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Porcine reproductive and respiratory syndrome virus seroprevalence in Scottish finishing pigs between 2006 and 2018

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Abstract
Background: Porcine reproductive and respiratory syndrome (PRRS) is a major endemic pig disease worldwide and is associated with considerable economic costs.

Methods: In Scotland, three abattoir surveys were conducted in 2006 (158 farms), 2012–2013 (94 farms) and 2017–2018 (97 farms) to estimate seroprevalence to PRRS virus (PRRSV) in commercial finishing pigs. These surveys covered around 79%, 59% and 66% of the Quality Meat Scotland assured farms slaughtering pigs in Scotland in 2006, 2012–13 and, 2017–18 respectively. In the 2006 survey, six pigs per farm were sampled and tested using the CIVTEST SUIS PRRS E/S test. In the 2012–2013 and 2017–2018 surveys, 10 pigs per farm were sampled and tested using the IDEXX PRRS X3 Ab test. A farm was considered positive if it had one or more seropositive samples.

Results: The prevalence of positive farms was 45.6% (95% CI: 38.0–53.4), 47.8% (95% CI: 38.1–57.9) and 45.4% (95% CI: 35.8–55.3) in the 2006, 2012–2013 and 2017–2018 surveys, respectively, and 70%–75.5% farms did not change their status between sampling periods.

Conclusion: The prevalence of PRRSV exposure in Scottish pig herds was high and changed little from 2006 to 2018. These surveys have informed planning for a prospective PRRS control programme in Scotland.

KEYWORDS
pigs, PRRS, seroprevalence, surveys

INTRODUCTION

Porcine reproductive and respiratory syndrome virus (PRRSV) is the major cause of reproductive and respiratory problems in pigs worldwide. PRRSV has two distinct genotypes: PRRSV-1, formerly known as type 1, a European genotype associated with a relatively milder form of the disease and PRRSV-2, formerly known as type 2, a North American genotype usually associated with more severe clinical signs. Transmission of PRRSV primarily occurs by direct contact between infected and naïve pigs, in semen from infected boars and by in utero infection.

PRRSV-1 emerged in the United Kingdom (UK) during the early 1990s and is now endemic. PRRSV-2 has not been detected in the UK pig population to date. All PRRSV-1 strains from cases diagnosed in Great Britain (GB) that have been sequenced are within a fairly distinct cluster in subtype 1. Scanning Surveillance Network data show that PRRS remains one of the most prominent diagnoses made in GB.

There have been few diagnostic surveys to estimate the prevalence of PRRSV infection in the UK. A cross-sectional study of 103 British pig herds was conducted in 2003–04 to investigate the between- and within-herd variability of PRRSV antibodies. It found that...
35 herds (34%) were seronegative, 41 (40%) were seropositive and not vaccinated, and 27 (26%) were vaccinated. More recently, a UK-wide survey in 2013 tested pigs at slaughter for PRRSV antibodies. They found a seroprevalence of 58.3% (95% confidence interval (CI): 53.1–63.4) for PRRSV. The lowest seroprevalence was found in pigs aged >12 months (32.1%, 95% CI: 15.0–49.3) and the highest in pigs aged <6 months (68.3%, 95% CI: 54.5–82.5). These results confirm that PRRSV is endemic in the UK, and costs to the UK pig industry were estimated to be around £80 per sow and £3.50 per finishing pig.

Some countries have PRRSV free status, for example, Argentina, Australia, Brazil, Chile, Finland, New Zealand, Norway, Sweden and Switzerland. Others have eliminated the virus at regional level, for example, Horne Peninsula in Denmark. PRRSV-free status in previously infected countries has been achieved by national disease elimination programmes, for example, Chile and PRRSV-free status maintained or restored by strict health controls for imported pigs and semen, for example, Switzerland. Overall, the disease is endemic in most European countries as suggested by a study based on the perception of the pig veterinary practitioners.

