Comparative epidemiology of scrapie outbreaks in individual sheep flocks

C. A. REDMAN[1]*, P. G. COEN[1]†, L. MATTHEWS[1], R. M. LEWIS[2],
W. S. DINGWALL[2], J. D. FOSTER[3], M. E. CHASE-TOPPING[1], N. HUNTER[3]
AND M. E. J. WOOLHOUSE[1]‡

[1] *Centre for Tropical Veterinary Medicine, University of Edinburgh, Easter Bush, Roslin, Midlothian EH25 9RG, UK*
[2] *Animal Biology Division, Scottish Agricultural College, Bush Estate, Penicuik, Midlothian EH26 0PH, UK*
[3] *Institute for Animal Health Neuropathogenesis Unit, Ogston Building, West Mains Road, Edinburgh EH9 3JF, UK*

SUMMARY

Data recording the course of scrapie outbreaks in 4 sheep flocks (2 in Cheviot sheep and 2 in Suffolks) are compared. For each outbreak the data on scrapie incidence and sheep demography and pedigrees cover periods of years or decades. A key finding is that the incidence of clinical cases peaks in sheep 2–3 years old, despite very different forces-of-infection. This is consistent with age-specific susceptibility of sheep to scrapie, as has been reported for cattle to bovine spongiform encephalopathy and for humans to variant Creutzfeldt–Jakob disease. Scrapie incidence was higher in ewes than rams and at certain times of years, though these effects were not consistent between flocks. There was no evidence for high levels of vertical transmission.

INTRODUCTION

Scrapie, like other transmissible spongiform encephalopathies (TSEs), is a progressive, degenerative and invariably fatal neurological disease. Scrapie naturally affects sheep and goats. Although the disease has been recognized since the mid-18th century [1], many aspects of the biology of scrapie remain poorly understood. These include the nature of the infectious agent, which is thought, but not formally confirmed, to be an infectious protein, a prion. How this agent is transmitted is also poorly understood: it is widely accepted that the disease can be transmitted hori-

zontally, from sheep to sheep, but the exact route or routes by which this occurs are unclear [2]; there is also some evidence for vertical transmission, from ewe to lamb [2]. However, in recent years, considerable progress has been made in establishing the genetics of susceptibility to scrapie [3, 4], identifying other risk factors for individual sheep [2], and identifying risk factors for the introduction of the disease into a flock [5]. In the United Kingdom, and elsewhere in Europe, there is also now better information on the distribution of scrapie in national flocks [6, 5], partly stimulated by growing concern over the possible spread of BSE into sheep [7]. One difficulty with studying the epidemiology of scrapie is the very long time scales characteristic of the disease. Scrapie has a long incubation period, of the order of 2 years, and until recently there was no means of detecting pre-clinical infections (although preclinical diagnostics

are now available [8], they have yet to be used in any large-scale epidemiological studies). As a consequence, experimental studies of scrapie can take many years and, so far, have provided limited information on the transmission of the disease. At the level of the flock, scrapie outbreaks have durations of years or decades [9–11]. As a consequence, few long term studies of scrapie outbreaks have been undertaken and data from such studies are particularly valuable.

Here, we analyse epidemiological patterns for 4 outbreaks for which there are long-term data. All 4 studies were carried out in Scotland: 2 of Cheviot sheep; 2 of Suffolk sheep. Information from each of these outbreaks has been published previously [9, 12–15] but there has been no analysis comparing outbreaks. Such an analysis has several advantages: it allows comparison between two breeds with different genetics of scrapie susceptibility, between flocks with different demographies, and between outbreaks with different incidences of infection. Both differences and similarities between outbreaks can provide information on the epidemiology of scrapie. Of particular interest here are temporal (including seasonal), age-related and sex-related patterns in the distributions of scrapie cases.

Data from individual outbreaks can also be compared with the output of mathematical models of the dynamics of scrapie. Mathematical models of scrapie have only recently been developed [16, 17], but have proven to be useful aids to the interpretation of field data [9, 11]. In this paper, existing mathematical theory is used to help interpret the complex dynamics described by the data.

METHODS

Study flocks

Three of the outbreaks were in flocks maintained by the Institute for Animal Health Neuropathogenesis Unit (NPU) and one by the Scottish Agricultural College (SAC). All four flocks were maintained for research purposes: the NPU flocks explicitly as sources of natural scrapie infections, the SAC flock to breed for lean meat. Two of the NPU outbreaks occurred in the same flock of Cheviot sheep; however, the outbreaks occurred at different times and, importantly, were separated by 4 years when scrapie was absent and during which the flock was moved to a different location, with concomitant changes in husbandry. Therefore, although it is possible that sub-

clinical infections from the first outbreak initiated the second [11], the outbreaks can be considered distinct events. The other NPU and the SAC outbreaks were both in flocks of Suffolk sheep. The origins and histories of the flocks are described in greater detail elsewhere. The breeding systems within flocks were variable and complex. All four flocks were (largely) closed. The NPU Cheviot flocks included lines bred for scrapie susceptibility and lines bred for scrapie resistance; the NPU Suffolk flock was bred for scrapie susceptibility; the SAC Suffolk flock was bred as different lines but without regard to scrapie susceptibility. The demography of the NPU flocks was influenced by the removal of sheep for experimental studies and by the need to retain a larger proportion of lambs than usual for this purpose. Otherwise, all four flocks were maintained in accord with contemporary commercial practice. Other relevant aspects of their husbandries are discussed below.

