Risk of scrapie in British sheep of different prion protein genotype

M. Baylis,1 C. Chihota,1 E. Stevenson,1 W. Goldmann,2 A. Smith,2 K. Sivam,3 S. Tongue3 and M. B. Gravenor1

Correspondence
M. Baylis
matthew.baylis@bbsrc.ac.uk

1Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berkshire RG20 7NN, UK
2Neuropathogenesis Unit, Institute for Animal Health, West Mains Road, Edinburgh, UK
3Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, UK

There is a well-established association between sheep prion protein (PrP) genotype and the risk of death from scrapie. Certain genotypes are clearly associated with susceptibility to the disease and others to resistance. However, there have been no attempts to quantify the disease risk for all 15 PrP genotypes. Here, datasets of the PrP genotypes of nearly 14,000 British sheep and of more than 1,500 confirmed scrapie cases were combined to yield an estimate of scrapie risk (reported cases per annum per million sheep of the genotype, or RCAM) for British sheep. The greatest scrapie risk by far, ranging from 225 to 545 RCAM, was for the VRQ-encoding genotypes ARQ/VRQ, ARH/VRQ and VRQ/VRQ. The next greatest risk (37 RCAM) was for the ARQ/ARQ genotype. The ARR/ARR genotype was the only numerically significant genotype for which no scrapie cases have been reported. The AHQ allele conferred resistance and the risk of scrapie in AHQ/VRQ sheep was very low (0.7 RCAM), although there was a higher and moderate risk for the AHQ homozygote (5 RCAM). The ARH allele appeared to confer susceptibility when encoded with VRQ, but possible resistance when encoded with other alleles. Scrapie risk varied with age: for VRQ/VRQ and ARH/VRQ the risk peaked at 2 years of age; that for ARQ/VRQ peaked at 3 years. There was some evidence that, following the lower risk at 4 and 5 years, a second rise occurred from about 6 years. Comparison with other published data indicated that the scrapie risk of certain PrP genotypes may differ between Great Britain and other countries.

INTRODUCTION

Scrapie is a transmissible neurodegenerative disease of sheep and goats characterized by changes in behaviour, trembling, ataxia, pruritis and fleece loss, proceeding to recumbency and death. The exact nature of the infectious agent is not known (Somerville, 2002), but it is widely believed that a major component is the host-encoded prion protein (Prusiner, 1982).

In sheep that have been exposed to the infectious agent of scrapie, the likelihood of progression to disease and the incubation period are very strongly linked to at least three polymorphisms in the PrP gene, which encodes the prion protein (Hunter, 1997). At codon 136, valine (V) is linked to scrapie susceptibility, while alanine (A) is linked to resistance (Hunter et al., 1994, 1996). At codon 154, arginine (R) is linked to susceptibility, while histidine (H) is linked to resistance (Laplanche et al., 1993). At codon 171, glutamine (Q) and histidine (H) are linked to susceptibility, while arginine (R) is linked to resistance (Hunter et al., 1997a; Ikeda et al., 1995; O’Rourke et al., 1996; Westaway et al., 1994). This apparent complexity is slightly misleading as, of the 12 possible combinations of these polymorphisms, only five appear to occur with any frequency (Belt et al., 1995; Ikeda et al., 1995; but see Kutzer et al., 2002). These five are A at codon 136, R at codon 154 and R at codon 171 (A136R154R171 or ARR for short) and, continuing this notation, ARQ, AHQ, ARH and VRQ. These five alleles combine to give a total of 15 PrP genotypes commonly found in sheep.

The 15 genotypes differ widely in their susceptibility to scrapie, ranging from complete resistance for the ARR/ARR genotype to extreme susceptibility for the VRQ/VRQ genotype. Previously, the genotypes have been assigned to different classes of susceptibility for the purpose of easing interpretation and facilitating the design of control programmes (Dawson et al., 1998; DEFRA, 2001). Generally, the criteria used for assigning a genotype to one class rather than another have not been given, and indeed different schemes may classify the same genotypes differently.
differently. Thus, in the UK, the AHQ/VRQ genotype is allocated to a class where ‘scrapie may be occasionally recorded’ (Dawson et al., 1998) but is defined as ‘highly susceptible to scrapie’ by the UK’s National Scrape Plan (DEFRA, 2001). There are several other possible examples.

