Scrapie epidemic in a fully PrP-genotyped sheep flock

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In scrapie-affected sheep flocks, host PrP genotype plays a vital role in determining which sheep will succumb to scrapie and the incubation period. Consequently, within-flock scrapie dynamics is best understood within the context of the genotype profile of the flock. Here we describe a 17 month epidemic of scrapie in a commercially farmed flock of 230 genotyped Texel sheep. At the start of the study, 70% of the sheep were of three genotypes only: ARR/ARQ, ARH/ARQ and ARQ/ARQ. Only 15% of sheep encoded the disease-associated VRQ allele and only a single sheep (0–4%) was of the most susceptible VRQ/VRQ genotype. For susceptible genotypes there was a marked deficit ($P<0.025$) of older animals ($>3$ years), implying that some cases of scrapie had occurred previously. In the ensuing 17 months, 18 sheep of known genotype were confirmed positive for the disease: seven VRQ/ARQ, six VRQ/ARH, two VRQ/ARR, three ARQ/ARQ. Median ages at death were 2–7, 2–8, 4–2 and 3–8 years respectively. Mortality rates were 55, 86, 13 and 3% respectively. Survival analysis revealed a highly significant effect of genotype on survivorship, but no difference between VRQ/ARQ and VRQ/ARH, or between VRQ/ARR and ARQ/ARQ. There was no difference in the survivorship of middle- and older-age cohorts of susceptible sheep. Scrapie risk group (as defined by PrP genotype) was not associated with submission as a scrapie suspect but later found to be negative, or with dying of unknown causes on the farm.

Introduction

Scrapie, an invariably fatal neurodegenerative disease of sheep, attacks animals in a flock in a non-random manner. After exposure to the scrapie-causing agent, susceptibility to the disease is linked to certain polymorphisms in the amino acid sequence of a host protein called PrP and, within a single flock of sheep, animals of a range of PrP genotypes (and, hence, various levels of susceptibility) are generally found (Hunter et al., 1997a, b; Baylis et al., 2000). While there have been numerous published studies of the PrP genotypes of sheep with both natural and experimentally acquired scrapie, few have examined the PrP genotypes of flocks as a whole. This is particularly true for commercial (non-experimental) sheep flocks. Studies of this type are required, however, to establish the patterns of scrapie attack in the natural (farmed) situation.

Three PrP polymorphisms have particularly strong linkage with the occurrence of both natural and experimental scrapie. These are valine (V) or alanine (A) at codon 136, arginine (R) or histidine (H) at codon 154, and glutamine (Q), arginine (R) or histidine (H) at codon 171 (reviewed by Hunter, 1997). Of the 12 possible alleles derivable from these polymorphisms, only five are commonly seen. These are: ARR, ARQ, VRQ, AHQ and ARH (Belt et al., 1995). The ARR allele is clearly associated with resistance to scrapie and VRQ is clearly associated with susceptibility (Belt et al., 1995; Hunter et al., 1996). Sheep of VRQ/VRQ genotype are highly susceptible to the disease (Belt et al., 1995; Hunter et al., 1996) whereas sheep of ARR/ARR genotype appear to be resistant. Susceptibility of the ARQ/ARQ genotype is more complex and varies with sheep breed: in Suffolk sheep this is the most common genotype of scrapie cases (Hunter et al., 1997b) while scrapie is rare in Texel sheep of this genotype (Dawson et al., 1998). The AHQ allele may be associated with resistance in some breeds but not in others and the ARH allele may be neutral (Dawson et al., 1998).

In August 1998 we took blood samples from all breeding animals in a pedigree flock of Texel sheep. At that time the farmer suspected that one sheep was showing signs of scrapie...
but had never previously reported a case to the relevant UK authorities. Over the next 17 months nearly 50 sheep were submitted to the authorities, and 20 cases of scrapie were confirmed. In December 1999 the farmer announced that the flock was to be dispersed, with the culling of susceptible individuals. Uniquely, therefore, we are able here to report the entire, official, epidemic of scrapie in this sheep flock and are able to answer several interesting questions. Was there evidence of the occurrence of scrapie in the flock prior to its first official reporting? What were the death rates from scrapie among the different susceptible PrP genotypes? And were particular PrP genotypes associated with any other (non-scrapie) fates?