Field data

The following data are available from all (or almost all) sheep in each outbreak: 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. Since all four flocks were maintained for research purposes, three of them for research into scrapie, the identification of scrapie suspects is believed to have been extremely good. Suspect scrapie cases were confirmed by histopathological detection of vacuolation of brain tissue. Only confirmed cases are included in the analyses presented here. For two of the outbreaks, the SAC Suffolk and the second NPU Cheviot, there is some information on PrP genotypes that determine susceptibility to scrapie. PrP genotypes were established by sequencing PCR products or using oligonucleotide probes, as previously described [13, 14]. The effect of PrP genotype on susceptibility to scrapie is well documented [3, 4] and has been previously analysed in some detail for both these flocks described [13, 14], and therefore receives limited attention here.

Statistical analysis

Demographic variables (mean flock size, sex ratio, and survivorship curve) were calculated for the set of affected birth cohorts involved in the full course of an outbreak. Mean life expectancies in the presence of

Table 1. *Demographic characteristics of study flocks*

Flock	Birth cohorts	No. sheep	Flock size (range)	Female:male sex ratio >1 year	Mean life expectancy (years)	Mean life expectancy >1 year (years)	Mean life expectancy >1 year with scrapie (years)	% bred	
								Males	Females
NPU Cheviot 1	1967–78	1321	273–751	1·3	3·9	4·3	4·0	21	74
NPU Cheviot 2	1982–92	1604	304–653	1·5	3·5	4·9	4·8	20	50
NPU Suffolk	1956–80	1658	43–597	1·7	4·8	5·3	3·7	2	17
SAC Suffolk	1988–94	2489	198–760	6·7	1·6	3·9	3·7	88	94

scrapie were calculated using survivorship data right-censored for sheep removed for experimental purposes and for sheep still alive at the end of the outbreak. Mean life expectancies in the absence of scrapie were calculated using data additionally right-censored for scrapie deaths.

The overall incidence of scrapie was calculated using the total number of sheep in the relevant birth cohorts as the denominator; incidence by birth cohort using the number born in that year; and incidence by age using the number surviving to the start of the age class. Note that these were whole populations, not samples. Relative risks were used to compare incidences in different subsets [18]. Comparisons of frequencies (e.g. numbers of cases) within or across outbreaks were made using χ^2 tests. Comparisons of continuous variables (e.g. ages of cases) were made using generalized linear models, inspecting for normally distributed residuals and transforming variables where appropriate. Statistical analyses were carried out using the software packages Excel, Minitab and SAS. Because four independent data sets were being analysed, statistical significance was set at $P = 0.01$ throughout.

RESULTS

Demography

Demographic information is summarized in Table 1. Flock sizes were variable but were generally in the range 300–600. Sex ratios were female biased (range 1·3–6·7). Survivorship curves are shown in Figure 1. Mean life expectancies in the absence of scrapie had a wide range (Table 1), but most of this variation was due to different proportions of lambs retained (Fig. 1), and life expectancies conditional on surviving the first year were less variable (Table 1). Maximum life span was 12·2 years (a NPU Cheviot ewe).

Scrapie incidence

Overall incidences in the sets of birth cohorts affected by the outbreak varied by more than an order of magnitude, 2–43 % (Table 2). The outbreaks could be ranked crudely in terms of incidence as follows: NPU Suffolk > first NPU Cheviot > SAC Suffolk > second NPU Cheviot. The time courses of the outbreaks are shown by calendar year in Figure 2 and by birth cohort in Figure 3. Outbreak durations ranged up to 24 years. Peak incidences by birth cohort were 5–76 % and by calendar year 1–49 %. The interval between the first case being reported and peak incidence by calendar year varied from 3 to over 17 years (Table 2). The reduction in life expectancy due to scrapie was 0·1–1·6 years (Table 1, Fig. 1).

