Polygenic variation and transmission factors involved in the resistance/susceptibility to scrapie in a Romanov flock

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Data from 4049 Romanov sheep belonging to a flock affected by natural scrapie were analysed by using survival-analysis techniques. Failure time was defined as the period of time between first exposure to infection and the date that animals left the flock with scrapie signs. Four hundred and forty-seven sheep were identified as ‘scrapie animals’. Several models, including level of exposure as a time-dependent effect, PrP genotype, sex, age at first exposure, litter size and factors related to vertical transmission, were tested. The best model was extended to a sire–dam frailty model, in order to estimate the polygenic variation in addition to that in the Prnp gene. A combined effect of rearing type and the dam’s disease status was detected. Thus, only sheep with a low degree of exposure to infection as lambs (lambs reared artificially and born out of a healthy dam) showed less risk than others. Animals first exposed to infection at older ages seemed to be less susceptible to scrapie. In this Romanov population, new genotypes (AHQ/AHQ, AHQ/VRQ, ARR/VRQ and ARR/ARQ) were associated with risk, suggesting the effect of genotypes on the incubation period of animals. Polygenic variance was responsible for 21 % of the total genetic variability that was related to susceptibility to scrapie. Therefore, the genetic susceptibility to scrapie may be explained by the joint effect of point mutations at the Prnp major gene and a number of genes that modulate its effect.

INTRODUCTION

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are a group of fatal degenerative disorders of the central nervous system. They comprise a group of diseases that affect many species. TSEs are characterized by the accumulation of an abnormal isoform of the cellular prion protein, named PrPSc. Scrapie is a TSE affecting sheep and goats.

TSE in sheep, as in mice and humans, is controlled genetically (Hunter et al., 1989; Hunter, 1997; Prusiner & Scott, 1997; Elsen et al., 1999). Susceptibility to scrapie, which may be evaluated by mortality rate and incubation period, is mainly controlled by polymorphisms in the Prnp gene. Several point mutations located at codons 136 (T, A, V), 154 (R, H) and 171 (R, Q, H, K) of the gene have been associated with natural scrapie (e.g. Hunter, 1997). The ARR allele is associated with resistance and VRQ is associated with susceptibility (e.g. Hunter et al., 1996; Elsen et al., 1999). However, there are alleles, such as ARQ, ARH and AHQ, that behave differently depending on the breed (e.g. Hunter, 1997; Thorgeirsdottir et al., 1999). It has also been demonstrated that there is an interaction between the genotype of the host and the scrapie strain (Goldmann et al., 1994; O’Rourke et al., 1997; Barron et al., 2003). In the mouse, the incubation period of the disease has been associated with the PrP genotype (Moore et al., 1998) and with the prion strain inoculated (Bruce et al., 1991). However, polymorphism of the Prnp gene does not explain the total variation in incubation period that is observed, indicating the existence of other environmental and genetic factors that are involved in the disease. Several studies have provided evidence for other genes that influence the TSE incubation period in inbred lines of mice (Stephenson et al., 2000; Lloyd et al., 2001, 2002; Manolakou et al., 2001; Moreno et al., 2003b) and in sheep (Moreno et al., 2002, 2003a) with defined PrP genotypes.

In general, the incidence of infectious diseases is influenced strongly by environmental factors (Soller & Andersson, 1998). In natural scrapie, non-genetic factors associated with susceptibility have been described (Elsen et al., 1999;
Baylis et al., 2002). In order to establish the association between PrP genotype and susceptibility to scrapie, transmission factors have been accounted for in the context of survival analysis (Elsen et al., 1999; Baylis et al., 2002).

The main objective of this paper was to detect and quantify polygenic variation involved in the resistance or susceptibility to scrapie, in addition to that in the Prnp gene, in a naturally infected flock. Simultaneously, several transmission factors related to the natural infection in the flock were identified and tested.

