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

BPEX-FUNDED PROJECT RDOR1035

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SUMMARY

- Faeces and serum samples collected from 91 wild boar culled from the Forest of Dean were tested for non-statutory endemic pathogens of GB pigs.
- Serological evidence of exposure to two *Leptospira* serovars was detected with a combined seroprevalence of nearly 18%.
- *Leptospira* Bratislava, the *Leptospira* serovar to which most seropositive wild boar had antibody, is pig-adapted and also occurs in a range of wildlife species. The seroprevalence detected was significantly higher in older pigs, and the results are consistent with endemic infection in the wild boar population.
- A lower seroprevalence was detected to *Leptospira* Javanica which is reported in the literature to be associated with a rodent reservoir and is zoonotic.
- One adult female was positive for antibody to porcine reproductive and respiratory syndrome virus by ELISA but active infection was not detected, indicating potential for infection but not clear evidence that the wild boar population is a reservoir of infection to domestic pigs.
- Infection with, or exposure to, other pathogens tested was not confirmed and with the given population and sample size this indicates at least 95% confidence that the prevalence of those pathogens in this wild boar population is less than 5%.
- No *Salmonella* species were detected, culture results for a proportion of faecal samples may have been affected by the time interval between collection and culture.
- These results 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 regarding future wild boar surveillance are given.

1. Background

The Forestry Commission England (FCE) undertook a wild boar cull in the Forest of Dean between September 2013 and March 2014. The aim was to cull approximately 100 animals from a population estimated by the FCE to be of the order of 400-600 animals, although this is not based on a systematic survey (Natural England Report to Defra FFG 2012/1). BPEX approached AHVLA about the possibility of using the cull as an opportunity to undertake some wild boar surveillance. BPEX also received a request from pig practitioners in the area and the National Pig Association was also very supportive. BPEX kindly agreed to provide funding for testing and epidemiological analysis.

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

Testing for 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:

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* and *Salmonella*, between wild boar and other species. *Salmonella* and *Leptospira* infections are also zoonotic.

b) Porcine reproductive and respiratory syndrome virus (PRRSv), swine dysentery and *Mycoplasma*

hyopneumoniae are three of the top four diseases identified for control by the Pig Health and Welfare Council 20:20 VISION launched in 2011 and embraced by BPEX's Pig Health Improvement Project. In regions where pig herd prevalence of these infections (especially PRRS) is low, identifying any other sources of infection has become more important, especially where regional eradication is being considered. Farmers think of wild boar as a potential source of infection, no testing of the wild boar population has been done to provide evidence to confirm or refute this.

c) Surveillance in wild boar elsewhere in Europe have included some of these pathogens (PRRSv, *Salmonella*, *Leptospira*, swine influenza) and has shown evidence of exposure to, or infection with, some. Testing at least 60 sera from a population of 600 should detect at least one positive wild boar if prevalence is 5% with a confidence level of 95%. Given the limited size of the population, this level of testing will also allow estimation of prevalence and for an expected prevalence of 5%, testing 60 animals would give a confidence of 95% and accuracy of 5.2% in the measured prevalence value.

d) The results may help in assessing wild boar as a risk pathway in pathogen transmission between pigs and wild boar in the region. This has become particularly relevant in the light of the role that wild boar are playing in the spread of African Swine Fever in Eastern Europe and Russia.

e) The risk posed by wild boar is not only to commercial herds as they may be as, or more, likely to have direct contact with pigs on small holdings where external biosecurity tends to be poorer. There are significant numbers of movements of small numbers of pigs between smallholder herds with potential for dissemination of pathogens between these and wild boar, and thence into the commercial sector.

f) As these pathogens are 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.

g) There is value in holding samples from culled wild boar as an archive.

h) Undertaking this survey would provide information regarding the logistics and feasibility of similar work in the future.

