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Spatio-temporal and risk factor analysis of alleles related to Scrapie resistance in sheep in Great Britain before, during and after a national breeding program

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Abstract

Certain genotypes of sheep have been identified to increase their susceptibility (the VRQ allele) or resistance (the ARR allele) to classical scrapie. This study's aim was to assess the spatio-temporal pattern of the ARR and VRQ alleles in GB and to explore the risk factors associated to their presence.

Data was collected from the GB scrapie active surveillance program, the sheep and goat inventory survey (GB census survey) and the agricultural survey for the period 2002-2015. Spatio-temporal trends of genotypes were assessed through the use of choropleth maps, spatial cluster and linear regression analyses. Multivariable mix-effect logistic regression analyses were performed to investigate the association between the resistant or susceptible genotypes, and breeds, farm purpose, animal purpose, surveillance stream, country location and herd size.

The results show a significant upward trend in the frequency of most resistant ARR alleles (1.15% per year, 95%CI: 0.76-1.53) and significant downward trend of most susceptible VRQ alleles (-0.40% per year; 95%CI: -0.69 to -0.10]. The trend continues after the termination of the national scrapie plan in 2009. Breeds such as Herdwick (OR=0,26; 95%CI: 0.14-0.46), Shetland (OR=0.22; 95%CI: 0.13-0.39), Swaledale (OR=0.58; 95%CI: 0.47-0.73), Scottish blackface (OR=0.54; 95%CI: 0.41-0.71) and Welsh Montain (OR: 0.59; 95%CI: 0.44-0.79) were identified with lower odds ratios of having the resistant ARR allele, while Beulah speckled face (OR=1.58; 95%CI: 1.04-2.41), Jacob (OR=2.91; 95%CI: 1.33-6.40), Lleyn (OR=2.94; 95%CI: 1.28-6.74) and Suffolk (OR=2.19; 95%CI: 1.69-2.84) had higher odds ratios of having the ARR allele. Other risk factors associated to presence of ARR allele were finishing farms (OR=1.15; 95%CI: 1.06-1.24) and farms in Scotland (OR=0,78; 95%CI: 0.73-0.83) and in Lowland grazing areas (OR=1.53; 95%CI: 1.39-1.67). Factors associated with presence the VRQ genotype were farms in Scotland (OR=0,85; 95%CI: 0.77-0.93) and breeds such as Herdwick (OR=2.2; 95%CI: 1.08-4.97), Shetland (OR=4.12; 95%CI: 2.20-7.73) and Sweledale (OR=1.51; 95%CI: 1.10-2.09). For the most resistant genotype, two significant spatial clusters were identified: a high-risk cluster in the south-west of GB (RR=1.51, p<0.001) and a low-risk cluster in northern GB (RR=0.65, p<0.001). For the most susceptible genotypes, one significant high-risk cluster was identified in Wales (RR = 2.89 and p=0.013).

Surveillance for classical scrapie could be improved with a risk-based approach by focussing on those areas and farm types identified to have higher frequency of VRQ alleles and less frequency of ARR alleles. Scrapie control strategies could focus on developing breeding programs on farms with Shetland, Herdwick and Swaledale breeds.

Keywords: Scrapie; PrP genotype; Risk factor; Sheep; Surveillance; cluster analysis

1. Introduction

Long-term national occurrence of scrapie can be reduced by selection of sheep carrying the resistant PrP gene (ARR - encode alanin at codon 136 and arginin at codon 154 and 171) and removal of susceptible genes (VRQ – encode valine at codon 136, arginine at codon 154 and glutamine at condon 171)(Goldmann, 2008; Fast and Groschup, 2013). Under this axiom, the implementation of classical scrapie (CS) eradication programmes for sheep in European countries proved that it is possible to significantly reduce the prevalence of this disease using polymorphisms of prion protein gene (PrP) approaches in conjunction with herd cull protocols (EFSA, 2014). Similar programs were implemented in several non-EU countries, including Canada and the United States of America (Scrapie-Canada, 2017; APHIS, 2018). Commission Regulation (EC) 2245/2003, which is an amendment of the (EC) 999/2001 regulation, requires that, in addition to each positive transmissible spongiform encephalopathy (TSE) case in sheep, the prion protein genotype shall be determined for a random subsample of those ovine animals tested negative under active surveillance. In Great Britain (GB), since the adult sheep population accounts for more than 750,000 animals, the active surveillance program requires a total of 20,000 sheep samples to be tested each year, with minimum sample for genotyping of at least 600 animals. The primary objective of genotyping is to estimate the prevalence of the most resistant and most susceptible genes in the national sheep flock.

Recent data for the prevalence of scrapie have shown that the number of CS cases in sheep is consistently falling in GB and other EU Member States(EFSA, 2014). The NSP in GB was implemented in July 2001 in a

huge effort to change the dominating genotypes of the national herd with the most scrapie resistant genotypes for breeding, and decrease the frequency of the most susceptible animals (Ortiz-Pelaez et al., 2014). This program ended in March 2009. The program is based on the fact that sheep with the ARR allele have a significantly reduced risk of developing scrapie compared with other genotypes, while presence of the VRQ allele increases it greatly the risk (Belt et al., 1995; Elsen et al., 1999; Baylis et al., 2004; Baylis and Goldmann, 2004). Probably, the NSP in GB has been the largest genotyping programme for animal disease control ever implemented in the world, with roughly 3 million sheep from 90 different breeds genotyped (Dawson et al., 2008) and with costs of approximately £86 million per year (Ortiz-Pelaez et al., 2014). As a result, CS incidence has decreased by over 90% since 2002 (with incidence of up to 0.25% in the fallen stock survey), and only two cases of CS were detected in the fallen stock survey (incidence <0.01%) between 2013 and 2016 (EFSA, 2016). However, since the selection for scrapie resistant genotypes was made voluntary (in the GB since 2009), active scrapie surveillance has become the main means of controlling and eradicating CS in sheep populations (EFSA, 2014). Farms with positive cases are also monitored for a period of two years through testing of their fallen stock and their sheep at slaughter. However, a CS eradication policy relying solely on current surveillance programs is unlikely to succeed (EFSA, 2014) and there is a risk of a future increase in CS, unless the sensitivity of the surveillance program for detection of CS is improved.

