

PROJECT REPORT TO DEFRA

1. BACKGROUND TO PROJECT

The National Scrapie Plan (NSP) was launched in 2001 following concerns over the theoretical possibility of the presence of BSE in small ruminant populations, and the uncertainty about the biological relationships between scrapie, BSE and 'new variant' Creutzfeldt-Jakob. The aim of the NSP has been to reduce and eventually eradicate small ruminant TSEs from the national sheep flock.

Given that the relative susceptibility to classical scrapie is jointly determined by the variability at codons 136, 154 and 171 of the PrP gene, the Ram Genotyping Scheme (RGS), a major component of the NSP, has comprised widespread genotyping of breeding rams for these three codons and subsequent selective breeding.

The focus on PrP genotype alone led to concerns among sheep breeders regarding potential impacts upon traits other than scrapie resistance. The concerns were that favoured PrP alleles could be antagonistic to other economically important traits or sufficiently rare that their selection increases the rate of inbreeding and consequently the rate at which genetic variability is lost. It was therefore necessary to consider selective breeding as advocated by the NSP in the context of overall genetic improvement, conservation and utilisation of UK sheep genetic resources, and to devise optimal strategies to achieve these objectives whilst minimising TSE risks.

On the other hand, Defra set up the NSP Semen Archive for preserving alleles being removed under the NSP. The Archive (a gene bank) can provide a valuable means for reducing the risks related to exclusive selection on PrP genotype. However, given the cost and commitment in both the establishment and the maintenance of the Archive, clear guidelines on sampling, access and release of the material stored needed to be established. Failure to do so would have translated in a waste of cost and effort.

2. OBJECTIVES OF THE PROJECT

Objective 1: To collect and analyse the information necessary to critically evaluate the impact of widespread selection on PrP genotype in the UK sheep population.

- 1.1. To assess possible associations between PrP genotype and other traits of economic importance, using currently available datasets.
- 1.2. To implement enhanced data collection to assess and monitor possible associations of PrP genotype with performance, survival and health characteristics, in representative breeds from each major sector of the UK sheep industry.
- 1.3. To obtain reliable estimates of allele frequencies for the PrP locus.

Objective 2: To devise optimal breeding strategies for scrapie resistance in the context of an overall genetic improvement programme with restrictions on the loss of genetic variability.

- 2.1. To determine optimal genotyping strategies.
- 2.2. To evaluate the effect of different breeding alternatives on the frequency of PrP alleles and on effective population size of individual breeds.
- 2.3. To determine the optimum balance of selection for resistance to scrapie *versus* selection for other important traits.
- 2.4. To evaluate the most effective strategy for increasing levels of scrapie resistance over time and identify its consequences for the endemic status of the disease.

Objective 3: To provide guidelines to the Semen Archive Management Board on objectives and practical sampling strategies in the Archive.

Objective 4: To make recommendations based on the outputs of objectives 1 and 2 and disseminate them to the sheep industry.

3. REVIEW OF OBJECTIVE 1: TO COLLECT AND ANALYSE THE INFORMATION NECESSARY TO CRITICALLY EVALUATE THE IMPACT OF WIDESPREAD SELECTION ON PrP GENOTYPE IN THE UK SHEEP POPULATION

Large datasets for representative breeds from each major sector of the UK sheep industry were collected. Data from experimental and commercial flocks were analysed in order to assess potential associations between PrP genotype and other traits of economic importance and to obtain breed-specific frequencies of PrP alleles. Datasets contained performance information of relevance to the respective industries, PrP genotype on the same animals, pedigree records, and were of a sufficient size to allow meaningful and robust genetic analyses. Initial work prior analyses included i) recruitment of commercial flocks; ii) merging NSP PrP genotype to existing sheep breeder data for analysis of performance in commercial flocks; iii) enhanced data collection for analysis of survival in commercial flocks; and iv) genotyping of experimental flocks.

3.1. Data collation and collection

3.1.1. Recruitment of commercial flocks: Breeders already undertaking performance and pedigree recording with Signet, who agreed to collect additional data on lamb and ewe survival, and who were also prepared to grant access to their data, were recruited to the project. The aim was to have sufficient participating flocks to allow at least 2,000 breeding ewes per breed, thus allowing datasets of statistically meaningful size to be obtained during the duration of the project. In total, 144 flocks from 11 breeds were recruited to the project. Data was successfully obtained from 127 flocks: 5 North Country Cheviot (Hill), 17 Scottish Blackface, 10 Welsh Mountain, 9 Rough Fell, 19 Charollais, 9 Poll Dorset, 22 Texel, 9 Beulah, 6 Blue Faced Leicester, 9 Lleyn and 13 North Country Cheviot (Park).

3.1.2. Matching PrP genotype to existing sheep breeder data for analysis of performance in commercial flocks: The National Scrapie Plan Administration Centre (NSPAC) supplied PrP genotypes obtained from animals using a variety of proprietary commercial genotyping methodologies, chiefly SNP assays. In all cases the ovine PrP gene was genotyped at polymorphic codons 136(A/V), 154(R/H) and 171(Q/R/H) to discriminate between the five major alleles: ARR, ARQ, ARH, AHQ and VRQ. Datasets for the 11 breeds were prepared by matching Signet performance data and NSPAC PrP genotypes in the commercial flocks participating in the project. 'Matched record' implies that we have animal identification with known parentage, weights, ultrasonic fat and muscle depth and the PrP genotype. Additionally, in each dataset a large number of matched flockmates without PrP genotype were also available, potentially strengthening some statistical analyses and allowing investigation of whether or not breeders preferentially genotype animals with better performance characteristics.

3.1.3. Enhanced data collection for analysis of survival in commercial flocks: Protocols were developed and implemented to capture data on lamb deaths and ewe disposal reasons in the commercial flocks participating in the project. In parallel, tissue collection protocols from dead animals were implemented, enabling genotype data to be obtained for dead animals. A comprehensive list of presumed reasons for death, and a genotype profile for dead animals were obtained across all breeds.

3.1.4. Genotyping of experimental flocks. PrP genotypes were obtained from blood samples for the ADAS Scottish Blackface (2,747 animals), the SAC/RI Scottish Blackface (7,291 animals) and the IRS/ADAS/SAC Longwool (1,106 animals) flocks. Also, genotypes were obtained from tissue samples for 380 dead lambs in the SAC/RI Scottish Blackface flocks to study potential associations between the PrP gene and survival in these flocks. Samples were genotyped for codons 136, 154, and 171 through Orchid Cellmark Europe Ltd, Oxfordshire, UK.

3.2. Associations of PrP genotype with lamb performance

3.2.1. Traits: Traits for which performance records were available were analysed. In commercial flocks, lamb performance traits were those recorded by Signet routinely: live body weights at birth, eight weeks and ~20 weeks (at the time of ultrasonic scanning), and ultrasonic muscle and fat depths. The data of ultrasonic fat depth were found to be skewed, based on the normal probability plots, and were therefore square root transformed. Experimental flocks (managed in a similar way to commercial flocks) are comprehensively recorded and extra traits were available, particularly, for Scottish Blackface flocks. Extra traits included slaughter and carcass traits. The specific traits analysed and the numbers of lambs for each trait are shown in Tables 1 and 2 for commercial and experimental flocks, respectively.

Table 1. Traits analysed and numbers of lambs per trait in commercial flocks.

Breed	Trait ¹					
	Birth weight (kg)	Eight-week weight (kg)	Scan weight (kg)	Ultrasonic muscle depth (mm)	Ultrasonic fat depth (mm)	Survival
North Country Cheviot (Hill)	1,366	8,163	6,374	6,115	6,113	2,188
Scottish Blackface	2,066	21,283	12,568	11,473	11,470	4,577
Welsh Mountain	4,701	14,439	8,086	6,270	6,247	2,386
Rough Fell		2,876	2,111	1,565	1,565	
Charollais	12,420	22,750	14,796	13,924	13,924	6,241
Poll Dorset	9,568	20,860	8,502	8,753	8,154	4,706
Texel	16,112	23,292	20,727	19,294	19,288	9,726
Beulah	2,564	10,115	9,086	8,034	8,034	1,806
Blue Faced Leicester	1,982	3,563	3,175	3,141	3,141	763
Lleyn	10,176	12,175	3,281	2,647	2,647	3,553
North Country Cheviot (Park)	2,821	5,080	4,038	3,829	3,829	2,477

¹Birth weight: actual weight at or within 24 hrs of birth; Eight-week weight: actual lamb weight at a target age of 56 days; Scan weight: actual lamb weight at ultrasonic scanning (ca. 20 weeks); Ultrasonic Muscle Depth: actual muscle depth measured at the third lumbar vertebra; Ultrasonic fat depth: square root transformation of fat depth measured at the third lumbar vertebra; Survival traits are described in section 3.2.1.