**METHODS**

**Data.** Data were obtained from the Langlade INRA experimental farm, located near Toulouse, France, and first described by Elsen et al. (1999). The present study includes data from animals of the prolific Romanov breed (average lambs per lambing, 3±1). At lambing, the usual management practice was to put dams and offspring in isolated lambing compartments. Two lambs at most were left with their dams and the others were fed by artificial feeding 24 h after receiving the colostrum. In 1991, Romanov animals were involved in a study to determine genetic susceptibility to Teladorsagia circumcincta, a gastrointestinal parasite (Laplanche et al., 1996). The two experiments carried out with lambs born in October (artificial oral challenge and natural infection on artificially infected pasture) were detailed by Elsen et al. (1999) and Gruner et al. (2004). These trials were suspected to be the starting point of the scrapie outbreak. The first signs of scrapie appeared in this group of animals in April 1993. In 1996 and 1998, new sets of the parasite experiments were run especially to search for a cause–effect relationship between this gastrointestinal infection and the susceptibility to scrapie. However, to date, no clear evidence or explanation (parasite as a cofactor or as a vector of prions) has been obtained.

The distribution of PrP genotypes in the flock has changed across time through selection (Elsen et al., 1999). Since 1993, appropriate matings of PrP-genotyped animals have been carried out in order to maintain a naturally scrapie-infected flock. Except for lambs culled at 3 months of age or lambs involved in specific experiments, animals were kept until their natural death. The flock was genetically closed between 1979 and 1996. In 1997 and 2001, several Romanov animals were brought into the flock from another INRA experimental farm. PrP genotyping started at the beginning of the scrapie outbreak in 1993 and has been done systematically since 1994. Suspect animals (based on clinical signs) were sent to the Veterinary School of Toulouse, France, for diagnosis by histology and immunohistochemistry (Andrèoletti et al., 2002a). Diagnosis by histology and immunohistochemistry was also done systematically for all culled animals and those that died from natural causes since 1997.

The data consisted of records from 4049 Romanov animals that were living in the Langlade flock between 1 April 1993 and 4 March 2002. Among them, 447 died from scrapie. Animals were born between 1983 and 2002.

**Statistical methods.** Survival analysis is a suitable framework to analyse this type of data (Elsen et al., 1999). This type of analysis allows use of information for all animals, even if they were not affected by scrapie by the time that the analysis was performed (censored records). Survival analysis models the hazard for an animal to show signs of scrapie at time t, provided that it has not been affected until then. Therefore, it describes the rate at which animals show signs of scrapie over time. Failure time was considered as duration of exposure (DOEX). DOEX was defined as the length of the period between an animal’s first exposure to infection and the date that it left the flock with scrapie signs, the diagnosis being confirmed afterwards with histology or immunohistochemistry. Animals were identified as ‘scrapie animals’ when they showed clinical signs together with positive histology. Accordingly, 447 animals out of 4049 in the dataset became ‘scrapie animals’ and therefore had uncensored records. Animals still alive in March 2002 or animals that died from other causes were included as censored records. In order to calculate DOEX, infection was assumed to start in January 1991. That date was chosen because the first scrapie animals (animals involved in the parasite experiment) were born in 1991. Thus, for animals born in the Langlade farm before 1991, the first exposure was assumed to occur at that date. For animals born at Langlade afterwards, first exposure was assumed to occur at birth. Finally, for animals brought in from outside, first exposure was assumed to occur on the date that they came into the farm.

Cox proportional-hazard models were fitted to the analysis. These models may include fixed (transmission factors and the PrP genotype effect) and random (an additional genetic component, named the polygenic effect) effects. Computations were performed by using the software package Survival Kit v3.12 (Ducrocq & Solkner, 1998).

In order to estimate the polygenic variance or the amount of variability explained by an additional genetic component, we first performed a number of analyses to assess the effects of several transmission factors to be included in the final model. The PrP genotype effect was always included in the analyses. Thus, taking previous results into account (Elsen et al., 1999), various recorded factors with a possible influence on susceptibility were studied. For each model, its effects were tested by using likelihood-ratio tests (LRTs) sequentially and marginally. Afterwards, all possible models that included only significant effects, as determined in the first step, were compared by using Bayesian information criteria (BIC). Finally, the best model (corresponding to the model with the smallest BIC value) was extended to a sire–dam frailty model to estimate the polygenic effect. The different factors included in the models were as follows.