It has been argued that "control and prevention of scrapie is beyond reach when knowledge about the pathways of transmission is absent" (Adams, 2016). This is also hindered by the inefficacy of current treatments for the decontamination and disinfection of farms following scrapie outbreaks (Acin, 2015; Hawkins et al., 2015; Gough et al., 2017). Furthermore, it is known that the scrapie prion can persist in the environment for several years (3 to +16 years) (Georgsson et al., 2006; Wiggins, 2009; Smith et al., 2011). Under this scenario, and given the risk of developing CS again through contamination from the environment, the main threat is considered to be a population level decrease in the ARR allele frequency and a re-emergence of the VRQ allele in the national flock (Ortiz-Pelaez et al., 2014). The minimum ARR allele frequency below which within-flock infection cannot be sustained is uncertain and may depend on the prevalence of local risk factors, such as breed, flock-type heterogeneity, management systems and trading practices (Melchior et al., 2010). Considering that the objective of the TSE surveillance program is to detect

and eradicate CS, a continuous adjustment process of this program is needed to increase the detection of cases, especially with the current low incidence levels. Therefore, additional knowledge is required of the spatio-temporal distribution of the resistant and susceptible allele frequencies in the population, together with identification of risk factors associated with these alleles, in order to develop and apply further risk-based surveillance strategies.

The aim of this study was twofold: firstly, to visualize and explore the spatio-temporal patterns and assess clustering of the resistant (ARR) and most susceptible (VRQ) PRNP alleles in sheep in GB since commissioning the NSP (from 2002 till 2015); and secondly, to identify risk factors associated with genotypes in order to assist in the development of targeted risk-based scrapie surveillance programs.

2. Materials and methods

2.1. Data source

Historical data, from 2002 until 2015, were extracted from three national databases: (1) the Scrapie Surveillance database, (2) the annual Agricultural Survey, and (3) the annual Sheep and Goat Inventory survey. The datasets were merged using the county (administrative area) parish holding (CPH) reference which is a unique identification number for each farm in GB.

2.1.1. Scrapie surveillance data

The structure of the scrapie surveillance program in GB is summarized in Figure 1. Passive surveillance (not explored in this study), provides testing of all sheep with clinical suspicion of scrapie (via the scrapie Notification Database; SND). Active surveillance is completed using three different routes: fallen stock survey (FSS), abattoir survey (AS) and dead in transit survey (DTS). With an adult population of over 750,000 sheep in the GB, active surveillance requires sampling of 20,000 sheep per year, and at least 600 animals per year need to be genotyped. However, a derogation in Annex III, Chapter II, paragraph C of the EU TSE Regulation permits Member States to replace up to 50% of their requirement for sheep tested for human consumption with the same number of fallen stock sheep. Animals sampled for active surveillance are selected from 20 to 27 fallen stock site per year (number varied per year) and about 14 abattoirs. Only abattoir slaughtering more than 40,000 sheep per year were ask to participate in the surveillance. The selection of abattoirs and fallen

stock site was done based on their geographical distribution. From each site, animals for sampled are preselected based on the following criteria: sheep are over 18 month age, an eartag is present and not to select more than two animals from the same holdings. The final selection of animals recruited for the surveillance is done randomly by selecting one out of every three of the pre-selected animals. The selection of the negative samples for genotyping is also based on a stratification method. Few samples per fallen stock site and abattoir site are selected each month for genotyping (per year 300 samples per each surveillance route are selected). Apart from this, only samples with good quality conditions for testing are selected. In addition, all scrapie positive cases are genotyped. In the years 2002, 2003 and 2012, a large number of sheep (>10,000) were genotyped as part of the NSP and research study (Ortiz-Pelaez et al., 2014). The samples collected were the obex region of the brainstem and the Cerebellum. In the fallen stock survey, the staff from the disposal sites collected the samples after receiving training from Animal and Plant Health Agency (APHA). In abattoirs, samples are collected by trained staff from the Food Standard Agency.

Genotyping of the prion protein gene were done for the codons 136, 141, 154 and 171. The methods used for the genotyping of the samples are described in the supplementary material. All genotypes were grouped in five categories, following the classification completed in the NSP. These were: type 1 (ARR/ARR), type 2 (ARR/AHQ, ARR/ARH, ARR/ARQ), type 3 (AHQ/AHQ, AHQ/ARH, AHQ/ARQ, ARH/ARH, ARH/ARQ, ARQ/ARQ), type 4 (ARR/VRQ) and type 5 (AHQ/VRQ, ARH/VRQ, ARQ/VRQ, VRQ/VRQ). They established decreasing levels of resistance to CS, with type 1 or genotype ARR/ARR being the most resistant and type 5 or genotypes with VRQ alleles and non-ARR alleles being the most susceptible. Linear regression analysis was carried out to evaluate the trends on the frequency of each group genotype (outcome variable) over the year (exposure variable). For this analysis, data for the year 2005 was excluded due the low number of observations for that year.

To assess the spatial distribution and risk factors, two binary outcome variables were created. The first variable indicates the presence of the resistant allele ARR, and therefore includes homozygous or heterozygous combinations of ARR allele, except the ARR/VRQ genotype (grouped NSP types 1 and 2). The

other outcome variable indicates the presence of the most susceptible genotype VRQ, including homozygous or heterozygous combinations of VRQ allele (corresponding with NSP type 4 and 5).

For each sample, data was also collected on farm CPH, the country and the year of sampling. Breed information was also collected, but was only available for sample from the fallen stock survey. Breed information was recorded at the sites by previously trained staff.

2.1.2. Agricultural Survey and Sheep and Goat Annual Inventory.

These dataset represent yearly large-scale surveys of sheep populations. The annual agricultural survey provides data on farm purpose (less favoured area (LFA) grazing, lowland grazing, dairy and others). This survey is conducted in June of each year and covers 80% of the farming population in GB (DEFRA, 2017). The annual inventory is a census survey and a legal requirement that provides estimates of the number of sheep and goats (herd size), together with the geographical coordinates for the location of the holdings, animal purpose (finishing, breeding, grazing, stores, dealer, wool, pet and other) and production type (dairy, meat, wool and others)(DEFRA, 2016a). Data for each survey for the period 2002-2015 was used and was matched for each animal in the scrapie surveillance system by CPH and year. When matching by year was not possible, matching was done by CPH only.

2.2. Statistical and spatial analysis

2.2.1. Spatial analysis

The annual distribution of most resistant and most susceptible alleles were calculated spatially in each administrative area. A choropleth map was generated in ArcMap 10.2.2 (ESRI, USA) showing the frequency of both allele groups for each administrative area. Spatial clusters in GB of NSP genotype group 1 (ARR/ARR) were investigated using Kulldorf's spatial scan statistic implemented using the SaTScan software (version 9.4.4) in order to identify potential areas for breeding and replacement of resistant genotypes. In addition, spatial clusters in GB of the NSP genotype group 5 (VRQ/non-ARR) and group 4 (VRQ/ARR) were also investigated in order to identify areas to target for eradication/surveillance schemes. Aggregated data for the period 2012-2015 were used to allow sufficient sample numbers for the identification of possible and current genotype clusters. For all genotype groups, 50% percent of the population must be at risk for a cluster to be detected.