Sex and age

Among sheep more than 1 year old there was a tendency for incidence to be greater in ewes than rams (Table 2), significantly so for the first NPU Cheviot ($\chi^2_{(1)} = 56·3$, $P < 0·001$) and the NPU Suffolk ($\chi^2_{(1)} = 7·4$, $P = 0·006$) outbreaks. For all four outbreaks the modal age class for scrapie deaths was 2–3 years; no cases were seen in sheep less than 1 year old and few in sheep more than 5 years old. The youngest case was 1·0 year (a NPU Suffolk ewe) and the oldest case was 7·7 years (also a NPU Suffolk ewe). Incidence (which controls for demography) also showed a decline among sheep more than 4 years old (Fig. 4). The mean age of scrapie cases did vary significantly between outbreaks ($F_{3,984} = 10·9$, $P < 0·001$) but the variation was slight, 2·4–2·9 years (Table 2). There was a tendency for a decline in the age of cases during the course of the outbreaks, although this was significant only for the NPU Suffolks ($F_{1,1462} = 10·1$, $P < 0·001$). However, for all four outbreaks, analysis of covariance found no difference between the trend
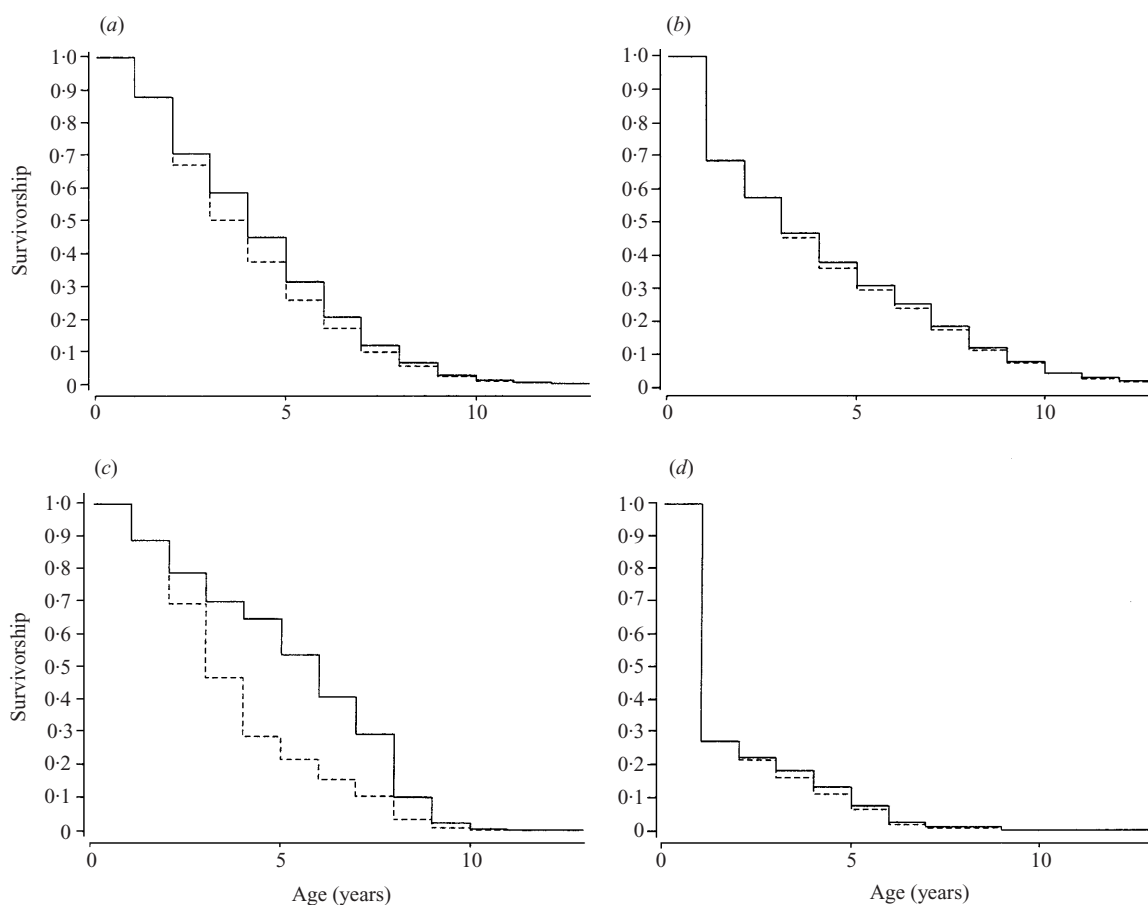
Fig. 1. Survivorship plots for the four study flocks during the outbreaks. Data are either right-censored for scrapie deaths, representing non-scrapie morality only (solid lines), or include scrapie mortality (broken lines). Numbers of sheep and mean life expectancies are shown in Table 1. (*a*) First NPU Cheviot flock. (*b*) Second NPU Cheviot flock. (*c*) NPU Suffolk flock. (*d*) SAC Suffolk flock.

Table 2. *Scrapie incidence in study flocks*

Flock	No. cases	Overall incidence (%)	Outbreak duration (years)	Period to peak incidence (years)	Max. % affected by year	Max. % affected by cohort
NPU Cheviot 1	137	10·4	12	3	5·3	23·4
NPU Cheviot 2	33	2·1	9	9	1·0	5·4
NPU Suffolk	710	43·0	24	17	49·3	76·1
SAC Suffolk	108	4·3	10	4	4·8	5·9

	Incidence by sex (>1 year old) (%)			Relative risk if parent(s) affected		
Flock	Males	Females	Mean age of cases (years)	Sire only	Dam only	Either or both
NPU Cheviot 1	4·0*	18·2*	2·4‡	2·4	3·2	3·3¶
NPU Cheviot 2	3·9	2·4	2·8‡	7·8	2·9	6·1¶
NPU Suffolk	44·5†	51·7†	2·9‡	1·3§	1·9§	2·0¶
SAC Suffolk	12·1	13·2	2·6‡	2·1	3·5	2·8¶

