Genotype-level variation in lifetime breeding success, litter size and survival of sheep in scrapie-affected flocks

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SUMMARY

Five different sheep flocks with natural outbreaks of scrapie were examined to determine associations between individual performance (lifetime breeding success (LBS), litter size and survival) and scrapie infection or PrP genotype. Despite different breed composition and forces of infection, consistent patterns were found among the flocks. Regardless of the flock, scrapie infected sheep produced on average 34% fewer offspring than non-scrapie infected sheep. The effect of scrapie on LBS appears to be a function of lifespan as opposed to fecundity. Analysis of litter size revealed no overall or genotype differences among the 5 sheep flocks. Survival, however, depends on the individual's scrapie status (infected or not) and its PrP genotype. Susceptible genotypes appear to perform less well in LBS and life-expectancy even if they are never affected with clinical scrapie. One possible explanation for these results is the effect of pre-clinical scrapie. Additional evidence supporting this hypothesis is discussed.

INTRODUCTION

Scrapie is a transmissible spongiform encephalopathy (TSE), a category of fatal and incurable diseases that includes bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD), transmissible mink encephalopathy (TME), feline spongiform encephalopathy (FSE), Kuru and variant Creutzfeldt-Jakob disease (vCJD). Scrapie has been reported world-wide and affects many sheep producing regions (Dawson *et al.* 1998). It has been present in the sheep population of Britain since the mid-18th century (Parry, 1983; Stamp, 1962) and remains widespread throughout the country.

Despite recent detailed studies of scrapie outbreaks within individual sheep flocks (Elsen *et al,* 1999; Hunter *et al,* 1996; Hunter *et al.* 1997) and comparative epidemiological analysis on multiple sheep flocks (Redman *et al.* 2002), key determinants of epidemiological and transmission dynamics of sheep scrapie are still poorly understood. In recent years considerable progress has been made in establishing the genetics of susceptibility of scrapie (Dawson *et al.* 1998; Hunter *et al.* 1997). It is known that resistance or susceptibility is largely under genetic control (Hunter, 1997), however, the effects of PrP genotype on scrapie susceptibility can vary between flocks and breeds of sheep (Dawson *et al.* 1998) and can also depend on scrapie isolates (Goldmann *et al.* 1994). To date, there have been few detailed within-flock studies of the effects of variation in PrP genotype at the individual level during natural scrapie outbreaks. Many studies

have been performed to determine the genetic status and variability of PrP genotype of sheep breeds in different countries (e.g. Germany: Drogemuller *et al.* 2001; Italy: Vaccari *et al.* 2001 and Spain: Acin *et al.* 2004), and a few studies have examined the PrP genotype profile of individual flocks (e.g. Baylis *et al.* 2000). Previous research that has examined genotype-level associations within flocks have generally focused on the relationship with scrapie infection, including incubation time (Goldmann *et al*,, 1991, 1994) and age of onset of scrapie (Baylis *et al.* 2002; Bossers *et al.* 1996; Clouscard *et al.* 1995; Elsen *et al.* 1999). Despite the extensive amount of research that exists on scrapie infection no study has attempted to quantify the effect of scrapie on significant performance parameters such as lifetime breeding success, litter size or survival. A few analyses have examined PrP genotype-level associations with performance parameters (e.g. Brandsma *et al.* 2004: litter size and 135 days weight; Barillet *et al.* 2002: dairy production traits, Prokopova *et al.* 2002: lean growth rate; and de Vries *et al.* 2004: muscle mass, liveweight gain, wool quality and fat depth). Overall these studies have found no significant association between PrP genotype and the trait examined, although some association between the resistant ARR and depth of muscle mass was found in German black-headed mutton sheep (de Vries et al. 2004). However, these studies examined the traits in the absence of scrapie infection, with a view to determining the effect of breeding for resistance, rather than the population dynamic and population genetic implications of a natural scrapie outbreak within a flock.

As with all TSEs, scrapie has a long incubation period between infection and onset of typical clinical signs. Although there is no explicit evidence to date for effects of pre-clinical scrapie, it has been identified as a possible cause for unexplained mortality within flocks (McLean *et al.* 1999). Furthermore, the focus of research on outbreaks of scrapie in sheep flocks has been on scrapie cases, and no study has considered individuals that did not develop clinical signs. Genotype-related differences in the performance of sheep manifesting no signs of scrapie may indicate the presence of pre-clinical scrapie within the flock. Identification and quantification of this phenomenon may result in changes in the incidence of scrapie deaths and the overall impact of scrapie as a disease within sheep.