2.2.2. Risk factor analyses

Two binary outcomes were created to investigate factors associated with presence of ARR allele (outcome 1) and presence of the VRQ (outcome 2) allele. Risk factors considered were: year, breed, herd size, animal purpose, farm purpose, testing stream (fallen stock, abattoir and death in transit), production type and country. For breed, all minor breed present in less than 20 animals in the dataset were removed from the analysis.

A univariable analysis was done using multilevel mix-effect logistic regression to identify significant independent associations between the outcome and all predictor variables, and with the variable year included as a fixed effect and the farm id as a random effect. For this, all farms with CPH or flock mark with missing values were assumed to be new farms. All variables significant ($p<0.10$) at the univariable level were then included in a multivariable mix-effect logistic regression model to assess the risk factors associated with the two outcomes. For each outcome, three models were developed: 1) Model with breed included, 2) Model without breed and without farm purpose, and 3) Model without breed and without country. The reason were that data on breed of animals were only collected in the fallen stock survey, reducing dramatically the number of observations in the multivariable models. Data on farm purpose was only collected in England, and therefore a model without country was needed to explore the possible association with this variable. For the multivariable models, a backward stepwise process was completed to retain those variables not significant at the 0.05 level. Models were run using Stata 12 (StataCorp, 2011. College Station, Texas, USA) and the command 'melogit'. To assess goodness of fit of the models, model predictions (probability of having an allele) were compared with actual outcomes. For this, model predictions were transformed into binary variable using 0.5 as the cut-off probability to classify a sheep into positive (having the allele) or negative (not having the allele).

3. Results

3.1. Descriptive statistics

From 2002 to 2015, a total of 435,159 sheep were TSE tested after the launch of the NSP. Under the active surveillance program, most frequently collected samples were for the abattoir survey (49.0%), followed by the fallen stock survey (41.0%) and death in transit survey (1.0%). The numbers of animals tested over time

are shown in Table 1. A change in the trend in the sampling streams was observed over time, going from over 90% animals tested through abattoirs in 2002-2003 to over 73% of sheep tested through the fallen stock survey in 2015.

A total of 65,666 sheep were genotyped, consisting of 54.3% from the abattoir survey, 42.6% from the fallen stock survey and 3.1% from the death in transit survey (Table 1). These originated from 18,590 different farms (an average of 2.68 samples per farm). However, for 16,944 (25.8%) sheep data on CPH or flock mark was missing. Breed information was obtained for 7,430 (11.3%) of sheep genotyped. A total of 48,194 (73.4%) sheep were matched to the Sheep and Goat inventory dataset. Only 18,795 (28.6%) sheep were matched to the agricultural survey.

Type 2 genotypes were the most frequent (63.6%), followed in decreasing order by NSP type 3 (25.2%), type 1 (21.5%), type 5 (6.0%) and type 4 (5.0%) (Figure 2). On average, the ARR allele accounted for 63.7% (n=42,133), while the VRQ allele accounted for 11.1% (n=7,312). Significant trends in the frequency of the NSP genotype categories were evident across all the types, except for type 2 (Figure 2). Significant frequency increase over time was observed for type 1 (b=1.15 95% CI [0.76 to 1.53], R^2=0.79, p<0.001). Significant frequency decrease over time was observed for type 3 (b=-0.72 95% CI [-1.01 to -0.43], R^2=0.73, p<0.01), type 4 (b=-0.24 95% CI [-0.37 to -0.12], R^2=0.62, p<0.001) and type 5 (b=-0.40 95% CI [-0.69 to -0.10], R^2=0.44, p<0.05). It is important to note that the results observed for year 2005 must be considered anecdotal since only 78 animals were genotyped (Table 1). The total number of CS cases from 2002 to 2015 were 53 (0.1%) out of 45,106 sheep with the ARR allele and 253 (3.5%) out of the 7,263 sheep having the VRQ allele. The total number of Atypical scrapie cases were 181 (0.4%) for sheep with the ARR allele and 2 cases (0.03%) for sheep with the VRQ allele.

3.2. Spatial Analysis

3.2.1. Choropleth Maps

The results indicate that the spatial distribution of the most resistant allele (ARR) has increased over time (Figure 3). While in 2002 and 2003, few counties had a proportion of sheep with ARR allele larger than 31%, during the following years this genotype dominated the national population. Conversely, in the first years of

the TSE active surveillance program, several counties presented a higher proportion (>10%) of the VRQ allele (Figure 4). However, for the period 2010-2015 after the NSP, the proportion of this allele has decreased in most counties.

3.2.2. Cluster Analysis

For the most resistant genotype (ARR/ARR), two significant clusters were identified through spatial cluster analysis ($p < 0.001$, Figure 5-A); one high- and one low-risk cluster. The high-risk cluster of ARR/ARR occurrence was in south-west GB (RR=1.51, $p<0.001$) and the low-risk cluster for ARR/ARR occurrence covered the entirety of northern GB (RR=0.65, $p<0.001$). For the most susceptible genotypes (VRQ and non-ARR) only one significant high-risk cluster was identified in Wales (RR = 2.89 and $p=0.013$, Figure 5-B); farms in this region were almost three times as likely to have animals with the susceptible genotypes VRQ and non-ARR, than farms outside Wales .

3.3. Univariable risk factor analyses

Findings from the univariable multilevel mix-effect logistic regression models are summarised in Tables A and B in the supplementary material. Six variables (year, testing route, country, breed, animal purpose and farm purpose) were independently associated with presence of ARR allele, while for presence of VRQ allele four potential risk factors were identified (year, testing route, country and breed). Examining the relationship between both alleles and year of sampling, there was a significantly increased odds from 2008 until 2015 for the most scrapie resistant genotypes, while the odds ratios across time for most scrapie susceptible genotypes have progressively decreased up to 2015.

3.4. Multivariate risk factor analyses

The final multivariable models that accounted for breed resulted in country and breed being associated with the presence of resistant or susceptible genotypes. Country was significant for both alleles, with Wales presenting higher odds of having the resistant ARR allele and Scotland presenting lower odds of having the susceptible VRQ allele (Table 2). Several breeds were associated with higher odds of having the resistant ARR allele. These were, in decreasing order, Jacob, Lleyn, Suffolk, Beulah speckled face and Suffolk crosses. Breeds associated with lower odds of having the resistant ARR allele were, in increasing order, Shetland, Herdwick,

Scottish Blackface, Swaledale and Welsh Mountain (Table 2). Breeds associated with higher odds of having the susceptible VRQ allele were, in decreasing order, Shetland, Border Leicester, Herdwick, Dorset Horn&Poll and Swaledale. Breeds associated with lower odds of having the VRQ allele were, in increasing order, Suffolk and Suffolk cross (Table 2). However, predictions from the multivariable models using VRQ outcome did not seem to predict well which sheep had the allele.