Methods

Flock history. At the end of July 1998 we were contacted by a farmer of pedigree Texel sheep who expressed interest in joining our scrapie field study. The farmer had never previously reported a case of scrapie to the relevant authorities but had a current suspect case. On 19 August 1998 we took blood samples from the entire breeding flock, comprising 233 sheep in total. There were 220 samples from females born between 1980 and 1997 and 13 samples from males born between 1993 and 1998. On 20 August 1998 the farmer notified the authorities of the suspect scrapie case and it was slaughtered. Scrapie in this animal was later confirmed. In August 1999 the farmer completed a detailed questionnaire about the flock. In September 1999 we revisited the farm and took blood samples (for PrP genotyping) from 73 ewe lambs born in 1998. We did not sample ram lambs; nevertheless, samples from three ram lambs suspected of having scrapie were sent to us for analysis. Data for these 76 ewe and ram lambs are ignored here, apart from the cases of scrapie which we report. In December 1999 we were notified of the farmer’s intention to disperse the flock, with the slaughter of all sheep carrying or presumed to be carrying the scrapie-associated VRQ allele. The last sheep left the farm in July 2000.

Tissue samples from suspect scrapie cases were subject to routine analysis for evidence of scrapie by the Veterinary Laboratories Agency, UK. Up to four methods were used: (i) histopathological examination of brain tissue for signs of vacuolation; detection of the disease-associated isoform of PrP (PrP^sc); (ii) immunocytochemistry (ICC) and (iii) Western blotting (WB); and (iv) detection of scrapie-associated fibrils (SAF).

For most analyses we consider only data collected between August 1998 and December 1999 (17 months). However, for survival analysis we include all available data, including deaths from scrapie between January and December 2000.

PrP genotype analysis. For each sheep, approximately 5 ml of blood was collected into an EDTA-vacutainer and stored at −20 °C prior to genotype analysis. Genotype analysis was performed by DNA sequencing using an ABI Prism 377 DNA sequencer as recommended by the manufacturer. In short: DNA was isolated from 100–500 µl blood using either a Nucleon DNA extraction kit (Anachem) followed by amplification/sequencing reactions as previously described in Baylis et al. (2000), or a Qiagen DNeasy tissue extraction kit, after which approximately 10–20% of the genomic DNA was subjected to 30 cycles of PCR amplification with oligonucleotide pair 218 CGGCTATCCACCTCAG GGA and 827 TTGCCCCITATCCTACTATGAGA. Following purification with Microcon columns (Amicon) PCR product was sequenced with oligonucleotides 4142 or 9012 (Baylis et al., 2000) in Big Dye Terminator reagent (ABI) diluted in Better-Buffer (Microzone). Control samples of known genotypes were run in parallel in all PCR and sequencing reactions. Most scrapie samples were genotyped more than once. Results obtained in this manner represent pairs/groups of amino acids at three separate codon positions: 136 (valine, V, and alanine, A); 154 (histidine, H, and arginine, R); and 171 (glutamine, Q, arginine, R, and histidine, H). From this we infer that the PrP genotype, in allelic format is, for example, V136R154Q171/A116R154R171, because this is the only possible genotype derivable from the five known PrP alleles. All of the PrP genotyping performed at the Institute is consistent with the assumption that there are only five alleles with regard to these three codons in British sheep and so, for ease of interpretation, we have presented all genotypes in allelic format.

Results

Farmer’s perception of scrapie

The farmer claimed that the first suspect case of scrapie on the farm was in ‘1997 but with hindsight earlier’. According to the farmer, the first suspect scrapie case was home-bred; subsequently, the disease incidence had increased to a concurrent rate of 20 deaths per year, with cases in purchased and homebred animals, rams and ewes, and sheep aged between 2 to 3 years of age and >3 years old. Typical signs were poor body condition, rubbing/scratching and recumbency. The farmer did not know how the flock first became infected with scrapie, but suspected it may be related to the purchase of a ram from The Netherlands in 1990.