In this paper, we focus on differences in individual performance associated with scrapie infection or PrP genotype in five different sheep flocks with natural outbreaks of scrapie. An outbreak of scrapie should exert substantial selection pressures against those PrP alleles associated with susceptibility. We illustrate the force of this selection by quantifying the effect of scrapie on individual fitness, assessed through estimates of individual lifetime breeding success (LBS). Differences in LBS due to scrapie are expected within each flock. Such differences may be the result of differential longevity and/or differential fecundity. We examine each component across five different sheep flocks. Measures of individual lifetime breeding success, litter size and survival are used to quantify: (1) the impact of scrapie; and (2) differences between PrP genotypes in scrapie and non-scrapie infected sheep.

METHODS

Study Flocks

Data were generated from five outbreaks of natural scrapie (Table 1). Three of the outbreaks were in flocks maintained by the Institute for Animal Health Neuropathogenesis Unit (NPU), one in a flock maintained by the Scottish Agricultural College (SAC) and one in a flock maintained by the Institut National de la Researche Agronomique (INRA) (Table 1). All flocks were maintained for research purposes. The origins and histories of the flocks are described in greater detail elsewhere (Elsen *et al.* 1999; Hunter *et al.* 1996, 1997; Redman *et al.* 2002).

Field Data

The following data are available for almost all individual sheep in each flock: date of birth; pedigrees; date of death or removal from flock; cause of death or reason for removal. Scrapie was suspected based on clinical signs, including loss of condition and rubbing. Suspect scrapie cases were confirmed by histopathological detection of vacuolation of brain tissue. Only confirmed cases of scrapie were used in the analysis.

For three of the outbreaks, the SAC Suffolk, the NPU Cheviot II and the INRA Romanov; there was some information on PrP genotypes, established by

sequencing PCR products or using oligonucleotide probes, as previously described (Elsen *et al.* 1999; Hunter *et al.* 1996, 1997). Data from these three flocks were used to examine genotype variation in lifetime breeding success, litter size and survival. For the INRA Romanov flock, genotype data were available for all animals in the flock since the onset of scrapie in 1993 whereas the genotyped individuals in the SAC Suffolk and the NPU Cheviot II consisted of most scrapie cases and approximately 50% of the non-scrapie infected sheep in each flock. As such, the focus of the genotype variation analysis was on the INRA Romanov flock. However, where possible, corresponding data were presented for the NPU Cheviot II and SAC Suffolk flocks.

Statistical Analysis

Data within each database was standardised to suit the analysis that was to be performed. For all flocks experimental, non-breeding animals and all males were excluded from the analysis. In the INRA Romanov flock the breeding practices with males were different: replacement sires were not used for long and experimental animals were mostly males and culled according to protocol. Males were therefore removed from the other flocks to standardise the data. Statistical analysis was first performed on each flock to determine differences associated with scrapie status (scrapie infected vs non-scrapie infected). For flocks with genotype information (INRA Romanov, NPU Cheviot II and SAC Suffolk) individuals were categorised as either susceptible (genotypes that were affected by scrapie) or non-affected (genotypes that were not affected by scrapie or scrapie

infection was low or suspect). Susceptible and non-affected genotypes within each flock are listed in Table 2.

Variation in lifetime breeding success. Lifetime breeding success (LBS) was calculated as the total number of live offspring produced by each breeding female, with and without scrapie, in each flock. Data analysis included all cohorts involved in the outbreak (Table 1) with the exception of the INRA Romanov flock. Data collection in the INRA Romanov flock is ongoing therefore there are living females that have yet to produce all their offspring. As such, lifetime breeding data is not available for these animals. Therefore, the analysis in the INRA Romanov flock was restricted to cohorts born between 1986 (first cohort involved in the outbreak) and 1993, excluding those which died prior to the 1993-1999 outbreak.

Mean (±SE) lifetime breeding success was estimated for each flock. Differences in the LBS of scrapie infected and non-scrapie infected sheep within each flock were analysed using a Student t-test. To determine if there were any differences in the effect of scrapie on LBS across the five flocks a comparison was performed using a Generalised Linear Model with negative binomial errors (S-Plus Version 6.0) and the significance of the flock*status interaction was assessed from the change in deviance on dropping that term from the model, distributed as $\chi^2_{(4)}$. For flocks with genotype information, two analyses were performed to examine differences in LBS; the first examined differences between scrapie infected and non-scrapie infected individuals within and across susceptible genotypes and the

second examined the LBS of non-scrapie infected individuals, looking for differences between susceptible and non-affected genotypes. Both analyses were performed using a General Linear Model (GLM) (SAS Version 8.2).

Variation in litter size . The size of all litters produced throughout the scrapie outbreak was calculated for scrapie and non-scrapie dams in each flock. Data from all flocks were standardised to include all litters born within the years of the scrapie outbreak (Table 1).