**Flock.** Flock (F), which represents the effect of the experimental groups, was divided into four groups: animals coming from outside Langlade (OF; n=33), animals from the main flock (MF; n=3820), animals coming from parasite experiments infected with T. circumcincta (PF; n=115) and animals in a special protocol where animals were left to older ages (SF; n=81).

**Age at first exposure (AFEX).** Sheep were classified into three age groups: 0–24 months (n=3807), >24–36 months (n=99) and >36 months (n=143). Accordingly, for animals born before 1991, AFEX corresponded to the age class that they belonged to in January 1991; for animals born afterwards, they were assigned to the first age class. Finally, for animals coming from outside, AFEX was the age class that they belonged to when they arrived at the farm.

**Sex (Sx).** The animals’ sex was registered, with 1785 males (M) and 2264 females (F).

**Level of exposure [LEX (p)].** This is a time-dependent class effect that describes how the hazard rate of a particular animal changes at time t, p being the duration of exposure for each animal until the beginning and the end of each lambing season. Thus, LEX describes the evolution of the level of exposure during infection and its effect on risk. To define changing points (p), years were divided into lambing and interlambing periods. Lambing periods were considered because recent studies (Andrèoletti et al., 2002b; Tuo et al., 2002) have shown that positive placentas are a major route of transmission of TSE. This division of time was made to acknowledge periods (lambings) where positive placentas (Andrèoletti et al., 2002b; Tuo
et al., 2002) may be a major operating route of infection versus other ways of transmission, which mostly occur between lambs. Therefore, under these models, animals were assumed to be exposed to doses of infection that changed at the beginning and at the end of each lambing season. As a consequence of these expected changes in level of exposure, a change in the hazard of animals was also expected from period to period.

Genotype of the animal (PrP). Ten PrP genotypes were considered. In total, 1310 individuals did not have a known genotype; however, they were kept in the dataset to obtain a better estimate of other risk factors in the models (Vitezica et al., 2005). To deal with non-genotyped individuals, we proceeded in two ways. Firstly, a class effect with 11 levels was defined. Ten levels corresponded to the genotypes found at Langlade and class 11 was the class of unknowns, to include all non-genotyped animals. Secondly, probabilities of genotypes were assigned to non-genotyped animals. Probabilities of genotypes refer to the probabilities for an animal of carrying each of the ten genotypes that were found in the flock. Probabilities were computed, depending on the family relationships of non-genotyped individuals with the genotyped animals. Probabilities were calculated for each non-genotyped animal and included in the model of analysis. Probabilities were computed by using an iterative-peeling approach (Fernando et al., 1993). In terms of estimation of risk associated with each genotype, genotype probabilities and classes of PrP genotypes provided similar results (Diaz et al., 2003; Vitezica, 2003). However, to estimate polygenic variance, probabilities of genotypes were used, because they were expected to provide additional information (Meuwissen & Goddard, 1997). For instance, these probabilities recognized the heterogeneity of genotypes of the non-genotyped group (Vitezica et al., 2005).

Litter size (Ls). This factor represents the size of the litter in which the animal was born. Three classes were defined: litters with one or two lambs (ST; n = 977), three lambs (T; n = 1492) and more than three lambs (MT; n = 1580).

Rearing type (Rt). Two levels were defined: lambs reared by their mothers (n = 2857) and lambs reared artificially (n = 1192).

Rt ls. This is a combined effect of the levels of rearing type and litter-size effects.

Rt DDS. This is a combined effect that involves rearing type and dam’s disease status. Four levels of the effect were defined. Thus, we had lambs that were fed maternally and born from healthy dams (MH; n = 2331), fed maternally and born from scrapie dams (MS; n = 526), lambs fed artificially and born out of healthy dams (AH; n = 985) and lambs fed artificially and born out of scrapie dams (AS; n = 207). Dams with positive histology without clinical signs were included in the scrapie group.