To date, there have been no published attempts to quantify fully the link between genotype and the risk of scrapie. One reason may be that quantifying disease risk requires knowledge of the genotypes of the sheep that develop scrapie and those that do not. Patterns are apparent from the few studies that have genotyped entire flocks (Baylis et al., 2002; Elsen et al., 1999; O’Doherty et al., 2002) but the patterns tend to differ from one flock to another and the data are too few to draw national-level conclusions.

Here we have combined two large datasets in order to calculate the risk of scrapie for UK sheep of all 15 genotypes. The first dataset was the Scrapie Notifications Database, held by the Veterinary Laboratories Agency (VLA), which holds details, including genotype, of all confirmed scrapie cases in Great Britain. The second is a genotype database held by the Institute for Animal Health (IAH), which holds the genotypes of nearly 14 000 UK sheep. In essence, we used the IAH data to estimate the number of sheep of each genotype present in Great Britain and the VLA data to obtain the proportion of each genotype that was confirmed with scrapie.

METHODS

Scrapie case data. The scrapie case dataset was derived from a subset of the Scrapie Notifications Database and comprised anonymous information on 1821 submissions of suspect scrapie cases to the VLA for which genotypes had been obtained. Sources were England, Scotland and Wales but not Northern Ireland. Dates of death ranged from August 1998 to April 2002 inclusive (45 months). For each animal, data included a unique identifier, a code for the local Animal Health Office, the breed, sex, date of birth, date of death, PrP genotype and whether scrapie was confirmed. In total, there were 1543 confirmed cases. Of these, five lacked breed data and 19 lacked a date of birth. In many cases, the accuracy of the date of birth, particularly to the level of day, must be questionable.

Genotype data. Since 1998, the IAH has been undertaking a farm-based study of scrapie in the UK. About 60 study farms are spread from Cornwall to the Shetland Islands; these comprise ‘case flocks’, with at least one case of scrapie statutorily confirmed by the VLA since 1997, and ‘control flocks’ matched by breed and size. Entire breeding flocks are blood sampled at the time of entry into the study, and new female lambs (and replacement rams) are sampled over the subsequent 2 years. All genotypes were obtained using the methods described by Baylis et al. (2000, 2002).

Here, we used 13 896 genotypes received by June 2002. These were obtained from 44 flocks, of which 26 had had at least one case of scrapie. In total, 28 breeds and 34 crossbreeds were represented.

As yet, there are few published datasets against which the IAH dataset can be compared. However, Arnold et al. (2002) recently published the allele frequencies for a number of different sheep breeds in the UK, obtained from institutes undertaking commercial genotyping, usually of rams. The means of these allele frequencies vary very favourably with those from the IAH dataset: ARR, 46·0 %, 43·9 %; AHQ, 9·6 %, 9·3 %; ARQ, 33·6 %, 33·0 %; ARH, 5·3 %, 3·8 %; VRQ, 5·4 %, 10·0 % (commercial versus IAH data, respectively). The greatest differential, which is for the VRQ allele, may result from under-representation of susceptible sheep in the commercial data.

Scrapie risk. Our estimate of scrapie risk was sensitive to the number of sheep in the UK. We used an estimate of 20 million sheep in England, Scotland and Wales; from the June 1997 census, these comprised 90 % breeding stock and 10 % ‘other sheep 1 year old and over’ (SEAC, 1998). We ignored lambs under 1 year old (which number another 20 million) as the majority do not live long enough to develop scrapie. From the IAH genotype data, we estimated the number of sheep of each genotype present in Great Britain simply by multiplying the proportion of each genotype in the IAH dataset by 20 million.

The VLA data present the number of confirmed scrapie cases, of known genotype, over a 45 month period. While there were data for 1543 genotyped confirmed cases, DEFRA statistics indicated that there was a total of 1879 reported cases (including animals for which genotypes were not obtained) between August 1998 and April 2002 (http://www.defra.gov.uk/animals/bse/bse-science/scrapie/scrapie_age.PDF). We therefore corrected for the failure to genotype all cases by multiplying the genotyped cases by 1·22 (1879/1543). For each of the 15 genotypes, we then obtained the mean number of reported cases per annum and then the mean number of reported cases per annum per million sheep of the genotype, as estimated from the IAH data. The mean reported cases per annum per million sheep (RCAM) was used here as the measure of scrapie risk.