Statistically significant differences shown (for details see main text): *females > males; †females > males; ‡differences between flocks; §dam only > sire only; ¶all > 1.
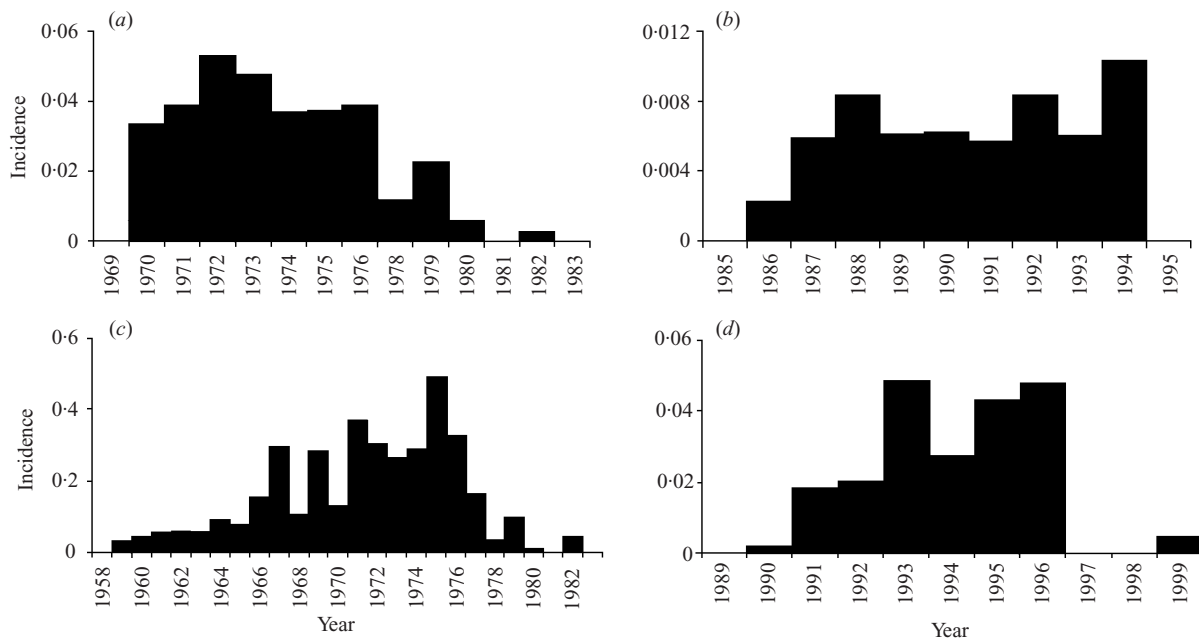
Fig. 2. Incidence (cases per sheep) of scrapie by calendar year for each outbreak. (*a*) to (*d*) as for Figure 1.
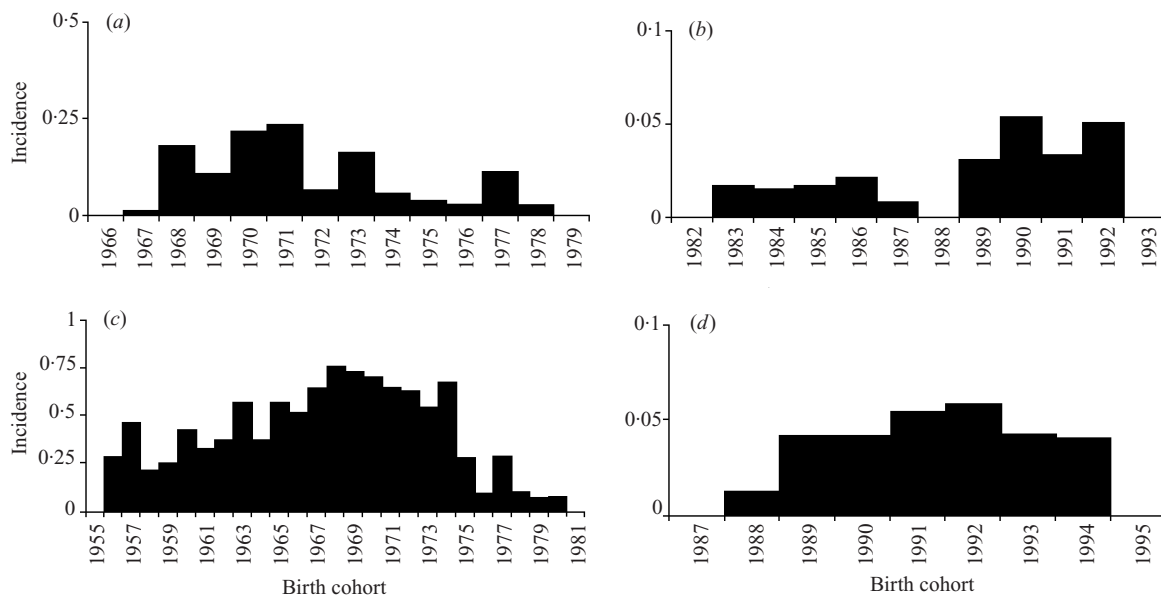

Fig. 3. Incidence (cases per sheep) by birth cohort for each outbreak. (*a*)–(*d*) as for Figure 1.

through time for the age of scrapie deaths and the age of non-scrapie deaths (Fig. 5), suggesting that any apparent decline in the age of cases was a demographic artifact.