PrP genotypes of flock

Fifteen genotypes can be derived from the five known PrP alleles. Of these, 13 were found in the Texel flock (Table 1). About 70% of the flock were of just three genotypes: ARQ/ARQ, ARQ/ARH and ARQ/ARQ. Only 15% of sheep carried the scrapie-associated VRQ allele. Significantly, there was only a single animal of the most highly susceptible VRQ/VRQ genotype. The genotype frequencies correspond to allele frequencies of: ARR, 22.7%; ARH, 1.3%; ARQ, 50.9%; ARQ, 17.4%; VRQ, 7.7%.

Evidence of the previous occurrence of scrapie in the flock

An indication of previous losses from scrapie can be obtained from the predicted and observed frequencies of the VRQ/ARR genotype. When VRQ-attacking scrapie occurs in a flock, the frequency of the VRQ allele is reduced (Woolhouse et al., 1999). As sheep of the VRQ/ARR genotype have a low susceptibility to the disease, the number of sheep of this genotype is more likely to reflect a past frequency of the VRQ allele than the present frequency. Thus, in our flock the VRQ/ARR genotype has an expected frequency (based on current allele frequencies) of 3.5% or eight sheep in a flock of 233 animals, but twice this number were observed (Table 1).

Similar patterns may be obtainable by breeding susceptible (VRQ-encoding) ewes with resistant (ARR/ARR) rams. More
convincing evidence of the prior occurrence of scrapie comes, therefore, from the age structure of the flock. The youngest sheep known to have died of scrapie in this flock were 2 years of age (next section). It is likely, then, that if there have been a substantial number of deaths from scrapie, there will be relatively fewer susceptible sheep over 3 years old than under 3 years old. This is the case (Table 2; \( \chi^2 = 7.44, \text{d.f.} = 4, P < 0.025 \)). Only 32% of sheep of the VRQ/ARQ, VRQ/ARH and VRQ/VRQ genotypes were between 3 and 8 years of age, compared to 61% for the other genotypes.

### Scrapie cases

During the 17 months from our first sampling (August 1998) to the decision to disperse the flock (December 1999), 20 animals were submitted as suspect scrapie cases and later confirmed to be positive by VLA (Table 3; #1–20). Of these, seven were VRQ/ARQ, six were VRQ/ARH, two were VRQ/ARR, three ARQ/ARQ and one ARR/ARR. The remaining case (#13) was genotyped from blood (taken when alive) to be ARR/ARR, a genotype believed to be entirely resistant to scrapie, but the tissue on which the scrapie confirmation was based was genotyped as ARQ/ARQ. The origin of this confusion is not known and this scrapie case is not considered any further.

There were three further cases of scrapie after the decision to disperse the flock, all with the VRQ/ARQ genotype; one (#21) had been sampled by us in 1998; two (#22, #23) were sampled by us as ewe lambs in 1999; the second of these was taken to an experimental sheep facility at the Institute for Animal Health at Compton in July 2000 and died of scrapie (confirmed by detection of PrPSc by Western blot) in November 2000.

The confirmation of scrapie in a sheep of genotype ARR/ARH (#12) is highly unusual. The genotype is consistent with its parentage (its sire was ARR/ARR and its dam had at least one ARH allele) and is likely to be correct. It is possible that the confirmation of scrapie is, in fact, incorrect. The confirmation was based on a positive result by SAF, despite inconclusive histopathology and a negative result by ICC (Table 3). Confirmations of scrapie on the basis of SAF have been made in other instances where, subsequently, it has been shown that scrapie was very unlikely (M. Baylis, unpublished observations). The sensitivity of SAF as a diagnostic method for scrapie has been shown to be less than certain other methods (Simmons et al., 2000) and, during 1999, VLA stopped confirmations of scrapie on the basis of a positive result by SAF alone. ICC and WB were introduced as alternatives (Table 3). Independent Western blotting of tissue from this animal at the Neuropathogenesis Unit of IAH failed to detect PrPSc in either medulla or spleen. It seems likely that the confirmation of scrapie in animal #12 is a misdiagnosis.

