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Modelling the spread of scrapie in a sheep flock: evidence for increased transmission during lambing seasons

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Summary. Presence of scrapie infectivity in the placenta suggests the possibility of increased transmission of scrapie during the lambing season. This hypothesis was explored here using a mathematical model of scrapie transmission dynamics which has previously been successfully used to study several scrapie outbreaks in Scottish sheep flocks. It was applied here to the Langlade experimental sheep flock (INRA Toulouse, France), in which a natural scrapie epidemic started in 1993. Extensive data were available, including pedigree, scrapie histopathological diagnoses and PrP genotypes. Detailed simulations of the scrapie outbreak reveal that the observed patterns of seasonality in incidence can not be accounted for by seasonality in demography alone and provide strong support for the hypothesis of increased transmission during lambing. Observations from several other scrapie outbreaks also showing seasonal incidence patterns support these conclusions.

Introduction

Scrapie is a naturally occurring transmissible spongiform encephalopathy of sheep. The disease is associated with a conformationally abnormal form of the prion protein PrP. Polymorphisms of the PrP gene encoding for this protein largely control the susceptibility and resistance of sheep to the disease [14]. The epidemiology of scrapie and particularly the transmission mechanisms are still incompletely understood [12]. Vertical transmission, i.e. maternal transmission to lamb, is thought to occur although it is possible that contamination occurs post-natally rather than in utero [2]. Horizontal transmission is likely to occur by the oral route since the earliest detection of scrapie infectivity in naturally infected individuals is in the digestive tract (infection of the Peyer's patches followed by replication in the gut-associated lymphoid tissues and spread to the central nervous system) [1]. Consequently, the presence of scrapie infectivity in the placenta [19] suggests the possibility of increased transmission during lambing via the ingestion of contaminated placental material.

Seasonality in scrapie incidence has been observed in several scrapie outbreaks, but the seasonal patterns do differ between outbreaks and a consistent explanation for this phenomenon has not yet been identified [20]. In this paper, we examine whether seasonality in transmission of scrapie (via increased transmission during lambing) can explain the observed pattern of seasonality in incidence in a natural scrapie outbreak in the Langlade experimental sheep flock.

In the past few years the development of mathematical models of the flock-to-flock spread of scrapie has provided a valuable tool with which to assess the force of infection and the efficiency of surveillance and control strategies [15, 9, 13]. Modelling in more details the dynamics of the within-flock transmission of scrapie has allowed us to improve our understanding of scrapie susceptibility, transmission and pathogenesis [17, 10, 11, 16]. In diseases such as scrapie with long incubation periods relative to lifespan, the epidemiology cannot be fully understood without reference to flock demography and management. Moreover, the confounding effects of incubation period, lifespan, age-dependent susceptibility and the changing force of infection make direct analyses of the case data difficult. A mathematical model of transmission dynamics provides a flexible tool for combining

epidemiological, demographic, genetic and management data, and exploring through simulation the consequences of alternative biological scenarios.

Here, we consider an outbreak of scrapie in the Langlade experimental sheep flock in which a natural scrapie outbreak started in 1993 and for which extensive demographic, genetic and scrapie case data are available. Several experiments and observations were conducted in this flock to study the scrapie pathogenesis [1, 2, 3], the PrP [7] and non PrP genetic susceptibility [18]. The data were also analysed to determine individual performances in a scrapie-affected flock [5]. Few modelling approaches were applied to these data. They were based on survival analysis, to assess the genetic susceptibility and transmission factors [7], as well as polygenic variation in the resistance to scrapie [6]. In this paper, we fit a model of transmission dynamics to the Langlade data set in order to explore and explain the natural scrapie incidence patterns observed.

We consider two alternative scenarios, one in which horizontal transmission occurs throughout the year, and a second, “seasonal model”, in which horizontal transmission is confined to the lambing period. Model outputs are compared to data on scrapie incidence, age at clinical onset, flock genetics and genotypes of scrapie cases. We also examine three additional data sets from outbreaks in Scottish sheep flocks for evidence of a connection between the timing of lambing and of scrapie deaths. The data and the model are described in the following sections, then the results are presented and discussed.