The final multivariable models that did not accounted for breed showed that higher odds of having the ARR allele were found in lowland grazing farms and in farms classified as 'finishing', 'grazing', 'pet' and 'wool' (Table 3). Lower odds of having ARR were found in farms located in Scotland and in farms with more than 250 sheep. Wales was no longer found as a significant risk factor associated to higher odds of ARR allele. Model predictions were more accurate in models without breed. Lower odds of having VRQ allele was found in Scotland and in farms classified as 'wool' producers, but with model predictions being unable to provide accurate estimates.

4. Discussion

The implementation of CS eradication programs for sheep in European countries have demonstrated that it is possible to reduce the prevalence of this disease using PrP genetic approaches in conjunction with herd cull protocols (EFSA, 2014). Recent data for the prevalence of scrapie have shown that the number of CS cases in sheep is consistently falling in GB and other EU Member States (EFSA, 2014). However, at EU level no decreasing trend has been observed (EFSA, 2016). The NSP was launched in 2001 as a voluntary programme until July 2004 where it became mandatory for all flocks with confirmed cases from that date (EC, 2001a, b, 2003a, b; Boden et al., 2010). The programme ended in 2009. Results of this study show clearly that over the 14-year study period (2002 to 2015), the impact of the implementation of statutory eradication measures and the use of genetic breeding programs has had a significant effect on the increase in ARR allele frequency and decrease of VRQ allele frequency in the sheep population. These results agree with previous studies (Tongue et al., 2008; Ortiz-Pelaez et al., 2014). Whereas the odds of the occurrence of the ARR allele has doubled, the occurrence of VRQ allele has halved. Furthermore, results show that proportion of sheep with the VRQ alleles has reduced in the vast majority of the counties since the earliest years of the NSP. Specifically, a significant upward trend for occurrence of allele ARR (61.5% to 77.2%) and downward trend

for allele VRQ (12.0% to 5.7%) between 2002-2003 and 2014-2015 was detected, in agreement with observations in other European countries where the actual frequency of the ARR allele is around 80% (the Netherlands: 78.5%, Hagenaars et al. (2010); and Belgium: 79.3%, Dobly et al. (2013)). In GB, a previous study compared the period between 2002–2003 and 2012–2013, and reported an absolute increase of 9% in the frequency of the ARR allele from 43.3% to 52.3% (Ortiz-Pelaez et al., 2014). This value reaches an absolute increase of 15.7% in 2015 in comparison with 2002-2003 and indicates, that despite the termination of the NSP six years ago, the trends have continued. The increase in the frequency of the ARR allele continues in 2016, as it was detected in 38% of sheep tested in the active surveillance (Data not shown). A possible explanation would be that many flock owners in GB are still replacing their sheep from farms where the ARR allele is known to be present as a measure to prevent the occurrence of future classical scrapie cases.

It has been described that while the presence of the ARR allele decreases significantly the number of CS cases, the presence of VRQ alleles increases it (Ortiz-Pelaez and Bianchini, 2011; Ortiz-Pelaez et al., 2014). Over the 14-years study period, only 0.1% of positive CS was detected in animals with ARR allele. The allele ARR is known to confer resistance to all strains of CS, although the genetic resistance of homozygous ARR genotype is not absolute (Groschup et al., 2007). Furthermore, in GB, a 90% reduction in the prevalence of CS between 2005 and 2012 has been reported, and only 2 cases detected through active surveillance in 2013-2015 despite 77,510 sheep tested (EFSA, 2016). The fact that the NSP targeted PrP genotype changes mainly on infected farm, might explain, to some extent, the large decrease in incidence of CS despite the moderate change in frequency of these genotypes in the population.

Our model showed that breed and country were associated with the most resistant and most susceptible genotypes. It is has been reported that there is considerable variation in the distribution of alleles between breeds (Eglin et al., 2005; McIntyre et al., 2006; Melchior et al., 2010; Hautaniemi et al., 2012; Dobly et al., 2013). Of particular interest in this study, were the breeds such as Shetland, Herdwick and Swaledale, which were identified to have high odds of having the VRQ allele and at the same time present low odds of having the ARR allele. Surprisingly, according to the GB farm animal genetic resources breed inventory, the population of Swaledale and Herdwick breeding females has fallen steeply between 2002 to 2015 (78% and 82%, respectively) (DEFRA, 2016b). It is unknown to what extent this reduction can be attributed to the NSP

breeding programme. At the present time, the largest female breeding population is the Swaledale breed. On the other hand, breeds such as Suffolk or Lleyn have higher odds of detecting ARR allele and low odds of having the VRQ allele. These results suggest that future scrapie control programs (surveillance and breeding strategies) could be breed-specific. However, the large number of British sheep breeds and production types compared with other European countries could make these breed-specific scrapie control programs more difficult to implement.

There have been previous attempts to compare the surveillance performance of the two active surveillance sources among EU countries (Bird, 2003; Del Rio Vilas et al., 2007). The present study shows, in the univariable analysis, that abattoir survey detects higher frequency of the VRQ allele relative to the Fallen stock survey, while this route is able to detect higher number of sheep with the ARR allele than the abattoir survey. Despite fallen stock survey being a higher risk-based source of classical scrapie (EC, 2001a; Del Rio Vilas et al., 2005; Del Rio Vilas et al., 2008), the current results suggest that the abattoir survey could potentially be covering areas with farms with increased presence of susceptible genotype (although this factor was not present in the final multivariable analysis). Similarly, breeding farms and those farms located in less favoured areas were found to have lower odds of having the ARR allele. Given that the better predictions where obtained from the multivariable models without breed, the risk factors identified in those models could be prioritized for improving the sensitivity of the scrapie surveillance system. Therefore, the surveillance could be modified to increase sampling of the breeding farms, those in less favored areas and those specialized in wool production. Nonetheless, breed remains an important factor to consider given the fact that some breeds have at the same time high odds of having the VRQ allele and very low odds of having the ARR allele, and vice versa. Careful consideration should be model on the models with VRQ, since the analysis of model residuals indicate poor prediction of animals with VRQ genotypes.

It is know that in GB some geographical regions have been associated with an increased risk of occurrence of CS (Tongue et al., 2006). A more recent study, based on data from 2001 to 2005, shows that the distribution of CS cases in GB exhibits a definite spatial pattern (Stevens et al., 2009). Specifically, South and central Wales were identified as areas with a generally higher occurrence of the disease than the rest of GB. In our study, the spatial scan statistic identified one significant cluster of VRQ alleles in Wales. This country was also

identified to have higher odds of detecting this allele in the multivariable model with breed. The results from the spatial analysis could also be used to informed areas to target to improve sensitivity of the surveillance.

