in the same pens, and between pens within compartments. The hypothesis was that the moment pigs become HEV infected depends on the level of HEV contamination in a pen by the previous batch, with a higher contamination resulting in earlier infection. A secondary aim was to investigate whether a PRRSv pre- or co-infection influences the latent period, or the duration of the infectious period, of HEV in a pen with fattening pigs under field conditions.

For the Italian trials, the effectiveness of cleaning and disinfection procedures on farms against *Salmonella* and HEV contamination will be evaluated both by IZSAM and IZSLER.

For the UK trials, the aim was to investigate the epidemiology of HEV on farms, with particular regards to infection dynamics and the mixing of pens/groups of pigs, and additionally to investigate environmental/wildlife reservoirs of infection.

Methods

Netherlands

Pilot studies were carried out to inform the methods for the longitudinal study. For pilot study 1, four farrow-to-finish farms were enrolled and individual pigs belonging to 24 pens across four age groups were sampled. To identify the most accurate method to sample pens for HEV detection, a further pilot study (pilot 2) was carried out on one farm with four ages of fattening pigs. Four different sampling methods were compared: individual rectal swabs; boot socks; pooled fresh faecal droppings; oral fluid.

Following this, two longitudinal trials were designed with evidence obtained from these pilot studies. Two farms were selected, one with a low transmission profile, and the other with a high transmission profile (based on an earlier prevalence study). On both farms, the last two weeks of a batch of fatteners, and the consecutive two batches were followed up. The observational unit for the longitudinal trial was the pen, instead of the individual animal. This decision was made based on the idea that the reproductive ratio of HEV within a homogeneous population is far above 1, so transmission within the pen will be fast and inevitable. In addition, the farmers' risk mitigation would likely be decided and performed on a pen, or even compartment level, so transmission should also be studied on pen level. From each batch of pigs studied, 36 pens were sampled across three compartments, with each pen sampled once a week. At the start and end of the batch period, serum samples were collected to identify contingent HEV infection prior to the fattening phase and to identify seroconversion during the fattening phase. In addition to faecal samples, an oral fluid sample was collected each week to test for infection with PRRSv. Between batches, all pens were sampled after cleaning and disinfection by the farmer.

Italy

In Italy, ten farms were selected in total; five per institution. Due to delays caused by the COVID-19 pandemic and the Italian African Swine Fever outbreak, farrow-to-wean or weaner farms were selected, as opposed to fattening farms as initially planned. Farms were allocated an ID as follows: farms sampled by IZSAM were identified as "IT_01" to "IT_05", and for IZSLER farm they were identified as "IT_06" to "IT_10". Farms were sampled pre- and post- cleaning and disinfection (C&D); individual cleaning regimes or disinfectant products were not investigated. Post C&D samples were collected within 24-96 hours after C&D had taken place. At each farm, two pens were sampled, from which a swab of the floor and feeder were collected, in addition to 500ml of water from a drinker inside the pen. The presence of *Enterobactericeae* (EB) was also enumerated as a proxy for residual faecal contamination. Samples for bacteriological analysis (*Salmonella* and *Enterobacteriaecae*) were

collected using a pre-moistened sponge (10ml sterile netutralising buffer) with a handle. For HEV testing dry cotton swabs were used. Table 1 displays the sampling scheme per trial farm.

Table 1: Sampling scheme for collection of samples on Italian trial farms

BEFORE C&D delivery room/weaning room			
	Swabbing point (100 cm^2)	Swabbing point (100 cm^2)	Sampling point (500 mL)
Box 1	floor	feeder	drinker
Box 2	floor	feeder	drinker
AFTER C&D delivery room/weaning room			
	Swabbing point (100 cm^2)	Swabbing point (100 cm^2)	Sampling point (500 mL)
Box 1	floor	feeder	drinker
Box 2	floor	feeder	drinker

UK

The inclusion criteria for trial farms was that they must be of commercial size, and be either farrow-to-finish or weaner-finisher farms. A list of current pig holdings in the UK was used to identify suitable farms, and these were contacted by email and phone. Two farms were recruited, identified as HEV-03 and HEV-04; HEV-03 was a two-stage breeder-finisher farm, with pigs moving from the breeder unit up to the finisher unit at approximately 12 weeks of age. HEV-04 was a single-site weaner-finisher unit. Each farm was visited four times, and the age groups sampled at each visit are described in Table 2.

