AHDB PORK

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SURVEILLANCE FOR NON-STATUTORY PATHOGENS IN WILD BOAR CULLED IN THE FOREST OF DEAN 2015-2016

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SUMMARY

- Faeces and serum samples collected from a subset of wild boar culled in the Forest of Dean in 2015-16 were tested for evidence of infection with, or exposure to, a selection of non-statutory endemic pathogens of GB pigs.
- The findings were broadly similar to those of a similar study performed in 2013-14 except that evidence of Hepatitis E virus infection was found; this pathogen was not included in previous testing.
- Serological evidence of exposure to *Leptospira* Bratislava was detected with a low estimated seroprevalence of 3.6%.
- Evidence of Hepatitis E virus infection or exposure was detected in nearly 6% of culled wild boar. One wild boar was excreting Hepatitis E virus (HEV) and the virus was identified as genotype HEV-3 and was not typical of HEV strains reported in domestic pigs or human cases.
- Analysis for spatial clustering showed that wild boar culled in one area had a significantly higher risk of being HEV positive than the risk outside of that area, suggesting a possible social group effect with HEV infection. No spatial clustering was identified in *L.* Bratislava positive wild boar.
- No *Salmonella* or *Brachyspira* species, or porcine epidemic diarrhoea virus were detected in faeces. No antibody to porcine reproductive and respiratory syndrome virus or *Mycoplasma hyopneumoniae* was detected in sera. With the given population and sample sizes this provides at least 95% confidence that the prevalence of those pathogens in this wild boar population is less than 4%.
- Although antibody to swine influenza or porcine epidemic diarrhoea was detected in a few wild boar in one assay for each pathogen, these results were not confirmed by other antibody assays.
- The results from this study are relevant for a long-established wild boar population in a forested region of England which has a relatively low commercial pig density and should not be extrapolated to wild boar populations which exist, or could establish, in other regions.
- Suggestions are given regarding future wild boar surveillance.

1. Background

The Forestry Commission England (FCE) undertook a wild boar cull from late 2015 to early 2016 in the Forest of Dean. The aim was to cull 400-500 animals from a population estimated by the FCE to be at least 1000 animals, based on a recent estimate (Gill and Ferryman, 2015). In the current study, AHDB Pork agreed to fund testing and epidemiological analysis of samples from 100-120 culled wild boar sampled by the Forestry Commission England in the Forest of Dean for an agreed selection of non-statutory contagious pig pathogens. The results can be compared with those from a similar study reported in 2014 (Williamson and others, 2014) and will indicate if the rise in the Forest of Dean's wild boar population in the intervening years coincides with any evidence of greater exposure of wild boar to endemic pig pathogens. AHDB Pork (as BPEX) funded the previous study to assess the prevalence of selected non-statutory pig pathogens in a subset of wild boar culled in 2013-14 (Williamson and others, 2014). This was in response to concerns of pig producers and practitioners in the Forest of Dean area and from the National Pig Association. In the previous study, there was no evidence of infection of culled wild boar with most non-statutory pig pathogens tested; there was a combined seroprevalence of 18% to leptospire (two serovars) and a single PRRSV-antibody positive, PRRS-virus negative, wild boar was detected (Williamson and others, 2014).

2. Rationale for testing culled wild boar for non-statutory pig pathogens

Testing for the selected high priority non-statutory pathogens identified in table 1 below was considered to be worthwhile surveillance in culled wild boar for the following reasons, most of which have become more relevant with the increase in the wild boar population in the region of the cull in recent years:

a) They are contagious endemic pig pathogens which can transmit between pigs and wild boar, within the wild boar population itself and, in the case of *Leptospira,* Hepatitis E virus and *Salmonella*, between wild boar and other species, including human as these pathogens are also zoonotic.

b) Porcine reproductive and respiratory syndrome virus (PRRSv), *Brachyspira hyodysenteriae* and *Mycoplasma hyopneumoniae* cause three of the top four diseases identified for control by the Pig Health and Welfare Council 20:20 VISION launched in 2011 (BPEX, 2011). In regions where pig herd prevalence of these infections (especially PRRS) is low, identifying sources of infection other than domestic pigs has become more important, especially where regional eradication is being considered.