This study contains some important biases and limitations. Sheep geographical source was determined in the fallen stock survey by the farm address where the sheep was collected and in the abattoir survey by the flock mark, which indicates the farm where the animal was born. Hence, if animals were moved to different farms, this information was not captured. Coverage of the current surveillance system is dependent on the location and through-put of participating abattoirs and fallen stock sampling sites. Therefore, some counties in GB may have a lower representation than others. In addition, large numbers of samples were genotyped in 2002, 2003 and 2012 for a previous project that aimed to determine the change in frequency of PrP genotype in the sheep population to assess the impact of the NSP (Ortiz-Pelaez et al., 2014). These large number of animals tested would have an important influence on the risk factors observed. The aggregation of year data in this study allowed the analysis to have sufficient power to detect trends and risk factors.

Although GB surveillance is already balanced in terms of geographical coverage, this study suggests that sensitivity of scrapie surveillance could be further improved by developing a risk-based approach focussing on genotype with more samples submitted from those areas and farms identified to have higher frequency of VRQ genotypes and less frequency of ARR genotypes. Of particular concern is Swaledale sheep, which are the largest female breeding population in GB, but detected in this study of having a high frequency of VRQ allele together with a low frequency of ARR allele.

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Figure 1. Description of the structure of the scrapie surveillance program in Great Britain (Abbreviations: IMC = Immunochemistry; MWB = Modified Western Blot; CSFS = Classical Scrapie Flock Scheme; ASM = Atypical Scrapie Monitoring scheme)

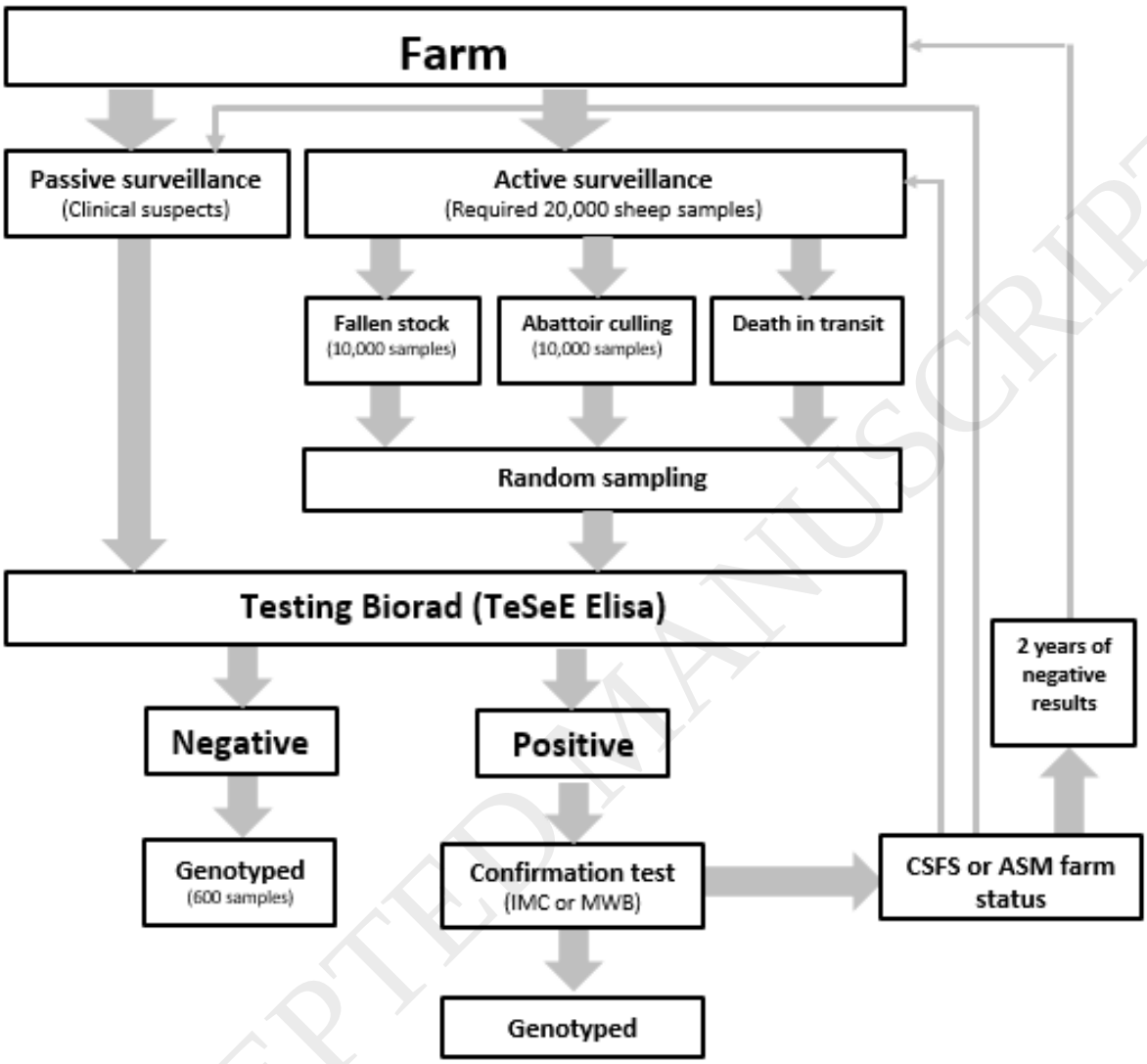


Figure 2. Annual frequency of genotypes of sheep in the Great Britain from 2002 to 2015. The prion protein gene (PrP) genotypes were defined as: type 1 (ARR/ARR), type 2 (ARR/AHQ, ARR/ARH, ARR/ARQ), type 3 (AHQ/AHQ, AHQ/ARH, AHQ/ARQ, ARH/ARH, ARH/ARQ, ARQ/ARQ), type 4 (ARR/VRQ) and type 5 (AHQ/VRQ, ARH/VRQ, ARQ/VRQ, VRQ/VRQ). They establish decreasing levels of resistant to classical scrapie with type 1 or genotype ARR/ARR being the most resistant and type 5 or genotypes with VRQ alleles and non-ARR alleles being the most susceptible.