3.2.2. Data analyses: Full details of the methods used are given in Moore *et al.* (2006), Man *et al.* (2006) and Sawalha *et al.* (2007b). Linear mixed model univariate analyses were performed using the statistical package ASReml, which utilises the restricted maximum likelihood method for estimating variance components. The statistical models varied among traits but in general they included the effect of the PrP genotype, the 'animal genetic' effect (which accounts for other genes affecting the performance trait), maternal (genetic and non-genetic) effects and non-genetic factors having a significant effect on the trait (e.g. sex, litter size, age of dam and date of recording). The analyses included full pedigree information, and hence accounted for all genetic relationships between animals, which is critical to remove potential confounded effects of background (non-PrP-linked) genetic effects. At least, five analyses differing in the PrP genotype classes were carried out for each trait. In the first analysis, animals were categorized by PrP genotype. In the other four analyses, animals were classified according to the number of copies they carry of each of the PrP alleles. For example, analysis 2 was based on the number of copies of the ARR allele (i.e., the classes compared were ARR homozygous, ARR heterozygous and ARR non-carriers). The Bonferroni correction was used, where appropriate, to adjust for multiple testing.

Table 2. Traits analysed and numbers of lambs or ewes per trait in experimental flocks.

Trait	Scottish Blackface SAC	Scottish Blackface ADAS	Swaledale ADAS ²	Suffolk SAC ³	Longwool SAC/IRS/ ADAS
Lamb¹					
Live weights at:					
Birth (kg)	7,114	6,350	1,465	3,301	
Marking (kg)	6,848	6,297	1,456		
Weaning (kg)	6,806	6,154	1,437	2,813	
150 days (kg)				2,062	
Slaughter (kg)	2,670		620		
Carcass weight (kg)	2,669		620		
Carcass conformation	2,675		620		
Age at slaughter (d)	2,675		620		
Ultrasonic muscle depth (mm)	6,774	1,448		2,062	
Ultrasonic fat depth (mm)	6,780	1,398		2,062	
CT predictors	411				
Survival	3,673-6,777				
Ewe⁴					
Maternal behaviour	2,100				
Litter size	1,899		760		1,071
Live weights (kg)	2,356				
Condition score	2,356				
Ultrasonic muscle and fat	2,344				
CT predictors	335				
Wool yield and quality	2,231-2,265				
Longevity	1,875		674		1,072

¹Birth weight: actual weight at or within 24 hrs of birth; Marking weight: actual weight at mid lactation (at an average age of 52 d); Weaning weight: actual weight at weaning (at an average age of 110 d for Scottish Blackface and 56 d for Suffolk); Carcass weight: dressed weight of carcass recorded at about 24 h after slaughter; Carcass conformation: visual appraisal (EUROP score). Ultrasonic Muscle Depth: actual muscle depth measured (around weaning) at the third lumbar vertebra; Ultrasonic fat depth: square root transformation of fat depth measured (around weaning) at the third lumbar vertebra. CT predictors: Computer tomography predicted weights of weights of muscle, carcass fat, internal fat, and bone tissues and total carcass, and killing out percentage. Slaughter traits were available for male lambs only. Survival traits are described in section 3.2.1.

²In Swaledale, marking weight was measured at an average age of 31 d and weaning weight was measured at an average age of 123 d.

³In Suffolk, live weight and ultrasonic fat and muscle depths were recorded at a fixed age of 150 days. Numbers presented include lambs with predicted genotypes (see section 3.2.2).

⁴Maternal behaviour: score (1 to 6) on dam's reaction and distance the dam retreats from its lamb(s) when they are being handled for the first time within 24 h of birth; Litter size: number of lambs born (dead and alive); Live weight, condition score and CT predictors of body composition (carcass muscle, carcass subcutaneous and inter-muscular fat, internal body cavity fat and bone) and body condition score were recorded at 4 focal production points each year (at pre-tupping, pregnancy scan, mid-lactation and weaning) over several years; Ultrasonic muscle and fat: depths recorded at mating time; Wool traits: Fleece weight, staple length, length of coat wool at birth, grey score of grey fibres (1 to 10), score of kemp (1 to 10), quality scores; Longevity: actual number of days after the first year until death or censoring.

Except if the Suffolk flock, genotyping in experimental flocks was non-selective (i.e. all animals in the flocks at a particular time were genotyped) and only genotyped animals were included in the analyses. In commercial flocks, only a proportion of the animals in each dataset were genotyped. The percentage of genotyped lambs was 32.4 in North Country Cheviot (Hill), 31.6 in Scottish

Blackface, 27.6 in Welsh Mountain, 51.1 in Rough Fell, 52.4 in Charollais, 31.5 in Poll Dorset, 45.1 in Texel, 39.7 in Beulah, 19.7 in Blue Faced Leicester, 42.8 in Lleyn and 43.8 in North Country Cheviot (Park). Exploratory data analysis indicated that genotyped lambs had superior trait values relative to ungenotyped lambs, possibly reflecting preferential genotyping of better performing lambs. In the estimation of trait means for genotype or allele classes, this effect was accounted for by fitting PrP genotype or allele class in combination with the fixed effect “genotyped or ungenotyped”, thus forming trait predictions for both categories of lambs.

The Suffolk dataset was relatively small (about 470 animals with both genotype and performance data) and genotypes were only available for codon 171 (alleles R, Q and H). Pedigree and genotypic records were used to predict the genotypes of 5,173 ungenotyped animals by performing segregation analysis with the software GENEPROB. Probabilities for the different genotypes were inferred for ungenotyped animals. Each inferred genotype was assigned a genotypic probability index (GPI) to reflect the accuracy of prediction. The GPI values range from 0 (for animals with no relatives with known or predicted genotypes) to 100 (for animals for which genotypes were predicted with 100% certainty). Animals with GPI = 0 were excluded from the association analyses. About 25% of the 5,173 predicted genotypes were inferred with at least GPI \geq 50. Genotypes of 235 animals were inferred with 100% certainty. Full details are given in Sawalha *et al.* (2008b).

3.2.3. Results of associations of PrP genotype with lamb performance: Some significant associations of PrP genotype and lamb performance traits were found and they are summarised in Table 3. However, these significant associations were found often in small group sizes and were seldom large. There were no consistent patterns across ages, across datasets of the same breed (i.e., Scottish Blackface) or across breeds. Importantly, selecting against the VRQ allele or in favour of the ARR allele would have had a negligible impact on performance at the sector level.

Table 3. Significant associations found for lamb performance traits.

Breed	Birth Weight	8-Week Weight	Scan Weight	Muscle Depth	Fat Depth	Slaughter	CT
North Country Cheviot (Hill)							
Scottish Blackface							
Commercial		*					
SAC experimental	*					*	
ADAS experimental							
Welsh Mountain			*	*			
Rough Fell							
Swaledale (experimental)							
Charollais				**			
Poll Dorset							
Texel	*	**	**				
Suffolk (experimental)							
Beulah		*	*	*			
Blue Faced Leicester							
Lleyn				*			
North Country Cheviot (Park)	*			*			

* $P < 0.05$, ** $P < 0.01$. Shaded cells indicate the specific traits analysed for each breed.

The specific significant results were:

Scottish Blackface – SAC experimental flocks

- Heterozygous ARQ lambs were 0.04 kg heavier than ARQ/ARQ lambs at birth.
- Heterozygous AHQ lambs were 0.5 kg heavier at slaughter and had 0.2 kg heavier carcasses than non-AHQ carriers.

- Heterozygous ARQ lambs were 0.4 kg heavier than ARQ/ARQ and non-ARQ carriers at slaughter.
- Heterozygous VRQ lambs had 0.5 kg heavier carcasses than non-VRQ carriers but needed ~ 10 d longer finishing time.

Scottish Blackface – Commercial flocks

- Trends follow those of experimental flocks although the only significant association found was for a different trait:
 - Heterozygous VRQ lambs were 0.5 kg heavier than non-carriers at eight weeks.

Welsh Mountain

- Homozygous ARR lambs were 0.72 kg lighter than non-ARR carriers at scanning.
- Heterozygous ARR lambs were 0.50 kg lighter than non-ARR carriers at scanning.
- ARR carrier lambs had 0.48 mm lower muscle depths than non-carriers and this was consistent with their lower scan weights.
- Data were re-analysed using scan weight as a covariate. Although this improved the model fit, the support for an association with PrP genotype dropped below significance, suggesting that the muscle depth associations reflected differences in animal size rather than underlying muscularity.

Charollais

- Heterozygous VRQ lambs had 0.57mm deeper muscle than non-VRQ carriers.

Texel

- Heterozygous ARR lambs were 0.8kg heavier than non-ARR carriers at birth.
- Heterozygous ARR lambs were 0.5kg heavier than non-ARR carriers at eight weeks.
- Homozygous ARR lambs were 0.41kg lighter than heterozygous ARR lambs at scanning.

