Study of Porcine Epidemic Diarrhoea virus in UK pigs at slaughter in 2013

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Summary

Using sera collected at abattoirs for a national prevalence study for food-borne pathogens in 2013, this survey detected evidence of exposure to Porcine Epidemic Diarrhoea virus (PEDV) infection in GB pigs. These results suggest that endemic strain(s) of PED virus are still present at a low level in GB but that only a small proportion of the GB pigs sampled were exposed to infection before they reached slaughter, although uncertainties exist regarding the performance of the serological test. There have been no reports in GB pigs of clinical outbreaks of PEDV resembling virulent PED seen in North America and testing of diagnostic diarrhoea submissions for PEDV by PCR at AHVLA has not detected virus. The serology results support other evidence that PEDV is not circulating widely in the GB national herd and suggest that there is a high degree of naivety to infection with PED virus.

Introduction

This report describes a seroprevalence study for Porcine Epidemic Diarrhoea (PED) virus in British pigs at slaughter. The work extends a prevalence study undertaken in 2013 for *Salmonella*, *Toxoplasma*, Hepatitis E virus (HEV), *Yersinia*, Porcine Reproductive and Respiratory Syndrome virus (PRRSv), Extended Spectrum Beta-Lactamase (ESBL) *Escherichia coli* and antimicrobial resistance (AMR) in *Campylobacter coli* in UK pigs, for which results have previously been reported (Powell *et al*., 2014).

PED is a gastrointestinal disease of pigs which was first reported in England in the 1970s (Wood, 1977). It is not a zoonotic infection and does not represent a public health risk. Cases in the UK have tended to be relatively mild, presenting with watery diarrhoea and mild systemic signs such as fever, anorexia and lethargy. Piglets are most often affected although the disease can be seen in finishers and adults. No diagnoses have been made by the Animal Health and Veterinary Laboratories Agency (AHVLA) or Scotland's Rural College (SRUC) in GB pigs since 2002 although very little virological or serological testing has been performed in recent years. Between 2007 and 2012, 94 of 206 sera tested for antibody at the AHVLA mainly for monitoring purposes were found to be antibody positive. This included eight positive sera in 2012 suggesting that the virus remains endemic in British pigs.

New virus strains have emerged in China in recent years causing significant disruption to Asian pig production (Wang *et al*., 2013). In May 2013, a virulent form of PEDV emerged in the USA causing mortality of 30-100% in neonatal pigs and significant morbidity with reduced growth in older growing pigs in affected herds. The virulent form of PED virus had been detected in 30 US States by May 2014 and has spread to Canada. The virus responsible for the US outbreak is reported to be 99.4% similar to the virulent Chinese strains (Sun *et al*., 2012) although the origin of infection for the US has still not been identified. It is not known whether immunity to endemic GB PEDV strains would protect against the virulent PEDV strain that has emerged in the USA and Asia, and there is concern that if this strain, or similar ones, were introduced to the UK they could have a severe impact on pig health, welfare and productivity leading to significant economic loss. There is thus interest in determining the current status of British pigs with respect to PED virus infection.

The samples already collected for the UK prevalence study under project OZ0150 provided an ideal opportunity to address this data gap and provide the first GB-wide seroprevalence estimate for PEDV in slaughter pigs.

Methods

The study design and sample collection methods for this study have previously been described in detail in the OZ0150 final report (Powell *et al*., 2014). A brief summary has been included below for reference together with the PEDV laboratory methods.

Study design

The PEDV testing utilised samples collected for a UK-wide prevalence study of *Salmonella*, *Toxoplasma*, HEV, *Yersinia*, PRRSv and ESBL *E. coli* and AMR in *Campylobacter coli* in pigs at slaughter. The study was designed to be consistent, where possible, with the technical specifications used for a previous study of *Salmonella* in slaughter pigs conducted in 2006/7 (Commission Decision 2006/668/EC). The target sample size for the UK as a whole was 600 pigs, although pigs reared and/or slaughtered in NI were not tested for PED virus.

Sampling schedule

Sampling took place between 14th January 2013 and 14th May 2013 at twelve high throughput abattoirs in England that together represent approximately 80% of all slaughtered finishing pigs in GB. Sampling was weighted by abattoir throughput and stratified by calendar month. The date of sampling and carcase to be sampled on a given day were both randomly selected. Further details are given in Powell *et al.* (2014).