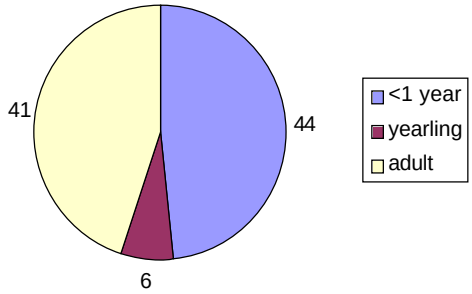
3. Wild boar sampling

Approximately 150 apparently healthy wild boar were culled by FCE between September 2013 and March 2014. Samples were collected by FCE staff from 91 wild boar culled between 2/09/13 and 4/02/14 after carcases were returned to game larders for evisceration. Clotted blood and faecal samples were collected, transported via ex-Fera staff to AHVLA Langford from where they were sent to AHVLA Bury St Edmunds grouped into 19 separate submissions. At Bury St Edmunds, sera were separated from the clotted bloods and 89 faeces and 89 serum samples were aliquoted and stored at minus 70 degrees Centigrade. From two boar only faeces were received while from two others only clotted bloods were received. Samples were received between one and 11 days after the wild boar had been culled, samples from 77 of the sampled boar reached Bury St Edmunds within seven days of culling. Samples 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 AHVLA (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 cull, estimated age (0 = less than one year, 1 = yearling, X = 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 AHVLA Bury St Edmunds, each set of samples received on a given day was given a unique AHVLA submission number and the FCE identification numbers were recorded for each sample. Data were received for 91 culled boar from an estimated 150 culled. Figure 1 shows the estimated age distribution.

Figure 1: Estimated age distribution of culled wild boar sampled



Approximately equal numbers of male and female culled wild boar were sampled: 47 female and 44 male. Figures 2 and 3 show the Forest of Dean and surrounding area together with an estimation of pig unit locations based on electronic pig movement (eAML2) data. The figures show premises recording at least one movement of live pigs on or off the unit in the two years up to September 2013 when the cull began. Pig premises in figure 2 are displayed according to the cumulative number of pigs moved, those in figure 3 are displayed according to the cumulative number of movements made.

5. Testing for non-statutory pathogens

Sera were tested for PRRS virus and antibody and for antibody to *Mycoplasma hyopneumoniae*, swine influenza virus, porcine epidemic diarrhoea virus (PEDv) and 19 *Leptospira* serovars. Faeces were tested for *Salmonella* serotypes and *Brachyspira* species. Culture for *Salmonella* was initiated on fresh faeces on the day on which it was received at Bury St Edmunds. Appendix 1 gives details of the tests used, numbers of samples tested for each pathogen, and whether pooling of samples was undertaken. Diluted sera were filtered (0.20• m) to improve serum quality for leptospira serology.

Leptospira MAT was performed on 84 sera to detect antibody to six *Leptospira* pools constituting 19 serovars. Fifteen sera tested positive; 11 to pool 3 only, two to pool 5 only and two to both pools 3 and 5. Individual serovar MATs were performed to determine which *Leptospira* serovar was most likely to have infected the seropositive wild boar. Twelve of the 13 pool 3-postive sera gave highest titres to *L*. Bratislava, one did not give titres to any individual pool 3 serovar. The four pool 5-positive sera all gave titres to *L*. Javanica.

No sera tested positive for antibody to *Mycoplasma hyopneumoniae* or porcine epidemic diarrhoea virus.

One serum sample had a low positive antibody ELISA result to PRRSv (0.66 S/P ratio) and when tested in the more specific IPMA tests for US and European PRRSv genotypes, gave negative (<1/10) results in both tests. No PRRS virus was detected by PCR in this, or any other of the serum samples. The ELISA positive serum was from an adult female culled in September 2013, this female was seronegative to *Leptospira* serovars.

Testing for antibody to four swine influenza serology strains detected no significant titres (less than 1/40) to avian-like H1N1, pandemic H1N1 and H1N2. Titres of 1/40 or more are generally considered to be positive. Seven sera had titres of 1/40 to H3N2 while six sera gave reactions of 1/20 and nine sera gave reactions of 1/10. All 22 sera with any titre to H3N2 were tested in the IDEXX influenza ELISA and all gave negative results.

No *Brachyspira* species DNA was detected in any of the 31 pools of three faeces each tested. No *Salmonella* serotypes were cultured from any of the 29 pools cultured.