Scarpie was confirmed in two other sheep on the basis of SAF only. Animal #6 was SAF-positive but negative by histopathology; it was ARQ/ARQ, a genotype in which scrapie occurs (albeit rarely) in this flock. This scrapie confirmation must be considered questionable. Animal #11 was SAF-positive but histopathology was inconclusive; it was VRQ/ARR, again a genotype in which scrapie occurs (rarely) in this flock. Independent Western blotting of tissue from the medulla of animal #11 at the Neuropathogenesis Unit of IAH detected PrPSc and the confirmation is considered valid.

Scarpie was confirmed in a second sheep of VRQ/ARR genotype (#14), on the basis of the detection of PrPSc by immunocytochemistry, despite negative results by histopathology and Western blotting. Independent Western blotting of tissue from the medulla of animal #14 at the
Table 3. Scrapie cases (confirmed by VLA) in a flock of Texel sheep

Hist, histopathology; ICC, immunocytochemistry; SAF, scrapie-associated fibrils; WB, Western Blot; +, positive; -, negative; x, not done. WB results in parentheses are from tissue supplied to IAH.

<table>
<thead>
<tr>
<th>#</th>
<th>Sex</th>
<th>Died</th>
<th>Age at death (months)</th>
<th>Hist</th>
<th>ICC</th>
<th>SAF</th>
<th>WB</th>
<th>Genotype</th>
<th>Scrapie?</th>
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<tr>
<td>1</td>
<td>F</td>
<td>Aug 1998</td>
<td>30</td>
<td>+</td>
<td>x</td>
<td>+</td>
<td>x</td>
<td>VRQ/ARQ</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>Aug 1998</td>
<td>30</td>
<td>+</td>
<td>x</td>
<td>+</td>
<td>x</td>
<td>VRQ/ARQ</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>Aug 1998</td>
<td>77</td>
<td>+</td>
<td>x</td>
<td>+</td>
<td>x</td>
<td>VRQ/ARH</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>Sep 1998</td>
<td>31</td>
<td>+</td>
<td>x</td>
<td>+</td>
<td>x</td>
<td>VRQ/ARQ</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>Oct 1998</td>
<td>55</td>
<td>+</td>
<td>x</td>
<td>-</td>
<td>x</td>
<td>VRQ/ARQ</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>Nov 1998</td>
<td>79</td>
<td>-</td>
<td>x</td>
<td>+</td>
<td>x</td>
<td>ARQ/ARQ</td>
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</tr>
<tr>
<td>7</td>
<td>F</td>
<td>Jan 1999</td>
<td>35</td>
<td>+</td>
<td>x</td>
<td>+</td>
<td>x</td>
<td>VRQ/ARH</td>
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</tr>
<tr>
<td>8</td>
<td>F</td>
<td>Jan 1999</td>
<td>82</td>
<td>+</td>
<td>x</td>
<td>+</td>
<td>x</td>
<td>VRQ/ARH</td>
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</tr>
<tr>
<td>9</td>
<td>F</td>
<td>Jan 1999</td>
<td>45</td>
<td>+</td>
<td>x</td>
<td>+</td>
<td>x</td>
<td>ARQ/ARQ</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>Feb 1999</td>
<td>35</td>
<td>+</td>
<td>x</td>
<td>+</td>
<td>x</td>
<td>VRQ/ARQ</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
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<td>47</td>
<td>?</td>
<td>x</td>
<td>+</td>
<td>x (+)</td>
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<tr>
<td>12</td>
<td>F</td>
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<td>48</td>
<td>?</td>
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<td>+</td>
<td>x (-)</td>
<td>ARR/ARH</td>
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<tr>
<td>13</td>
<td>F</td>
<td>Mar 1999</td>
<td>84</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>x</td>
<td></td>
<td></td>
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<td>14</td>
<td>F</td>
<td>Jun 1999</td>
<td>52</td>
<td>-</td>
<td>+</td>
<td>?</td>
<td>- (-)</td>
<td>VRQ/ARR</td>
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</tr>
<tr>
<td>15</td>
<td>M</td>
<td>Jun 1999</td>
<td>27</td>
<td>+</td>
<td>+</td>
<td>x</td>
<td>- (+)</td>
<td>VRQ/ARQ</td>
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</tr>
<tr>
<td>16</td>
<td>M</td>
<td>Jul 1999</td>
<td>28</td>
<td>+</td>
<td>+</td>
<td>x</td>
<td>+ (+)</td>
<td>ARQ/ARQ</td>
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</tr>
<tr>
<td>17</td>
<td>F</td>
<td>Sep 1999</td>
<td>29</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>- (+)</td>
<td>VRQ/ARH</td>
<td>Yes</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>Sep 1999</td>
<td>31</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+ (+)</td>
<td>VRQ/ARH</td>
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</tr>
<tr>
<td>19</td>
<td>F</td>
<td>Nov 1999</td>
<td>32</td>
<td>+</td>
<td>+</td>
<td>x</td>
<td>+</td>
<td>VRQ/ARQ</td>
<td>Yes</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>Dec 1999</td>
<td>34</td>
<td>+</td>
<td>+</td>
<td>x</td>
<td>+ (+)</td>
<td>VRQ/ARQ</td>
<td>Yes</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>Jul 2000</td>
<td>40</td>
<td>+</td>
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</tr>
<tr>
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<td>F</td>
<td>Jul 2000</td>
<td>28</td>
<td>+</td>
<td>+</td>
<td>x</td>
<td>+ (+)</td>
<td>VRQ/ARQ</td>
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<tr>
<td>23</td>
<td>F</td>
<td>Nov 2000</td>
<td>32</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x (+)</td>
<td>VRQ/ARQ</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Genotype mismatch between blood taken from live sheep #13 (ARR/ARR), and the tissue of the confirmed scrapie case, believed originally to be the same animal (ARQ/ARQ).