c) Surveillance in wild boar elsewhere in Europe have included some of these pathogens (PRRSv, *Salmonella*, *Leptospira*, swine influenza) and there has been evidence of exposure to, or infection with, some.

d) The results may help in assessing wild boar and risk pathways in pathogen transmission between pigs and wild boar in the region. These risk pathways are explored in a publication on feral wild boar in England in relation to notifiable disease (Defra, 2008). This has become particularly relevant in the light of the role that wild boar are playing in the spread of ASF in Eastern Europe and Russia (EFSA, 2017).

e) The risk posed by wild boar to domestic pigs is not only to commercial herds as wild boar may be more likely to have direct contact with pigs on small holdings where external biosecurity tends to be poorer. If there is evidence of transmission of pathogens between these small herds and wild boar, the significant numbers of movements of small numbers of pigs between smallholder herds would facilitate wider dissemination, including into the commercial sector.

f) Hepatitis E virus was included this time as the status of GB wild boar for hepatitis E virus is not known and is of interest as, although the virus is not pathogenic in pigs or wild boar, it is zoonotic. The culled wild boar which pass meat inspection are sold for consumption, following *Trichinella* testing. The virus is highly prevalent in domestic pigs in GB (Grierson and others, 2015).

g) As most of the pathogens tested were ones causing disease in pigs, their presence in wild boar could cause morbidity and mortality and is likely to be of interest to those involved in wildlife disease and conservation.

h) There is value in holding these wild boar samples as an archive for future use.

3. Wild boar sampling

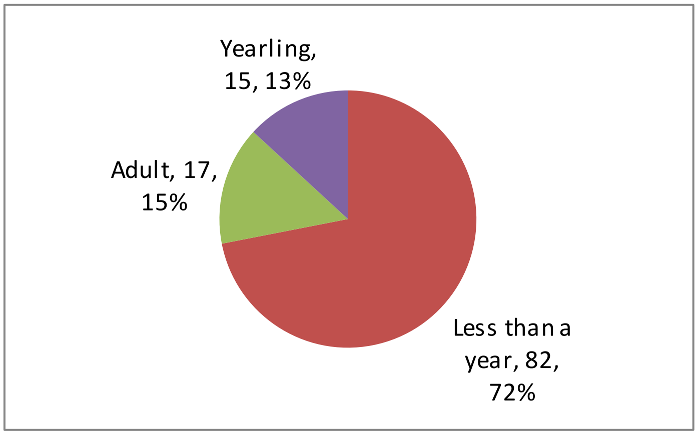
Training was provided to FCE staff collecting the samples to ensure that serum quality from blood samples was good with minimal haemolysis and contamination and maximise the number in a suitable condition for testing. Approximately 450 apparently healthy wild boar were culled by FCE between September 2015 and March 2016. Clotted blood and faecal samples were collected by FCE staff from 114 wild boar culled between 20/12/2015 and 4/02/115/03/2016 as soon as possible after carcases were returned to game larders for evisceration. Samples were labelled with the culled wild boar identity and each set of paired samples was immediately sent in a pre-paid first class post pack to APHA Bury St Edmunds where 112 faeces and 110 clotted blood samples were received. *Salmonella* cultures were set up on fresh faeces, sera were separated from the clotted bloods and serum samples were aliquoted and stored at minus 70 degrees Centigrade until they were tested further. From six wild boar, only faeces were received while from four others only clotted bloods were received. Samples from 111 of the sampled boar reached Bury St Edmunds within four days of culling. Samples from three wild boar took 5-9 days to reach the laboratory. Samples for this study were only collected from wild boar which did not have suspect TB lesions at meat inspection. The culled wild boar were also tested for *Trichinella* according to FSA requirements under a separate contract with APHA (OG0123).

4. Epidemiological details

Each culled wild boar had a unique FCE identification number against which background information was recorded. The information included the date and map reference of the cull, estimated age (less than one year, yearling, adult), dressed weight (after removal of head, viscera and lower legs, there is an estimated 25% to 30% reduction from live to dressed weight) and sex. On receipt at APHA Bury St Edmunds, samples from each individual boar received were given a unique APHA submission number and the FCE identification numbers were recorded for each sample.

Data were received for 114 culled boar, one had an incorrect identifier and so was omitted from the epidemiological analysis as it was not possible to link to denominator information. Figure 1 shows the estimated age distribution. More males were culled than females (65 male and 49 female), with a higher proportion of males being less than a year old than in the females.