6. Analysis

The estimated combined *Leptospira* seroprevalence was 17.9% (with a 95% confidence interval of 9.7-26.1%). The estimated seroprevalences for *L*. Bratislava and *L*. Javanica individually were 14.3% and 4.8% respectively. Appendix 2 shows the analysis undertaken of leptospire serology against the various parameters recorded to determine whether any parameter was associated with a wild boar being more likely to be seropositive. Only age was found to have an association; being a yearling or an adult was significantly positively associated with being *Leptospira* seropositive. This association was strong for adults but the association with being a yearling had a relatively high standard error as there were only six

yearling wild boar and was only just above the significant 0.05 p-value. There was no association with month of cull, sex or weight. The estimated PRRSv seroprevalence based on ELISA results was 1.1% (with a 95% confidence interval of 0.0-3.3%).

7. DISCUSSION

This is the first substantial surveillance undertaken on a wild boar population in GB for exposure to, or infection with, non-statutory pathogens. The Forest of Dean population has established from animals derived from two releases of captive wild boar in the 1990s and again in 2004. The FCE 2013 estimated the population of wild boar in the Forest to be 535 animals (FCE 2014) and testing nearly 90 culled wild boar from the 2013-14 cull provides sufficiently robust results from which to infer the status of the Forest of Dean population with respect to the pathogens tested.

Exposure of wild boar to just one of the non-statutory pathogens, *Leptospira*, was detected by this survey with the antibody results indicating that two different serovars were infecting them; *L*. Bratislava and *L*. Javanica. On the basis of the 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 wildlife, 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. However, the seroprevalence of 14.3% is sufficiently high to indicate likely endemic infection in the wild boar population as Ellis (1992) suggested that a seroprevalence of 10% or more based on use of the MAT in pigs was consistent with endemic infection. The wild boar may themselves be acting as a wildlife reservoir of *L*. Bratislava infection and, as infection persists in the urogenital tract of pigs and infection can be transmitted in semen during service or in urine, the infection may be being maintained within the wild boar population. The significantly higher seroprevalence in adults in particular could suggest that venereal transmission is playing a part once the wild boar become reproductively active. Exposure to *L*. Javanica has not been detected in domestic pigs in GB but low-level seroprevalence was reported in pigs in Greece as well as in goats (Burriel and others, 2003) and this serovar has been associated with a rat reservoir elsewhere in the world associated with disease in cattle and humans (Natarajaseenivasan and others, 2011). *L*. Javanica has also been isolated from squirrels and rats in GB (Charlie Dalley, personal communication). The seroprevalence to *L*. Javanica is low in these wild boar and they may be an incidental host for this serovar associated with infection in another wildlife species. Zoonotic infection due to *L*. Javanica is reported and evidence of its presence emphasizes the need for good personal hygiene and protective clothing in those contacting wild boar, for example during culling. 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.

Another zoonotic pathogen which was tested for, *Salmonella* species, was not isolated from faeces of any of the sampled wild boar. 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). In the Italian survey, large intestinal contents were collected and delivered daily to the laboratory while in the Swiss survey, *Salmonella* were detected in 12% of tonsils but not in faeces from the same boars. In the Spanish study, only serology was performed indicating exposure to, but not assessing current infection with, *Salmonella* species. In this study, faeces were cultured on arrival using a method suitable for detecting low numbers of organism, however, the delay in some faeces reaching the laboratory may have meant that organisms were no longer viable in a proportion of samples and tonsils were not collected. The Italian survey identified a variety of *Salmonella* serotypes consistent with a range of sources of infection including from the wild boar population itself, 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 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 using this method which was used in Australia and detected *Brachyspira hyodysenteriae* and *pilosicoli* in the faeces of wild boar (Phillips and others, 2009).