Beulah

- Homozygous ARR lambs were 0.32kg heavier than non-ARR carriers at eight weeks.
- Heterozygous ARR lambs were 0.47kg lighter than non-ARR carriers at eight weeks.
- Homozygous ARR lambs had 0.22mm deeper muscle than heterozygous ARR lambs.

Lleyn

- Heterozygous VRQ lambs had 2.0mm deeper muscle than non-VRQ carriers.

North Country Cheviot (Park)

- Heterozygous VRQ lambs were 0.34kg heavier than non-VRQ carriers at birth.
- Heterozygous ARR lambs had 0.47mm deeper muscle than non ARR carriers.

3.3. Associations of PrP genotype with lamb survival

Full details on traits analysed, method of analysis and results for the experimental flocks are given in Sawalha *et al.* (2007a).

3.2.1. Traits and data analyses: Lamb survival traits considered were viability at birth, survival from 1 to 14 days, survival from 15 days to weaning and survival from weaning to slaughter, and, in commercial flocks, survival from birth (0 days) to weaning (120 days). Viability at birth was analysed as a binary trait (i.e. alive or dead at 24 hr after birth) using a non-linear sire model with ASReml. Postnatal survival traits were defined using actual days of survival before death or censoring. Records were considered censored if the lambs were still alive at the end of the period or were dead or removed from the flock during the period due to reasons not related to their viability. In experimental flocks, the data of actual time of survival were analysed using a Weibull proportional hazard model which included sire and maternal environmental litter effects. The software Survival Kit was used for postnatal survival analyses. In commercial flocks, survival times were modelled using Cox proportional hazard models, with the baseline hazard stratified by

flock and including the animal additive genetic effect, and the models were implemented in R using the kinship package. The hazard ratios for *PrP* genotypes were compared using a likelihood ratio test.

3.2.2. Results of associations of PrP genotype with lamb survival: Table 4 shows the flocks analysed for survival traits and a summary of the significant associations found. The PrP genotype showed no significant association with viability at birth in any of the datasets analysed. However, in the Scottish Blackface experimental flocks a potentially important association was found with postnatal survival. The PrP genotype significantly influenced the hazard rate (i.e. relative likelihood of death) during all postnatal periods. The largest effect of the PrP genotype was associated with the presence or absence of ARR and ARQ alleles and, to a lesser extent, to the AHQ allele. Generally, the presence of the ARQ allele was associated with lower hazard rate while the presence of two ARR alleles or one AHQ allele was mostly associated with increased hazard rate.

Table 4. Significant associations found for lamb survival traits.

Breed	0 days	1-14 days	15-120 days	>120 days	0-120 days
North Country Cheviot (Hill)					
Scottish Blackface					
Commercial					
SAC experimental		*	**	*	
Welsh Mountain					
Charollais					*
Poll Dorset					
Texel					
Beulah					
Blue Faced Leicester					
Lleyn					
North Country Cheviot (Park)					

* P<0.05, ** P<0.01. Shaded cells indicate the specific traits analysed for each breed.

The specific significant results were:

Scottish Blackface – SAC experimental flocks

- The hazard rate for ARR/ARR lambs was over twice that for ARR heterozygous lambs during the periods from 1 to 14 d and from 121 to 180 d.
- Comparatively, the hazard rate for ARQ heterozygous lambs was half, to a third, of that for ARQ non-carriers during the same periods.
- The hazard rate for AHQ carrier lambs was twice that for non-carriers during the period from 15 to 120 d.
- The estimated lamb loss from 1 d to 180 d due to higher postnatal mortality for the ARR/ARR genotype compared with the ARR/ARQ genotype was 2.20%.
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Charollais – Commercial flocks

- The hazard rate for ARR/VRQ lambs was significantly higher than that for ARR/ARR lambs.

3.2.3. Factors affecting lamb mortality: A range of factors were found to affect lamb mortality. The effect of each factor was similar across breeds, though whether or not a factor was significant, varied amongst breeds. In general, factors affecting mortality included birth weight, number of lambs at birth, sex and age of dam. The genetic merit of dams had more influence on viability at birth than the genetic merit of lambs themselves. Estimates of heritability for postnatal survival traits were significantly greater than zero. These results indicate that lamb survival can

be improved through farm management practices and genetic selection. Both animal and maternal genetic effects should be considered in breeding programmes for improving viability at birth. Full details for the experimental flocks are given in Sawalha *et al.* (2007c).

3.4. Associations of PrP genotype with ewe traits

Analyses of maternal traits were performed with datasets from experimental flocks, particularly the SAC Scottish Blackface flocks which were the most comprehensively recorded. The traits analysed are described in Table 2.

3.4.1. Data analyses: Most ewe traits had repeated records as ewes were recorded at each parity. Preliminary multi-trait analyses of litter size and maternal behaviour score (i.e., litter sizes or scores at different parities are considered different traits) showed that the different measurements reflected what is genetically the same trait (i.e., the genetic correlation between litter sizes or scores at different parities was very high). Thus both traits were analysed with ASReml using repeatability animal models.

As described in Table 2, live weight, CT predictors of body composition (carcass muscle, carcass subcutaneous and inter-muscular fat, internal body cavity fat and bone) and body condition score were recorded at 4 focal production points each year over several years. Because of the continuous nature of these traits and their change within and across years, the data were analysed, also with ASReml, using a linear mixed random regression model and assuming that the direct additive genetic effect was a 2nd order Legendre polynomial function of time. This method of analysis enables not only testing the effect of PrP genotype on the overall trait mean, but also testing if the shape of the curve (e.g., growth curve) is different for different PrP genotypes, which allows studying the seasonal changes in tissue weights. Full details of analyses and results for these traits are given in Sawalha *et al.* (2008a).

Ewe longevity (survival from after one year of age) was analysed as described above for lamb survival. Wool traits (fleece weight, staple length, grey and kemp contents, birth coat, wool quality and British Wool Marketing Board score) were analysed as described above for lamb performance traits.

3.4.2. Results of associations of PrP genotype with ewe traits: Very few significant associations were found and they are summarised in Table 5. As with lamb performance traits, these significant results were not consistent across breeds. Also, selecting against the VRQ allele or in favour of the ARR allele would have had a negligible impact on ewe performance.

Table 5. Significant associations found for ewe traits.

Trait	Scottish Blackface SAC	Swaledale ADAS	Longwool SAC/IRS/ADAS
Weight	*		
Body condition score			
Ultrasonic muscle and fat			
CT predictors			
Litter size		**	
Longevity			
Wool	*		
Maternal behaviour score			

* P<0.05, ** P<0.01. Shaded cells indicate the specific traits analysed for each breed.

The specific significant results were:

Scottish Blackface – SAC experimental flocks

- Homozygous ARQ/ARQ ewes had about 2% less grey fibres than ARQ non-carriers.

- ARQ carrier ewes (i.e. homozygous and heterozygous ARQ) were about 0.5 kg lighter than non-ARQ carriers at all ages.
- There was a significant interaction between PrP genotype and age of the ewe (i.e. the effect of PrP genotype was not the same at different ages) for CT carcass muscle and fat, internal body cavity fat and bone, body condition score, and body weight. In general, ARQ carrier ewes mobilized more fat reserves at times of nutrient deficiency such as during lactation and gained it back more quickly by the mating season (when nutrients became abundant) than non-ARQ carriers.

Swaledale – ADAS experimental flock

- Homozygous ARR/ARR ewes had significantly higher total number of lambs born than heterozygous ARR ewes.

Summary of associations of PrP genotype with other economically important traits

- Analyses of large experimental and commercial datasets did not reveal consistent significant associations of the PrP genotype with a wide range of performance traits.
- Analyses of experimental Scottish Blackface flocks presented evidence that the ARQ allele is associated with higher postnatal survival rates than the more resistant ARR or AHQ alleles.
- These results are potentially important for the industry but have not been replicated in any of the 10 commercial datasets.
- Selection aimed at eliminating the VRQ allele would have a negligible impact on performance and survival at the sector level.

3.5. Estimates of PrP frequencies

Estimates of allelic and genotypic frequencies for the PrP locus were obtained for each breed. Initially, less bias in favour of ARR animals was expected in the commercial flocks participating in the project than in national estimates as project breeders were asked to genotype all lambs born rather than just lambs to be retained for breeding. Thus, in order to evaluate potential bias in national estimates, allelic frequencies obtained from the NSP were compared with those obtained from flocks participating in the project (Table 6).

Contrary to what was expected, the frequency of the ARR allele was higher in commercial project flocks than the frequency estimated at the national level. Also, in general, the frequencies of ARQ and VRQ alleles were lower in project flocks. This suggests that breeders participating in the project may have started PrP selection earlier or have been selecting more actively in favour of the resistant allele and against susceptible alleles than other NSP flocks. It was therefore concluded that the datasets available were not appropriate for investigating potential bias in estimates of PrP frequencies.