Differences between scrapie and non-scrapie individuals and between PrP genotypes in the number of live lambs per litter ("litter size") produced by dams at each breeding event were tested. Linear mixed effects models with dam identity fitted as a random effect were used to account for the repeated measures made on individual sheep over multiple breeding attempts. PrP genotype or scrapie status was used as a fixed effect. Models were fitted with Poisson errors using the procedure glmmPQL (S-Plus Version 6). For all flocks, we initially tested for effects of breeding year (as a multilevel factor) and dam age (as a quadratic function). These variables, if significant, ($P<0.05$) were retained in the models, as follows: NPU Cheviot II, dam age; SAC Suffolks, breeding year; INRA Romanovs, NPU Cheviot I and NPU Suffolks, dam age and breeding year. Analyses of associations between litter size and PrP genotype were restricted to the INRA Romanov and SAC Suffolk flocks due to insufficient genotype data in the other flocks.

Variation in survival . Survival analyses were performed on the female population considering the age at removal from flock as the survival measurement. Removal includes animals that died naturally as well as those culled for non-experimental reasons. Data analysed included only cohorts which were exposed to scrapie (Table 1). All survival analyses were performed using Proc lifetest and Proc Phreg (SAS Version 8.2). Median life expectancies (± 95% confidence intervals) were calculated using survival data censored for sheep culled at less than 1 year of age and those still alive. Data were stratified by genotype (VRQ/VRQ, ARQ/VRQ, ARQ/ARQ, Non-affected) and scrapie status (scrapie infected, non-scrapie infected). The following null hypotheses were tested in Proc Lifetest: (1) there are no differences in the overall mean life expectancy of scrapie infected versus non-scrapie infected individuals within each of the 5 flocks; and (2) there are no differences in the mean life expectancy of non-scrapie infected individuals among the susceptible and non-affected genotypes in the NPU Cheviot II, SAC Suffolk and INRA Romanov flocks. Differences between survivorship curves were tested using Kaplan-Meier estimator and the log-rank test. Significance was set at $p \leq 0.05$, and where multiple comparisons were performed the Bonferroni correction was applied.

In addition to the Kaplan-Meier procedure, Cox proportional hazard models were run using Proc Phreg (SAS Version 8.2) to determine the significance of any variables other than genotype in the survivorship of non-scrapie infected individuals. Selection of variables was made by looking for significant changes in

the log likelihood (χ^2) after using a hierarchical method of variable selection (Collett, 2003). The following variables were tested for significance and model improvement: year of birth, mode of feeding (maternal vs. artificial), and breeding status (breeder, non-breeder). Genotype was added into the model last after other significant variables were adjusted for. Significance was set at $p \leq 0.05$. Goodness of fit of all models was examined by looking at the residuals.

Variation in cause of removal. Managers of the INRA Romanov flock kept records on the reason for removal from the flock in addition to the date of removal. The data can be grouped into the following three categories: Poor Health (e.g., mastitis, arthritis, septicaemia, lungs, diarrhoea, toxaemia), Accidental (e.g., drowning, fracture, wound) and Management (e.g., culled for meat, sold, age-related culling). Such data may provide information to indicate whether or not there are any removals that may be attributed to pre-clinical scrapie. We hypothesise that effects of pre-clinical scrapie would result in sheep with the susceptible genotypes being removed significantly more for health-related causes than sheep with non-affected genotypes. To test this hypothesis we examined the causes of removal in the three most susceptible genotypes (ARQ/VRQ, VRQ/VRQ and ARQ/ARQ) as well as the non-affected genotypes. Comparisons of the number of removals of susceptible and non-affected genotypes within each removal category were made using a χ^2 test or Fishers Exact test (if $n<5$). Analysis of frequency data was carried out in StatXact (Version 5.0). Statistical significance was set at $p \leq 0.05$.

RESULTS

Variation in lifetime breeding success

Association with scrapie status. For both scrapie and non-scrapie infected sheep the LBS was highest in the INRA Romanov sheep and lowest in the NPU Cheviot I sheep (Table 3). For all flocks the lifetime breeding success of females that developed scrapie was significantly lower than non-scrapie infected sheep ($p \leq 0.001$), with the exception of the NPU Cheviot II flock (n=10, Table 3). However, the power to detect differences in LBS within the NPU Cheviot II flock was low .

Despite differences between flocks in the average number of offspring, the percentage difference in the LBS between scrapie and non-scrapie infected ewes was similar across all 5 flocks, with the scrapie ewes producing on average 34% fewer offspring (Table 3). Combining the data from all five flocks, no significant interaction between flock and scrapie status was found ($p=0.637$), implying no difference between flocks in the reduction in breeding success due to scrapie.