Since the proportion of each genotype in the national flock was estimated from the large sample in the field study, we based our confidence intervals (CI) on the case data only. A 95 % CI for the proportion of cases occurring for each genotype was obtained (Agresti & Coull, 1998), giving upper and lower limits for the numbers of cases of each genotype per year consistent with the observed numbers obtained out of the total sample of 1543. This method reproduced the greater uncertainty in risk for rare genotypes compared with common ones.

To compare statistically the scrapie risk for different genotypes, we considered the data in a case–control context (i.e. a comparison of the cases of each genotype out of a total at risk for each genotype over the relevant period) and expressed the comparative risk as odds ratios.

Estimates of scrapie risk were also obtained for sheep that died at the age of 1 year, 2 years, etc. up to 8 years old (only 5 of the 1543 cases died of scrapie aged 9 years or older). The total number of sheep in Great Britain of each age was estimated from the age distribution of sheep in the IAH database, with an assumed total national flock of 20 million breeding sheep.

RESULTS

The results are presented in Table 1. The high degree of susceptibility associated with the VRQ allele is immediately apparent. The greatest scrapie risk by far was for the ARQ/VRQ, ARH/VRQ and VRQ/VRQ genotypes, with 225–545 RCAM. In addition, the resistance conferred by the ARR allele is also clear. The ARR/ARR genotype was the only numerically significant genotype (~21 % of UK sheep) for which no scrapie cases have been reported and the scrapie risk for the ARR/VRQ genotype (6 RCAM) was only a fraction of that of the three VRQ-encoding genotypes listed above.
After these three VRQ-encoding genotypes, the next most susceptible genotype (averaging across all sheep breeds in the UK) appeared to be ARQ/ARQ, with a scrapie risk of 37 RCAM. Numerically, this genotype is one of the commonest in the UK and, overall, the actual number of reported scrapie cases that are ARQ/ARQ is very similar to the number that are VRQ/VRQ. The susceptibility associated with the ARQ allele was also apparent from the high attack rate of the ARQ/VRQ genotype and, given the relative frequency of ARQ/VRQ in the UK, more than 50% of reported scrapie cases in the UK are of this genotype. However, this susceptibility was reduced by ARR: the ARR/ARQ genotype is the most common sheep genotype in the UK and yet there have been very few reported scrapie cases, resulting in an estimated scrapie risk of less than 0.5 RCAM.

The effects of the AHQ and ARH alleles are subtle. The AHQ allele appeared to confer resistance and this was most apparent from the remarkably low scrapie risk of the AHQ/VRQ genotype (upper 95% confidence limit of scrapie risk was 3.7 RCAM). Although the frequency of this allele in the UK is relatively low and, accordingly, there may be considerable effects of sampling error in the calculations, it may be significant that, for this allele only, the scrapie risk of the homozygote (AHQ/AHQ) was greater than that of the VRQ heterozygote (AHQ/VRQ) (odds ratio 7.8, 95% CI 0.95–65, P = 0.06).