Estimates from the experimental flocks reflect particular breeding decisions in these flocks. (e.g., the experimental Swaledale flock was bred intentionally to increase the frequency of AHQ and VRQ alleles for scrapie genetics research and the experimental Scottish Blackface flocks were selected against the VRQ allele). As mentioned above, the Suffolk flock was only genotyped for codon 171. Allelic frequencies were 46.2, 2.1 and 51.7 for R, H and Q, respectively.

Table 6: Allelic frequencies estimated from the project datasets compared with those estimated at the national level (in brackets)¹.

Breed	ARR	AHQ	ARH	ARQ	VRQ
North Country Cheviot (Hill) ²	54.5 (45.5)	14.8 (11.5)	(0.2)	27.8 (36.3)	2.9 (6.5)
Scottish Blackface	(29.5)	(7.8)	(0.0)	(60.2)	(2.6)
Commercial	43.9	6.2	0.0	48.1	1.8
SAC experimental	31.0	8.3	0.04	59.5	1.1
ADAS experimental	40.2	4.9	0.0	54.8	0.0
Welsh Mountain	58.5 (37.1)	7.2 (22.9)	(0.1)	22.9 (33.4)	11.3 (6.4)
Rough Fell	45.5 (41.0)	2.4 (1.8)	0.0 (0.0)	51.0 (55.0)	2.4 (2.2)
Swaledale (experimental)	39.0 (41.1)	24.0 (16.3)	0.0 (0.0)	27.0 (36.9)	11.0 (5.6)
Charollais	83.0 (60.4)	0.0 (0.1)	0.0 (0.2)	15.0 (35.1)	1.0 (4.2)
Poll Dorset ³	77.0 (60.5)	0.0 (0.9)	0.0 (0.1)	17.0 (28.4)	5.0 (10.2)
Texel	56.0 (33.4)	2.0 (4.2)	29.0 (43.7)	11.0 (15.3)	1.0 (3.4)
Beulah	69.4 (53.4)	2.4 (3.5)	0.0 (0.0)	26.6 (39.8)	1.6 (3.3)
Blue Faced Leicester	69.2 (61.3)	14.9 (17.5)	0.0 (0.0)	15.9 (20.9)	0.0 (0.3)
Lleyn	74.4 (56.0)	7.0 (11.1)	7.2 (9.5)	10.9 (21.7)	0.5 (1.8)
North Country Cheviot (Park) ²	58.9 (45.5)	30.4 (11.5)	0.2 (0.2)	30.4 (36.3)	1.5 (6.5)

¹Estimates for national Great Britain's populations are those from Eglin *et al.* (2005; Vet. Rec. 156: 433-437).

²Eglin *et al.* (2005) did not distinguish between Hill and Park

³Eglin *et al.* (2005) did not distinguish between Horn and Poll Dorset

4. REVIEW OF OBJECTIVE 2: TO DEVISE OPTIMAL BREEDING STRATEGIES FOR SCRAPIE RESISTANCE IN THE CONTEXT OF AN OVERALL GENETIC IMPROVEMENT PROGRAMME WITH RESTRICTIONS ON THE LOSS OF GENETIC VARIABILITY

This objective was completed using computer modelling. Stochastic computer simulations programs (developed in Fortran 90) were used to model realistic schemes in practice in the UK for both mainstream and rare breeds. Scenarios under PrP selection were compared to that where there was no selection on PrP, but animals were selected on performance. At least 100 hundred replicates were run for each scenario.

The models were used to investigate the effect of selection on PrP genotype on PrP allele frequencies, rates of inbreeding and genetic gain in a performance trait under selection and risk of scrapie outbreaks. It is important to note that the rate of inbreeding determines the genetic size of a population which is described by the 'effective population size' (N_e) as $N_e = 1/2\Delta F$ and also the rate at which genetic variability is lost.

The computer simulations allowed us to i) determine optimal genotyping strategies; ii) evaluate the effect of different breeding alternatives on the frequency of PrP alleles and on effective population size of individual breeds; iii) determine the optimum balance of selection for resistance to scrapie *versus* selection for other important traits; and iv) evaluate the most effective strategy for increasing levels of scrapie resistance over time and identify its consequences for the endemic status of the disease.

4.1. Simulations of mainstream breeds: Sheep flocks participating in sire-referencing schemes (SRS), where selection is applied on performance, were simulated for the three major breed types in the UK (i.e. terminal sire, crossing sire and hill sheep). Table 7 summarises the most relevant parameters used in the simulation of the three breed types and a detail description of the simulations is given in Man *et al.* (2007). Briefly, flocks of different sizes were simulated and genetic links were created by using reference sires. A number of ewes (varying among breed types) were mated to each of three (terminal sire) or two (crossing sire and hill) reference sires in every flock and year. Surplus ewes were mated to rams born within the flock (within-flock sires).

Artificial insemination was assumed for reference sires and natural matings were assumed for within-flock sires.

Table 7. Simulation parameters for terminal sire, crossing sire, and hill breeds

	Terminal sire	Crossing sire	Hill
Number of flocks	15	13	17
Number of ewes per flock	40-140	30-90	100-700
Total number of ewes per year	1030	600	6800
Percentage of within-flock sires replaced per year	50	50	60
Number of reference sires replaced per year	3	2	2
Number of reference sires used per year per flock	3	2	2
Percentage of ewes producing lambs from reference sires	31	30	8
Within-flock sire:ewe ratio	1:20	1:20	1:40
Generation interval			
Male	3.0	2.8	2.2
Female	3.5	3.4	3.8
Average litter size			
at birth	1.7	2.2	1.5
at weaning	1.5	1.6	1.3

The performance trait under selection was assumed to be controlled by a very large number of loci, each of small effect (i.e., the 'infinitesimal model') and to have a heritability of 0.25. This trait was recorded in both sexes at 150 days. The PrP gene was assumed to have no direct impact on the trait and to be unlinked with genes that influence this trait. Selection on performance was based on BLUP (Best Linear Unbiased Predictor) estimated breeding values.

Populations were simulated over a 30 year period. Sire referencing started in year six, after five years of random selection. Selection on PrP genotype started in year 16, after ten years of sire referencing. Year $t = 0$ refer to the year PrP selection began, so that year $t = -15$ refer to the base population (in which all animals were unrelated), and year $t = 15$ refer to the last year of the simulation.

Initial allele frequencies in the ranges of 5% to 90% for ARR (f_{ARR}) and 5% to 30% for VRQ (f_{VRQ}) were simulated. The other alleles (xxx) made up for the remainder in the population. The PrP selection strategies modelled were: i) only animals with no VRQ can be used for breeding (strategy S1); ii) only animals with at least one ARR and no VRQ can be used for breeding (strategy S2); and iii) all animals can be used for breeding, but they were sequentially selected on their genotype using the following priority - ARR/ARR, ARR/xxx, xxx/xxx (i.e. those without ARR or VRQ), ARR/VRQ, VRQ/xxx, and VRQ/VRQ (strategy S3). These three PrP selection strategies were targeted to i) reference sires only; ii) all sires; or iii) all breeding animals (i.e., sires and dams). In addition, a fourth strategy modelling the potential future 'Ewe Genotyping Scheme' was considered. Under this strategy, rams and ewes carrying the VRQ allele and homozygous ARQ/ARQ rams were not allowed to be used for breeding.

4.2. Simulation of rare breeds: In comparison with mainstream breeds, simulations for rare breeds modelled i) smaller population and flock sizes (population sizes of 100 to 400 ewes; the minimum flock size was 20 ewes); ii) lower mating ratios (1M:5F or 1M:10F); iii) lower replacement rates; (iv) sires used across flocks; (v) only natural matings; and, for most breeds, (vi) no selection on performance. Realistic population parameters were obtained from the Rare Breeds Survival Trust.

4.3. Prediction of rates of genetic gain and inbreeding: Average true breeding value for the performance trait (G_i) and average inbreeding (F_i) of animals born at each year i were computed. Rates of gain (ΔG_i) and inbreeding (ΔF_i) were obtained every year as $\Delta G_i = G_i - G_{i-1}$ and $\Delta F_i = (F_i$

$-F_{i-1})/(1 - F_{i-1})$, respectively. Annual rates between years i and j (ΔG_{i-j} and ΔF_{i-j} , where $j > i$) were obtained by averaging the individual annual rates.

4.4. Prediction of risk of scrapie outbreaks: The risk of occurrence of an epidemic was measured by the basic reproduction ratio (R_0), which is defined as the average number of secondary infections produced by one infected animal when that animal is introduced into a previously unexposed susceptible population. When R_0 is less than one, the risk of an outbreak occurring is minimal, and if one occurs, it is expected to die out. If R_0 is greater than one then there is a risk that the outbreak becomes epidemic. The framework adopted for computing R_0 is described in Box 1 (Appendix 1).