Association with PrP genotype. For the INRA Romanov flock, we compared LBS in scrapie and non-scrapie infected sheep within each of the three susceptible genotypes (ARQ/ARQ, ARQ/VRQ and VRQ/VRQ) (Fig. 1). The INRA Romanov flock had genotype information on all the scrapie infected sheep (n=202) and the

majority (67%; n=330/491) of non-scrapie infected sheep. Amongst susceptible genotypes a GLM revealed significant effects of both status (scrapie infected versus non-scrapie infected: $F_{1,360}$=50.36, p<0.001) and of genotype (ARQ/ARQ, ARQ/VRQ and VRQ/VRQ; $F_{2,360}$, p=0.004) on LBS. There was no interaction between the two factors (p=0.709), indicating that the proportionate reduction in LBS due to scrapie did not differ between genotypes. As observed across the entire flock, the LBS of scrapie infected sheep was significantly less than non-scrapie infected sheep. Regardless of status, multiple comparison tests (with Bonferroni correction) revealed that the LBS of VRQ/VRQ was significantly lower than both ARQ/ARQ (p=0.003) and ARQ/VRQ (p=0.022) but there was no significant difference between ARQ/ARQ and ARQ/VRQ (p=0.815).

Considering only non-scrapie sheep, there were differences between susceptible and non-affected genotypes. A GLM analysis revealed significant genotype effects ($F_{3,325}$=3.70, p=0.012). Multiple comparison (with Bonferroni correction) revealed that the LBS of non-scrapie infected VRQ/VRQ sheep was significantly less than the non-affected genotypes (p=0.022) and only marginally not significantly different from the ARQ/ARQ non-scrapie infected sheep (p=0.071). No other comparison was significant or approaching significance (p>0.10).

The NPU Cheviot II flock had genotype information on all scrapie infected sheep (n=10), however, very few (18%; n=41/225) non-scrapie infected sheep were genotyped. Despite the small sample size a GLM analysis of status (scrapie

infected versus non-scrapie infected) and genotype (ARQ/VRQ versus VRQ/VRQ) was performed amongst susceptible genotypes. There was no interaction between the two factors ($p=0.696$) and no significant status ($p=0.083$) or genotype differences ($p=0.057$) although genotype tended towards significance, with the LBS of VRQ/VRQ sheep less than that of sheep with the ARQ/VRQ genotype. Considering only non-scrapie infected sheep, comparison of the LBS of the three susceptible and non-affected genotypes revealed significant genotype effects ($p=0.002$). Multiple comparisons (with Bonferroni correction) revealed that the LBS of non-scrapie infected VRQ/VRQ sheep was significantly less than the non-affected genotypes ($p=0.0036$).

Within the SAC Suffolk flock there was only one susceptible genotype (ARQ/ARQ). As with the NPU Cheviot II flock, genotyping information was limited. All scrapie infected sheep were genotyped, however, only 39% ($n=211/537$) of the non-scrapie infected sheep were genotyped. Despite the small sample size, a one-way ANOVA on differences in the LBS of scrapie infected versus non-scrapie infected amongst ARQ/ARQ genotypes revealed no significant difference between scrapie infected and non-scrapie infected sheep within the susceptible genotype ARQ/ARQ ($p=0.563$). Considering only non-scrapie infected sheep, there were significant differences between susceptible (ARQ/ARQ) and non-affected genotypes ($p<0.001$) where ARQ/ARQ sheep had a significantly lower LBS than the non-affected sheep.

Variation in Litter Size

Association with scrapie status. The largest litter sizes were observed in the INRA Romanov flock and the smallest in the NPU Cheviot I flock (Table 4). There were no significant differences between the size of litters from scrapie infected and non-scrapie infected dams in each flock (Table 4).

Association with PrP genotype. Amongst susceptible genotypes in the INRA Romanov and SAC Suffolk flocks, there were no differences in litter size between sheep that developed scrapie and those that did not (INRA: $F_{1,302}$=0.973, p=0.325; SAC: $F_{1,76}$=1.584, p=0.212). Considering only sheep that never developed scrapie, there were also no significant differences between non-affected and susceptible genotypes (INRA: $F_{1,693}$=0.90, p=0.346; SAC: $F_{1,76}$=1.584, p=0.212).

Variation in Survival

Association with scrapie status. For all 5 flocks there was a significant reduction in the survival time (age at removal) of scrapie infected individuals relative to non-scrapie infected individuals (Table 5). The INRA Romanov had the largest difference between median survival of scrapie infected and non-scrapie infected sheep (4.3 years) whereas the NPU Cheviot I had the lowest (1.4 years).

Association with PrP genotype (non-scrapie infected sheep only). For the INRA Romanov flock both the Kaplan Meier (χ^2=39.23, df=3, p<0.001; Figure 2A) and Cox proportional hazards genotype-only model revealed significant differences among the 4 genotype groups ((VRQ/VRQ = ARQ/VRQ) < (ARQ/ARQ = non-affected)) in the age at removal of non-scrapie infected sheep. As such, the following groups of genotypes were formed: highly susceptible (VRQ/VRQ + ARQ/VRQ) and other (ARQ/ARQ + non-affected). This was done to increase the power of the analysis as the sample size of VRQ/VRQ non-scrapie infected individuals was very low. Diagnostic checks on the Cox proportional hazards model with covariates revealed a violation of the assumption of proportional hazards. This appeared to be the result of increased risk of early death for the highly susceptible VRQ/VRQ and ARQ/VRQ individuals after 2 years. As such, a piecewise Cox model was applied, comparing age at removal for the different genotype groups (highly susceptible, other) before and after 2 years. The results show that there is a significant genotype effect even after adjustment for significant variables: year of birth, breeding status, and breeding status by genotype interaction (Table 6), however, only for individuals after 2 years. There was no difference in the risk of removal between the genotype groups prior to 2 years. Sheep with the highly susceptible genotypes, VRQ/VRQ and ARQ/VRQ, had a 14x higher risk of an early death.