PrP DDS. This is an effect that combines the scrapie status of the dams with their PrP genotype. The scrapie group included dams with positive histology without clinical signs. Dams’ genotypes were classified into four groups: resistant (ARR/ARR and ARR/AHQ), intermediate (AHQ/AHQ, AHQ/VRQ, ARQ/AHQ, ARR/ARQ and ARR/VRQ), susceptible (ARQ/ARQ, ARQ/VRQ and VRQ/VRQ) and unknown. Seven levels were defined; thus, we had animals born from healthy dams with unknown genotype (n = 725), with resistant genotype (n = 472), with susceptible genotype (n = 626) and with intermediate genotype (n = 1518). Similarly, we had animals born out of scrapie dams with resistant genotype (n = 8), with susceptible genotype (n = 594) and with intermediate genotype (n = 106).

BICs were computed for all candidate models. BIC was chosen as a criterion of selection because it does not require nested models to be compared. To calculate BICs, the number of uncensored records was taken into account. The model with the smallest value of BIC was chosen and extended to account for polygenic variation. Therefore, a Cox genetic-frailty model was fitted to the data under the context of the so-called ‘sire–dam model’. Thus, an additional polygenic effect (g) was added to the model of choice, which already included transmission factors (TF) and PrP genotype. Such a model recognizes that the hazard of animals differs from the average depending on TF, PrP genotype and the effect of g. The effect g represents an additional source of genetic susceptibility caused by the joint effect of a number of other genes (polygene). This polygene is inherited by each animal from its sire and dam. Then, let g contain the sires and dams of the individuals. g was assumed to follow a multivariate normal distribution with a mean of zero and a (co)variance matrix of $\sigma^2_g$, where A is the relationship matrix among the individuals and $\sigma^2_g$ represents the polygenic variance due to the effect of the polygene.

In order to quantify the magnitude of the polygenic variance ($\sigma^2_{PrP}$), the overall genetic variability ($\sigma^2_{T,T}$) associated with resistance/susceptibility was estimated. To do this, the effect of the PrP genotype was removed from the previous model. Thus, the new model only contained the effects of TF and g. Under this simplified model, g captures the overall genetic variability among individuals. Afterwards, the ratio of polygenic variance to overall genetic variance was calculated. Heritability was not used, because of the controversy on how to define heritability in the context of survival analysis (Ducrocq & Casella, 1996; Damgaard et al., 2003).

RESULTS AND DISCUSSION

In order to avoid biasing the estimate of polygenic variation by confounding genetic susceptibility and environmental-transmission factors, several effects were tested. Partial $\chi^2$ statistics from the LRT for each effect under each model are shown in Table 1. Corresponding BICs are also shown in Table 1. Only models with significant effects are included. Regardless of the model, the PrP genotype of animals, together with LEX, made the largest contribution to explain

<table>
<thead>
<tr>
<th>Model</th>
<th>F</th>
<th>AFEX</th>
<th>Sx</th>
<th>LEX</th>
<th>PrP</th>
<th>Ls</th>
<th>Rt</th>
<th>Rt ls</th>
<th>Rt DDS</th>
<th>PrP DDS</th>
<th>BIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model_1</td>
<td>67·4 (3)</td>
<td>29·3 (2)</td>
<td>7·4 (1)</td>
<td>541·5 (33)</td>
<td>730·5 (8)</td>
<td>7·2 (2)</td>
<td>–</td>
<td>–</td>
<td>20·6 (3)</td>
<td>–</td>
<td>5004-13402</td>
</tr>
<tr>
<td>Model_2</td>
<td>68·4 (3)</td>
<td>32·1 (2)</td>
<td>7·4 (1)</td>
<td>545·6 (33)</td>
<td>630·9 (8)</td>
<td>7·4 (2)</td>
<td>14·3 (1)</td>
<td>11·6 (5)</td>
<td>5016-75801</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model_3</td>
<td>69·0 (3)</td>
<td>32·9 (2)</td>
<td>7·2 (1)</td>
<td>544·7 (33)</td>
<td>630·5 (8)</td>
<td>20·0 (8)</td>
<td>12·2 (5)</td>
<td>5043-50045</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Value of the $\chi^2$ statistic, degrees of freedom (n) for each effect under each model and the corresponding BICs for different Cox models