Figure 1: Estimated age distribution of culled wild boar sampled



5. Results of testing for infection with or exposure to non-statutory pig pathogens

Table 1 summarises the results of testing. Appendix 1 gives details of the tests used, numbers of samples tested for each pathogen, whether pooling of samples was undertaken and other testing details.

Table 1: Results of testing for infection with or exposure to non-statutory pig pathogens

Pathogen	**Results**	**Interpretation**
Salmonella serotypes	No *Salmonella* isolated	No evidence of *Salmonella* excretion
Porcine respiratory and reproductive syndrome virus (PRRSv)	108 sera were antibody negative. One serum was inconclusive in the antibody ELISA and tested negative in the PRRSV PCR and IPMA for both antibody to both genotypes 1 and 2	No evidence of exposure to PRRSV
Brachyspira species	No DNA to *Brachyspira* species detected	No evidence of *Brachyspira* species excretion
Mycoplasma hyopneumoniae (enzootic pneumonia)	No antibody detected	No evidence of exposure to *Mycoplasma hyopneumoniae*
Swine influenza virus	All sera negative in IDVET ELISA. Eight sera antibody positive in IDEXX ELISA were tested by HAIT to four swine influenza strains and were negative.	Seropositivity to GB-endemic swine influenza not confirmed. Equivocal ELISA results in one test to be investigated further using other influenza strains.

Pathogen	Results	Interpretation
Porcine epidemic diarrhoea virus (PEDv)	Results of antibody detection using three different ELISA did not agree; all sera tested negative in one (IDVet), five sera were positive in the second (BioVet) and two were positive in the in-house ELISA but these two sera were different from the five BioVet positive sera. No PEDV was detected by PCR in faeces	Inconclusive serological testing for PEDV with conflicting results from three ELISAs – all tested negative in one ELISA. No evidence of PEDV excretion
Leptospira serovars	Four sera tested antibody positive; all to *Leptospira* pool 3 only. Individual serovar MATs were performed to determine which *Leptospira* serovar was most likely to have infected the seropositive wild boar. Two of the four pool 3-postive sera gave highest titres to *L*. Bratislava at 1/1600 and one at 1/200. One did not give titres to any individual pool 3 serovar and one gave a titre of 1/100 to both *L*. Bratislava and *L*. Australis	Evidence of exposure to *Leptospira* serovars in pool 3, antibody detected *Leptospira* serovar Bratislava
Hepatitis E virus	Four sera tested antibody positive. One faeces from an antibody-negative wild boar tested PCR-positive, serum from this boar was also PCR-positive	Evidence of exposure to HEV. In one animal, evidence of infection with, and excretion of, HEV

In summary; no wild boar tested positive for antibody to PRRSV or *M. hyopneumoniae*. No wild boar were found to be excreting *Salmonella* or *Brachyspira* species, or PEDV in faeces. Results for swine influenza did not confirm exposure to swine influenza strains in the HAIT panel, further testing is planned to investigate seropositivity in a few sera in one of the influenza A ELISA assays used. PEDV serology using three different ELISAs gave conflicting results and the results are considered inconclusive. As a result, the PED PCR was undertaken to check that there was no PEDV excretion with negative results.

Positive results were obtained for antibody to *Leptospira* Bratislava and Hepatitis E virus and one wild boar tested positive for the presence of HEV in both faeces and serum by PCR, this virus-positive boar was negative for HEV antibody.

6. Molecular analysis of HEV detected

The HEV detected in one wild boar was partially sequenced. The sequence obtained was a partial fragment of ORF2 and phylogenetic analysis demonstrated that it is genotype HEV-3 but interestingly it appears to be an outlier to that circulating in pigs and detected in human cases. When blasted against publically available sequence data in GenBank there was only about 83% identity match. Full genome sequencing is being attempted outside this study.

7. Epidemiological Analysis

The number of samples (107-112) tested for *Salmonella*, PRRSV, *M. hyopneumoniae* and PEDV (PCR) was sufficient to detect a positive animal if the pathogen was present with at least a 3% prevalence with 95% confidence. The number of samples tested for swine influenza, PEDV antibody and *Brachyspira* species (81-90) was sufficient to detect a positive animal if present at a 4% prevalence or greater with 95% confidence.