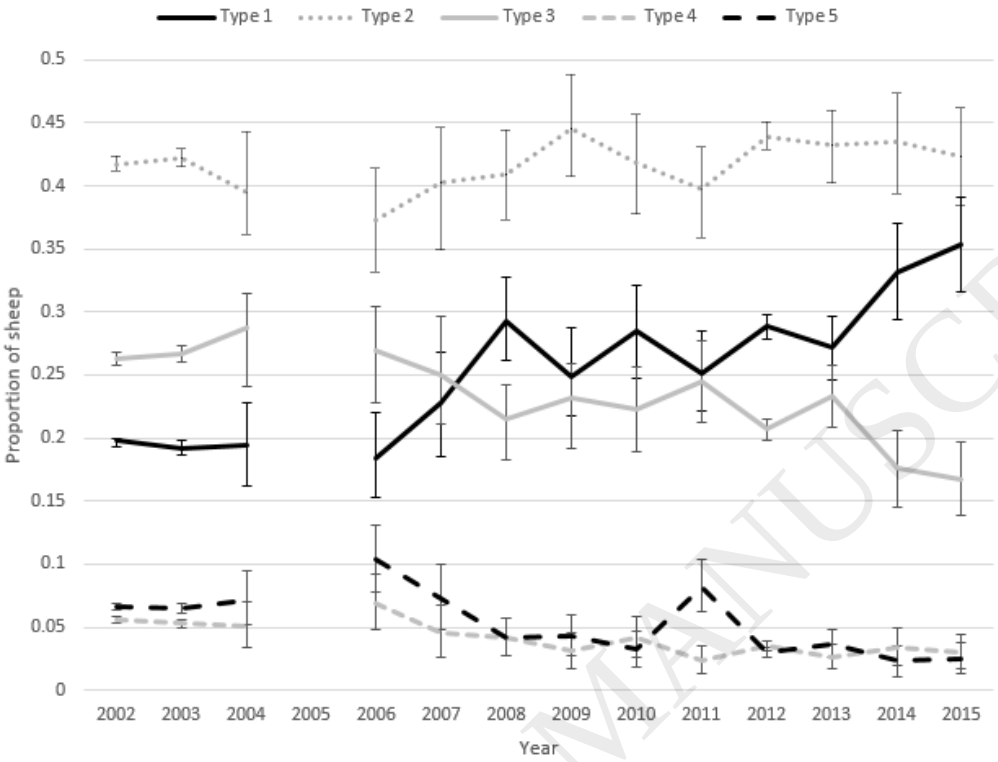
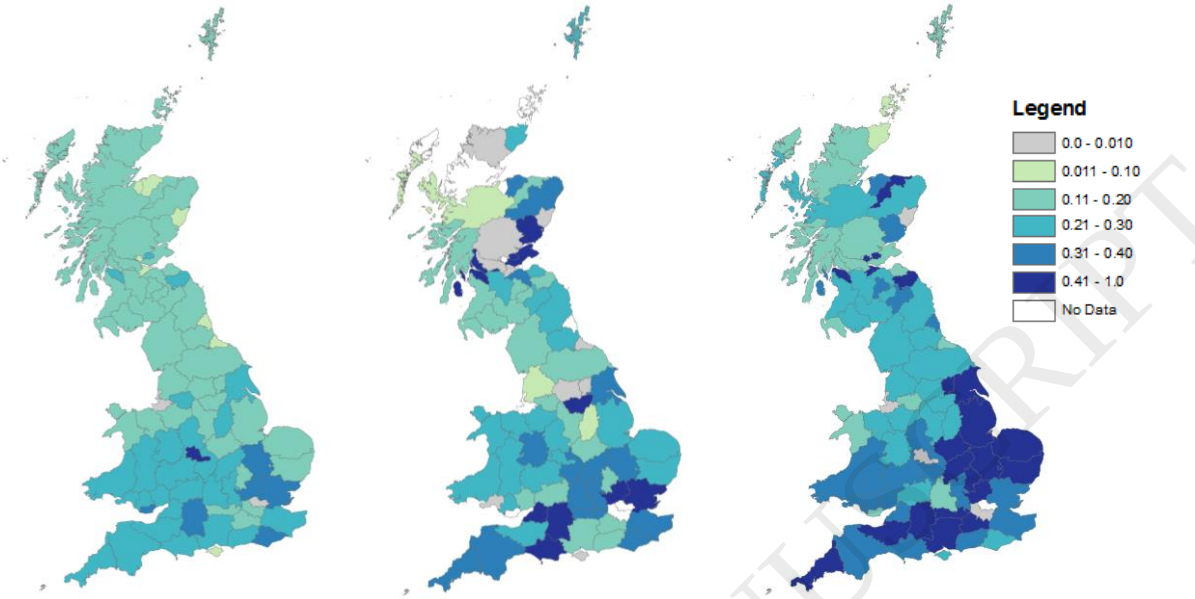


Figure 3. Geographic distribution of sheep with “most resistant” (ARR) genotype to classical scrapie by administrative area from 2002 to 2015 in Great Britain.



2002 - 2003
(n = 35,457)

2004 - 2009
(n = 2,323)

2010 - 2015
(n = 10.369)

Figure 4. Geographic distribution of sheep "most susceptible" including homozygous or heterozygous combinations of VRQ allele and excluding ARR allele to classical scrapie by administrative area from 2002 to 2015 in Great Britain.