In combination with VRQ, the ARH allele clearly conferred susceptibility and, indeed, the ARH/VRQ scrapie risk was the second greatest. However, in other genotypes it appeared to confer resistance. Thus, the scrapie risk of ARQ/ARH was less than that of ARQ/ARQ (odds ratio 1.7, 95% CI 0.68–4.41, P = 0.3) and that of AHQ/ARH was less than that of AHQ/AHQ, although in neither case were the differences statistically significant.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases per year (n)</th>
<th>Percentage of sheep</th>
<th>Cases per year per million (n)</th>
<th>95% CI (lower–upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARR/ARR</td>
<td>0</td>
<td>21.3</td>
<td>0</td>
<td>0.0–0.3</td>
</tr>
<tr>
<td>ARR/AHQ</td>
<td>0.3</td>
<td>5.6</td>
<td>0.3</td>
<td>0.0–1.6</td>
</tr>
<tr>
<td>ARR/ARQ</td>
<td>2.0</td>
<td>28.0</td>
<td>0.4</td>
<td>0.2–0.8</td>
</tr>
<tr>
<td>ARR/ARH</td>
<td>0</td>
<td>2.1</td>
<td>0</td>
<td>0.0–2.9</td>
</tr>
<tr>
<td>AHQ/AHQ</td>
<td>2.0</td>
<td>1.9</td>
<td>5.0</td>
<td>2.3–10.9</td>
</tr>
<tr>
<td>ARQ/AHQ</td>
<td>11.0</td>
<td>6.3</td>
<td>8.7</td>
<td>6.2–12.1</td>
</tr>
<tr>
<td>AHQ/ARH</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>0.0–20.6</td>
</tr>
<tr>
<td>ARH/ARH</td>
<td>0.7</td>
<td>1.6</td>
<td>2.0</td>
<td>0.5–7.3</td>
</tr>
<tr>
<td>ARQ/ARH</td>
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<td>1.6</td>
<td>5.2</td>
<td>2.2–12.1</td>
</tr>
<tr>
<td>ARQ/ARQ</td>
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<td>12.2</td>
<td>36.9</td>
<td>33.1–41.0</td>
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<td>ARR/VRQ</td>
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<td>9.6</td>
<td>6.3</td>
<td>4.5–8.6</td>
</tr>
<tr>
<td>AHQ/VRQ</td>
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<td>2.5</td>
<td>0.7</td>
<td>0.0–3.7</td>
</tr>
<tr>
<td>ARQ/VRQ</td>
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<td>5.9</td>
<td>225.4</td>
<td>214.7–236.0</td>
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<tr>
<td>ARH/VRQ</td>
<td>22.7</td>
<td>0.3</td>
<td>405.0</td>
<td>321.9–508.2</td>
</tr>
<tr>
<td>VRQ/VRQ</td>
<td>94.8</td>
<td>0.9</td>
<td>544.5</td>
<td>490.5–602.9</td>
</tr>
</tbody>
</table>

The frequency distribution of ages at death is shown in Fig. 1. Eighty per cent of cases died aged 2–4 years. By contrast, only 3% of cases died aged 7 or 8 years. Does this small number of cases in older animals reflect a lower rate of transmission or less susceptibility or are there simply very few of them around? Estimates of scrapie risk, by age and genotype, are shown in Fig. 2(a). For VRQ/VRQ, there was a marked peak in scrapie risk at 2 years of age. For ARH/VRQ, there was also a peak at 2 years, but it was less marked, with a relatively high risk at 3 years. For ARQ/VRQ, there was a small peak at 3 years. For the latter two genotypes, the risk dropped until age 5 or 6 years, whereupon it appeared to rise again. For ARQ/VRQ, the scrapie risk at 8 years actually exceeded that at any younger age. For the other genotypes, the patterns were harder to discern as the risk was low at all ages. When risk was presented on a logarithmic scale (Fig. 2b), it appeared that for ARQ/ARQ the risk of scrapie
was relatively constant with age, while for ARR/VRQ there was a marked rise with age. For both genotypes, there were signs of an early peak and a fall at age 5 years. For ARQ/AHQ, the risk of scrapie also appeared to increase with age.

**DISCUSSION**

The estimates of scrapie risk were derived from datasets that almost certainly harbour biases. The IAH genotype dataset was obtained from the relatively small number of farms that have taken part in the field study. The SND dataset is supposed to be equally representative of all scrapie-affected sheep farms in Great Britain (as it contains data on cases of a notifiable disease), but we have to consider that there may be different levels of reporting in different sectors of the industry. Furthermore, there are likely to be differences between the two datasets in terms of representation of different regions, farm types (hill, upland or lowland), flock types (pedigree, pure-bred or commercial) and sheep breeds. Finally, in our calculations we have assumed that the chance of successfully confirming disease in a ‘true’ scrapie case, or of obtaining a genotype from it, is independent of genotype, neither of which is proven. We envisage, then, that the estimates will be improved as larger, more accurate datasets become available.