Table 4. Characteristics of 21 scrapie cases grouped by genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Age at death (months)</th>
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<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Median</td>
</tr>
<tr>
<td>ARQ/ARQ</td>
<td>3</td>
<td>28</td>
</tr>
<tr>
<td>VRQ/ARR</td>
<td>2</td>
<td>47</td>
</tr>
<tr>
<td>VRQ/ARH</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>VRQ/ARQ</td>
<td>10</td>
<td>27</td>
</tr>
</tbody>
</table>

* Scrapie confirmed on the basis of SAF only and considered a possible misdiagnosis. Numbers in parentheses give the median and maximum if the possible misdiagnosis is excluded.

Neuropathogenesis Unit of IAH also failed to detect PrPSc. The true scrapie status of this animal must be considered ambiguous. For the purposes here we consider the animal to have died of scrapie, but accept that this may be incorrect. Our conclusions are not significantly affected under either scenario.

Given the ambiguity in the true scrapie status of some of the confirmed cases, Table 3 indicates which cases we conclude to be true scrapie and include in further analyses. Of the original 20 confirmed cases (#1–20), 18 are considered to be scrapie although one (#6) is ambiguous. Two cases (#12, #13) are excluded from analyses.

Characteristics of the scrapie cases, grouped by genotype, are given in Table 4. The lowest median age of death, at 2–5 to 3 years, was for the VRQ/ARH and VRQ/ARQ genotypes;
Scrapie epidemic in PrP genotyped sheep flock

Fig. 1. Kaplan–Meier survival distribution functions for deaths from scrapie in a flock of Texel sheep. (A) Survival functions for four susceptible genotypes. The survival function for ARR/ARH is not shown, as the single scrapie case was probably a misdiagnosis (see text). Survival times are measured from the start of the study until the date of submission as a scrapie suspect. Censored events (i.e. deaths of animals from causes other than scrapie) are shown as diamonds. (B) Survival functions for sheep of three age cohorts (at the start of the study). Thick lines, VRQ/ARQ and VRQ/ARR (high susceptibility); thin lines, ARQ/ARQ and VRQ/ARR (low susceptibility).

by contrast, the median age of death for the VRQ/ARR and ARQ/ARQ genotypes was nearly 4 years. Nevertheless, patterns relating genotype and age at death were not clear (for example, the range in age is almost identical for VRQ/ARH and ARQ/ARQ) and, overall, there was not a significant effect of genotype on the age at death (Mood median test, $\chi^2 = 3.9$, df = 3, not significant). This reflects, presumably, the range in age at which the sheep were first infected or the dose of infectivity received.

The single sheep of VRQ/VRQ genotype, which was more than 4 years old in August 1998, did not succumb to scrapie. Three months after the start of the study, it was found with a broken hind leg and culled by the farmer.