The effectiveness of different strategies for eradicating scrapie was mainly assessed by the percentage of flocks with a basic reproduction ratio of less than one ($P_{R_0 < 1}$). The mean and distribution (including standard deviation and the 5% threshold value, which corresponds to the cut-off value for the highest 5% of the distribution) of R_0 were also considered when comparing different scenarios in terms of risk of outbreaks.

4.5. Effect of selecting on PrP genotype on genetic gain in performance and on inbreeding in mainstream breeds: A detailed description of the research aimed at quantifying the impact of various PrP selection strategies on PrP allelic frequencies, inbreeding and genetic gain in performance traits in mainstream breeds is given in Man *et al.* (2007). Results are only summarised here for terminal sire breeds. Results for crossing sire and hill breeds followed similar patterns to those for terminal sire breeds.

Figures 1 and 2 show, respectively, the annual rates of genetic gain in the performance trait and annual rates of inbreeding for terminal sire populations under different selection strategies targeted to all sires. It should be noted that the lowest f_{ARR} found in UK mainstream breeds is 0.30 and the highest f_{VRQ} is 0.15. When compared with the scenario where selection was only on the performance trait, the reduction in genetic gain observed when selecting on PrP genotype was very small when targeting only the VRQ allele (strategy S1) or favouring ARR carriers (strategy S2). This was true even when f_{VRQ} was relatively high (0.30) or f_{ARR} was relatively low (0.30). Selection favouring homozygous ARR sires (strategy S3) also had a small impact on rates of gain in the performance trait when the initial f_{ARR} was high (0.70) but led to a substantial slowing in gain in both breeds when the initial f_{ARR} was 0.30 or lower. Over the 15 years of selection, S3 led to about one and a half years (when initial $f_{ARR} = 0.30$) or three years (when initial $f_{ARR} = 0.05$) of loss in cumulative gain compared with that obtained with the scenario with no PrP selection.

The rate of inbreeding in scenarios undergoing PrP selection was equal to or less than the rate of inbreeding obtained with selection exclusively on the performance trait (Figure 2). In general, the greater the loss in genetic gain for the performance trait, the greater was the reduction in the rate of inbreeding. This is because selection on PrP genotype resulted in less intense selection on the performance trait, so that the animals selected for breeding tended to be less closely related than when selection was solely on the performance trait.

Figure 3 shows average true breeding value and inbreeding plotted against f_{ARR} for terminal sire populations with initial $f_{ARR} = 0.30$ and $f_{VRQ} = 0.15$ when strategies S2 and S3 were targeted at reference sires only, all sires or all breeding animals. Changes in gain and inbreeding with f_{ARR} followed very similar patterns within each strategy. The loss in gain was negligible when targeting reference sires only under strategy S2 ($S2_R$) but f_{ARR} was just over 0.5 at the end of PrP-based selection ($t = 15$). Results for strategy S2 targeting all sires ($S2_{RW}$) and for strategy S3 targeting reference sires ($S3_R$) were very similar. The losses in gain under these two strategies were small and f_{ARR} increased up to 0.7 by $t = 15$. The increase in f_{ARR} was rapid in the initial years under strategy S2 targeted to all breeding animals ($S2_{RWD}$) and f_{ARR} reached 0.85 at the end of the selection period. However, during the 15 years of PrP selection, this strategy led to about one year loss of cumulative gain when compared with the scenario where there is no selection of PrP

genotype, and this loss occurred in the first five years. Strategy S3 targeted to all sires and to all breeding animals (S3_{RW} and S3_{RWD}) led to the fastest increases in f_{ARR} (particularly S3_{RWD} which led to fixation by $t = 10$) but also to the highest losses in rates of gain in the performance trait. For instance, during the 15 years of selection, S3_{RWD} led to loss of two years of cumulative genetic progress in performance when compared with no PrP selection and, as with other strategies, most of this loss occurred during the first five years.

The main results for mainstream breeds can be summarised as follows:

- Assuming realistic breed frequencies, selection against the VRQ allele had a minimal impact on genetic progress for performance traits when compared with the scenario where there was no selection on PrP genotype.
- More extreme PrP selection strategies aimed at increasing the frequency of the ARR allele and decreasing the frequencies of ARQ and VRQ alleles led to decreases in the rate of genetic progress for performance when the ARR frequency was low or when the ARQ frequency was high.
- In general, the rate of inbreeding not only did not increase with PrP selection, but decreased in most scenarios as PrP selection was counter-balanced by a weaker selection on performance when compared with the scenario with no selection on PrP genotypes.
- Eradication of the VRQ allele or fixation of the ARR allele within 15 years of selection was possible only with PrP selection targeting all breeding animals.

Figure 1. Average annual rate of genetic gain in performance (in initial phenotypic standard deviation units) after 15 years of selection when different PrP selection strategies are applied on all sires in terminal sire populations assuming $f_{VRQ} = 0.15$ and different f_{ARR} . Results are also presented for the scenario where there is no selection of PrP genotypes.

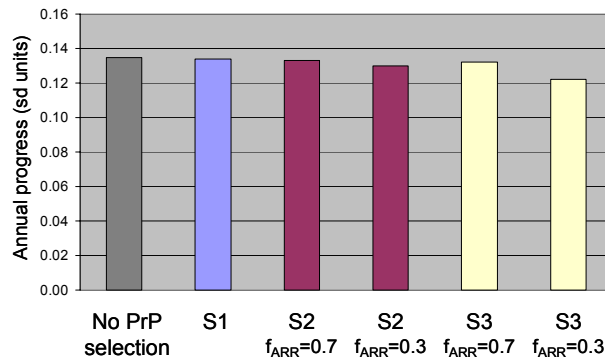


Figure 2. Average annual rate of inbreeding (in %) after 15 years of selection when different PrP selection strategies are applied on all sires in terminal sire populations assuming $f_{ARR} = 0.15$ and different f_{ARR} . Results are also presented for the scenario where there is no selection of PrP genotypes.

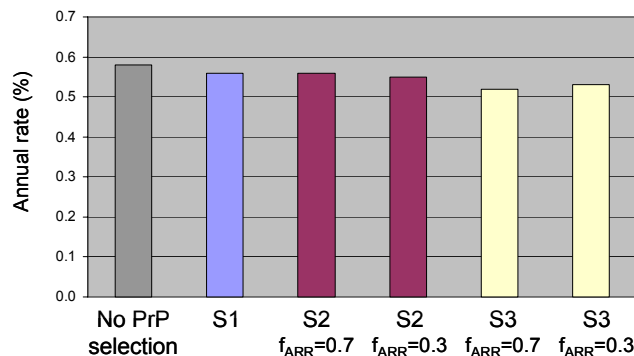
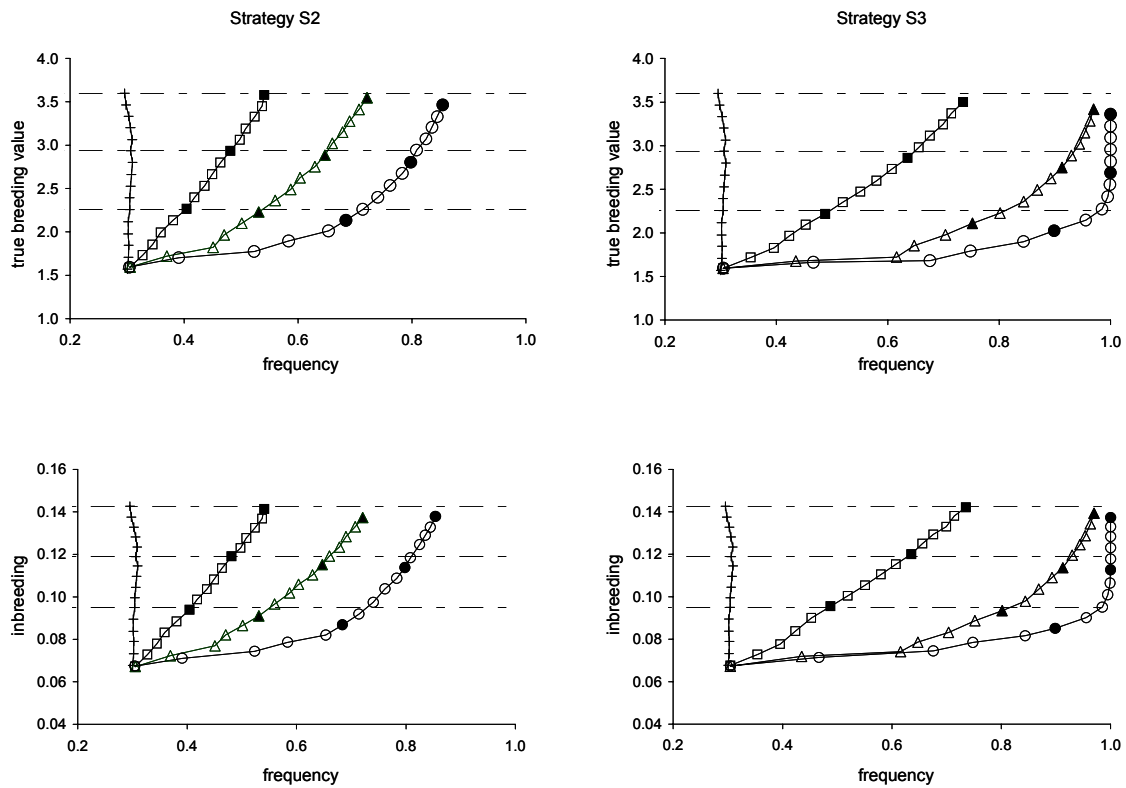
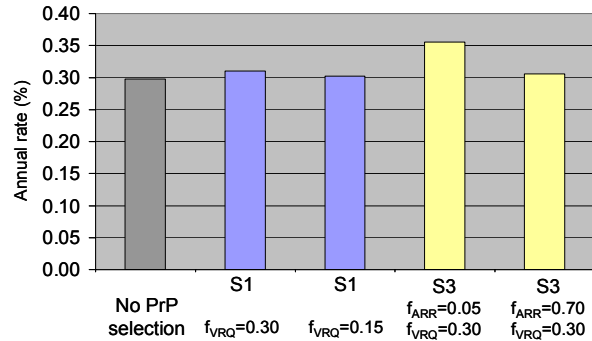