All effects are significant with at least $P<0·05$. Statistics correspond to the marginal LRT. For definition of effects, see Methods.
Table 2. Relative hazards and 95% confidence intervals (CI) of transmission factors other than PrP and LEX included in model_1

<table>
<thead>
<tr>
<th>Effect</th>
<th>Relative risk</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flock*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OF</td>
<td>0.103</td>
<td>0.0134–0.7716</td>
</tr>
<tr>
<td>MF</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>2.416</td>
<td>1.5480–3.7720</td>
</tr>
<tr>
<td>SF</td>
<td>0.035</td>
<td>0.0047–0.2437</td>
</tr>
<tr>
<td>Age at first exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24 months</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>&gt;24–36 months</td>
<td>0.303</td>
<td>0.1645–0.5684</td>
</tr>
<tr>
<td>&gt;36 months</td>
<td>0.297</td>
<td>0.1492–0.5919</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>0.648</td>
<td>0.4711–0.8926</td>
</tr>
<tr>
<td>F</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Rearing type combined with dam’s disease status†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>1.221</td>
<td>0.9404–1.5858</td>
</tr>
<tr>
<td>AS</td>
<td>0.931</td>
<td>0.6374–1.3584</td>
</tr>
<tr>
<td>MH</td>
<td>1.000</td>
<td>–</td>
</tr>
<tr>
<td>AH</td>
<td>0.607</td>
<td>0.4632–0.7966</td>
</tr>
<tr>
<td>Litter size‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>0.811</td>
<td>0.6281–1.0480</td>
</tr>
<tr>
<td>T</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>MT</td>
<td>1.182</td>
<td>0.9440–1.4794</td>
</tr>
</tbody>
</table>

*OF, Animals from outside; MF, main group; PF, parasite group; SF, special group.
†MS, Reared maternally by a scrapie dam; AS, reared artificially and born from a scrapie dam; MH, reared maternally by a healthy dam; AH, reared artificially and born from a healthy dam.
‡ST, One or two lambs; T, three lambs; MT, more than three lambs.

the differences in risk among animals. The smallest value of BIC (Table 1) indicated that model_1 was the best to estimate the polygenic variance. Model_1 included the effect of flock, sex, age at first exposure, LEX, the combination effect of rearing type and dam’s disease status, litter size and PrP genotype. Table 2 shows relative risks of levels of factors other than LEX and PrP genotype.

Level of exposure

Relative risks for animals to show scrapie signs according to LEX are shown in Fig. 1. LEX was introduced into the model as a time-dependent effect. Therefore, LEX describes how the risk of animals achieving symptomatic disease is expected to change across time as a function of the evolution of the level of exposure. In this specific model, the hazard of an animal was assumed to change at the beginning and at the end of each lambing period (Fig. 1), i.e. the risk between lambing periods was assumed to be different from that within lambing periods. Thus, for each animal, LEX recognizes how its hazard departs from the average when this animal is first exposed to infection and how this risk changes with the different infection levels over time. In our model, the definition of the changing point is important. In this respect, LEX is biologically relevant, as it allows us to identify specific periods when animals share the environment more closely than in others. LEX also allows us to distinguish the effect of biological events, such as lambing, where the routes of infection are already known and may occur in addition to other sources of infection. Vectors of infection (Wisniewski et al., 1996; Post et al., 1999) and mechanisms of infectivity (Andreóletti et al., 2002b; Tuo et al., 2002) have been described in the literature.