Mortality and survival rates

In the 17 month period from August 1998 to December 1999, death rates from scrapie were: ARQ/ARQ, 3% (2/58); VRQ/ARR, 13% (2/16); VRQ/ARQ, 55% (6/11) and VRQ/ARH, 86% (6/7). Note that these rates exclude the two ram lambs that died of scrapie (#15, #16) and that were not included in the initial 233 sheep sampled in August 1998. Survival times of the sheep of these genotypes were subjected to survival analysis (Collett, 1994) using SAS (Allison, 1995). The survival measure was time (in months) from August 1998 to the date of submission as a scrapie suspect (which was then confirmed at later date). Kaplan–Meier survival distribution functions for the four genotypes are shown in Fig. 1(A). There was a highly significant effect of genotype on survival function ($\chi^2 = 56.4$, df = 3, $P < 0.0001$), with survivorships of VRQ/ARQ and VRQ/ARH decreasing more rapidly than those of ARQ/ARQ and VRQ/ARR. Within these pairs, however, there were no significant differences in survivor function. It is noteworthy that the VRQ/ARQ and VRQ/ARH survivorships decrease to or close to zero, suggesting that the ‘censored’ animals of those genotypes probably would have died from scrapie if they had not been lost from other causes.
Hazard ratios were obtained from a proportional hazards model, with the ARQ/ARQ hazard (i.e. the death rate from scrapie) as the model baseline. The VRQ/ARR hazard was not significantly different from the baseline. However, the VRQ/ARQ hazard was 21.8 times greater than the baseline ($\chi^2 = 14.5$, d.f. = 1, $P<0.001$) while that of VRQ/ARH was 45.1 times greater ($\chi^2 = 20.9$, d.f. = 1, $P<0.0001$).

The genotypes ARH/ARQ and ARQ/ARQ have elsewhere been categorized as having similar risks of scrapie as ARQ/ARQ and VRQ/ARR (Dawson et al., 1998). It is perhaps noteworthy that there were four scrapie cases out of 74 sheep encoding ARQ/ARQ and VRQ/ARR, but none out of 54 sheep encoding ARH/ARH and ARQ/ARQ. The difference approaches, but does not reach, significance ($\chi^2 = 3.0$, d.f. = 1, $P<0.1$).

**Age-dependent survival**

The oldest sheep of scrapie-susceptible genotype sampled in August 1998 was an ARQ/ARQ and almost 90 months old. Susceptible sheep were therefore divided into three groups according to their age in August 1998: 6–30 months old (note that we did not sample any sheep under 6 months old); 30–60 months old; and 60–90 months old. These age groupings were chosen arbitrarily. Because of small sample sizes, sheep of ARQ/ARQ and VRQ/ARR genotypes and ARQ/ARQ and VRQ/ARH genotypes were combined to give two groups of differing susceptibility to scrapie (low and high, respectively). We then used survival analysis to examine whether different cohorts had different survivorships (Fig. 1B). There were no deaths from scrapie in the youngest age cohort of the less susceptible sheep (thin solid line). Note that this analysis excludes the young ARQ/ARQ male that died of scrapie (#16) as it was not one of the 233 sheep sampled at the start of the study. Similarly, there were no losses from scrapie in the youngest age cohort of the more susceptible sheep (thick solid line) for the first 13 months. This was followed over the ensuing 10 months, however, by heavy losses from scrapie until there were no survivors. The middle and oldest age cohorts of the less susceptible sheep experienced some losses from scrapie in the early months of the study but then there were no others (thin dashed and dotted lines). The same age cohorts of the more susceptible sheep also experienced most losses in the early months of the study (thick dashed and dotted lines). Significantly, there appear to be no differences in the survival functions of the middle- and older-aged cohorts in either group of sheep. In other words, the different ages of the cohorts and, possibly, their different durations of exposure to scrapie infectivity (depending on when the flock first became infected) do not appear to have affected their relative survivorships.

**Unconfirmed suspect cases**

During the 17 month study period, 30 animals were slaughtered and submitted to the authorities as scrapie suspects, but evidence of scrapie was not detected. One further negative case occurred after the decision to disperse the flock.