For the NPU Cheviot II Flock both the Kaplan Meier (χ^2=23.7, df=2, p<0.001; Figure 2B) and Cox proportional hazards genotype-only model revealed

significant differences among the 3 genotype groups (VRQ/VRQ, ARQ/VRQ and non-affected) in the age at removal of nonscrapie infected sheep. The risk of early death for sheep with genotype VRQ/VRQ was 4.2x higher than for non-affected sheep ($p<0.001$). The risk of early death for sheep with genotype ARQ/VRQ was 2.7x higher than for non-affected sheep ($p=0.001$). The only other variable that was significant was year of birth. Addition of this variable did not change the significance of genotype in the model.

In the SAC Suffolk flock both the Kaplan Meier ($\chi^2=3.90$, df=1, $p=0.048$; Figure 2C) and Cox proportional hazards genotype-only model revealed significant differences among the 2 genotype groups (ARQ/ARQ and non-affected) in the age at removal of nonscrapie infected sheep. The risk of early death for sheep with genotype ARQ/ARQ was 1.5x higher than non-affected sheep but the significance was marginal ($p=0.049$). However, adjusting for significant variables (i) year of birth and (ii) breeding status revealed that differences between genotypes ARQ/ARQ and non-affected were significant ($p=0.010$).

Variation in cause of removal

For all 3 flocks examined there were genotype differences in the life expectancy of the sheep. Overall, sheep with highly susceptible genotypes did not live as long as sheep with non-affected and/or less susceptible genotypes. Examination of the distribution of age at death from scrapie (Figure 2A-C) revealed similarity

between the three flocks. The peak in scrapie deaths approximates the point at which 50% of the susceptible yet non-scrapie infected animals in the flock are being removed (Figure 2A-C). For example, in the INRA Romanov flock mean age of scrapie deaths is approximately 2 years of age, with all scrapie deaths occurring before age 4. In the survival graph for non-scrapie infected deaths all VRQ/VRQ and ARQ/VRQ die within 4 years, whereas the less susceptible ARQ/ARQ and non-affected genotypes have a maximum lifespan of 9 years (Figure 2A). A similar pattern can be observed for VRQ/VRQ and ARQ/VRQ sheep in the NPU Cheviot II flock and the ARQ/ARQ in the SAC Suffolk flock.

For the INRA Romanov flock the presence of the VRQ allele appears to be a significant factor in the age at removal of non-scrapie infected sheep in flocks affected by scrapie. The cause of this lower mean life expectancy in ARQ/VRQ and VRQ/VRQ sheep in the presence of scrapie suggests pre-clinical scrapie amongst the most susceptible genotypes. To explore this hypothesis further we examined the causes of death in non-scrapie infected sheep with the highly susceptible genotypes (VRQ/VRQ and ARQ/VRQ) versus other non-scrapie infected sheep with ARQ/ARQ and non-affected genotypes . A greater proportion of animals with the highly susceptible genotypes were removed for health-related reasons (χ^2=41.11, df=1, p<0.001), whereas animals with ARQ/ARQ and non-affected genotypes were more likely to be removed for management reasons (χ^2=38.56, df=1, p<0.001). There was no significant difference between the

genotype groups for the proportion of animals removed for accidental causes ($p>0.05$).

DISCUSSION

We have used detailed individual-level analyses of outbreaks of natural scrapie in five sheep flocks to quantify the effects of scrapie and of PrP genotype on individual fitness. Despite different breed composition and scrapie incidence, we found consistent patterns in lifetime breeding success, litter size and sheep survival among the flocks.

There were significant differences in lifetime breeding success (LBS) of scrapie and non-scrapie infected sheep within the 4 flocks where there was sufficient data to examine the comparison, with scrapie sheep producing on average 34% fewer offspring than non-scrapie infected sheep. However, despite differences in the average LBS measured in each flock, there was no evidence of any difference between flocks in proportionate reduction in LBS due to scrapie. There is therefore no indication of any variation between sheep breeds in loss of fitness due to scrapie infection. In addition to the overall effect of scrapie, there were also genotype differences in the LBS of scrapie and non-scrapie sheep which correlated with the susceptibility of the genotype (VRQ/VRQ < ARQ/VRQ < ARQ/ARQ < non-affected). This could only be examined in detail for the INRA

Romanov flock, but a similar pattern was apparent in the NPU Cheviot II and SAC Suffolk flocks.