Four sera out of 82 were positive for antibody to HEV and one faeces out of 107 was positive by PCR (as was the serum from that boar). The estimated HEV seroprevalence was 4.9% (CI 0.2-9.6%), and the prevalence of faecal virus excretion (PCR positive) was 0.9% (CI 0.0-2.8%). Combining antibody and virus results gives an estimated prevalence of 6.3% (CI 0.9-11.6%). Four samples out of 110 were positive for *Leptospira* Bratislava, providing a seroprevalence estimate of 3.6% (CI 0.1-7.2%).

A logistic regression was used to assess whether significant associations (P-value<0.05) were present between either HEV or *Leptospira* status and the explanatory data (sex, age, weight (categorised and as continuous variable) and month of death). No significant associations were detected for increased or reduced risk of a wild boar sample being positive to Hepatitis E. However, samples collected in February were significantly less likely to be *Leptospira* positive than those collected in December, with samples from March also less likely but just above the significance cut off (P=0.055). The results of the risk factor analysis were affected by the small number of positive samples, meaning that many categories had no positives and only a small population, meaning that no estimate of risk could be generated. With this in mind, it should be noted that no *Leptospira* positive samples were found in males, in adult or yearlings or in wild boars weighing less than 30 kgs and more than 60 kgs. The age results differ from the findings in the 2014 report which found an increased risk of a sample being *Leptospira* antibody positive for adults and yearlings compared to those less than a year, although there were more seropositive wild boar on that occasion.

Analysis for significant spatial clustering of Hepatitis E antibody or virus positive wild boar was undertaken using SaTScan software. A case-control method was used to look for circular spatial areas that had significantly higher risk of being HEV positive than the risk outside of the circle. This analysis detected that the area where there were four HEV cases and a negative wild boar was a significant cluster (p-value 0.001). In contrast, the same analysis on the *Leptospira* results indicated that the most likely high risk cluster was not statistically significant (P=0.410).

8. DISCUSSION

This is the second time surveillance has been undertaken on a wild boar population in GB for exposure to, or infection with, non-statutory pathogens. The Forest of Dean population established from animals derived from two releases of captive wild boar in the 1990s and again in 2004. The population of wild boar in the Forest was estimated in 2015 to be at least 1000 animals and testing between 80 and 110 wild boar from the 2015-16 cull provides robust results from which to infer the status of the Forest of Dean population with respect to most of the pathogens tested. Infection and/or exposure of wild boar with two of the non-statutory pathogens tested for, *Leptospira* Bratislava and Hepatitis E virus, was confirmed, both of which are potential zoonoses.

Testing at least 80 samples from an estimated population of 1000 wild boar should detect at least one positive wild boar if prevalence is 4% with a confidence level of at least 95%. One can infer from the testing done that, at the time of the cull, there had been no or very low exposure to PRRSV, GB-endemic strains of swine influenza and *Mycoplasma hyopneumoniae* in the population and there was no or very low faecal excretion of *Salmonella* and *Brachyspira* species, and PEDV, and that the wild boar population was not sustaining endemic infection with these pathogens. There was thus no evidence of significant transmission from the pig population to wild boar at the time of sampling. Nor was there evidence that the wild boar were a reservoir of infection for pigs for the above pathogens.

The prevalence of HEV (antibody and virus) of 6% is low compared to that detected in UK pigs at the time of slaughter (Grierson and others, 2015). Testing for HEV was not performed in the previous study. Hepatitis E virus (HEV) is a zoonotic pathogen with a worldwide distribution, and infects several mammalian species, including pigs and wild boars. Prevalence estimates of virus infection in wild boar, reported recently in Italy, are variable but tend to be higher than found in this study (Montagnaro and others, 2015; Aprea and others, 2017). However, in both studies, HEV PCR was undertaken on liver samples rather than faeces. A higher seroprevalence of 42% was detected in wild boar in Japan (Hara and others, 2014). The four wild boar with antibody and no virus detected are not likely to pose a risk of zoonotic infection. However, one wild boar (0.9%) was viraemic and excreting virus in faeces, this animal had no HEV antibody indicating that it was in the acute stages of infection. Interestingly, this virus-positive wild boar was classed as less than one-year-old. Analysis for spatial clustering showed that wild boar culled in one area had a significantly higher risk of being HEV positive than the risk outside of that area, suggesting a possible social group effect with HEV infection possibly transmitting between a number of wild boar in a group, or obtained from a common source. Wild boar and pig products (especially those containing liver) are considered a potential source of HEV infection for humans mainly when eaten poorly cooked or uncooked (Colson and others, 2010). Those consuming wild boar products should take the same precautions as when consuming pork or other meat and meat products, and ensure that they are thoroughly cooked before consumption. Partial sequencing detected genotype HEV3 which is the genotype detected in European pigs and in indigenous UK human infections, however the virus appears to be an outlier and is not similar to HEV3 recorded in pigs and humans which fall into two groups within HEV3 (Grierson and others, 2015). Full genome sequencing of this HEV is being attempted.