In Scotland, three abattoir surveys were conducted in 2006, 2012–13 and 2017–18 to estimate PRRSV seroprevalence in commercial finishing pigs. This study presents data and other findings from these surveys and how the results will contribute to the development of a prospective control and elimination programme for PRRSV in Scotland.

The pig industry in Scotland is a small but very well organised industry which focuses on assured production of high quality farrow-to-finish pigs, with a large proportion of outdoor herds. The Scottish pig sector, in 2018, comprised over 332,600 pigs (nearly 7% of the UK pig herd) and the industry contributes about 3.2% of the Scottish Agricultural Output (approximately £101.1 million). There were around 210 holdings with more than nine fattening pigs, which contain around 195 thousand fattening pigs.

METHODS

Description of serological surveys

Three PRRSV serosurveys have been conducted in Scotland: the first in 2006, the second in 2012–2013 and the third in 2017–2018. The primary aim of the surveys was to establish historic banks of serum samples from all commercial pig herds that slaughtered finished pigs in Scotland at that time point. In each survey, blood samples were taken from commercial finishing pigs immediately after slaughter during exsanguination. Samples were collected into 30 ml tubes with no anticoagulant and processed within 24 h of collection. Blood tubes were centrifuged, then serum collected and stored in aliquots at −20°C until required for testing. Healthy pigs from consignments of approximately 5-month-old pigs were surveyed. From each consignment of pigs within each slapmark, every second pig was sampled until six (2006 survey) or 10 (2012–13 and 2017–18 surveys) samples per slapmark were taken. These sample sizes were determined presuming a perfect test, that is, 100% sensitivity and specificity, to detect at least one infected animal in a batch of 100 animals, with a 95% confidence level, if the prevalence at batch level was ≥40% (2006 survey) or ≥30% (2012–2013 and 2017–2018 surveys). No information was collected about vaccination for PRRSV in the herds sampled.

A slapmark is a herd-specific mark that is, tattooed on a pig to identify farm of origin. In the UK, it is a legally required official reference for each pig farm. In some cases, a farm may have more than one slapmark. The samplers were aware of this and, to the extent possible, avoided multiple sampling from the same farm in each survey. Therefore, in our analysis we presumed that each slapmark represented a unique farm. Samples were taken from pigs originating from farms belonging to the Quality Meat Scotland Pig Health Scheme (also known as Wholesome Pigs Scotland Scheme). The Scheme represents 100% of the commercial pig herds in Scotland that produce finished pigs at bacon weight. As part of the Scheme, producers consented for samples to be taken from their animals at slaughter and tested.

The first survey was conducted from April 2006 to December 2006. Samples were stored at SRUC-Disease Surveillance Centre (DSC) Edinburgh as described above and were tested for antibodies against PRRSV by Sci-Tech Laboratories using a commercial ELISA test (CIVTEST SUIS PRRS E/S – Hipra test). Twelve samples from two farms were re-tested by SRUC-DSC Edinburgh using the IDEXX PRRS X3 Ab test due to unexpected results at first testing, that is, positive results for farms believed by their veterinarian not to be infected. For these twelve samples, the result for the IDEXX test, not the Hipra test, was used for the analyses reported in this manuscript.

The second survey was conducted from July 2012 to July 2013. Ten pigs were sampled per slapmark. For the eight farms that submitted fewer than 10 pigs per batch, all pigs in the batch were sampled. Samples were stored at SRUC-DSC Edinburgh as described above and were tested by SRUC-DSC Edinburgh using the IDEXX PRRS X3 Ab test. This survey has been described previously.

The third survey was conducted from June 2017 to December 2018. Ten pigs were sampled per farm. Samples were stored at SRUC-DSC Edinburgh as described above and were tested SRUC-DSC Edinburgh using IDEXX PRRS X3 Ab test.