Figure 3. Average true breeding value (in initial phenotypic standard deviation units) and inbreeding coefficient (in %) plotted against f_{ARR} in terminal sire breeds when strategies S2 and S3 are targeted at reference sires only (\square), all sires (Δ) or all breeding animals (\circ). Initial frequencies were $f_{ARR} = 0.30$ and $f_{VRQ} = 0.15$. For a given scenario, the different plotted values in the figure correspond to different years of selection. Filled symbols correspond to years 5, 10 and 15 of PrP selection. Results are also presented for the scenario where there is no selection of PrP genotype (+). The parallel broken grid lines are given for reference and are drawn at the values obtained with the scenario with no PrP selection at years 5, 10 and 15.



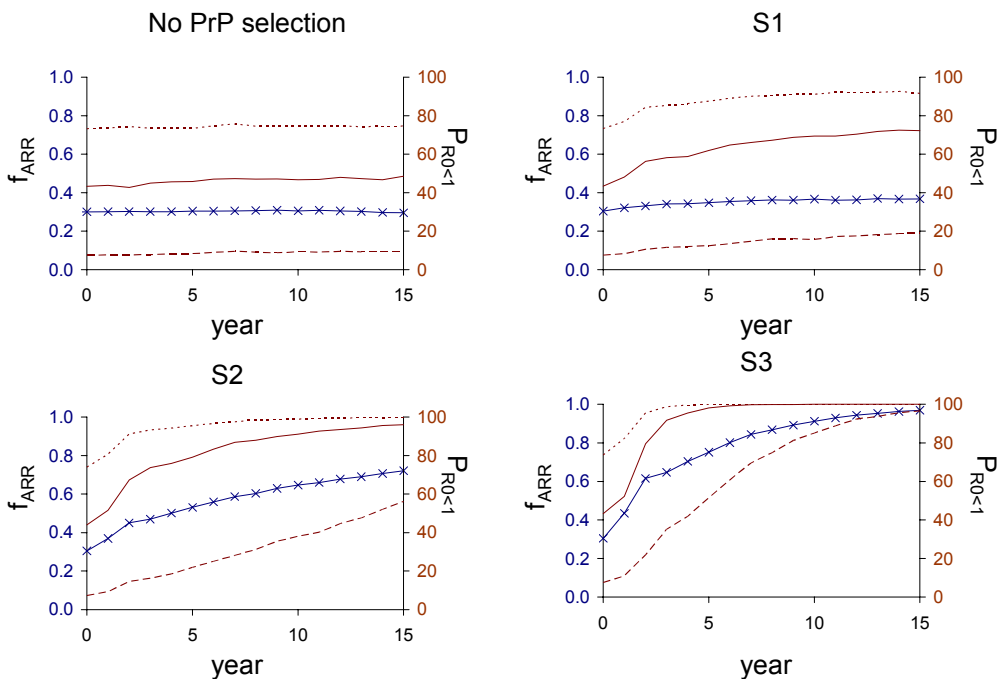
4.6. Effect of selecting on PrP genotype on inbreeding in rare breeds: When compared with the scenario where there was no selection on PrP genotype, increases in the rate of inbreeding (i.e., decreases in effective population size) when selecting only against the VRQ allele were small even when the initial frequency of this allele was relatively high ($f_{VRQ} = 0.30$; Figure 4). However, selection in favour of the ARR allele led to substantial increases in the rate of inbreeding when the f_{ARR} was 0.30 or lower. Under some scenarios, PrP selection strategies were not feasible due to a lack of breeding animals of the required genotypes.

Figure 4. Average annual rate of inbreeding (in %) when different PrP selection strategies are applied on all sires in rare breeds assuming different f_{VRQ} and f_{ARR} . Results are also presented for the scenario where there is no selection of PrP genotypes. The population consists of 100 ewes and the mating ratio was 1M:10F.



4.7. Effect of selecting on PrP genotype on risk of scrapie outbreaks in mainstream and rare breeds: Figure 5 shows frequencies and percentage of flocks with $R_0 < 1$ ($P_{R_0 < 1}$) under the various selection strategies in the worst case scenario where when initial frequencies were $f_{ARR} = 0.30$ and $f_{VRQ} = 0.15$. With the default benchmark R_0 ($C_{R_0^b}=2.5$), $P_{R_0 < 1}$ was still less than 80% under strategy S1 when initial $f_{ARR} = 0.30$. Although S1 was successful in decreasing f_{VRQ} , the frequency of other susceptible genotypes (e.g., ARQ/ARQ) was still quite high in the population. This was also the case when selection was targeted to both males and females (not shown). However, the 5% threshold value corresponding to the cut-off value for the highest 5% of the distribution decreased from $R_0 = 7$ at $t = 0$ to $R_0 = 4$ at $t = 15$. This indicates that the epidemic is expected to be minor if an outbreak occurs. Strategies S2 and S3 were able to achieve $R_0 < 1$ in all flocks even when selection was targeted only to males (Figure 5). This occurred at years 10 (S2) and 5 (S3), when the frequency of the ARR allele reached values between 0.60 and 0.70.

Figure 5. Frequency of the ARR allele (f_{ARR} ; —x—) under no PrP selection and under different selection strategies targeted to all sires and average percentage of flocks with $R_0 < 1$ ($P_{R_0 < 1}$) when the initial frequencies were $f_{ARR} = 0.30$ and $f_{VRQ} = 0.15$. $P_{R_0 < 1}$ is given for different benchmark values of R_0 (1: —, 2.5: 20: - - - -).



Summary of the effect of PrP selection on rates of inbreeding and genetic gain in performance and on risk of scrapie outbreaks in mainstream breeds

- Selection against VRQ
 - has a minimal impact on genetic gain in performance and inbreeding
 - is not sufficient to eliminate the risk of outbreaks. The risk still exist after 15 years of selection but epidemics would be minor if an outbreak occurs
- More extreme strategies in favour of ARR or against ARQ
 - lead to reductions in rates of genetic gain, mostly in the first five years of selection. These reductions are only large if the initial f_{ARR} is relatively low
 - lead to reductions in rates of inbreeding
 - reduce the risk of outbreaks substantially. The risk is small when $f_{ARR} > 0.7$

Summary of the effect of PrP selection on rates of inbreeding and on risk of scrapie outbreaks in rare breeds

- Selection against VRQ
 - lead to small increase in rate of inbreeding if the initial f_{VRQ} is high (≥ 0.30)
 - is not sufficient to eliminate the risk of outbreaks
- Selection in favour of ARR
 - lead to large increase in rate of inbreeding if f_{ARR} is low (≤ 0.30)
 - reduce the risk of outbreaks substantially. The risk is small when f_{ARR} reaches 0.7 but takes longer to achieve this than in mainstream breeds (e.g., $f_{ARR} > 0.7$ may not be achievable within 15 years if initial f_{ARR} is low; e.g., 0.05)

4.8. Mating strategies for reducing susceptibility to scrapie

Given the high level of uncertainty surrounding scrapie, fixation of the ARR allele or elimination of the VRQ allele may not be advisable. For instance, atypical scrapie has been detected in animals with the ARR/ARR genotype, the most resistant genotype to classical scrapie. Also, the VRQ allele is rare in animals affected by atypical scrapie. Instead of selecting on PrP genotype, an alternative way of reducing susceptibility to scrapie in the population is to allow any genotype to be selected but to mate the selected animals in a way that offspring with susceptible genotypes is avoided. This strategy could allow PrP frequencies to remain at the current levels.