All of these cases showed some signs that occur with scrapie, most notably ‘wasting’. It is possible that at least some of these sheep may have had scrapie and were showing behavioural or physiological signs, but for unknown reasons confirmation in the laboratory was not achieved. If this occurred to any significant degree, we would expect there to be an association between failure to confirm scrapie and PrP genotype. This is not the case. Taking the survival measure to be time (in months) from August 1998 to the date of submission as a scrapie suspect (which was later not confirmed), there was no significant difference between the survival functions of sheep of the different scrapie risk categories given in Table 2 (Log rank test, $\chi^2 = 0.11$, d.f. = 2, $P>0.5$).

**Deaths from other and unknown causes**

Scrapie-affected farms report having more sheep that die of unknown causes than scrapie-free farms (McLean et al., 1999) and a high proportion of sheep that die from unknown causes may, in fact, show signs of scrapie when examined using histopathology (Clark et al., 1994). It is interesting to ask, therefore, whether in our study farm there was an association between PrP genotype and the risk of being found dead.

During the 17 month study period, 18 sheep were found dead from unknown causes. Taking the survival measure to be time (in months) from August 1998 to the date of being found dead, there was no significant difference between the survival functions of sheep of the different scrapie risk categories given in Table 2 (Log rank test, $\chi^2 = 1.22$, d.f. = 2, $P>0.5$).

**Discussion**

This paper presents a detailed account of an epidemic of natural scrapie in a flock of sheep. The account is unique in several respects: the sheep flock was commercially farmed; we observed the epidemic from the first reporting of a case of scrapie in the flock to its dispersal more than a year later; and the entire breeding flock was sampled for PrP genotyping immediately prior to the first report. Furthermore, the breed of sheep (Texel) encodes all five known PrP alleles that have proven linkage to the occurrence of scrapie. The major limitations of the study are that we do not know when the flock first became infected with scrapie, how many cases occurred prior to the study, nor whether any recent ancestors of the cases themselves were infected with, or died from, scrapie.

In responses to a questionnaire the farmer implied that there had been cases of scrapie in the flock prior to the start of our study. We have previously shown that scrapie leaves a statistically detectable signature in the age-genotype structure of scrapie-affected sheep flocks (Baylis et al., 2000). Here we confirm the presence of a signature in the study flock; namely, there was a significant paucity of older sheep of the most susceptible genotypes. One implication is that we were (at least in theory) able to conclude the likely occurrence of scrapie.
in the flock, even had information from the farmer not been made available.

A second signature was apparent in the observed and predicted frequencies of sheep of the VRQ/ARR genotype. The high level of susceptibility of VRQ-containing genotypes other than VRQ/ARR means that, as deaths from scrapie occurred in the flock, the frequency of the VRQ allele was reduced to a level too low to account for the observed number of surviving sheep of VRQ/ARR genotype. It remains to be seen whether this measure is a useful marker for the occurrence of VRQ-attacking scrapie in other sheep flocks.

If the flock had not been dispersed, it is likely that a high proportion of future scrapie cases would have been produced by either sires or dams of VRQ/ARR genotype, as sheep of the other VRQ-containing genotypes would be expected to die before reproducing many times. It is worth considering, therefore, that the high level of resistance of the VRQ/ARR genotype might have significantly extended the duration of the scrapie epidemic in the flock. This raises the intriguing possibility that the use of ARR/ARR (resistant) rams, with no consideration of ewe genotype, is not necessarily the most rapid method of eliminating scrapie from affected flocks.

There has been one previous study of scrapie in Texel sheep. Genotype frequencies were obtained by Belt et al. (1995) for scrapie-affected and unaffected Texel sheep collected from over 30 different flocks in The Netherlands. Scrapie was recorded in five different genotypes. It has been suggested by one of us previously that this large range in tropism is a result of the multi-flock nature of the study, because of variable host genetics or more than one scrapie strain, and that a simpler picture would be obtained in single flock studies (Hunter, 1997). Our new data disagree with this assertion. In our single flock study, scrapie was recorded in four genotypes, and would be expected in a fifth (VRQ/VRQ). It is more likely, therefore, that the wide tropisms reported by Belt et al. (1995) and here are characteristics of Texel sheep.