According to the manufacturer’s information, both tests detected antibodies to PRRSV-1 and PRRSV-2 but did not differentiate antibodies against these genotypes. Other studies have reported that the specificity of tests used was close to 100% but sensitivity was below 100%. The IDEXX test is widely used in the UK for health assurance monitoring, and the agreement between the IDEXX test and the Hipra test was considered good. The sensitivity of the IDEXX

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ELISA was estimated to be 98.8%, and the specificity 99.9% in England in previous work (J.-P. Frossard, personal communication). However, the sensitivity and specificity of these tests specifically for pigs originating from Scottish farms has not been assessed.

Data analysis

For the three surveys, farms were classified as PRRSV seropositive if they had one or more positive ELISA results and were classified as seronegative if all samples yielded negative ELISA results.

All statistical analyses were done using R,9 24 95% CI calculated using the binconf function in the Hmisc package,25 and plots created using the ggplot2 and ggalluvial packages.26,27 Farms, identified by slapmarks, with location defined by eastings and northings, were matched to NUTS 2 regions28 using the sf package.29 It was possible to obtain a location for all farms that participated in the 2017–18 survey. Location data were not available for nine farms in the 2006 survey and 19 farms in the 2012–13 survey.

RESULTS

The number of finishing farms from which pigs were tested is given in Table 1. Most of the farms sampled were located in the North East Scotland region (61.1%, 57.3% and 62.9% in the 2006, 2012–2013 and 2017–2018 surveys, respectively), where most Scottish commercial pig production is concentrated. The number of seropositive farms per sampling period is shown in Table 1.

The percentage of seropositive farms was similar over the three surveys (Figure 1).

Among pigs from positive farms, the mean and median number of pigs (and percentage) per batch testing positive were 3.8 (64.4%) and 4 (66.7%) for the 2006 survey, 8.7 (87.6%) and 9 (90%) for the 2012–13 survey and 9.1 (90.7%) and 10 (100%) for the 2017–18 survey, respectively (Figure 2).

Seropositive farms were mostly located in the North East Scotland region in the three surveys (30.9%, 41.3% and 35.1% in the 2006, 2012–2013 and 2017–2018 surveys, respectively).

Comparing farm results between sampling periods, that is, from 2006 to 2012–2013 and from 2012–2013 to 2017–2018 (Tables 2 and 3 and Figure 3), 30 of 43 (70%) farms from the 2006 survey to the 2012–2013 survey and 37 of 49 (75.5%) farms from the 2012–2013 survey to the 2017–2018 survey did not change status between survey periods. From 2006 to 2012–2013 and from 2012–2013 to 2017–2018, 32.6% and 38.8% of farms, respectively, that were seronegative in the initial survey were also seronegative in the subsequent survey; and 37.2% and 36.7% farms, respectively, that were seropositive in the initial survey were also seropositive in the subsequent survey. In contrast, over the same periods, 9.3% and 18.4% of farms, respectively, changed from seropositive to seronegative, and 20.9% and 6.1% of farms, respectively, changed from seronegative to seropositive.

Expected herd sensitivity and herd specificity, based on sample size calculations and assumed sensitivity and specificity of the test, for the 2012–2013 and 2017–2018 surveys were 97.1% and 99%, respectively, and for the 2006 survey they were 68% and 99%, respectively.30,31

DISCUSSION

This paper reports results from three abattoir surveys of PRRSV seropositivity conducted in Scotland in 2006, 2012–2013 and 2017–2018, and how they contributed to the assessment of commercial pig units in Scotland. The diagnostic tests used were not able to differentiate antibodies resulting from vaccine usage from those arising from infection with field strains, which, therefore, could lead to an overestimation of the prevalence of infection with field strains.