We have developed a method to optimise matings in such a way that the risk of producing susceptible genotypes is minimised. We constructed a 'risk matrix' measuring the mean level of susceptibility of the offspring of each of the 15 x 15 possible combinations of sire/dam genotype. This matrix was based on the values given by Baylis *et al.* (2004) for the risk of scrapie for different genotypes. A 'simulated annealing' algorithm was used to obtain the optimal solution. Results show that optimisation of matings leads to a reduction in susceptibility to scrapie while maintaining PrP frequencies when compared with random mating.

4.9. Determining optimal genotyping strategies

The computer simulation program described above was extended to investigate the effect of genotyping different proportions of animals. All sires were assumed to have known genotypes but the percentage of ewes being genotyped varied (10, 20, 50, 80 and 100%). The ewes to be genotyped were chosen based on BLUP estimated breeding values for performance or at random. Seven PrP selection strategies were simulated for terminal sires. Six of these strategies

did not allow the use of VRQ carriers and differ in i) whether or not selection in favour of the ARR allele was applied; ii) whether or not ewes with unknown genotypes were used; and iii) whether or not selection against the ARQ allele was applied. The seventh strategy implied sequential selection of sires using the following priority: ARR carriers, AHQ and ARH carriers, ARQ carriers, non-genotyped and VRQ carriers. Three initial PrP allele frequencies were simulated based on high, moderate and low frequencies for ARR and VRQ alleles. The genotyping and PrP selection strategies were compared with the scenario where selection was only for performance (no selection on PrP genotype). Comparisons were in terms of change in allele frequencies, genetic gain for the performance trait and inbreeding over 15 years of PrP selection.

The main results obtained were the following:

- In most PrP selection strategies, genotyping 80% (rather than 100%) of the ewes resulted in only a slightly slower rate of change of allele frequency.
- Genotyping 10 or 20% of the ewes led to very similar rates of change of allele frequency.
- The VRQ allele was lost under all strategies but this was achieved earlier (2 to 5 years, depending on the scenarios) when only genotyped ewes were used.
- The ARR allele was fixed under all strategies except when selection was only against the VRQ allele.
- The lowest genetic gain was obtained when 50% of the ewes were genotyped and the highest inbreeding was obtained when all ewes were genotyped.
- It would not be possible to apply the PrP selection strategies when only 20 or 10% of the ewes are genotyped and only genotyped ewes are used.

4.10. Predicting genotypes

Simulations were also run to estimate the proportion of genotypes that can be predicted with either 100 or 80% certainty when only a proportion of animals are genotyped. The population consists of 12,000 animals born in six generations (2,000 animals per generation created from 200 males and 200 females selected at random). Four scenarios varying in the proportion of animals genotyped (10, 20, 50 and 80%) were simulated. The proportion of predicted genotypes was estimated assuming different PrP allelic frequencies (ranging from 0.0 to 0.7 for different alleles). The genotyped animals were chosen at random using stratified sampling within sire and dam families. Genotypes were predicted using an 'iterative peeling' algorithm.

Results shown that, in general, only a small proportion of genotypes can be predicted (not more than 8%) with 100% certainty. The highest proportion of predicted genotypes (4.9 to 7.3%, depending on the PrP frequencies) was obtained when 50% of the animals had known genotypes and the smallest (0.2 to 0.3%) were when 10% of animals were genotyped. With less restricting conditions (80% certainty) the extra proportion of predicted genotypes is small (less than 1%). The PrP frequencies had a moderate effect on the proportion of the genotypes that can be predicted.

5. REVIEW OF OBJECTIVE 3: TO PROVIDE GUIDELINES TO THE SEMEN ARCHIVE MANAGEMENT BOARD ON OBJECTIVES AND PRACTICAL SAMPLING STRATEGIES IN THE ARCHIVE

Tasks under this objective included to 1) identify the risks posed to animal genetic resources by the NSP and TSE and the achievable objectives for the Semen Archive to address those risks; 2) determine practical strategies for creating the Archive; and 3) provide guidelines for accessing and releasing material from the Archive. All these tasks have been completed successfully.

Two reports were produced to provide advice to the Semen Archive Management Board (SAMB). The first report (Roughsedge *et al.*, 2004) addressed tasks 1 and 2 and the second report (Roughsedge *et al.*, 2007) addressed task 3.

5.1. Potential risks of NSP and achievable objectives for the Semen Archive

Risk 1 ('Novel TSE'): A new TSE, potentially more threatening than classical scrapie, could emerge for which the currently favoured PrP allele, ARR, is the most susceptible and a PrP allele that is currently disfavoured may be found to be resistant.

- *The Archive can assist in the management of future TSEs by holding stocks of semen that carry the alleles currently disfavoured.*

Risk 2 ('Lost attributes'): There could be a loss of desirable attributes due to conflicting associations of PrP genotype scrapie resistance and other important traits (e.g., performance, health or survival).

- *The Archive can assist in the restoration of lost attributes in the event of a policy reversal by holding stocks of semen that carry the alleles currently disfavoured.*

Risk 3 ('Genetic bottlenecks'): A severe reduction in the number of rams available to breed replacements could lead to genetic bottlenecks .

- *The Archive can assist in managing genetic bottlenecks arising from implementing the NSP by holding stocks of semen that carry the ARR allele. This is, however, not a primary Archive objective as those breeds most threatened by such a scenario have also had semen archived by the Rare Breeds Survival Trust.*

5.2. Practical sampling strategies for individual breeds to manage Risks 1 and 2: Risk 1 (novel TSE) and 2 (lost attributes) imply that the bank will be used to restore or increase the frequency of a removed PrP allele. Deterministic and stochastic computer simulations were performed to quantify the restorative potential (in terms of number of semen straws) of the Archive to achieve a future re-introduction of a particular allele being currently disfavoured in terminal, crossing and hill populations. For a fixed amount of semen stored in the bank, the restorative potential varied between breed types due to differences in mating ratios and levels of fertility and fecundity. It was shown that terminal breeds would require two and a half times as many semen straws as hill breeds to produce the same frequency of ewes homozygous for the desired allele in the population after a ten year re-introduction period. Requirements for crossing sire breeds were between those for terminal sire and hill breeds but closer to the former. Full details of this work are given in Roughsedge *et al.* (2006).

A piece of software, the 'Semen Calculator' (with a User Guide) was developed and provided to the SAMB to allow flexible and informed planning of objectives.

5.3. Optimal sampling strategies to manage Risk 3: The previous sub-objective (5.2) concentrated on the restorative potential of the Archive to re-introduce an allele removed by the NSP but did not consider the genetic relationships between contributing rams. This is an issue of great importance as these relationships will in large part determine the genetic variability in future populations. In order to address this issue a method to minimize genetic relationships (specifically, group coancestry) should be adopted when selecting the donor rams and deciding the contribution that they each make to the bank. Using the optimisation technique 'simulated annealing', an algorithm was derived to obtain the optimal contributions of candidate rams to achieve target allelic frequencies in the Archive while maintaining at the same time genetic variability (global or within PrP alleles) in other loci unlinked to the PrP locus. A detailed description of the algorithm is given in Fernandez *et al.* (2006).

5.4. Access and release of material from the Archive: A full set of draft guidelines were provided to the SAMB given a clear outline approach to the release of semen from the Archive. They consider the circumstances that would trigger consideration of a release (i.e., research on TSE and trait associations and re-establishment of particular alleles) and outline the approach to be taken to such a consideration. Full details are given in the report (Roughsedge *et al.*, 2007).

Summary of strategies for creating and using the Semen Archive

- The Archive can assist in managing the risk of future TSE, loss of desirable attributes and genetic bottlenecks.
- The archive stands as a long term resource for the mitigation of the risks identified above and as such it is a valuable resource to be maintained.
- The restorative potential of the Archive depends on reproductive factors and population structure for specific breeds.
 - Terminal sire breeds are the most demanding in terms of numbers of straws while hill breeds are the least demanding.
- An algorithm for managing genetic variability in future populations has been developed.
 - Optimal contributions of candidate rams to the bank takes into account genetic relationships between them.
- Guidelines are available for sampling, access and release of material in the Archive are available.
- The 'Semen Calculator' can be used for relate straws to objectives in the Archive to allow informed sampling.