According to Fig. 1, the model located the highest risk to have occurred at some point in 1995, between the second and third lambing periods. Risk was reduced after the first lambing in 1997 and showed a new increase in 2001. This is a picture of the evolution of the epidemic in the flock. Secondly, the increase in risk was mostly associated with lambings. Several interpretations could be suggested, based on this result: it could simply reflect an increased observation of the flock during the lambing periods, it may indicate an additional stress associated with lambings on the onset of clinical signs after achieving a level of exposure, or even a seasonal trend at the time at which animal shows clinical signs. Although most peaks occurred at lambing periods, the highest point occurred in a between-lambing period in 1995 (Fig. 1). The latter observed peak of risk was mostly associated with sheep that were born between September and October 1991, i.e. the animals involved in the parasite experiment that died at the beginning of the scrapie outbreak. Elsen et al. (1999) have already shown a high risk associated with this group of animals and postulated that nematodes may only be a cofactor for infection. In addition to this group, animals born in the first lambing seasons in 1993 and 1994 were also at high risk (i.e. during lambings surrounding the outbreak). Stringer et al. (1998), modelling the dynamics of scrapie...
in a sheep flock, found that 80–90% of cases occurred at the beginning of the outbreak through horizontal transmission, the effect of which changes over time. In the Langlade flock, 71% of scrapie animals appeared between 1993 and 1997, when high infection pressure was expected.

It is known that an increasing level of exposure to prion-disease agents increases the risk of infection (Gravenor et al., 2003). As scrapie is a disease with a long incubation period, it is expected that animals undergo multiple exposures until the disease becomes apparent. Gravenor et al. (2003) pointed out that incubation period is affected not only by the total dose, but also by the number of and the time between challenges. In naturally infected populations, doses, subsequent doses and level of exposure are unknown. With regard to this, LEX was an attempt to account for the effect of the evolution of the underlying infection on the risk of animals to show signs of scrapie. As LEX recognizes how the risks of animals change over time, LEX is likely to take into consideration how, in naturally infected flocks, sheep are exposed to a range of doses and how this may affect the likelihood of animals to show scrapie signs.

**Age at first exposure**

Relative risks associated with the effect of age at first exposure are shown in Table 2. Relative risk of the first age group of animals (0–24 months) was over three times larger than the risk of sheep in the other two age groups (>24–36 and >36 months of age). Thus, animals facing infection for the first time early in life seemed to be more likely to show scrapie signs than animals facing that first infection at older ages. On average, if animals were first exposed to infection after 24 months of age, they required over 200 days longer than younger sheep to show scrapie signs, provided that DOEX estimates the time required for an animal to show scrapie signs. Similar results have been suggested by Matthews et al. (2001) in a modelling of population dynamics of scrapie. It seems to be clear that the effect of age at first exposure may be explained by a higher degree of susceptibility per se of younger, compared with older, animals (Matthews et al., 2001). However, there is also a relationship between age at infection and age at which scrapie symptoms appear (Woolhouse et al., 1998). Thus, if animals are exposed early in life, they may have sufficient time to show scrapie signs, whereas animals exposed at older ages may not. In other words, animals facing infection at older ages may have been culled or died for other reasons, and that may bias the estimation of the hazard assigned to older animals.

**Effect of rearing type combined with dam’s scrapie status**

Relative risks associated with the combined effect of the rearing type and the dam’s disease status are shown in Table 2. Relative risk of the different groups was expressed in relation to risk of the animals reared by non-scrapie dams (MH). Sheep that, as lambs, were fed maternally by scrapie dams (MS) showed twice as high a risk to become scrapie animals than sheep that, as lambs, were reared artificially and born out of healthy dams (AH). However, lambs that were subject to artificial feeding and born out of scrapie dams (AS) had a similar risk to those that were fed maternally and born out of healthy ewes (MH). Both groups showed a higher risk than lambs that were fed artificially and born out of healthy dams (AH). These results suggest that transmission was perhaps due to events occurring in the first 24 h of life and that there was additional risk for maternally fed animals, which shared the environment closely with their dams during the nursing period (Elsen et al., 1999). Within the group of scrapie

![Fig. 2. Survivor function according to PrP genotype.](http://vir.sgmjournals.org)
animals, time required for the animal to show scrapie signs (DOEX) was analysed in a linear context. MS animals tended to show scrapie signs 100 days before animals from the lowest-risk group (AH). Animals from the AS and MH groups showed scrapie signs 66 and 68 days before those in the AH group, respectively. These results were consistent with the estimated relative risks. The result shows that the lowest-risk group consisted of lambs that were less exposed to infection. These lambs were fed artificially and born out of healthy dams.