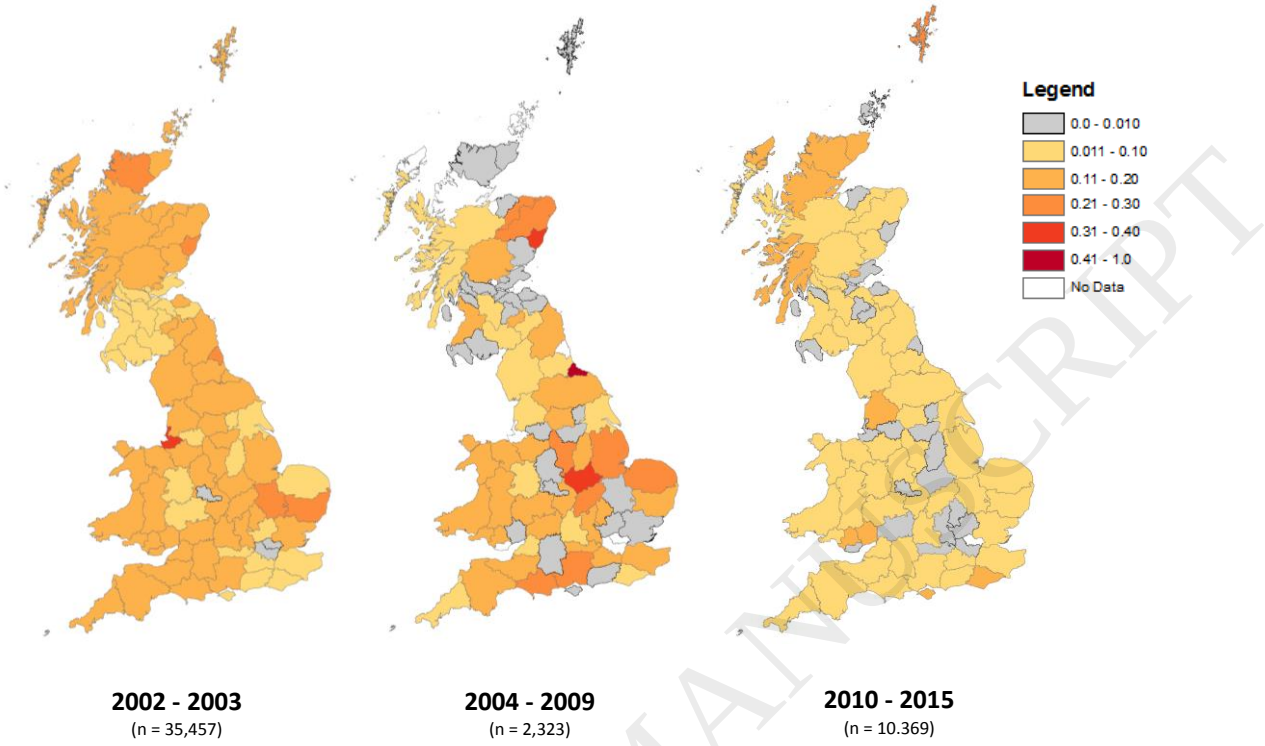


Figure 5. Spatial clusters of sheep with ARR/ARR genotype (left) and of the VRQ allele (right) using sheep samples from 2012 to 2015 in Great Britain. Relative risks (RR) of genotype or allele presence are show in red for RR>1 and low for RR<1.

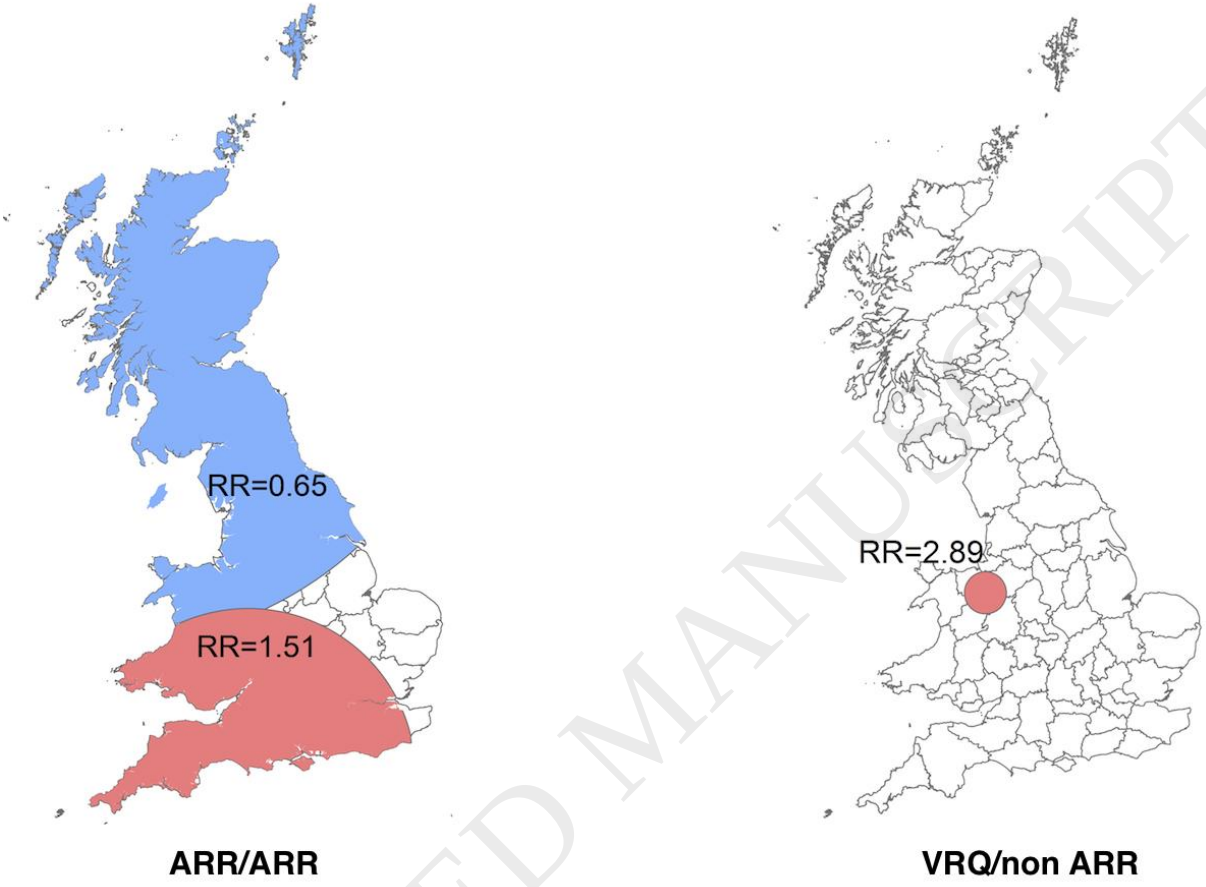


Table 1. Summary of the number of samples tested and the frequency distribution of active scrapie surveillance program and genotyping between 2002 and 2015 in sheep population in Great Britain.

Summary	Testing route	Year													
		2002	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**
Surveillance	Data numbers	33,657	80,988	16,941	21,506	75,370	45,472	24,175	22,107	18,228	19,040	19,538	19,482	19,287	19,368
	Abattoir (%)	97.0	93.9	67.8	54.1	65.4	57.3	45.2	49.6	43.7	36.3	32.9	35.4	35.3	25.7
	Fallen stock (%)	3.0	5.4	29.4	42.8	34.2	40.7	51.2	47.4	56.3	62.9	66.1	62.9	63.9	73.4
	Dead in transit (%)	0.0	0.7	2.8	3.1	1.2	1.9	3.6	0.0	0.8	1.0	1.7	0.9	0.9	1.0
Genotyping	Data numbers	30,095	20,333	558	85	515	402	734	599	587	699	8,639	1,192	607	626
	Abattoir (%)	97.70	93.83	44.95	38.46	55.93	17.38	0.96	52.51	55.29	48.64	67.37	81.48	50.00	50.32
	Fallen stock (%)	2.27	5.81	51.38	57.69	40.32	69.77	97.27	35.45	49.10	51.20	31.80	13.19	49.83	49.68
	Dead in transit (%)	0.03	0.36	3.67	3.85	3.75	12.85	1.78	12.04	0.00	0.16	0.83	3.70	0.17	0.00

Table 2. Multivariate mix-effect logistic regression model showing risk factors associated with "most resistant" (homozygous or heterozygous combinations of ARR allele and excluding VRQ allele) and "most susceptible" (homozygous or heterozygous combinations of VRQ allele) to classical scrapie including breed as exposure variable. Year has been included as fixed effect and farm as random effect.