Antibody to PRRSv was detected in a single wild boar by ELISA representing a very low estimated seroprevalence. In Switzerland (Wu and others, 2011) and Germany (Sattler and others, 2012), antibody to PRRSv was detected in 0.43% and 1.2% wild boar respectively which also equated to one boar testing positive in each survey. In the German study the ELISA positive result was not confirmed by further testing, in the Swiss study, the ELISA positive result was confirmed using the immunofluorescent antibody test. In this study, the wild boar which was PRRSv ELISA positive tested negative in the genotype 1 and genotype 2 IPMAs and by PCR – this does not mean the ELISA result is necessarily a false positive and indicates the potential for infection. This is most likely to have occurred through contact with infected domestic pigs or PRRSv-contaminated fomites. However, overall the results do not point to widespread or active PRRSv infection in the wild boar population when sampled or suggest that they represented a significant source of infection to domestic pigs at the time of sampling.

No exposure to swine influenza strains avian-like H1N1, pandemic H1N1 2009 or H1N2 was detected. Although a few sera had low positive HAIT titres to strain H3N2, this strain has not been detected in the UK since 1997 and the AHVLA swine influenza disease consultant decided that, as these sera tested negative in the swine influenza ELISA, that the HAIT titres were not sufficient evidence of exposure to H3N2 virus (Ian Brown, personal communication). 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.

The absence of antibody to PEDv, a virus which is highly contagious by the faeco-oral route, provides evidence that no endemic or other PEDv strain was circulating in this wild boar population.

The logistic and practical difficulties associated with obtaining good quality samples from wildlife are acknowledged by others (Arenas-Montes and others, 2013) and, in this survey, there was also local sensitivity to the cull which made sampling at the deer larder preferable. Collection of blood at the same time as evisceration and the delay before separating sera from blood clots did affect the quality of some samples. A proportion of blood samples were noted to be haemolysed and it was expected that this might limit the number of sera which were in a suitable condition for testing. In the event, most sera were used for most tests but, when calculating the confidence intervals for the results, a conservative estimate of 60 sera being of good quality was used. Testing 60 sera from this population of wild boar should detect at least one positive wild boar if prevalence is 5% with a confidence level of at least 95%. One can infer from the testing that there had been no or very low exposure to PRRSv, swine influenza, *Mycoplasma hyopneumoniae* and PEDv in the population and no or very low active infection with PRRSv and *Brachyspira* species and that the population was not sustaining significant endemic infection with these pathogens. However, as testing for *Salmonella* by culture may have been affected by delays in submission of some samples, the negative results do not reliably indicate absence of infection.

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 small pig units (see Figures 2 and 3; Fran Baird, personal communication) 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 populations of mainly older pigs are present, endemic infection with some pathogens, especially viruses like swine influenza and PRRSv, may not establish or persist and these units may thus be less likely to be a source of infection to wild boar. For other pathogens, such as swine dysentery, endemic infection can persist at low level even in older pigs. 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.

8. Future surveillance

Further testing on the samples collected can be considered if funding is available or sought from elsewhere by other parties, subject to agreement from appropriate Defra/AHVLA policy and departments. Food-borne zoonoses which infect, but do not cause disease in, wild boar which could be tested for include *Toxoplasma gondii*, *Yersinia* and *Campylobacter* species and hepatitis E virus. The wild boar sampled have already been tested for *Trichinella* species (muscle, project OG0123) and *Salmonella* serotypes.

Defra/AHVLA may have interest in testing for statutory pathogens. Any statutory testing would have to be considered and agreed by Defra/AHVLA policy. None of the wild boar sampled had visible tuberculous lesions at meat inspection. A Wildtech project at AHVLA incorporating novel technology to test for pathogens includes some statutory/notifiable pathogens and may be suitable, depending on Defra/AHVLA policy agreement. For early detection of notifiable disease, investigation of wild boar mortality and detection of new and emerging disease, wild boar surveillance based on testing for pathogens in found dead or euthanased sick wild boar is more appropriate than testing of 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 and Germany (Vicente and others, 2002; Sattler and others, 2012). Collection of other samples (e.g. tonsils) 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. This testing could include serology for erysipelas, porcine circovirus 2 and porcine parvovirus and pathogen detection (PCR, culture) for porcine circovirus 2 and various bacterial pathogens (enteropathogenic *Escherichia coli*, *Streptococcus suis, Haemophilus parasuis, Pasteurella multocida, Actinobacillus pleuropneumoniae*). The antimicrobial sensitivity patterns of any bacterial pathogens isolated would also be of value.