**Effect of PrP genotype**

Relative risk of animals due to their PrP genotypes and its 95% confidence interval, number of scrapie animals and the percentage of scrapie cases among those animals exposed for at least 365 days are shown in Table 3. Risks are relative to that of ARQ/VRQ animals. Relative risks among PrP genotypes were consistent with the results of Elsen et al. (1999). Thus, VRQ/VRQ sheep appeared on average to have a three times higher risk than sheep with the ARQ/ARQ genotype, with ARQ/VRQ sheep having an intermediate status. These analyses confirm the results reported by Elsen et al. (1999). However, the current results showed new genotypes and a new allele associated with the hazard of animals to show signs of scrapie in the Romanov population at Langlade. Thus, significant risk was also associated with AHQ/AHQ, ARR/VRQ, AHQ/VRQ and ARR/ARQ genotypes, with relative risks ranging from 0.033 to 0.84 (Table 3). The new allele was ARR. This allele has been found in a heterozygote form, together with the VRQ and ARQ alleles. Thus, ARR/VRQ showed on average a three times higher risk than ARR/ARQ. In addition, the frequency of AHQ among scrapie animals has increased from 0.5 to 3%, compared with the results of Elsen et al. (1999). In contrast, in the period of time evaluated, sheep with the ARR/ARR and ARR/AHQ genotypes did not show susceptibility to scrapie. The association between PrP genotype and susceptibility to scrapie has been described in several sheep breeds, with different behaviour (e.g. Belt et al., 1995; Hunter et al., 1996; Elsen et al., 1999; Thorgeirsdottir et al., 1999, 2002; Tranulis et al., 1999; Baylis et al., 2002; Billinis et al., 2004).

Variation in susceptibility causes variation of mortality rate and incubation period. Incubation period is difficult to determine in a naturally infected population, except for animals born or animals introduced into the flock when infection is already apparent. Our variable, DOEX, is an estimate of the incubation period. In Fig. 2, changes in survival rates according to time (DOEX) for the ten genotypes found in the Langlade flock are shown. Genotypes in the legend are ranked from the least to the most susceptible. Different patterns were found for each genotype. Survivorship decreased with time for all genotypes except ARR/ARR and ARR/AHQ. Survivorship decreased more dramatically for the most susceptible genotypes (VRQ/VRQ, VRQ/ARQ and ARQ/ARQ), decreasing to almost zero for VRQ/VRQ animals. This indicates that censored animals of the VRQ/VRQ group would have been likely to become scrapie animals if they had had the time opportunity to show scrapie signs. In addition to the mortality rate, Fig. 2 gives an illustration of weighted average differences in incubation period according to genotype. VRQ/VRQ animals seemed to have shorter incubation period than those of other genotypes. Moreover, 97% of them showed scrapie signs before 900 days of exposure whereas, at the same time, only 1.4% of ARR/ARQ individuals became scrapie animals. As compared to the results obtained in the previous analysis of this population (Elsen et al., 1999), the increase in the number of genotypes at risk is also an observation that is consistent with the differences in length of incubation period among the susceptible genotypes. To say this, we have assumed that the strain causing the infection has not changed. Our results are also consistent with those of a number of other studies. In sheep, the relationship between the length of incubation period and the PrP genotype has been shown experimentally (Goldmann et al., 1994; Houston et al., 2002). The results of Houston et al. (2002) indicate differences in average incubation period among different genotypes for Cheviot and Dorset animals that were infected