The effect of scrapie on lifetime breeding success appears to be a function of lifespan as opposed to fecundity. Analysis of litter size revealed no overall or genotype differences among the 5 sheep flocks. However, significant differences in survival of sheep were identified in this study. In general, age at removal from the flock depends on individual status (i.e. scrapie infection) and PrP genotype. For the five flocks examined, the median age at which scrapie infected sheep were removed from the flock was significantly less than that for non-scrapie infected sheep. Reduced survival in scrapie sheep was expected based on previous research where lower life expectancies were observed for the most susceptible sheep in the flocks (Bossers *et al.* 1996; Clouscard *et al.* 1995; Elsen *et al.* 1999; Thorgeirsdottir *et al.* 2002). As such, differences in the survival of scrapie affected sheep was not analysed in detail in this study. The focus of the survival analysis in this study was on non-scrapie infected sheep. The results of the Survival analysis and Cox Proportional Hazard model indicated significant genotype differences in the pattern of survival among the non-scrapie infected individuals for the flocks examined. Even when adjustment is made for significant covariates, there was an increased risk of removal associated with susceptible genotypes. For the INRA Romanov flock this seemed to depend on genotype or genotype susceptibility. VRQ/VRQ and ARQ/VRQ genotype individuals had

significantly lower life-expectancies, whereas the life-expectancy of ARQ/ARQ genotyped sheep were not significantly different from non-affected sheep.

The distribution of removals from each flock approximates the age distribution of scrapie deaths. This distribution suggests that although scrapie was not diagnosed, these sheep were removed because of scrapie that was not detected or other health-related causes associated with scrapie incubating within the sheep. Reports from other field studies are inconsistent. McLean *et al.* (1999) reported having more sheep die of unknown causes on scrapie affected farms than scrapie-free farms. Baylis *et al.* (2002) also observed in scrapie-affected sheep flocks a number of sheep that were found dead of unknown causes (8% of entire flock) but there was not a significant association with scrapie risk. In a recent study, however, a high prevalence of scrapie (6%) was observed amongst sheep that were found-dead in Shetland where scrapie is very common (Humphry *et al.* 2004).

For the INRA Romanov flock a significantly higher proportion of ARQ/VRQ and VRQ/VRQ sheep died of poor health in comparison to ARQ/ARQ and the non-affected genotypes. One would have expected that if removals were the result of pre-clinical scrapie that sheep with the ARQ/ARQ genotype would also have a high proportion of removal as a result of health-related illness. It appears that there may be a deleterious effect of the presence of the VRQ allele in the presence of scrapie in the flock. Unfortunately there were no equivalent data from the other

flocks with which to test this idea. The results of this study suggest that, across different flocks of different sheep breeds, susceptible PrP genotypes appear to perform less well in overall fecundity and life-expectancy even if they do not contract scrapie. This effect is more apparent in the most susceptible genotype: VRQ/VRQ performed consistently worse in relation to lifetime breeding success and survival even amongst apparently uninfected individuals.

There are two possible explanations for these findings. The first is that susceptible genotypes are in relatively poorer condition and are removed at younger ages. Unfortunately lack of data makes this hypothesis difficult to examine, although research to date suggests that there are no PrP genotype-related performance traits (Roden *et al.* 2001; Barillet *et al.* 2002; Brandsma *et al.* 2004; DeVries *et al.* 2004). The second hypothesis is that they are suffering from effects of pre-clinical scrapie, which is manifesting itself in terms of reduced lifespan even though typical clinical signs of scrapie are yet to develop. If this hypothesis were true we might expect: (1) most deaths in years 2-4 when most scrapie cases occur; and (2) the cause of death for susceptible genotypes to be different (i.e. more health-related). Both expectations are confirmed by the results reported within this paper, although the results for the susceptible genotype ARQ/ARQ in the INRA Romanov flock are not as clear. Physiological evidence of pre-clinical scrapie does exist. Changes in behaviour that appear to consistently precede clinical signs have been observed (Parry, 1983). Studies have shown that there was reduced rumination in sheep with scrapie and cattle with BSE (Austin & Simmons, 1993).

This reduced rumination may provide an explanation for the observations of loss of weight or body condition that has been reported for scrapie (Clark & Moar, 1992), BSE (Wilesmith *et al.* 1992) and chronic wasting disease (Williams & Young, 1982).

Scrapie has become the target of control measures and eradication programs world wide. The identification of infected sheep is crucial for the success of these programs. After initial infection, the disease has a long incubation period during which time infected sheep may be able to transmit disease to non-infected sheep. Evidence of scrapie can now be detected in sheep before the clinical signs occur (e.g. Schreuder *et al.* 1998) but it is unknown whether or not sheep are affected during this 'pre-clinical' phase. This study has suggested the possibility that reduced lifespan in susceptible PrP genotypes may be the result of pre-clinical scrapie. If pre-clinical scrapie does exist amongst susceptible genotypes we may underestimate levels of scrapie-related mortality in sheep flocks. The results presented here highlight the need for further research on performance of different sheep PrP genotypes both in the presence and absence of scrapie.