Seasonality

All four flocks had highly seasonal distributions of births, although the mode varied from the 3rd to 16th week of the year. The distribution of scrapie deaths through the year also showed seasonal (quarterly) variation which, when compared with seasonal distributions of non-scrapie deaths, was statistically significant for all except the second NPU Cheviot outbreak (Fig. 6). The seasonal distribution of scrapie deaths differed significantly between outbreaks ($\chi^2_{(9)} = 29 \cdot 7$, $P = 0 \cdot 001$).

Breeding and genetics

All flocks were (largely) closed and inbred, only a minority of rams being used for breeding (Table 1)
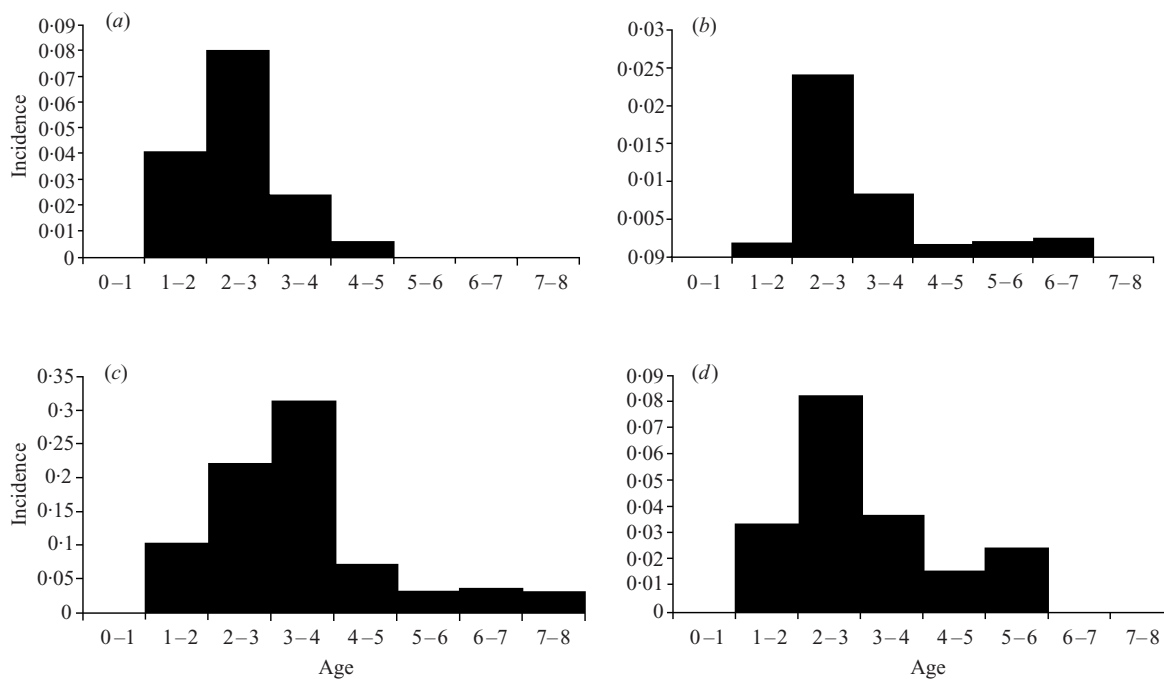
Fig. 4. Incidence (cases per sheep) by age class (years) for each outbreak. (*a*)–(*d*) as for Figure 1.
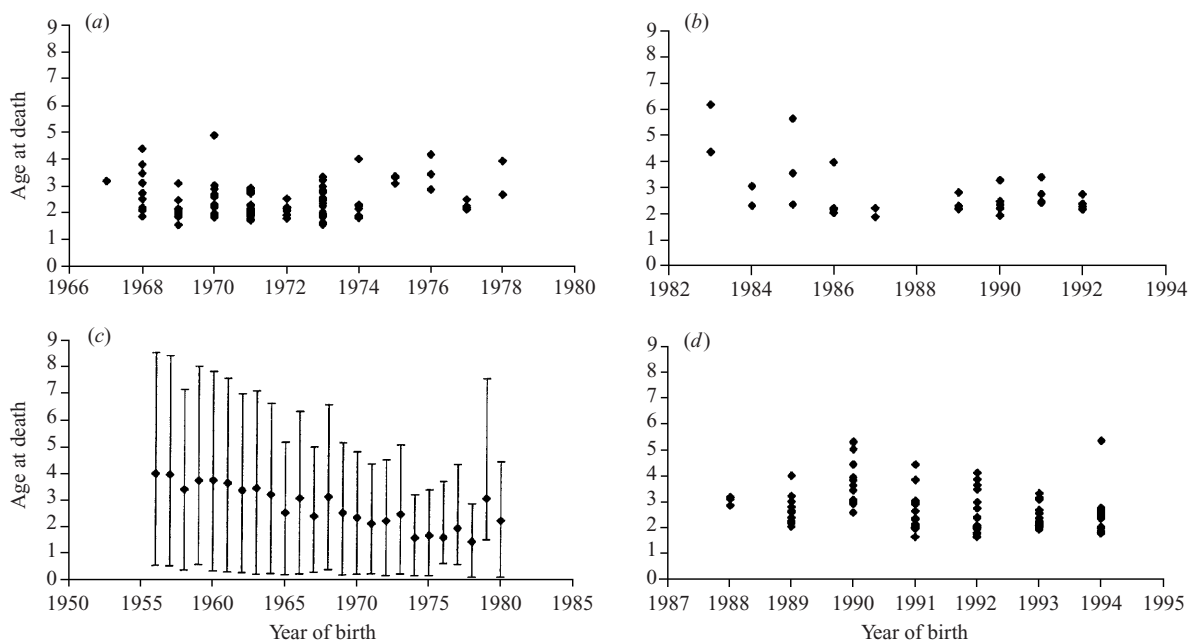