**Table 3. Number of scrapie cases, risk relative to that of the ARQ/VRQ genotype (95% CI) and incidence of scrapie among all individuals exposed for at least 365 days, according to PrP genotype**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. scrapie animals</th>
<th>Relative risk</th>
<th>No. animals</th>
<th>Scrapie animals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VRQ/VRQ</td>
<td>112</td>
<td>2.9568</td>
<td>169</td>
<td>66.2</td>
</tr>
<tr>
<td>ARQ/VRQ</td>
<td>227</td>
<td>1.000</td>
<td>439</td>
<td>52.0</td>
</tr>
<tr>
<td>ARQ/ARQ</td>
<td>86</td>
<td>0.514</td>
<td>228</td>
<td>37.7</td>
</tr>
<tr>
<td>AHQ/AHQ</td>
<td>1</td>
<td>0.042</td>
<td>13</td>
<td>7.7</td>
</tr>
<tr>
<td>ARR/VRQ</td>
<td>6</td>
<td>0.035</td>
<td>160</td>
<td>3.8</td>
</tr>
<tr>
<td>AHQ/ARQ</td>
<td>6</td>
<td>0.033</td>
<td>99</td>
<td>6.0</td>
</tr>
<tr>
<td>AHQ/VRQ</td>
<td>2</td>
<td>0.015</td>
<td>105</td>
<td>1.9</td>
</tr>
<tr>
<td>ARR/ARQ</td>
<td>2</td>
<td>0.012</td>
<td>108</td>
<td>1.8</td>
</tr>
<tr>
<td>ARR/AHQ</td>
<td>0</td>
<td>0.000</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>ARR/ARR</td>
<td>0</td>
<td>0.000</td>
<td>145</td>
<td>0</td>
</tr>
</tbody>
</table>

**Note:** The result shows that the lowest-risk group consisted of lambs that were less exposed to infection. These lambs were fed artificially and born out of healthy dams.
artificially by a subcutaneous inoculation with SSBP/1. In addition, Moore et al. (1998) found an association between the genotype of PrP and incubation period in mice.

**Polygenic variation**

Mean, mode, SD and skewness coefficient of the marginal posterior densities of the polygenic variance ($\sigma^2_{\text{PrP}}$) and of the overall genetic variance ($\sigma^2_{\text{T}}$) are shown in Table 4. Mode and mean of the marginal posterior densities of polygenic variance ($\sigma^2_{\text{PrP}}$) were not zero, indicating the existence of polygenic variation that is involved in the resistance/susceptibility to scrapie. This polygenic variance is an extra genetic variability that is not explained by the point mutations in the Prnp gene found in this Romanov population. There is some evidence from several sheep populations that supports this result. All individuals with the same expected degree of susceptibility do not acquire symptomatic disease. In our population, among susceptible genotypes, the proportion of scrapie animals among those exposed for at least 365 days varied from 1.8 to 66.2% (Table 3). Among those animals that died of scrapie, the time required to show scrapie signs varied not only between individuals across genotypes, but also within genotypes (Fig. 2). The ratio between polygenic variance and total genetic variability is also shown in Table 4. The polygenic variance represented approximately 21% of the total genetic variation that is involved in the resistance/susceptibility to scrapie. It is then reasonable to think that a major gene variation that is involved in the resistance/susceptibility variance represented approximately 21% of the total genetic variability is also shown in Table 4. The polygenic variance may also need to be considered to understand the existing genetic susceptibility in many sheep populations.

The existence of polygenic variation explaining additional variability in the susceptibility to scrapie does not seem to be negligible. Therefore, polygenic variation may need to be taken into account to establish control measures for the incidence of scrapie. Polygenic variation may also need to be considered when breeding decisions are available, and it can be made by selecting sheep with high accuracy, this lack of accuracy being a consequence of the small size of the dataset, together with the high censoring rate (Ducrocq et al., 2000). The marginal posterior density was not symmetrical and confidence intervals for the estimators could not be obtained with the information provided by the software used. On the other hand, the model fitted to the data has identified an additional source of genetic variation that has been assumed to be homogeneous across genotypes. However, heterogeneity of variance would not be unexpected if, to some extent, the existing genetic variability within and between genotypes depends on the strain of prion, doses and time between doses. In the future, it may be of major interest to develop new models to accommodate such effects.

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