Sample collection

Nine samples were collected from each pig, of which two were subsequently used for PEDV testing: one EDTA plasma sample (collected post-bleed) and caecal contents (collected at the evisceration point). The plasma samples were tested for PEDV antibody using an in-house antibody ELISA. Nucleic acid extracted from the caecal contents was also tested for PED virus by PCR. All testing was undertaken at AHVLA Weybridge.

Data collection

A standardized data collection form was completed by trained personnel after sampling to gather information relating to the abattoir processes and sampled carcase, including the name and address of the farm of origin.

Eligibility criteria

The study design had a number of exclusion criteria (Powell *et al*., 2014), including bacteriological examination being carried out within 96 hours of sample collection. This criterion was not applied to the PED analysis based on expert opinion that this delay in testing was not likely to adversely affect the tests.

Porcine epidemic diarrhoea antibody ELISA

All available plasma samples from GB pigs were tested for antibodies to PED virus using a blocking ELISA in which antibodies in the pig plasma sample bind to viral antigen and block antigen binding to a capture monoclonal antibody on a microtitre plate, addition of a different conjugated monoclonal antibody yields a colour reaction for negative plasma

samples and no colour reaction of positive plasma samples (van Nieuwstadt & Zetstra, 1991). Sera were tested at a single dilution of 1 in 5. Plasma samples producing >49% reduction in optical density compared to controls are positive and those with <50% reduction are negative; all samples with a result of 25%-49% are retested.

Porcine epidemic diarrhoea polymerase chain reaction

Nucleic acid extracted from caecal contents of pigs which tested positive for PED antibody was used in a diagnostic PCR to check for PED virus nucleic acid. The PCR was composed of QuantiFast SYBR Green RT-PCR Master Mix (Qiagen), forward and reverse primers, QuantiFast RT Mix, extracted nucleic acid and water to a volume of 25 µl. The primers targeted a highly conserved region in the S gene of PED virus. The mix was then run in a Mx3005P thermal cycler (Agilent Technologies) for 42 cycles followed by a dissociation curve analysis.

Statistical analysis

Questionnaire data and laboratory test results were data entered or imported into Microsoft Access. Data were cleansed and analysed using Microsoft Excel and Stata v.12 (StataCorp, USA).

Data were checked for implausible and/or inconsistent values. The prevalence was calculated using the survey command (svy) in Stata to take account of clustering of pigs within farms. Variation in seropositivity by age, region and farm production system were examined using chi-squared tests while taking into account within-farm clustering. Associations between PED and the other infectious organisms that had been previously tested for in this study were also investigated via the same method.

Results

In total, 564 British pigs were scheduled for sampling during the study period, and samples were collected from 558 of those pigs. The samples from sixteen pigs arrived at AHVLA Bury St Edmunds more than 96 hours after collection; these pigs have been included in all the subsequent analyses with the exception of testing for associations between PED and *Salmonella, Yersinia*, PRRSv or *Toxoplasma*.

The 558 eligible pigs originated from 395 farms, with between 1 and 8 pigs sampled from each of those farms. Almost two-thirds of the pigs were from farms located in either Yorkshire and the Humber (208 pigs; 37.3%) or the East of England (151 pigs; 27.1%) (Table 1). Nearly two-thirds (61.1%) of the pigs were from finishing only farms with a further 14.7% reported as from breeding and finishing farms whilst the source for the remaining quarter was either not known (12.9%) or not reported (11.3%) (Table 2). Most of the pigs were aged under 12 months (87.6%) with 10.4% reported as over 12 months old; the age was not recorded for 11 (2.0%) pigs. Almost three-quarters (72.0%) of the pigs were kept in controlled housing systems. Only a small proportion were reported as born outdoors and reared/kept in controlled housing since weaning (3.9%) or kept fully outdoors (0.5%) which is lower than expected although housing information was not provided for 23.5% of the pigs. Most of the pigs that were aged 12 months or older were from breeding and finishing farms and, as expected, none were reported to have originated from finishing only farms (Table 2).