Fig. 5. Age at death (years) by birth cohort for all scrapie cases during each outbreak. (*a*)–(*d*) as for Figure 1. Ages at death show no statistically significant trend through time when compared to trends in ages at death for non-scrapie cases (> 1 year only) using analysis of covariance ($F_{1,1159} = 0.1$, $P = 0.77$; $F_{1,1026} = 1.0$, $P = 0.31$; $F_{1,1462} = 0.2$, $P = 0.63$; $F_{1,779} = 2.0$, $P = 0.16$ respectively for the cause of death by time interaction term).

which had a greater impact on the effective population size than the larger (but much more variable) proportions of ewes used for breeding. For two flocks there was information on PrP genotype. For the second NPU Cheviot flock the PrP alleles present were VRQ, ARQ, ARR and AHQ; 35 out of 49 genotyped scrapie cases were in VRQ/VRQ homozygotes, 14 in VRQ/ARQ heterozygotes and none in other genotypes. For the SAC Suffolk flock the alleles present were ARQ, ARR and ARH; 77 out of 79
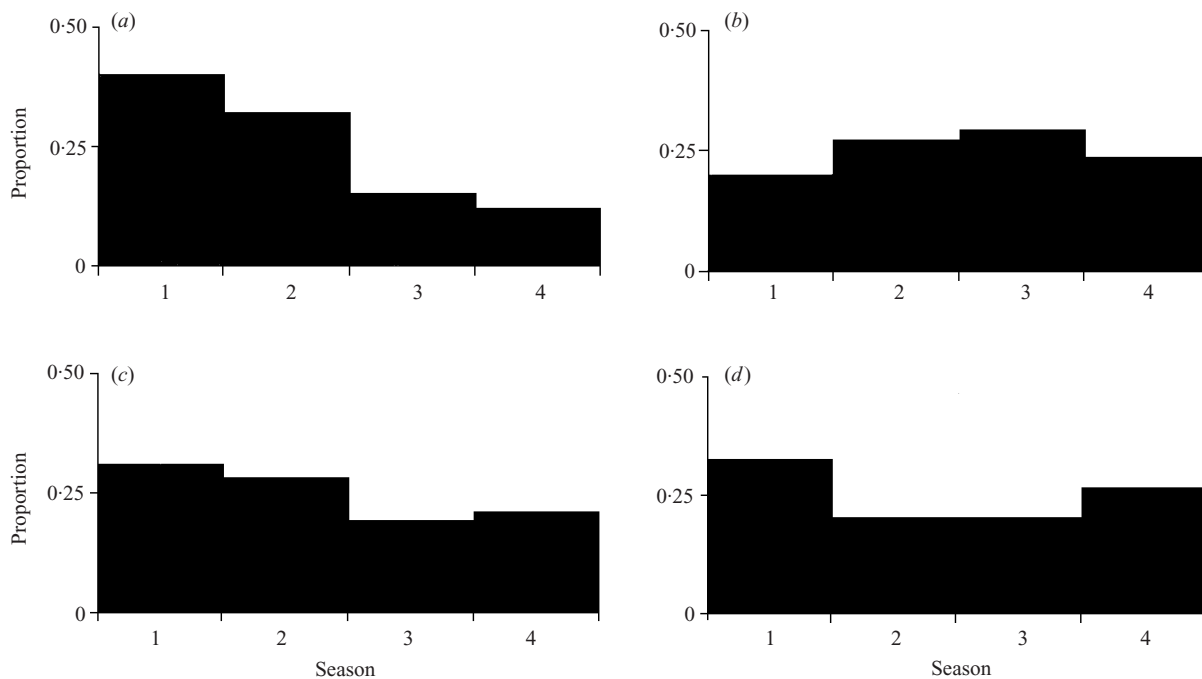
Fig. 6. Proportion of deaths due to scrapie by time of year (3-month intervals) for each outbreak. (a)–(d) as for Figure 1. Comparison of the numbers of cases by time of year compared to numbers of non-scrapie deaths gave $\chi^2_{(3)} = 77\cdot8$, $P < 0\cdot001$; $\chi^2_{(3)} = 5\cdot3$, $P = 0\cdot15$; $\chi^2_{(3)} = 19\cdot8$, $P < 0\cdot001$; $\chi^2_{(3)} = 13\cdot9$, $P = 0\cdot003$ for (a)–(d) respectively.

genotyped cases were in ARQ/ARQ homozygotes, 2 in ARQ/ARR heterozygotes and none in other genotypes.