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**Table 1** Number of farms and pigs from which samples were taken per survey and survey seroprevalence results

| Year    | Number of farms tested | Number of farms seropositive (per cent; per cent 95% CI) | Number of pigs tested | Number of pigs seropositive (per cent; per cent 95% CI) |
|---------|------------------------|----------------------------------------------------------|-----------------------|----------------------------------------------------------|
| 2006    | 158                    | 72 (45.6; 38.0–53.4)                                       | 948                   | 278 (29.3; 26.5–32.4)                                      |
| 2012–13 | 94                     | 45 (47.8; 38.1–57.9)                                       | 885                   | 394 (44.5; 41.2–47.9)                                      |
| 2017–18 | 97                     | 44 (45.4; 35.8–55.3)                                       | 978                   | 399 (40.8; 37.7–43.9)                                      |

**Table 2** Comparison of results between 2006 and 2012–2013 surveys for farms that were sampled in both surveys

|       | Negative | Positive | Total |
|-------|----------|----------|-------|
| 2006  | 14       | 9        | 23    |
| Positive | 4       | 16       | 20    |
| Total  | 18       | 25       | 43    |

**Table 3** Comparison of results between 2012–2013 and 2017–2018 surveys for farms that were sampled in both surveys

|       | Negative | Positive | Total |
|-------|----------|----------|-------|
| 2012/13 Negative | 19       | 3        | 22    |
| Positive | 9        | 18       | 27    |
| Total  | 28       | 21       | 49    |
The proportion of farms seropositive to PRRSV over the three surveys was consistently around 45%, suggesting that the prevalence of herd infection in Scotland is stable. Few studies have been done to assess prevalence of PRRSV infection in Europe and particularly in the UK. However, comparison of results from the 2013 UK survey with those from the 2012–2013 Scottish survey undertaken at a similar time indicates that the proportion of PRRSV seropositive farms was lower in Scotland. Compared with the median seroprevalence reported from 2010 to 2014 in Denmark, the proportion of farms infected with PRRSV inferred from all three surveys is higher in Scotland. However, these studies used different methods which compromise a direct comparison.

The results show that most farms, which were tested in more than one survey, did not change status between survey periods (Figure 3). A smaller proportion of farms changed status from seropositive to seronegative. The reasons for changes in the PRRSV status from seropositive to seronegative between surveys are not known but are likely to be due to either PRRS control strategies applied at farm level or depopulation of stock for controlling other diseases. Reasons for change in status from seronegative to seropositive in finisher pigs include the introduction of PRRSV onto the farm, destabilisation of immunity in the breeding herd and implementation of vaccination in growing pigs.

The high sensitivity and specificity of the antibody tests and the apparent prevalence of seropositive pigs provides assurance that test predictive values are high: for a within-herd prevalence of 30% and a test sensitivity of 98.8% and specificity of 99.9%, the positive predictive value, that is, the probability that an animal with a positive screening test truly has the disease, is 99.4%, while the negative predictive value, that is, the probability that an animal with a negative screening test truly does not have the disease, is 99.8%.

There was a higher proportion of samples collected which tested positive per herd in the 2012–2013 and 2017–2018 surveys compared with the 2006 survey. This might be due to the different tests used or to the vaccination strategy at farm level, for example, vaccinating more growing pigs. Reported sensitivity for the IDEXX test is higher than for the Hipra test but the agreement between the IDEXX test and the Hipra test was considered good by Biernacka et al. Furthermore, different sample sizes between the surveys, that is, six samples and 10 samples per farm, resulted in different herd sensitivities. However, what is clear is that there were a very low number of farms with only one positive sample (Figure 2) and this, associated with the high specificity of both tests, suggests that the risk of
classifying a farm as seropositive when it is not is low, that is, low risk of false seropositive farms.

Seropositive farms are mainly concentrated in the North East Scotland region where there is a high concentration of pig farms, and it is known that proximity to other pig herds is a risk factor for PRRS. In a country where a disease is endemic, establishment of free zones—or almost free zones—may assist in progressive control and eradication of the disease. Therefore, this geographical hotspot suggests disease zoning should be considered when developing a control programme for PRRS in Scotland. Zoning may encourage the more efficient use of resources within certain parts of a country, for example, to eradicate the disease in geographical zones where the prevalence is very low—as in the Highlands and Islands region—but having a
Limitations of the study

No information about vaccination for PRRSV in the herds sampled was collected in these surveys. There is little information about the proportion of farms vaccinating for PRRS. Data from a study in 2013 in England suggest that only 11% of farms vaccinate pigs around weaning for PRRS and, therefore, those pigs could be showing antibodies at slaughter. Additionally, anecdotal evidence suggests that farms that are vaccinating in Scotland are doing so because they have an underlying PRRS problem. Therefore, the results obtained here are very likely to be an accurate reflection of the true prevalence of farms that are PRRS positive.