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Figure Legends

Fig. 1. Differences in Lifetime Breeding Success (LBS) within scrapie susceptible genotypes (ARQ/ARQ, ARQ/VRQ, VRQ/VRQ) and non-affected genotypes in the INRA Romanov flock.

Fig. 2. Foreground: Survivorship. Age at removal for female non-scrapie infected sheep. A. INRA Romanov flock for susceptible (VRQ/VRQ, ARQ/VRQ, ARQ/ARQ) and non-affected genotypes; B. NPU Cheviot II flock for the 2 susceptible (VRQ/VRQ and ARQ/VRQ) and non-affected genotypes; C. SAC Suffolk flock for the susceptible (ARQ/ARQ) genotype and the non-affected genotypes. Background: Distribution of the age of scrapie deaths for females in A. the INRA Romanov flock; B. the NPU Cheviot II flock; and C. the SAC Suffolk flock. VRQ/VRQ: bold, black; VRQ/ARQ: bold, grey; ARQ/ARQ: normal, black; Non-affected: normal, grey.

Table 1. Demographic characteristics of the study flocks with outbreaks of natural scrapie. Outbreak, calendar years over which cases of natural scrapie were observed. Cohorts, birth cohorts involved in the outbreak of natural scrapie.

Flock	Organisation	Country	Breed	Research	Outbreak (years)	Cohorts (years)	Range of flock size per year *	No. sheep*	No. cases*
NPU Cheviot I	Institute of Animal Health, Neuropathogenesis Unit (NPU)	Scotland	Cheviot	Scrapie	1970-1982	1967-1978	273-751	1321	137
NPU Cheviot II	Institute of Animal Health, Neuropathogenesis Unit (NPU)	Scotland	Cheviot	Scrapie	1986-1994	1982-1994†	304-653	1604	33
NPU Suffolk	Institute of Animal Health, Neuropathogenesis Unit (NPU)	Scotland	Suffolk	Scrapie	1959-1982	1956-1980	43-597	1658	710
SAC Suffolk	Scottish Agricultural College (SAC)	Scotland	Suffolk	Meat	1990-1996	1988-1994	198-760	2489	108
INRA Romanov	Institut National de la Researche Agronomique (INRA)	France	Romanov	Fecundity Meat Scrapie‡	1993-1999	1986-1999†	390-792	5841	448

*, all values except INRA Romanov are from Redman *et al.* 2002. INRA Romanov data was calculated from INRA database.

†, data collection in these flock are ongoing. For the purpose of this research the database was closed in 1994 and 1999 for the NPU Cheviot II and INRA Romanov flocks respectively.

‡, research on scrapie began in 1993 after the first case of scrapie was observed in the flock.

Table 2. Susceptible and non-affected genotypes within the INRA Romanov, NPU Cheviot II and SAC Suffolk flocks. Susceptible genotypes are presented in order of deceasing susceptibility. Scrapie susceptibility, expressed as % of genotype affected, is shown in brackets.

NPU Chevoit II		**SAC Suffolk**		**INRA Romanov**	
Susceptible	Non-affected	Susceptible	Non-affected	Susceptible*	Non-affected†
VRQ/VRQ (56%)	AHQ/AHQ	ARQ/ARQ (58%)	ARQ/ARH	VRQ/VRQ (76%)	AHQ/AHQ
ARQ/VRQ (33%)	AHQ/VRQ		ARR/ARH	ARQ/VRQ (52%)	AHQ/VRQ
	ARQ/AHQ		ARR/ARQ	ARQ/ARQ (42%)	ARQ/AHQ
	ARQ/ARQ		ARR/ARR		ARR/AHQ
	ARR/AHQ				ARR/ARQ
	ARR/ARQ				ARR/ARR
	ARR/ARR				ARR/VRQ
	ARR/VRQ				

*, Data on scrapie susceptibility from Elsen *et al*. 1999.
†, Includes some suspect scrapie cases in all genotypes except ARR/ARR and ARR/AHQ (Elsen *et al.* 1999)

Table 3. Summary of the Lifetime Breeding Success (LBS) of scrapie and non-scrapie infected females in each flock, t statistic and corresponding p-value to test for differences between the two categories and the difference between scrapie and non-scrapie infected individuals within each flock. Cohorts used in the analysis are in brackets.