In all four outbreaks, the risk of disease was significantly higher among sheep born to cases than non-cases (Table 2: all $\chi^2_{(1)} > 20$; all $P < 0\cdot001$). For 3 of the 4 outbreaks, the relative risk was greater among sheep born to ewes which became cases and rams which did not than among sheep born to rams which became cases and ewes which did not (Table 2), but the effect was significant only for the NPU Suffolk flock ($\chi^2_{(1)} = 13\cdot3$, $P = 0\cdot001$).

DISCUSSION

The results confirm that scrapie outbreaks have very long durations (years or decades) even in a single sheep flock and can cause very high mortality, especially in particular birth cohorts. Scrapie reduced mean life expectancy within the flocks by up to 1·6 years. The study, albeit based on only four outbreaks, does not suggest any systematic differences in the dynamics of scrapie outbreaks between Cheviots and Suffolks despite the differing genetics of susceptibility in the two breeds. However, there were other differences between the outbreaks.

The outbreaks showed very large differences in overall incidences (2–43 %). These differences could reflect differences in the transmission rate of the infection, differences in the fraction of sheep that were genetically susceptible, and differences in the demography. The latter applies particularly to the SAC Suffolks where the large number of lambs removed at less than 1 year old had no opportunity to develop clinical scrapie – incidences in older sheep were relatively higher (Fig. 4). For all outbreaks, the number of infections is expected to be greater than the number of cases because of the long incubation period (of the order of 2 years), which means that only a fraction of infected sheep survive to show clinical signs. This fraction is determined by the distribution of ages at infection, the distribution of the incubation period and the survivorship curve. It is therefore difficult to estimate precisely but rough calculations (assuming exponentially distributed survivorships and a fixed 2 year, incubation period) suggest that only in the SAC Suffolks was it likely to have been below 50 % and in some flocks, may have been as high as 70 %. It was the SAC Suffolks, however, that had a demography most similar to commercial flocks.

The differences between the incidence of scrapie in ewes and rams more than 1 year old found in two of the flocks are also of interest. It is possible that higher

incidences in ewes reflect a longer life expectancy than for rams (given their survival to 1 year). This difference is substantial (more than 1 year in all four flocks) and could account for relative differences in incidence of the order of 10–30%. However, it is likely that apparent differences between the sexes were compounded by differences in genetics, since breeding in all flocks was non-random and there were large differences in the pattern of use of rams and ewes for breeding (Table 1). Slightly higher incidence of scrapie was reported in ewes than in rams during an outbreak in a Romanov flock once genetic and demographic differences had been accounted for [10].

There are also differences between flocks in the age of scrapie cases. Again, several different factors could influence this: demography, differences in incubation periods (both mean and variance) between outbreaks, and the distribution of age at infection. The latter will at least partly reflect the overall incidence during the outbreak: sheep in flocks with high exposure to scrapie will, on average, become infected earlier than sheep in flocks with low exposure. However, a striking feature of the differences in ages of cases between outbreaks is that these are very small (range 2·4–2·9 years, with a mode consistently in the 2–3 year age class) in comparison with the very large differences in incidence (2–43%). There are several possible explanations. First, any effect may be confounded by differences in demography: indeed, the age-specific incidences do show more variability (Fig. 4), although the flock with the highest incidence shows peak incidence in an older, not younger, age class. On the other hand, there may be differences in the incubation period; this cannot be excluded, especially if different scrapie strains are involved in the outbreaks. Alternatively, it may be that incidences in genetically susceptible sheep are, in fact, very high so that all susceptible sheep do become infected when young; however, this interpretation is not consistent with the results of modelling studies of the NPU Cheviot outbreaks [9, 11]. Finally, it is possible that younger sheep are more susceptible (or more exposed) than older sheep, which would constrain the distribution of the age of cases: this is consistent with the observed decline in the age-specific incidence in older sheep, although older sheep can still become cases (Fig. 4). Not dissimilar patterns of age-specific incidence have been reported for BSE in cattle [19] and variant CJD in humans [20] and have also been interpreted as reflecting age-specific exposure or susceptibility. Another approach to this question is to look for changes in the ages of cases during the course of the outbreak. Mathematical models incorporating direct horizontal transmission and assuming lifelong susceptibility predict that the age of cases should fall during the early stages of an outbreak [17]. However, no such fall is apparent in the data presented here, once the trends are corrected for changes in background demography (Fig. 5), even if the age of case data alone do show a significant change [12]. This result is also consistent with there being a restricted age range where sheep are more susceptible [11], although there may be alternative explanations.

All four outbreaks also show evidence of seasonality in the distribution of scrapie deaths, which differs from the distribution of non-scrapie deaths (Fig. 6). The seasonal patterns, however, differ between outbreaks, and in a manner which does not reflect differences in the timing of lambing. Seasonality of cases is, therefore, not obviously related to demography and must presumably reflect seasonality in the risk of infection and/or in the progression to clinical disease. Any seasonality in the risk of infection is of considerable interest as it may indicate possible routes of transmission, which remain very poorly understood. There has been some discussion of the possibility that transmission is related to the time and method of lambing: this could only be consistent with the data presented here if there were also differences in incubation periods between outbreaks.