Table 2: Description of sampling schedule for two UK longitudinal trials

Farm ID	**Visit No.**	**Age group sampled**
HEV-03	1	Sows and sucklers
	2	Weaners
	3	Growers
	4	Sows and Finishers
HEV-04	1	Weaners
	2	Weaners
	3	Growers
	4	Finishers

At each visit, individual faecal samples were collected from the floor of pig housing. The quantity of samples collected was determined by using a sample size calculation to ensure enough samples were collected to estimate prevalence at an age group level (95% confidence, 10% precision), using an estimated true proportion based on evidence from a previous HEV trial carried out at the APHA, and to enable the demonstration of disease freedom at a pen level (unit and population sensitivity 95%). Up to 21 environmental samples were also collected from the vicinity of pig housing, consisting of either dust, bird faeces, rodent faeces or swabs of cleaned and disinfected pens, drinkers or feeders. On the fourth visit to HEV-03, blood samples were collected from sows at the breeding site, and

finishers at the finisher site. Faecal samples were tested for the presence of HEV RNA using quantitative real-time PCR with an internal control RNA (QIAGEN). Blood serum samples were tested for the presence of HEV antibodies using an ELISA kit (ID Screen® Hepatitis E Indirect Multi-Species ELISA (ID-VET)).

Results

Netherlands pilot studies

In the first pilot study, all samples from weaner pigs were negative, while all farms were HEV positive. This evidence was used to inform the selection of fattening pigs as the age group of focus for the longitudinal trial.

In the second pilot study, four sample types were compared; individual rectal swabs per pen were seen as the reference standard and the three pen-level sampling methods were compared to that reference standard to calculate the sensitivity and specificity. Bootsocks had the highest sensitivity, identifying positive pens even when only one pig was shedding HEV. However, specificity was below 30%, probably due to old faeces being collected with bootsocks. Faecal droppings had the highest combination of sensitivity and specificity. This evidence was used to inform the types of samples collected in the longitudinal study; in the first week after the start of the fattening phase both boot socks and pooled faecal dropping should be collected for early detection of a positive pen. After that, only faecal droppings need to be collected to measure the duration of the infectious period.

Netherlands longitudinal study

On the low transmission farm, all pens sampled to date were negative for HEV, although boot socks of the first batch of pigs were positive in the last week prior to slaughter, demonstrating that HEV is still present on the farm and transmission is possible. On the high transmission farm, all pens have become positive. The moment of infection in a pen is dependent on the compartment the pen is in, and presence of a positive adjacent pen.

Italian study

Due to delays caused by the COVID-19 and African Swine Fever outbreaks in Italy only results from ISZLER are available for this report. Table 3 displays the summary characteristics of the five farms, consisting of two breeding, two farrow-to-wean and one weaning farm.

Table 3: Summary of farm characteristics sampled by ISZLER

Farm ID	Farm type	Estimated N° of pigs in pen	Pig stage sampled	Piglets estimated age (weeks)	Parity (sows only)
IT_06	breeding	10-12	Weaning	4	2-7

IT_07	farrow to wean	11-13	Delivery	4	2-7
IT_08	breeding	10-12	Delivery	4	2-7
IT_09	weaning	30	Weaning	11-12	1-8
IT_10	farrow to wean	11-13	Delivery	4	2-7

All samples were negative for *Salmonella* and HEV (Table 4). EB colony counts ranged from negative to 10^7 cfu/cm^2 (mL). Some surprising results were observed in post cleaning and disinfection samples, with EB cfu/cm^2 increasing in feeder and floor samples from IT_06 and IT_09, and feeder/floor samples from IT_08. These results are likely due to a potential poor cleaning & disinfection process carried out on those farms.

Table 4: Preliminary results of *Salmonella,* HEV and *Enterobacteriacae* pre- and post-cleaning and disinfection on ISZLER farms[1]

Farm ID	SA BC	SA AC	HEV BC	HEV AC	EB BC (cfu/cm^2)	EB AC (cfu/cm^2)
IT_06 feeder (box1/box2)	- / -	- / -	- / -	- / -	100,000/80,000	90,000,000/350,000
IT_06 floor (box1/box2)	- / -	- / -	- / -	- / -	8,000/1,700	100/20,000
IT_06 drinker			- / -	- / -		
IT_07 feeder (box1/box2)	- / -	- / -	- / -	- / -	450,000/260,000	90,000/1,700
IT_07 floor (box1/box2)	- / -	- / -	- / -	- / -	730,000/21,000	5,000/<10
IT_07 drinker			- / -	- / -		
IT_08 feeder (box1/box2)	- / -	- / -	- / -	- / -	460,000/1,500	14,000/12,000
IT_08 floor (box1/box2)	- / -	- / -	- / -	- / -	100/900	700/<10
IT_08 drinker			- / -	- / -		
IT_09 feeder (box1/box2)	- / -	- / -	- / -	- / -	<10/<10	<10/1,200
IT_09 floor (box1/box2)	- / -	- / -	- / -	- / -	<10/<10	<10/50,000
IT_09 drinker			- / -	- / -		
IT_10 feeder (box1/box2)			- / -	- / -		
IT_10 floor (box1/box2)			- / -	- / -		
IT_10 drinker			- / -	- / -		