One source of bias that merits immediate investigation is the over-representation of scrapie-affected flocks in the IAH genotype database compared with the national flock it is used to model and the suggested differences in the genotype profile of such flocks and their scrapie-free counterparts (Baylis et al., 2000). This suggestion arose from a pilot study of four flocks and indicated that a lower proportion of resistant sheep, or a higher proportion of susceptible sheep, may be risk factors for flocks acquiring scrapie. Once affected, however, scrapie-affected flocks lose susceptible animals to scrapie and it is likely that, over time, they will have fewer susceptible animals than unaffected counterparts. Thus, the sheep from scrapie-affected and scrapie-free flocks used in our study were of the following scrapie risk groups, respectively (ranged from the most resistant to the most susceptible; Dawson et al., 1998): R1, 19±8 %, 24±6 %; R2, 8±5 %, 5±4 %; R3, 37±2 %, 35±7 %; R4, 28 %, 26±3 %; R5, 6±5 %, 8±0 %. There was a good degree of similarity and the percentages of the most susceptible sheep differed by only 1–2 %. The greatest difference was in the percentage of resistant (ARR/ARR) sheep, which was nearly 5 %. However, as the risk associated with this genotype is near zero, our estimate of RCAM was not affected and the upper limit of the 95 % CI would change very little.

Nevertheless, the estimates presented here provide a clear first indication of the relative risk of scrapie in all 15 PrP genotypes at a national level. The risks (defined as reported cases per year per million sheep of each genotype) cannot be considered as absolute, given the significant under-reporting of scrapie in Great Britain. The true level of reporting is difficult to define and has been estimated to be 13 % (Hoinville et al., 1999) and 38 % (Sivam et al., 2004) in recent postal surveys. If correct, this would suggest that the true risk of scrapie in the VRQ/VRQ genotype lies in the range 1400–4000 cases per annum per million VRQ/VRQs. Furthermore, as these cases occur in the 1 % (2002 survey) to 2±7 % (1998 survey) of flocks that have scrapie, the risk of scrapie in the VRQ/VRQ genotype in an affected flock is between 140 000 and 160 000 cases per annum per million, or about 1 in 6 or 7 per annum. This seems a reasonable estimate, given the long incubation period and the low incidence of scrapie in many affected flocks.

The relative risks are broadly consistent with those obtained from detailed studies of some individual flocks. Baylis et al. (2002) described the epidemic of scrapie in a commercially farmed flock of 230 Texel sheep in the UK. At the time of
Scrapie risk in British sheep

blood sampling, the flock had only a single sheep of the VRQ/VRQ genotype, many others probably having died of scrapie, and it was not possible to draw conclusions about scrapie risk in that genotype. Considering other genotypes, the greatest risk was for ARH/VRQ followed by ARQ/VRQ; the same pattern is reported here. There appeared to be a lower risk of scrapie in the ARH/ARH and ARQ/ARH genotypes than ARQ/ARQ. Results presented here confirm this assertion.

The genotypes of scrapie cases were compared with those of their healthy flock mates for 11 flocks in Ireland (O’Doherty et al., 2002). Significant differences were observed between flocks, but the analyses confirmed the resistance associated with the ARR allele and susceptibility associated with the VRQ allele. Sheep of the VRQ/VRQ genotype were very rare (only 3 out of 2675 animals) and no scrapie cases were observed. There were only two (12 %) scrapie cases of the ARH/VRQ genotype, compared with 15 healthy flock mates. For ARQ/VRQ, the equivalent estimate of risk was three times greater. In this paper, by contrast, we found the risk for ARH/VRQ genotype, compared with 15 healthy flock mates. There were only two (12 %) scrapie cases of the ARH/VRQ genotype, compared with 15 healthy flock mates. For ARQ/VRQ, the equivalent estimate of risk was three times greater. In this paper, by contrast, we found the risk for ARH/VRQ to be 1·8 times that of ARQ/VRQ. The disparity may relate to sampling, error, differences in the frequency of ARH-encoding breeds in the two studies or the presence of different scrapie strains in Ireland and Great Britain. A particularly interesting feature of the Irish scrapie concerns the ARQ/ARH genotype, for which there were 21 cases and only 67 healthy flock mates (24 %), which indicated that its level of risk was second only to ARQ/VRQ (with VRQ/VRQ excluded) and twice that of ARQ/ARQ. In this paper, we found the risk to be medium (5±2 RCAM), less than for six other genotypes, and only one seventh that of ARQ/ARQ. Indeed, in the Irish data, ARQ/ARH comprised 15·7 % of all scrapie cases, compared with 0·3 % in the UK’s SND.