5. REVIEW OF OBJECTIVE 4: TO MAKE RECOMMENDATIONS BASED ON THE OUTPUTS OF OBJECTIVES 1 AND 2 AND DISSEMINATE THEM TO THE SHEEP INDUSTRY

Considerable effort has been devoted in the project to dissemination of the results. Information has been channelled firstly through the project steering group which has comprised government and industry representatives. Seven steering group meetings were hold along the four years of the project.

In addition, results and recommendations have been communicated through other channels:

- Eight articles have been published in peer reviewed journals.
- Eighteen papers have been presented at scientific conferences.
- Seven presentations have been given at NSA events.
- Two reports and a piece of software have been prepared for the SAMB to help them in their decisions when creating the Archive and releasing samples.
- Contributions have been made to the report of an Industry Focus Group on the future of the National Scrapie Plan Ram Genotyping Scheme.
- Eight newsletters have been sent to breeders participating in the project, Breed Societies involved in the project, and all Animal Health Officers involved in collecting blood samples for PrP Genotyping.

All publications generated by this project are included in Appendix 2.

6. STATE OF THE ART IN OBJECTIVES AND FUTURE WORK

Objective 1: *To collect and analyse the information necessary to critically evaluate the impact of widespread selection on PrP genotype in the UK sheep population*

This objective was successfully completed by collecting analysing large amount of data for representative breeds from each major sector of the UK sheep industry. Extensive analyses of experimental and commercial datasets did not reveal consistent significant associations of the

PrP genotype with a wide range of lamb performance traits. It can be concluded that selection aimed at decreasing the frequency of the most susceptible allele (the VRQ) and increasing the frequency of the most resistant one (the ARR) will have a negligible impact on performance at the sector level. However, analyses of experimental Scottish Blackface flocks presents evidence that sheep encoding the ARQ allele have higher post-natal survival rates than sheep encoding the more resistant ARR or AHQ alleles. There was some evidence that differences in mortality of different genotypes could be due to differences in susceptibility to infectious disease, but datasets were too small to test this hypothesis. Given the importance of lamb survival for the UK sheep industry, this is an important area for future development.

- Understand, using dense SNPs (Single Nucleotide Polymorphisms) along chromosome 13, the underlying causes of different mortality rates for different PrP genotypes.

This project has focused on PrP polymorphisms known to be associated with classical scrapie. However, emerging data on atypical scrapie is further complicating policy decisions dealing with TSE in sheep given that susceptibility to the disease for different PrP genotypes differs to that for classical scrapie. It is therefore critical to understand the current situation with this new form of scrapie.

- Establish the frequencies of variants at codons potentially associated with atypical scrapie for major UK breeds.

Objective 2: *To devise optimal breeding strategies for scrapie resistance in the context of an overall genetic improvement programme with restrictions on the loss of genetic variability*

This objective was successfully completed by running extensive computer simulations for mainstream and rare breeds. The consequences on loss of genetic variability (rate of inbreeding) and rate of genetic progress on other economically important traits were evaluated for different selection strategies based on PrP polymorphism at codons on which current eradication programmes are based (i.e., 136, 154 and 171). Modelling on the risk of disease outbreaks was also based on these polymorphisms and therefore determines the risk of classical scrapie. New information on atypical scrapie needs to be incorporated into the models to

- Assess the impact of current selection programmes aimed at eradicating classical scrapie on the prevalence of atypical scrapie
- Determine the breeding strategy that minimises the joint risks from classical and atypical scrapie.

Objective 3: *To provide guidelines to the Semen Archive Management Board on objectives and practical sampling strategies in the Archive*

This objective has been achieved and advice has been provided to underpin the formation of the Archive and to release material from it. However, currently there is no account of the genetic variability across the genome stored in the bank. A narrow base of genetic variability could have drastic consequences in the future if semen stored is used to re-introduce particular alleles in living populations. It is also unknown which PrP variants associated with atypical scrapie the Archive host. Given the important investment of Defra in establishing the Archive, it is essential that a comprehensive characterisation is undertaken to determine its future value.

- Characterise atypical scrapie resistant haplotypes in the Archive.
- Characterise genetic variability stored in the Archive.
- Evaluate the future utility of the Archive taking into account the genetic variability and PrP haplotype profile stored.

APPENDIX 1

Box 1. Calculation of the basic reproductive ratio

Assuming homogeneity of environment across flocks, the basic reproductive ratio for flock k in a particular year was estimated as:

$$R_{0(k)}^* = \frac{C}{N_k} \sum_{i=1}^{15} \sum_{j=1}^3 n_{ijk} S_{ij} \quad [1]$$

where S_{ij} is the relative susceptibility of an animal with genotype i and age j (0-1 years, 1-2 years or >2), n_{ijk} is the number of animals of genotype i and age j in flock k , N_k is the total number of animals in flock k and C is a scaling constant. The relative susceptibility of an animal with genotype i (x_i) was obtained from the French Langlade experimental flock of Romanov sheep (Touzeau *et al.*, 2006). As the ARH allele was not present in this breed, the relative susceptibilities for genotypes including this allele were extrapolated from the number of reported cases per annum per million sheep by genotype estimated by Baylis *et al.* (2004; J. Gen. Virol. 85: 2735-2740) for the UK population. The relative susceptibility (y_j) of an animal of age j (y_j) was obtained from a flock of Cheviot sheep (St Rose *et al.*, 2006). For animals of age 0-1 years ($j = 1$), 1-2 years ($j = 2$) or >2 years ($j = 3$), the relative susceptibilities were 0.61, 0.18 or 0.03, respectively. Finally, given x_i and y_j , the relative susceptibility of an animal with genotype i and age j (S_{ij}) was simply obtained as $S_{ij} = x_i y_j$. Three scaling constants were considered: $C_{R_0^b=1.0} = 18.6$, $C_{R_0^b=2.5} = 46.4$ and $C_{R_0^b=20.0} = 371.2$. These values were obtained from [1] assuming, respectively, benchmark values of $R_0^b = 1$, $R_0^b = 2.5$ and $R_0^b = 20$, and using the relative susceptibilities (S_{ij}) described above and N and n_{ij} obtained from the Langlade data. The benchmark values 2.5 and 20 were respectively the lower and upper limit for the basic reproductive ratio estimated by Hagenaars *et al.* (2003; Epidem. Infect. 131: 1015-1022) for the Langlade flock.

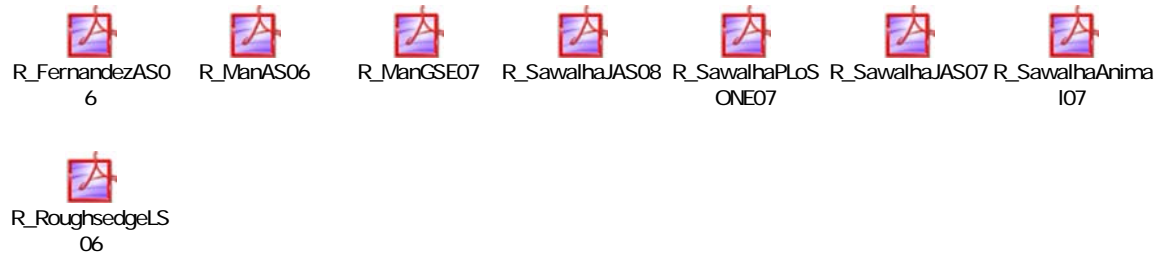
The environmental conditions for the transmission of scrapie are expected to vary for each flock (independent of the factors that were already parameterised in the model) and therefore, the basic reproductive ratio is expected to vary for each flock. The basic reproductive ratio for flock k that takes account of this variation for flock k was estimated as:

$$R_{0(k)} = \gamma_k R_{0(k)}^*$$

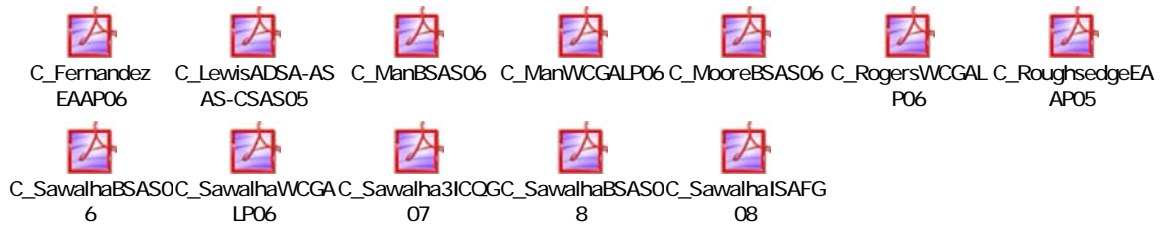
where γ_k is a random number taken from a gamma distribution with scale and shape parameter equal to one (and its mean and variance also equal one).

APPENDIX 2

Refereed Publications



Conference Proceedings



Technical Reports



Presentations/Posters



Newsletters