A total of 554 plasma samples were available to test for antibody to PED virus, of which 50 tested positive in the antibody ELISA. Therefore, after accounting for clustering of pigs within farms the seroprevalence was 9.0% (95% CI 6.3-11.7). The 50 seropositive pigs were from 43 different pig farms. For 22 of those farms, only a single pig had been tested for antibody to PEDV. Between two and five pigs were tested for antibody to PEDV from each of the other 21 farms, although only five of those farms had more than one seropositive pig.

Seropositivity was found to be higher in pigs aged less than six months (9.3%) and 6-12 months (9.5%) than in pigs aged >12 months (3.4%) although this variation was not statistically significant (p=0.31) (Table 3). There was no statistical evidence that seropositivity varied significantly between the regions listed in Table 1 (p=0.21). Data concerning the use of all-in, all-out (AIAO) or continuous production systems on the source farm was only available for 178 of the tested pigs. Of those, a higher proportion of the pigs from continuous systems tested positive for PED antibody than pigs from AIAO systems although the difference was not statistically significant (15.2% vs. 7.1%; p=0.14) (Table 4). However, it should be borne in mind that the study was not designed with sufficient power to investigate any of these associations, especially at this low seroprevalence.

Only the caecal contents from seropositive pigs were tested for the presence of PED virus by PCR. The caecal content from one seropositive pig was not available. PED viral nucleic acid was not detected in any of the 49 caecal content samples that were tested (Table 5).

The PED-seropositive pigs were significantly more likely to also test seropositive for PRRSv than PED-seronegative pigs (80.9% vs. 58.7%; p=0.005) (Table 6). However, the proportion of pigs in which PRRSv nucleic acid was detected by PCR was similar in PED-seropositive and PED-seronegative pigs (p=0.99; Table 7). A higher proportion of the PED-seropositive pigs were found to carry *Salmonella* in the caecum (42.6%) than in the PED-seronegative pigs (31.6%) (Table 8), whereas the opposite was true for *Toxoplasma* (Table 12) although there was no statistical evidence of an association between the PED serology results and either *Salmonella* carriage (p=0.13) or antibody to *Toxoplasma* (p=0.15), nor with *Yersinia* carriage (p=0.94; Table 9), antibody to HEV (p=0.39; Table10) or presence of HEV nucleic acid (p=0.92; Table11). However, PED-seropositive pigs were found to be significantly more likely to carry ESBL *E. coli* than pigs which did not have antibody to PED (38.0% vs. 22.7%; p=0.04) (Table 13).

Discussion

The PED serology results provide evidence that endemic strain(s) of PED virus are still present in GB but suggest that only a small proportion of the GB pigs sampled were exposed to infection before they reached slaughter. There have been no reports of clinical outbreaks of PEDV resembling virulent PEDV seen in North America. The results suggest that the majority of the pigs sampled were not exposed to PED virus infection and that there is a high degree of naivety in the national herd to infection with both resident (endemic) and exotic PED virus strains.

Pigs seroconvert to PEDV within 10 to 14 days post infection and actively acquired antibody may persist for some months. Antibody was detected by ELISA for 27 weeks following

experimental infection (van Nieuwstadt & Zetstra, 1991). If the same is true of field infection, it is likely that most pigs raised for meat (which represent the majority of pigs sampled in this study) would still be seropositive at the time of slaughter if infected by PEDV during their growing period. As colostral antibodies may persist up to five months of age (Tizard, 2012), it cannot be excluded that the antibody detected in a few of the youngest seropositive pigs in this study actually represent residual maternal antibodies, albeit unlikely. Nevertheless, this would still indicate the presence of PEDV on the unit on which the pig was bred.

The low prevalence of antibody does mean that serology is potentially more useful for diagnosis, for example by using paired serology, than it would be if there was high antibody prevalence. However, PEDV PCR on faeces remains the diagnostic method of choice during acute infection.

Several areas of uncertainty exist with respect to the antibody results. The first relates to the fact that the ELISA was developed with, and uses controls based on a virus isolate, CV777, which has been in culture since the 1980s and there is no information available regarding how closely this strain relates to current GB PEDV strains. Van Nieuwstadt and Zetstra (1991) report that the ELISA was successfully used in pigs in several field outbreaks and considered that the monoclonal antibody epitopes appeared to be well conserved in PEDV circulating at the time. Enhanced surveillance at AHVLA involving testing 143 samples in diagnostic submissions from diarrhoeic pigs on 68 pig units collected between June 2013 and April 2014 for PEDV by PCR has not yet detected PCR-positive samples. If any PCR-positive cases are detected, it will be vital to obtain sera from the affected cohort of pigs and herd to use in the antibody ELISA. The fact that no PED virus has been detected in recent diagnostic samples suggests that PEDV is not currently a significant cause of diarrhoea outbreaks in pigs in England and Wales.