O’Doherty et al. (2002) did not specify the breeds of the flocks that they studied, but it is well established that there are significant differences among breeds in which genotypes are attacked by scrapie. The most frequently cited example concerns the Suffolk breed. Suffolk sheep appear to lack the VRQ allele and the ARQ/ARQ genotype is the most susceptible to scrapie (Hunter et al., 1997b; O’Rourke et al., 1996). Breed effects are not restricted to comparisons of breeds that do or do not encode VRQ, however. Both the ARQ/ARQ and ARR/VRQ genotypes appear to be resistant to scrapie in the NPU flock of Cheviot sheep (Hunter et al., 1996), in French Romanov sheep the VRQ/ARR genotype is resistant but the ARQ/ARQ genotype is susceptible (Elsen et al., 1999) and in Texel sheep in the UK both ARR/VRQ and ARQ/ARQ genotypes are susceptible (Baylis et al., 2002).

It is unknown whether breed effects are a consequence of genetic differences among breeds that modulate the susceptibility encoded by the PrP gene or an effect of different strains of scrapie tending to circulate within individual breeds. Several different strains of natural scrapie have been identified on the basis of their characteristics in mice (Bruce et al., 1994, 2002) and some strains appear to attack genotypes differently. NPU Cheviot sheep of the ARQ/ARQ genotype are considered to be resistant to scrapie following challenge with the scrapie strain SSBP/1 (Goldmann et al., 1994) and are resistant to the natural scrapie that circulates in the flock (Hunter et al., 1996). However, they have been shown to be susceptible to the CH1641 isolate of scrapie, isolated from a natural scrapie case in the same Cheviot flock in 1970 (Foster et al., 2001). In this instance, therefore, sheep of the same genotype and breed, and in the same flock, are susceptible to some strains of scrapie but resistant to others.

Recently, a novel strain of scrapie was detected in Norway that resulted in a different pattern of PrP^Sc deposition and Western blot glycoprotein profile from that usually observed in the country (Benestad et al., 2003). Natural scrapie in Norway has been reported previously to be strongly associated with the VRQ allele (Tranulis et al., 1999), while the new strain has so far affected only the AHQ/AHQ and ARQ/ARQ alleles. Of the five genotypes that encode the AHQ allele, these two appear to have the greatest risk of scrapie from the UK data presented here. In Norway, many of the flock mates of the cases encoded the VRQ allele and yet have not succumbed to scrapie. This highlights once more an important limitation of our risk analysis – it presents a ‘national average’ and is expected to break down within specific breeds or individual flocks.

An intriguing result of our analysis is the evidence that the risk of a sheep dying of scrapie appears to rise with age, or peaks, drops and then rises again. This result is sensitive to our estimate of the age structure of the UK national flock. However, the same result was obtained from the age structure estimated from the 1998 postal survey (McLean et al., 1999). There are many possible explanations for this pattern. A gene other than PrP may contribute to control of the incubation period of scrapie and exert its effect in older animals. There may be different incubation periods of scrapie for sheep infected by different routes. Alternatively, there may be a bimodal distribution in the age at which sheep are infected with scrapie, with peaks at birth and at later lambing times. Finally, different strains of scrapie may have different incubation periods in sheep of the same genotype. This is well established in experimental scrapie in sheep and mouse models.

The very strong association of scrapie with PrP genotype described here, and by many authors previously, substantiates the possibility of controlling the disease, at a national level, by selective breeding (Schreuder et al., 1997) and, indeed, genetic control programmes are now under way in several countries, including The Netherlands, France, the UK and the USA. The UK government’s control programme, termed the National Scrapie Plan or NSP, was launched in 2001 (DEFFRA, 2001). The long-term aim is to eliminate all scrapie by increasing the frequency of the ARR allele in the national sheep flock to a level where the disease cannot persist. In the shorter term, the aim is to reduce the amount of scrapie by increasing the use of ARR-encoding
ACKNOWLEDGEMENTS

We thank the many farmers who have participated in our scrapie field study. This work was funded in part by BBSRC grant BS309857 awarded to the Institute for Animal Health.

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