The estimated *L.* Bratislava seroprevalence of 3.6% (CI 0.1-7.2%) is lower than that detected in the 2014 report (14.3%) and does not point to likely endemic infection in the wild boar population according to Ellis (1992) who suggested that a seroprevalence of 10% or more based on use of the MAT in pigs was consistent with endemic infection. On the basis of a relatively high seroprevalence of *L*. Bratislava and reports of its identification in association with reproductive disease, domestic pigs are considered to be a maintenance host and domestic animal reservoir of this serovar in GB (Williamson and others, 2004) and there are also reports of infection in horses (Smith and Dalley, 2006) and dogs. However, strains of *L*. Bratsilava have also been isolated from a range of wildlife species, including hedgehogs, rats, wood mice, voles and badgers; infections in some of these hosts may be incidental and, in predatory species, may relate to contact with wildlife reservoirs such as rodents and hedgehogs. Any of these species could potentially be a source of *L*. Bratislava serovar infection in wild boar and the scavenging behaviour of wild boar increases their exposure to known wildlife reservoirs. No spatial clustering was found in the *L.* Bratislava seropositive wild boar which supports incidental infections in wild boar from other hosts. *L*. Bratislava is potentially zoonotic although it is not a serovar recorded in association with human infections in recent UK Zoonoses reports. However, as for other leptospires, this presence of *L*. Bratislava infection emphasizes the need for good personal hygiene and protective clothing in those contacting wild boar, for example during evisceration of carcases. No evidence was found of exposure of wild boar to *L*. Pomona, Grippotyphosa or Tarassovi which are exotic to the UK and are pathogenic leptospire serovars present in pig populations elsewhere in the world.

No *Salmonella* species, another zoonotic pathogen, were isolated from faeces, despite cultures being performed promptly on individual fresh faeces using a sensitive method. Infection with a variety of serotypes has been detected in wild boar in surveys in Italy (Chiari and others, 2013), Switzerland (Wacheck and others, 2010) and Spain (Vicente and others, 2002). The Italian survey identified a variety of *Salmonella* serotypes in large intestinal contents consistent with a range of sources of infection including other wild boar, domestic livestock, waste, other wildlife species including birds and the environment. Although some of these sources of infection exist for wild boar in the Forest of Dean, the lower boar density than in some other European countries makes it less likely that *Salmonella* serotypes will establish as adapted strains in the wild boar population and may, in part, explain the perhaps surprising lack of *Salmonella* isolations.

Testing for *Brachyspira* species was undertaken by PCR which can detect non-viable organisms and no *Brachyspira* species were found in faeces by this method which was also used in Australia where *Brachyspira hyodysenteriae* and *pilosicoli* were detected in the faeces of free-living wild boar (Phillips and others, 2009). A recent study reported from Spain did not detect *Brachyspira* species in faeces from adult wild boar (Vadillo and others, 2017) using bacteriological culture.

There was no evidence of exposure to PRRSV. In Switzerland (Wu and others, 2011) and Germany (Sattler and others, 2012), antibody to PRRSv was detected in 0.43% and 1.2% of wild boar respectively. This equated to just one boar testing positive in each survey and there was no further confirmation of the ELISA positive results in the German study. In the Swiss study, the ELISA positive result was confirmed using the immunofluorescent antibody test.
No exposure to swine influenza strains endemic to pigs in GB (avian-like H1N1, pandemic H1N1 2009 or H1N2) or to H3N2 was detected. However, a few sera had antibody to influenza A in one of the two ELISA assays used. Possible reasons for this will be investigated and, if sufficient sample remains, the ELISA-positive sera will be tested against wider influenza strains. Pigs have been known occasionally to be infected with other influenza A viruses such as H9N2, however H5 and H7 infections are rare in pigs. Antibodies to swine influenza have been detected at low levels in wild boar in Germany (Sattler and others, 2012) and Spain (Vicente and others, 2002), both countries where there are significant wild boar populations.