If future surveys are planned, collecting samples in the field immediately after death would be preferable, logistics permitting, and provision of pre-paid sample kits for posting would allow prompt dispatch to the laboratory and improve sample quality. 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 will also help monitor the potential for, and risk of, interaction between the two species.

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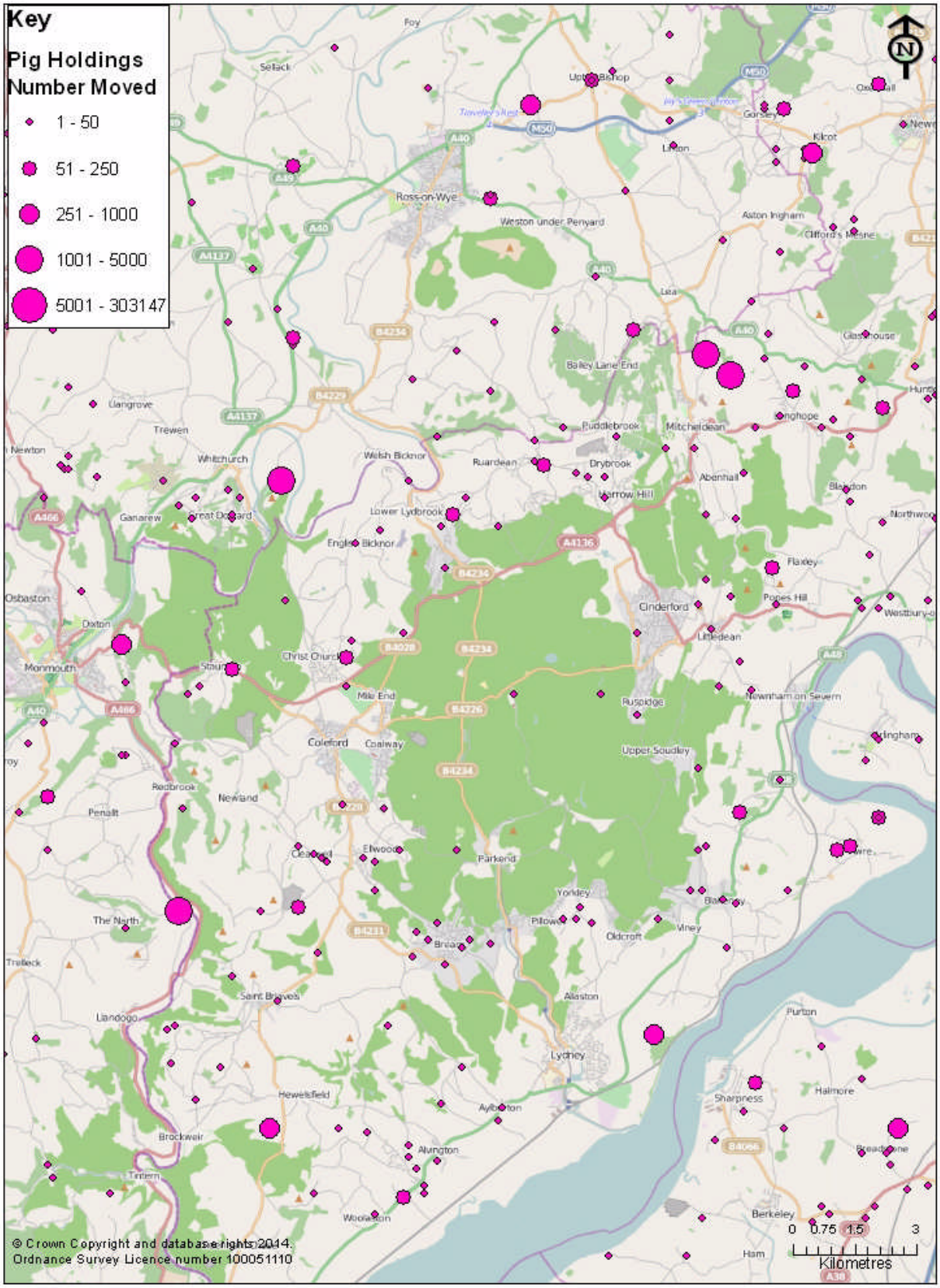
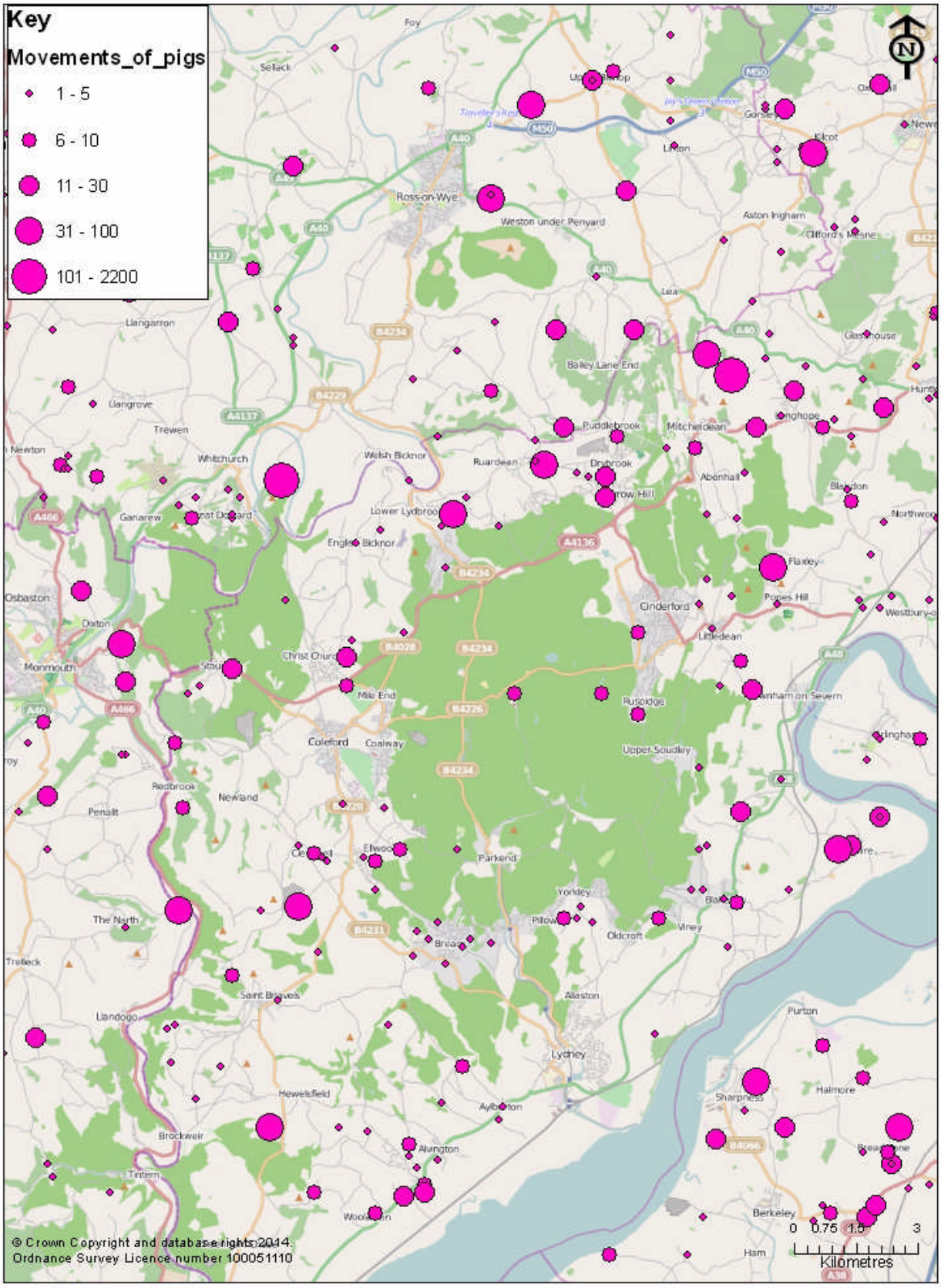
Figure 2 Pig premises with movements between Oct2011-Sept 2013 (cumulative number of pigs moved)