The PEDv antibody ELISA has been used successfully in the past at AHVLA (formerly Veterinary Laboratories Agency) during investigation of outbreaks of PEDv. This involved use of the test on a herd-level basis rather than on at individual pig-level as in this study, and with further testing of the herd possible as necessary. Interpretation of the significance of single positive PEDv ELISA results in isolation is more difficult.

Lastly, experience from the US suggests that antibody levels may wane quite rapidly (7-9 weeks) after infection. This would mean that pigs undergoing primary infection prior to weaning may not be seropositive at slaughter unless rechallenged during rear. Van Nieuwstadt and Zetstra (1991) reported detection of antibody by ELISA for 27 weeks following experimental infection in a single pig, but how this relates to field infections with current PED viruses is not known. Establishing reliable serological tests has reportedly been more problematic than establishing PCR tests for PEDV. In the US another serological test, namely the IFAT has been established, which we do not have not sufficient validation data/experience at AHVLA to reproduce at present. Based on multiple in silico analysis and exchange of information with our US colleagues, we are confident that the PCR used at AHVLA is comparable to those used in the US, there is more uncertainty around serological tests, in the UK and elsewhere.

No PED virus was detected in caecal contents of the seropositive pigs. Using the antigen ELISA method of detection, the virus was reported to be excreted for up to 11 days following experimental infection (Carvajal *et al*., 1995). PCR may detect the virus for longer than this but virus excretion is limited as immunity develops and no carrier state is recognized. Re-challenge of pigs in which immunity is waning could lead to re-excretion of virus for a shorter period without clinical signs. The fact that no PEDV was detected in pigs which have antibody to PED virus is thus not surprising, especially as pigs were tested at slaughter when they are less likely to have recently undergone primary infection; in endemically infected herds active infection tends to occur postweaning as passive immunity wanes. For the same reason, it is unlikely that pigs would have been in the very early stages of infection at the time of slaughter and not detected by testing only caecal samples from seropositive pigs by PCR.

This typical age distribution of primary infections, with a peak postweaning and waning of immunity over several months thereafter, could also offer a possible explanation for the marginally higher seroprevalence observed in pigs aged less than 12 months. However, given the small sample size in both the youngest and oldest age categories, further data would be required in order to fully explore this trend.

PED virus is transmitted via the faeco-oral route and strict biosecurity procedures and effective cleaning and disinfection are of utmost importance in preventing introduction of the virus and minimizing spread and/or re-circulation or carry-over of infection. All-in all-out management, when combined with stringent cleaning and disinfection between batches, can play a key role in controlling many infectious diseases by breaking cycles of transmission. Hence, it is not surprising that from the limited data available there appeared to be some indication that pigs managed on a continuous basis were more likely to have been infected with PEDV. Similar results were also obtained for PRRSv although both should be treated with caution given the limited subset of pigs for which this data was supplied.

The greater likelihood of PEDV seropositive pigs having been infected with PRRSv may reflect pig flow, management and hygiene practices, biosecurity and other features on particular units favouring persistence of active infection with these pathogens, rather than any interaction between them. PEDV has been reported to co-exist with various other enteric viruses, with co-infections more common among diarrhoeic than among healthy pigs (Zhang *et al.*, 2013). As this study sampled healthy pigs at slaughter and the timing of each infection was not known, it is not possible to determine whether these mixed or sequential infections affect clinical disease outcomes. One possible explanation for the apparent association of PEDV exposure with the presence of ESBL *E. coli* might be that sites with PEDV have more clinical enteric disease which influences antimicrobial use. These associations do not necessarily indicate a causal link and more detailed multivariable analyses on larger sample sizes would be required to corroborate the validity of these co-infection findings.