No faecal excretion of PEDV, a virus which is highly contagious by the faeco-oral route was detected. In the last survey, no PED antibody was detected using the in-house ELISA. In view of the recognised issue of possible false positive results with PED serological tests, three antibody assays were used, in one of which all the sera were negative. The low level seropositivity detected in the other two tests did not correlate with one another and it is suspected that they represent cross-reactivity and will be investigated further. A national seroprevalence of 9% was detected using the in-house ELISA in pigs sampled in 2013 (Cheney and others, 2014) suggesting that there was low-level endemic PEDV infection. However, since then, no PEDV has been detected in diagnostic submissions to APHA from diarrhoeic pigs which are being tested by PCR.

The wild boar population of the Forest of Dean is in an area which has a low density of commercial pig units and a high proportion of smaller pig units (see Figures 2 and 3) which influences the risks of wild boar becoming infected with pig pathogens and/or transmitting pathogens on to domestic pigs. One might expect that wild boar may be more likely to have contact with pigs or pig manure on small holdings where external biosecurity tends to be poorer, pigs are often kept outdoors allowing nose to nose contact with wild boar and fencing may be adequate to keep pigs in but not to keep wild boar out, particularly if the wild boar are seeking food, or sows in oestrus are present. Whilst this may be true, where small stable groups of mainly older pigs are present without regular introductions of naïve pigs, endemic infection with some pathogens, especially viruses like swine influenza and PRRSv, may not establish or persist and these pigs may thus be less likely to be a source of infection to wild boar. In Switzerland, Batista Linhares and others (2015) detected *Mycoplasma hyopneumoniae* infection in wild boar but proposed that spillover from domestic pigs to wild boar was more likely than transmission from wild boar to pigs. Wild boar density, occurrence of EP outbreaks in domestic pigs and young age were identified as risk factors for infection in the wild boar in their study.

The multiple factors which affect the probability of transmission of pathogens between a wild boar population and domestic pigs in the vicinity mean that the results of this study should not be extrapolated to wild boar populations which exist, or could establish, in other regions.

9. Future surveillance

Further testing on the samples collected in 2015-16 can be considered if funding is available or sought from elsewhere by other parties, subject to agreement from appropriate Defra/APHA policy departments.

Testing for other zoonoses, some of which are not associated with disease in wild boar, could include serology for *Toxoplasma gondii* and culture of faeces for *Yersinia* or *Campylobacter* species. Metagenomic studies could be considered for bacterial species in the faeces and include assessment of antimicrobial resistance genes. Barth and others (2015) suggest that faecal *Escherichia coli* could be used as biological indicator of contact between wild boar and domestic pig, although molecular studies did not reveal markers that would identify the direction of transmission. Looking globally, *Ruiz-Fon (2015) identified* viruses such as hepatitis E virus, Japanese encephalitis virus, Influenza virus and Nipah virus, and bacteria such as *Salmonella* spp., Shiga toxin-producing *Escherichia coli*, *Campylobacter* spp. and *Leptospira* spp. as the most prone to be transmitted from wild swine to humans, not all of these are relevant to the UK.

Any testing for statutory pathogens would have to be considered and agreed by Defra policy/APHA. The UK is declared free of *Brucella suis* and Aujeszky's disease virus which are known to be present in free-living wild boar populations elsewhere in the world, including Europe (Pedersen and others, 2014). None of the wild boar sampled for this study had visible tuberculous lesions at meat inspection. For early detection of notifiable disease, investigation of wild boar mortality and detection of new and emerging disease, wild boar surveillance based on examination and testing of found dead or euthanased sick wild boar is more appropriate and sensitive than testing culled healthy wild boar.