Flock	Status	n	Lifetime Breeding Success			t	p	% Difference
			Mean	SE	Range			
NPU Cheviot I (1967-1978)	Non-scrapie	208	2.9	0.1	1 - 8	5.14	<0.001	-27.0
	Scrapie	66	1.8	0.1	1 - 5			
NPU Cheviot II (1982-1994)	Non-scrapie	225	4.8	0.2	1 - 20	1.19	0.237	-34.0
	Scrapie	10	3.3	0.6	1 - 7			
NPU Suffolk (1956-1980)	Non-scrapie	191	3.2	0.2	1 - 10	5.43	<0.001	-40.0
	Scrapie	270	2.1	0.1	1 - 7			
SAC Suffolk (1988-1994)	Non-scrapie	537	4.8	0.1	1 - 27	4.17	<0.001	-31.0
	Scrapie	56	3.5	0.4	1 - 17			
INRA Romanov (1986-1993)	Non-scrapie	491	16.5	0.4	1 - 40	8.76	<0.001	-38.0
	Scrapie	202	10.3	0.6	1 - 37			

Table 4. Summary of the mean size of all litters born to scrapie and non-scrapie infected dams in each flock during the scrapie outbreak (years are in brackets). n_d is number of dams; n_l is number of litters. F statistic is from generalised linear mixed effects model with dam identity as random effect and Poisson errors (with corresponding degrees of freedom, df, and p-value).

Flock	Status	n_d (n_l)	Mean Litter Size		F (df)	p
			Mean	SE		
NPU Cheviot I (1970-1982)	Non-scrapie	248 (472)	1.34	0.02	0.060 (1,311)	0.807
	Scrapie	65 (90)	1.23	0.04		
NPU Cheviot II (1986-1994)	Non-scrapie	242 (566)	1.62	0.02	0.001 (1,233)	0.993
	Scrapie	10 (19)	1.58	0.12		
NPU Suffolk (1959-1982)	Non-scrapie	274 (870)	1.78	0.02	2.383 (1,535)	0.123
	Scrapie	292 (606)	1.65	0.02		
SAC Suffolk (1990-1996)	Non-scrapie	694 (1561)	1.76	0.02	0.455 (1,749)	0.500
	Scrapie	57 (107)	1.81	0.09		
INRA Romanov (1993-1999)	Non-scrapie	547 (917)	3.28	0.03	1.430 (1,661)	0.232
	Scrapie	114 (150)	3.43	0.08		

Table 5. Median survival times (± 95% CI) for scrapie and non-scrapie infected sheep in each flock. Cohorts used in the analysis are in brackets.

Flock	Status	Median	95% CI	χ^2	p
NPU Cheviot I (1970-1778)	Scrapie	2.24	2.14 – 2.49	129	<0.001
	Non-scrapie	3.80	3.53 – 4.46		
NPU Cheviot II (1986-1994)	Scrapie	2.41	2.17 – 3.32	31	<0.001
	Non-scrapie	6.07	5.74 – 6.57		
NPU Suffolk (1959-1980)	Scrapie	2.82	2.67 – 2.90	226	<0.001
	Non-scrapie	5.04	4.35 – 5.26		
SAC Suffolk (1990-1996)	Scrapie	2.63	2.07 – 2.60	164	<0.001
	Non-scrapie	4.64	4.19 – 4.89		
INRA Romanov (1993-1999)	Scrapie	1.76	1.75 – 1.82	484	<0.001
	Non-scrapie	6.02	5.35 – 6.30		

Table 6. Piecewise Cox Proportional Hazard model for mean life expectancy of non-scrapie infected sheep in the INRA Romanov flock. Risk ratio, exp(parameter estimate). 95% CI, exp(parameter estimate ± 1.96 (SE)). YOB, year of birth. NB, non-breeder. Other, ARQ/ARQ and non-affected genotypes. Highly susceptible, VRQ/VRQ and ARQ/VRQ genotypes. Baseline, genotype other, breeder, YOB 1993.

Model 2: Genotype + covariates						
Variable	Parameter	SE	Wald Chi square	p	Risk ratio	95% CI
Non-Breeder (NB)	1.566	0.252	38.57	<0.001	4.79	2.92 – 7.85
YOB 1994	0.022	0.203	0.01	0.913	1.02	0.687 – 1.52
YOB 1995	0.093	0.262	0.13	0.722	1.10	0.656 – 1.84
YOB 1996	0.302	0.227	1.77	0.183	1.35	0.867 – 2.11
YOB 1997	-0.249	0.293	0.73	0.394	0.78	0.439 – 1.38
YOB 1998	-1.197	0.376	10.2	0.001	0.30	0.145– 0.631
YOB 1999	-0.495	0.364	1.85	0.174	0.61	0.298 – 1.24
Non-Breeder, Other	-0.888	0.366	5.89	0.015	0.41	0.201 – 0.843
Highly susceptible < 2yrs	0.400	0.351	1.29	0.255	1.49	0.749 – 2.97
Highly susceptible > 2yrs	2.702	0.320	71.3	<0.001	14.91	7.96 – 27.9