The number of farms tested decreased from the 2006 survey to subsequent surveys. In 2012, the closure of one of the main abattoirs in Scotland led to the slaughter of finished pigs from some farms at abattoirs outside Scotland, and these pigs were not accessible for sampling. Thus, results for the 2012–13 and 2017–18 surveys could under- or over-estimate the actual sero-prevalence in Scotland. Furthermore, around 2013, the Scottish pig industry contracted to fewer farms and lower sow numbers for economic reasons which reduced the size of the pig industry in Scotland.

These surveys show that sampling finisher pigs at slaughter and serological testing for antibody to PRRSV is an easy and practical way of assessing herd PRRS status if no vaccination is used. If vaccination is used, seropositivity is not definitive, and supplementary testing should be considered, such as PCR to detect the virus, to help assess PRRS status.

A sample size of 10 is enough to detect at least one animal infected if there is a within-herd prevalence of at least 30% and considering a perfect test. The 30% within-herd prevalence assumption is similar to the figure reported by UK pig veterinarians, for the within-herd prevalence of PRRS infected pigs without clinical signs and was therefore considered acceptable. We calculated herd sensitivity and specificity based on the sample size and tests characteristics. Herd sensitivity (HSe) is the probability that a positive (diseased, infected, seropositive, exposed or immune) herd yields a positive herd-test result, and herd specificity (HSp) is the probability that a negative (non-diseased, non-infected, seronegative, non-exposed or non-immune)
herd yields a negative herd-test result. The high values of HSE and HSP suggest that the sample size used for the 2012/13 and 2017/18 surveys was adequate to establish farm serological status for the disease. Furthermore, with such a high within-herd seroprevalence on positive farms (Figure 2) to achieve the same HSE and HSP (97% and 99% respectively), the sample size could even be reduced to five animals per farm if we assume a within-herd prevalence of at least 50%.

These surveys increased situational awareness and led the Scottish pig industry to set up a working group to look at the feasibility of developing a control programme for PRRS in Scotland. Preliminary advice from this group included extending testing to assess the status of farms sending pigs to be slaughtered outside Scotland and farms that are not producing finisher pigs, collection of information regarding vaccination practices for PRRS on all commercial pig farms in Scotland, and establishing an interactive mapping system which allows PRRSV test results to be tracked over time and helps define regions for PRRSV control. These surveys and activities are essential steps to provide baseline information on the PRRS situation in Scotland and for assessing the feasibility of implementing a control programme for this disease. Seropositivity to PRRSV in surveys at several time points indicates that PRRSV herd infection is prevalent in Scotland and suggests that the prevalence of herd infection is stable. The results of these surveys have contributed to the planning of a control programme for PRRS in Scotland.

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CONFLICT OF INTEREST
The authors declare no competing interest.

AUTHOR CONTRIBUTIONS
Carla Correia-Gomes drafted and wrote the manuscript. She was involved in the analysis of the results of the three surveys. Andrew Duncan analysed the data and contributed to the manuscript. Allan Ward devised and organized the formation of the serum archives for the Scottish pig sector and collected all the samples used in the three surveys. Lysan Eppink contributed to manuscript review. Grace Webster contributed to manuscript review. Andy McGowan coordinated the sampling programme and provided farm descriptions. Michael Pearce contributed to manuscript preparation and review. Jill Thomson organised sample processing, storage, testing for surveys 2 and 3, with interpretation and collation of results and contributed to manuscript review. All authors have read and approved the final manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from Allan Ward (award@qmscotland.co.uk) upon reasonable request.

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