[1] SA= *Salmonella*; HEV= Hepatitis E Virus; BC=Before cleaning and disinfection; AC= after cleaning and disinfection; EB= Enterobacteriacae; cfu= colony forming units

UK study

Due to unexpected delays, only the results of HEV_03 samples were available to present in this report. Of the 376 faecal samples collected over four visits, 27.4% (103/376) samples were positive for HEV ribonucleic acid (RNA). None of the 26 faecal samples collected from farrowing sows were positive for HEV, and only 2.6% of suckler pig faecal samples were positive (2/78). There was a peak in the presence of HEV in pigs of weaner age (54.0%, 54/100), declining towards slaughter age (Figure 1). Pigs on this farm were mixed at weaning and remained in consistent groups throughout the rest of their fattening period. Further analysis and results from trial HEV-04 will assist in determining whether mixing events have an influence on HEV epidemiology and transmission.

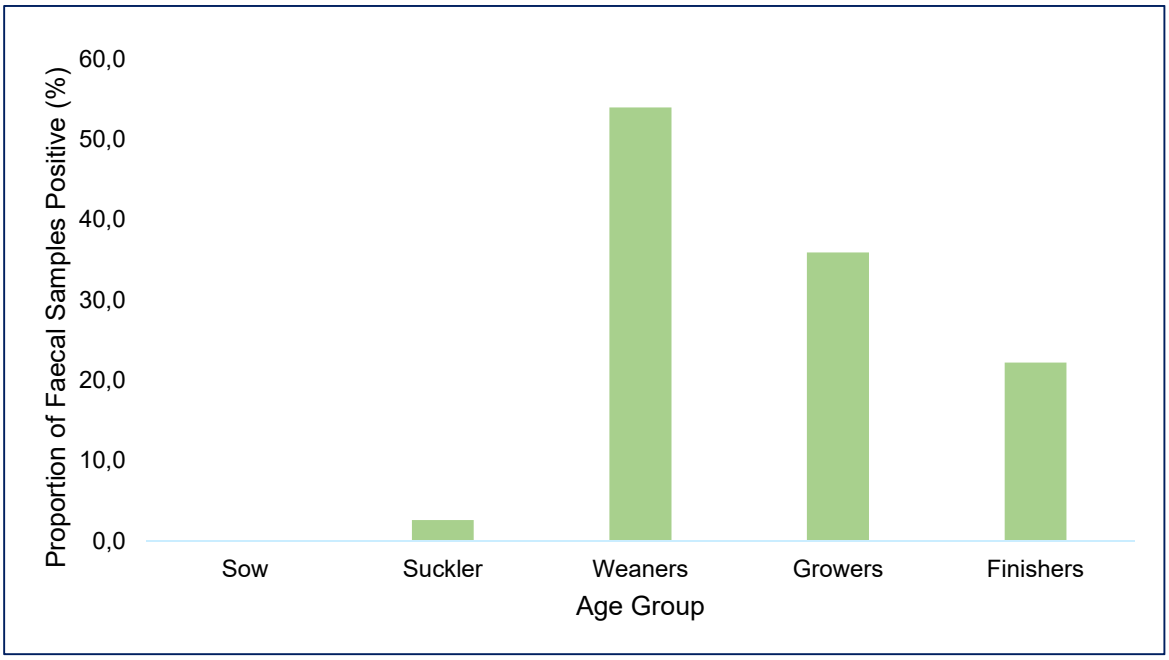

Figure 1: Proportion of faecal samples positive for hepatitis E virus by age group

Of the 24 blood samples collected from finisher pigs, 79.2% (19/24) were positive for HEV antibodies, and 84.2% (16/19) of those collected from sows were HEV positive. HEV RNA was also detected in 54% of environmental samples collected across the four visits (44/81), ranging from 10% at visit 1 to 90% of samples positive from visit 3.

These studies, although not yet complete, have provided further evidence on the transmission of HEV through the pen and house environment, and the presence of HEV in pig faeces, HEV antibodies in sows and finisher pigs, and of HEV in the wider environment, which may act as a reservoir for HEV incursion into the pig herd.

Future work

In the Netherlands, data collection will continue until the end of November 2022, with laboratory testing and analysis to follow. Oral fluid samples are due to be tested for PRRSv in June. In Italy, sample collection will continue at the ISZAM institute, and testing to continue at ISZLER, with analysis to be completed to determine the effect of cleaning and disinfection on *Salmonella* and HEV contamination. Sample testing will also continue at APHA and analysis will be completed to determine the influence of mixing pigs on HEV epidemiology, and to identify potential environmental reservoirs of HEV.

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