There are further interesting similarities between the results of the two studies. Belt et al. (1995) recorded four cases of scrapie out of 18 animals of ARQ/ARQ and VRQ/ARR genotype, but none in 20 animals of ARQ/ARH or ARH/ARH genotype. These proportions differ significantly ($\chi^2 = 5.0$, d.f. = 1, $P < 0.05$). We observed the same pattern, although the results were not quite significant ($0.05 < P < 0.1$). These observations suggest that sheep of ARQ/ARH or ARH/ARH genotypes may be largely or entirely resistant to scrapie. This is in disagreement with the classification of these genotypes by Dawson et al. (1998) and the UK Government's National Scrapie Plan (DEFRA, 2001). The observed pattern might occur if, in Texel sheep, VRQ and ARQ are associated with scrapie and ARH is associated with resistance, and VRQ is dominant to both ARH and ARQ, while ARH is dominant to ARQ. Hence, scrapie would occur in VRQ/ARQ, VRQ/ARH and ARQ/ARQ genotypes, but not ARQ/ARH or ARH/ARH genotypes.

The wide scrapie tropism observed in Texel sheep differs significantly from that reported in other breeds. Scrapie has not been reported in the ARQ/ARQ and VRQ/ARR genotypes in the NPU flock of Cheviot sheep (Hunter et al., 1996). In a flock of Romanov sheep in France there were no confirmed cases in VRQ/ARR sheep but there were many in ARQ/ARQ sheep and the scrapie hazard for ARQ/ARQ was only one-third that of VRQ/VRQ and half that of VRQ/ARQ (Elsen et al., 1999). By contrast, in our Texel flock scrapie was confirmed in both ARQ/ARQ and VRQ/ARR genotypes, but the hazard was about one-twentieth that of VRQ/ARQ.

There is experimental evidence for different scrapie incubation periods in different genotypes of the same breed of sheep (Goldmann et al., 1994), and this assumption has been a requirement for successful modelling of a within-flock scrapie epidemic (Matthews et al., 2001). Generally, the shortest incubation periods are associated with the most susceptible genotypes. In the field, this pattern should be manifested as a negative relationship between susceptibility and average age at death, and this was observed in the epidemic in Romanov sheep (Elsen et al., 1999). A similar pattern is apparent in our Texel sheep (Table 4). The median age at death is lower for VRQ/ARQ and VRQ/ARH than ARQ/ARQ and VRQ/ARR cases. The difference was not significant, however, and this is because of the death of a single, young ARQ/ARQ male at 28 months (#16) and the deaths of two, old VRQ/ARH ewes at 77 and 82 months (#3, #8). The latter may simply be the result of infections acquired by the adult sheep. However, there can be no doubt that the case in the young ARQ/ARQ male suggests that incubation periods in this genotype of Texel sheep can be short, even though the level of susceptibility is low. An alternative explanation is that this case was caused by a second scrapie strain with different attack characteristics from the main strain attacking the flock.

There are several reasons for expecting the survival function of older sheep in a scrapie-affected flock to differ from that of younger sheep. First, younger sheep may be more susceptible to infection than older sheep (Matthews et al., 2001). Secondly, older and younger cohorts will be born at different stages in the epidemic and will probably be exposed, as lambs, to different levels of infectious agent. Higher infectious doses are generally associated with shorter incubation periods. With this in mind, it seems remarkable that in our Texel sheep the survival functions for middle and older age cohorts were almost identical, suggesting similar levels of susceptibility and time-courses to death.

Several studies have suggested that, in scrapie-affected sheep flocks, a number of the sheep found dead of unknown causes may have had undiagnosed scrapie. Were this the case, an association between the likelihood of being found dead of unknown causes and PrP genotype would be expected. In the current study there was a remarkable number of such sheep during the 17 month study period (8% of the entire flock), but there was not a significant association with scrapie risk.
This work was supported by Biotechnology and Biological Sciences Research Council grant BS309857. We thank the farmer for access to the sheep and husbandry records, Michael Gravenor for showing M.B. how to do survival analysis, and VLA for providing data on the suspect scrapie cases submitted from the flock.

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Received 26 March 2002; Accepted 9 July 2002