CREATOR: DSG GIS DATE: 13/06/2014	Culled wild boar and pig holdings at the Forest of Dean	AHVLA Animal Health and Veterinary Laboratories Agency

Figure 3 Pig premises with movements between Oct2011-Sept 2013 (cumulative number of movements)



CREATOR: DSG GIS DATE: 13/06/2014	Culled wild boar and pig holdings at the Forest of Dean	AHVLA Animal Health and Veterinary Laboratories Agency

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

Pathogen	Sample	Test details	Number tested	Test Reference if available	Comments
Salmonella serotypes	Faeces tested in pools of 1-5	M.S.R.V. selective culture for *Salmonella* isolation where low numbers of organism may be present	29	Modified Semi-Solid Rappaport-Vassiliadis (MSRV) medium used	Faeces tested in pools of 1-5. Fully identify and antimicrobial sensitivity testing of any positive cultures.
Porcine respiratory and reproductive syndrome virus (PRRSv)	Serum	Antibody ELISA IPMA	89 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 any ELISA positive sera
		RT-PCR for viral nucleic acid detection (ORF 7 gene)	89	Frossard and others (2012)	Sequence ORF 5 gene of any PCR-positive samples
Brachyspira hyodysenteriae (swine dysentery)	Faeces tested in pools of 3	*Brachyspira* species PCR ‡	31	23s RNA/RFLP PCR detects and differentiates *B. hyodysenteriae*, *B. pilosicoli* and *B. innnocens* group.	Faeces tested in pools of three.
Mycoplasma hyopneumoniae (enzootic pneumonia)	Serum	Antibody ELISA	89	Blocking ELISA commercially available from DAKO	
Swine influenza virus	Serum	Antibody HAIT Antibody ELISA	89 22	OIE (2010) IDEXX swine influenza A ELISA http://www.idexx.co.uk/livestock-poultry/swine/swine-influenza-virus.html	Tests for antibody to four strains (avian-like H1N1, pandemic H1N1, H1N2 and H3N2). Equivocal results followed up with influenza ELISA
Porcine epidemic diarrhoea virus (PEDv)	Serum	Antibody ELISA	89	van Nieuwstadt and Zetstra (1991)	ELISA does not distinguish antibody to virulent PEDv from endemic PEDv
Leptospira serovars	Serum	Antibody – Microagglutination test (MAT) 6 pools Pool 3 Pool 5	84 13 4	OIE (2014), Ellis (1992)	19 serovars tested in 6 pools, positive results followed up to identify serovar with highest titre

Appendix 2 Analysis of *Leptospira* serology for association with recorded wild boar parameters

91 samples from 19 submissions, 7 samples not tested for lepto

Estimated prevalence of Lepto in wild boar

17.9%	prevalence
9.7%	lower 95% confidence interval
26.1%	upper 95% confidence interval

Due to multiple samples from the same submission a mixed-effects model was used with submission ID as the random effect. This was not found to significantly improve the model and so it was discarded and a logistic regression used

Sex	Positive	Negative	% pos	OR	P-value
F	9	34	20.9	1.00	baseline
M	6	35	14.6	0.65	0.453

Age	Positive	Negative	% pos	OR	P-value
0 = less than one year	2	36	5.3	1.00	baseline
1 = yearling	2	4	33.3	9.00	0.052
X = adult	11	29	27.5	6.83	0.017

Month	Positive	Negative	% pos	OR	P-value
Nov	5	2	71.4	7.50	0.085
Oct	1	10	9.1	0.30	0.365
Sep	5	29	14.7	0.52	0.487
Dec	2	6	25.0	1.00	baseline
Feb	0	7	0.0	(omitted)	
Jan	2	15	11.8	0.40	0.409

Weight (kg)	Lepto binary

Min	Avg	Max	
11.1	45.7	103.4	0
30.0	56.5	91.6	1

OR	P-value
1.02	0.116

These are spatial coordinates - to examine spatial relationships

AvgOfX	AvgOfY	Lepto binary
362033	211865	0
359733	211780	1

Variable	OR	P-value
x	1.00	0.159
y	1.00	0.249