The association of scrapie with PrP genotype has been extensively discussed elsewhere [3, 4]. However, where PrP genotype data are absent or incomplete, it is still possible to relate the incidence of scrapie to the presence or absence of the disease in the parents. In these four outbreaks, there is a consistent association between one or both parents having scrapie and the offspring having scrapie (Table 2), which is expected given the known genetic factors. The same effect, including a tendency for cases to be more closely associated with scrapie in the dam than in the sire has been reported previously [2]. This observation is consistent with some transmission of the disease from ewe to lamb, either true vertical transmission or enhanced horizontal transmission associated with close contact. The latter result was found here for just 1 of the 4 flocks (Table 2). However, any association may well be confounded by differences in the demography and genetics of the sexes (see above) and a very detailed analysis would be needed to confirm or refute the possibility of low level vertical transmission – this is not attempted here. A higher risk of

scrapie was reported in lambs which had been suckled by their dams as compared with those reared artificially [10].

The biological and epidemiological processes underlying the patterns reported here remain poorly understood. These include the way in which scrapie is transmitted from infected sheep to susceptible sheep, why susceptibility or exposure might vary with age, why the incidence of scrapie cases is seasonal, the possibility of vertical transmission, the length the incubation period in natural infections and how this might vary within and between outbreaks and whether there is any effect of preclinical scrapie on mortality. These questions will continue to be best addressed by using a combination of experimental studies, field data and mathematical models to provide quantitative insights into the dynamics of this complex disease.

ACKNOWLEDGEMENTS

REFERENCES

1. Stamp JT. Scrapie: a transmissible disease of sheep. Vet Rec 1962; **74**: 357–62.
2. Hoinville L. A review of the epidemiology of scrapie in sheep. Rev Sci Tech Off Int Epiz 1996; **15**: 827–52.
3. Hunter N, Goldmann W, Foster JD, Cairns D, Smith G. Natural scrapie and PrP genotype: case-control studies in British sheep. Vet Rec 1997b; **141**: 137–40.
4. Dawson M, Hoinville LJ, Hosie BD, Hunter N. Guidance on the use of PrP genotyping as an aid to the control of clinical scrapie. Vet Rec 1998; **142**: 623–5.
5. Hoinville LJ, Hoek A, Gravenor MB, McLean AR. Descriptive epidemiology of scrapie in Great Britain: results of a postal survey. Vet Rec 2000; **146**: 455–61.
6. Hoinville LJ, Hoek A, Gravenor MB, McLean AR. Scrapie occurrence in Great Britain. Vet Rec 1999; **145**: 405–6.
7. Kao RR, Bravenor MB, Baylis M, et al. The potential size and duration of an epidemic of Bovine Spongiform Encephalopathy in British sheep. Science 2002; **295**: 332–5.
8. Schreuder BEC, van Keulen LJM, Vromans MEW, Langeveld JPM, Smits MA. Preclinical test for prion diseases. Nature 1996; **381**: 563.
9. Woolhouse MEJ, Matthews L, Coen P, Stringer SM, Foster JD, Hunter N. Population dynamics of scrapie in a sheep flock. Philos T Roy Soc B 1999; **354**: 751–6.
10. Elsen JM, Amigues Y, Schelcher F, et al. Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov. Arch Virol 1999; **144**: 431–45.
11. Matthews L, Coen PG, Foster JD, Hunter N, Woolhouse MEJ. Population dynamics of a scrapie outbreak. Arch Virol 2001; **146**: 1173–86.
12. Foster JD, Dickinson AG. Age at death from natural scrapie in a flock of Suffolk sheep. Vet Rec 1989; **125**: 415–7.
13. Hunter N, Foster JD, Goldmann W, Stear MJ, Hope J, Bostock C. Natural scrapie in a closed flock of Cheviot sheep occurs only in specific PrP genotypes. Arch Virol 1996; **141**: 809–24.
14. Hunter N, Moore L, Hosie BD, Dingwall WS, Greig A. Association between natural scrapie and PrP genotype in a flock of Suffolk sheep in Scotland. Vet Rec 1997a; **140**: 59–63.
15. Simm G, Lewis RM, Grudy B, Dingwall WS. Responses to selection for lean growth in sheep. Anim Sci 2001. In press.
16. Stringer SM, Hunter N, Woolhouse MEJ. A mathematical model of the dynamics of scrapie in a sheep flock. Math Biosci 1998; **153**: 79–98.
17. Woolhouse MEJ, Stringer SM, Matthews L, Hunter N, Anderson RM. Epidemiology and control of scrapie within a sheep flock. Proc R Soc London B 1998; **265**: 1205–10.
18. Armitage P, Berry G. Statistical methods in medical research, 2nd edn. Cambridge: Blackwell Scientific Press, 1987.
19. Anderson RM, Donnelly CA, Ferguson NM, et al. Transmission dynamics and epidemiology of BSE in British cattle. Nature 1996; **382**: 779–88.
20. Ghani AC, Ferguson NM, Donnelly CA, Hagenaars TJ, Anderson RM. Epidemiological determinants of the pattern and magnitude of the vCJD epidemic in Great Britain. Proc R Soc London B 1998; **265**: 2443–52.