Lower priority non-statutory endemic pathogens of GB pigs could be included in future surveys, funding allowing, and some have been part of studies in Spain, Germany and Japan (Vicente and others, 2002; González-Barrio and others, 2015; Sattler and others, 2012; Abe and others, 2011). Collection of other samples (e.g. tonsils, liver) in addition to blood would extend the range of pathogens that could be considered for future surveillance, provided that training could be provided to those collecting samples. Serology and/or pathogen detection for any of following pathogens could be considered; porcine circovirus 2, porcine parvovirus, porcine enteroviruses, porcine sapelovirus and various bacterial pathogens (for example, *Erysipelothrix* sp., enteropathogenic *Escherichia coli*, *Streptococcus suis, Haemophilus parasuis, Pasteurella* *multocida, Actinobacillus pleuropneumoniae*). Antimicrobial sensitivities of any pathogens isolated would also be of interest.

The training in sample collection and provision of pre-paid sample kits for immediate dispatch of samples to the laboratory improved the quality of serum samples, and the validity of *Salmonella* culture results and a similar approach is recommended for any future sampling initiative. This survey was reliant on FCE staff for sample collection and in future surveys, their collaboration would again be essential.

Alongside any surveillance for pathogens, regular geographic mapping of pig units and wild boar distribution, and assessment of wild boar populations is important to monitor the potential for, and risk of, interaction between the two species and transmission of pathogens and to gauge the need, scale and success of population control measures over time.

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Appendix 1 Details of testing of wild boar samples for non-statutory pig pathogens
All tests performed by APHA except *Brachyspira* species PCR‡ which was subcontracted to SAC CVS Edinburgh

Pathogen	Sample	Test details	Number tested	Test Reference if available	Comments
Salmonella serotypes	Faeces	TC0699	112	Modified Semi-Solid Rappaport-Vassiliadis (MSRV) medium used for *Salmonella* isolation where low numbers of organism may be present	Each fresh faeces cultured individually immediately on receipt, 85% within three days of cull
Porcine respiratory and reproductive syndrome virus (PRRSv)	Serum	TC0412 Antibody ELISA TC0413 and TC0323 genotypes 1 and 2 IPMA	109 1	IDEXX PRRS X3 enzyme-linked immunoassay http://www.idexx.co.uk/livestock-poultry/swine/prrs.html	IPMA for both North American and European genotypes on the one ELISA inconclusive serum
		TC0718 RT-PCR for viral nucleic acid detection (ORF 7 gene)	1	Frossard and others (2012)	PCR undertaken on the one ELISA inconclusive serum
Brachyspira species	Faeces	TC0495 *Brachyspira* species PCR ‡	90 as 30 pools	23s RNA/RFLP PCR detects and differentiates *B. hyodysenteriae*, *B. pilosicoli* and *B. innocens* group.	Faeces tested in pools of three. Tested at SAC CVS Edinburgh
Mycoplasma hyopneumoniae enzootic pneumonia	Serum	TC0456 Antibody ELISA	109	Blocking ELISA commercially available from DAKO	
Swine influenza virus	Serum	Antibody ELISA x 2 TC0160 Antibody HAIT	81 10	IDEXX and IDVet influenza A ELISAs OIE (2010)	Positive or inconclusive ELISA sera tested in influenza HAIT for antibody to four strains (avian-like H1N1, pandemic H1N1, H1N2 & H3N2).
Porcine epidemic diarrhoea virus (PEDv)	Serum Faeces	a) Antibody ELISA 1 b) Antibody ELISA 2 c) TC0377 In-house antibody ELISA TC0398 PCR	86 86 20 107	https://www.id-vet.com/produit/id-screen-pedv-indirect/ http://www.biovet.ca/wp-content/uploads/doc/product/PEDV%20ELISA%20kit%20EN.pdf van Nieuwstadt and Zetstra (1991) PED/TGE qRT-PCR QIAGEN kit, Germany	Antibody ELISAs do not distinguish antibody to virulent PEDV from endemic PEDV
Leptospira serovars	Serum	TC0399 Antibody – Microagglutination test (MAT) 6 pools TC0451 MAT Pool 3	110 4	OIE (2014), Ellis (1992)	19 serovars tested in 6 pools, positive sera tested vs. individual serovars to identify serovar with highest titre
Hepatitis E virus	Serum Faeces Serum	Hepatitis E virus antibody ELISA RT-PCR	82 107 1	Wantai Total HEV Antibody kit (Fortress Diagnostics Ltd., Antrim, UK) Berto and others (2012)	Virus in positive samples partially sequenced