Risk factor		Most resistant n = 5,479			Most susceptible n = 5,764		
		Odds ratio	**95% CI**	**p-value**	**Odds ratio**	**95% CI**	**p-value**
Year		1.03	1.01-1.06	0.002	0.97	0.94-1.01	0.170
Country	England	Ref.			Ref.		
	Scotland	1.09	0.90-1.31	0.380	0.70	0.52-0.95	0.020
	Wales	1.30	1.05-1.61	0.018	0.97	0.71-1.32	0.833
Breed	Mule	Ref.			Ref.		
	Beulah speckled face	1.58	1.04-2.41	0.033	0.69	0.36-1.33	0.268
	Bluefaced Leicester	1.54	0.90-2.62	0.112	0.83	0.36-1.91	0.654
	Border Leicester	1.14	0.55-2.36	0.750	2.51	1.03-6.08	0.042
	Charollais	1.61	0.83-3.11	0.155	0.77	0.31-1.90	0.573
	Cheviot	0.78	0.57-1.05	0.106	1.51	0.96-2.37	0.074
	Dorset Horn & Poll	1.17	0.83-1.64	0.366	1.64	1.06-2.53	0.026
	English Leicester	0.92	0.54-1.56	0.743	1.86	0.88-3.92	0.103
	Herdwick	0.26	0.14-0.46	0.000	2.20	1.08-4.97	0.030
	Jacob	2.91	1.33-6.40	0.008	1.00*		
	Lleyn	2.94	1.28-6.74	0.011	0.15	0.02-1.15	0.069
	Scottish Blackface	0.54	0.41-0.71	0.000	0.81	0.51-1.28	0.362
	Shetland	0.22	0.13-0.39	0.000	4.12	2.20-7.73	0.000
	Suffolk	2.19	1.69-2.84	0.000	0.44	0.28-0.68	0.000
	Suffolk cross	1.55	1.25-1.93	0.000	0.74	0.56-1.02	0.068
	Swaledale	0.58	0.47-0.73	0.000	1.51	1.10-2.09	0.012
	Texel	0.79	0.62-1.01	0.056	0.70	0.46-1.07	0.102
	Texel cross	0.95	0.73-1.23	0.710	1.24	0.85-1.82	0.263
	Welsh Mountain	0.59	0.44-0.79	0.000	1.41	0.93-2.16	0.143
Animal purpose	Breeding	Ref.					
	Dealer	1.03	0.82-1.30	0.787			
	Finishing	1.03	0.85-1.24	0.764			
	Grazing	1.25	0.97-1.62	0.080			
	Pet	2.07	0.83-5.13	0.118			
	Producer/processor	0.55	0.14-2.11	0.385			
	Stores	1.04	0.82-1.31	0.767			
	Wool	0.67	0.48-0.93	0.018			
Random effect – Farm cons		0.33	0.17-0.64		0.54	0.26-1.11	
Model prediction (prob>= 0.5%)		79.7% of non-ARR sheep 96.1% of ARR sheep Chi-square p<0.001				100% of non-VRQ sheep 0% of VRQ sheep	

Ref.:Reference ; **CI**: Confidence interval; * lack of convergence

Table 3. Multivariate mix-effect logistic regression model showing risk factor associated with "most resistant" genotypes (homozygous or heterozygous combinations of ARR allele and excluding VRQ allele) and "most susceptible" (homozygous or heterozygous combinations of VRQ allele) genotypes to classical scrapie excluding breed as an exposure variable. Year has been included as fixed effect and farm was include as a random effect.

Model including country and excluding farm purpose							
Risk factor		**Most resistant n = 46,421**			**Most susceptible n = 47,895**		
		Odds ratio	**95% CI**	**p-value**	**Odds ratio**	**95% CI**	**p-value**
Year		1.06	1.05-1.06	0.000	0.93	0.92-0.94	0.000
Country	England	Ref.			Ref.		
	Scotland	0.78	0.73-0.83	0.000	0.85	0.77-0.93	0.001
	Wales	1.00	0.95-1.06	0.964	0.98	0.91-1.06	0.613
Animal purpose	Breeding	Ref.			Ref.		
	Dealer	1.11	1.01-1.23	0.035	0.97	0.83-1.10	0.537
	Finishing	1.15	1.06-1.24	0.000	1.00	0.89-1.11	0.942
	Grazing	1.15	1.05-1.26	0.003	0.94	0.83-1.08	0.387
	Pet	1.43	1.06-1.91	0.017	0.82	0.54-1.25	0.363
	Producer/processor	1.43	0.70-2.92	0.323	1.53	0.64-3.69	0.343
	Stores	1.12	1.02-1.24	0.017	0.89	0.77-1.02	0.099
	Wool	1.32	1.17-1.49	0.000	0.80	0.67-0.95	0.011
Herd size	<250	Ref.					
	250-1000	0.93	0.89-0.99	0.020			
	>1000	0.86	0.81-0.92	0.000			
Random effect – Farm cons		0.53	0.50-0.58		0.38	0.30-0.46	
Model prediction (prob>=0.5%)		91.4% of non-ARR sheep 98.5% of ARR sheep Chi-square p<0.001				100% of non-VRQ sheep 0% of VRQ sheep	
Model excluding country and including farm purpose							
		Most resistant n = 17,766			**Most susceptible[1]**		
		Odds ratio	**95% CI**	**p-value**	**Odds ratio**	**95% CI**	**p-value**
Year		1.07	1.06-1.08	0.000			
Animal purpose	Breeding	Ref.					
	Dealer	1.09	0.96-1.24	0.179			
	Finishing	1.18	1.06-1.32	0.003			
	Grazing	1.37	1.09-1.71	0.006			
	Pet	1.33	0.73-2.43	0.348			
	Producer/processor	0.98	0.44-2.14	0.953			
	Stores	1.11	0.97-1.27	0.115			
	Wool	1.47	1.17-1.82	0.001			
Farm purpose	LFA grazing	Ref.					
	Lowland grazing	1.53	1.39-1.67	0.000			
	Dairy	1.30	1.07-1.56	0.007			
	Other	1.50	1.36-1.66	0.000			
Random effect – Farm cons		0.33	0.26-0.42				
Model prediction (prob>=0.5%)		88.5% of non-ARR sheep 98.5% of ARR sheep Chi-square p<0.001					

Ref.:Reference ; **CI**: Confidence interval; * lack of convergence, [1]Farm purpose non-significant, hence model not considered