The degree of immunological naivety in the GB national herd suggested by the findings of this study and the highly transmissible nature of PED virus revealed by the recent outbreaks in Asia and North America highlight the potential threat posed to British pigs. This emphasises the need for strict biosecurity and effective cleaning and disinfection not only on pig units but also by allied industries, and the risk posed by live pigs imported from

countries with virulent PEDV. Initiatives such as the BPEX 'Cleaner Lorries Campaign' are making progress in reviewing current practices, identifying potential weak points and improving standards. These efforts will also assist with mitigating the risks posed by other contagious pathogens of pigs. Continuing to raise awareness of PEDV and maintaining vigilance for the signs of disease should continue to be encouraged to facilitate prompt detection of the virus should incursion of more virulent strains occur.

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Tables & Figures:

Table 1: Number of pigs sampled by region of origin (n=558)

Region of source farm	Number of pigs	% of pigs
North East England	13	2.3%
North West England	20	3.6%
Yorkshire and the Humber	208	37.3%
East Midlands	63	11.3%
West Midlands	10	1.8%
East of England	151	27.1%
South East	13	2.3%
South West	50	9.0%
Wales	2	0.4%
Scotland	28	5.0%

Table 2: Number of pigs sampled by age and production type (n=558)

Production type	Age				Total
	<6 months	6-12 months	>12 months	Not known	
Breeding and finishing	4	32	46	0	82
Finishing only	20	315	0	6	341
Not known	31	87	12	5	135
Total	55	434	58	11	558

Table 3: PED seroprevalence by age (n=543)

Age	Number tested	Number seropositive	Seroprevalence
<6 months	54	5	9.3% (95% CI 1.3-17.2)
6-12 months	431	41	9.5% (95% CI 6.4-12.6)
>12 months	58	2	3.4% (95% CI 0.0-8.4)

p=0.31 (p=0.26 with test for trend)

Table 4: PED seroprevalence by production system (n=178*)

System	Number tested	Number seropositive	Seroprevalence
AIAO	99	7	7.1% (95% CI 1.3-12.8)
Continuous	79	12	15.2% (95% CI 5.4-25.0)

p=0.14

*For 203 pigs this information was recorded as “not known” and for 173 pigs the question was not answered.

Table 5: PED ELISA and PCR results (n=558)

Testing method		PCR		
		Positive	Negative	Not tested
ELISA	Positive	0	49	1
	Negative	0	0	504
	Not tested	0	0	4

Table 6: Association between PED and PRRSv seropositivity (n=538)

		Number tested for PRRSv antibody	Number (%) seropositive
PED	Seropositive	47	38 (80.9%)
	Seronegative	491	288 (58.7%)

p=0.005

Table 7: Association between PED seropositivity and PRRSv RNA detection (n=348)

		Number tested for PRRSv RNA	Number (%) positive
PED	Seropositive	39	3 (7.7%)
	Seronegative	309	24 (7.8%)

p=0.99

Table 8: Association between PED seropositivity and *Salmonella* carriage (in the caecum) (n=532)

		Number tested for *Salmonella* carriage	Number (%) positive
PED	Seropositive	47	20 (42.6%)
	Seronegative	485	153 (31.6%)

p=0.13

Table 9: Association between PED seropositivity and *Yersinia* carriage (in the tonsils) (n=533)

		Number tested for *Yersinia* carriage	Number (%) positive
PED	Seropositive	46	16 (34.8%)
	Seronegative	487	167 (34.3%)

p=0.94

Table 10: Association between PED and HEV seropositivity (n=554)

		Number tested for HEV antibody	Number (%) seropositive
PED	Seropositive	50	48 (96.0%)
	Seronegative	504	468 (92.9%)

p=0.39

Table 11: Association between PED seropositivity and HEV RNA detection (n=554)

		Number tested for HEV RNA	Number (%) positive
PED	**Seropositive**	50	3 (6.0%)
	Seronegative	504	32 (6.4%)

p=0.92

Table 12: Association between PED and *Toxoplasma* seropositivity (n=532)

		Number tested for *Toxoplasma* antibody	Number (%) seropositive
PED	**Seropositive**	47	1 (2.1%)
	Seronegative	485	39 (8.0%)

p=0.15

Table 13: Association between PED seropositivity and ESBL *E. coli* (n=547)

		Number tested for ESBL *E. coli*	Number (%) positive
PED	**Seropositive**	50	19 (38.0%)
	Seronegative	497	113 (22.7%)

p=0.04