Field data

Langlade flock

The Langlade experimental sheep flock was founded in 1971 by INRA near Toulouse. It is attached to the SAGA, *Station d’Amélioration Génétique des Animaux*, and is used as a tool to study the genetics of reproduction, the resistance to internal parasites and to compare sire breeds. Before 1996, most animals belonged to the Romanov breed, a prolific breed with a mean litter size of 3.1 in adults, ranging between 1 and 6 lambs. From 1979 to 1996, the Romanov flock was composed of 600-800 ewes and was genetically closed.

A natural scrapie outbreak started in the flock in 1993 and has been studied ever since. It is probable that the disease was introduced in the flock by animals from a particular cohort (born in October 1991) involved in parasitological experiments, although no formal association could be found between the parasites and the onset of scrapie [7]. It constitutes a remarkable scrapie cluster: this cohort was the first to show scrapie clinical signs and all individuals had been removed by August 1993.

Extensive data are available on sheep born between 1983 and 2001. Pedigree data include: sex, breed, date of birth, date of death, reason for death, sire and dam ID. The PrP genotypes of most breeding animals since 1993 are also known. Genetic susceptibility to scrapie is determined by polymorphisms of the PrP genotype at codons 136, 154 and 171. Four alleles were identified in the flock: VRQ, ARQ, AHQ and ARR. Cases have been observed in eight of the ten resulting genotypes (all except AHQ/ARR and ARR/ARR), but the majority of cases occur within the three most susceptible genotypes: VRQ/VRQ,

ARQ/VRQ and ARQ/ARQ. When scrapie was suspected, the diagnosis was confirmed by a histopathological test [7].

As the flock management changed over the course of time, this study was conducted on a shorter 4-year period, from January 1992 until January 1996. The period covers the introduction of the animals involved in the parasitological experiment into the flock, to the time when breeding practices changed. No selection scheme on the PrP genotype was conducted prior to 1996, so breeding and culling were *a priori* independent of the PrP genotype.

Far more ewes than rams were maintained in the flock, so only female Romanovs were considered in this study. Furthermore, many lambs were culled under the age of 8 months, as only a fraction of the population was retained for breeding purposes. It was assumed that these lambs did not play a major role in the transmission of scrapie within the flock, and so they were also excluded from the analysis. The study flock therefore corresponds to the Romanov replacement ewes. During the 4 years of this study, replacement ewes were produced once a year during a short lambing period of 40 days in January-February.

Scottish flocks

In addition, data from three Scottish flocks, where evidence of seasonal incidence of scrapie was observed [20], were used to examine the relationship between the distribution of births (lambing) and the distribution of scrapie deaths in each flock.

Modelling the Langlade flock

The deterministic mathematical model on which this study is based is detailed elsewhere [21]. Only a summary outlining the main changes and developments is given here (see Appendix for an overview of the mathematical model).

In the model, the flock is structured according to scrapie status (susceptible or infected), PrP genotype, sheep age and, for the infected sheep only, transmission route (horizontal or vertical) and infectiousness. The infectiousness is a measure of the relative capacity of an infected animal to contaminate a susceptible one. When a susceptible sheep becomes infected, it is given an initial infectiousness, which is assumed to grow during the incubation period until clinical signs appear and the sheep is culled (see Pathogenesis). The model does not discriminate by sex; since only a small number of breeding rams are kept, we consider only the population of ewes.

Time, age and infectiousness are continuous variables in the model. Hence, to represent the evolution through time of the resulting population densities (e.g. $H_g(t, a, i)$, the horizontal infected sheep of genotype g , age a , infectiousness i at time t) with respect to age and infectiousness, the model consists of a set of partial differential equations.

The model incorporates the following components: seasonal breeding and routine culling, genetic susceptibility, a long and variable incubation period, and (seasonal) horizontal and vertical transmission. A schematic representation is given in Fig. 1.

When possible, parameters were directly estimated from the Romanov data restricted to the study flock, i.e. to the replacement ewes present at Langlade between January 1992

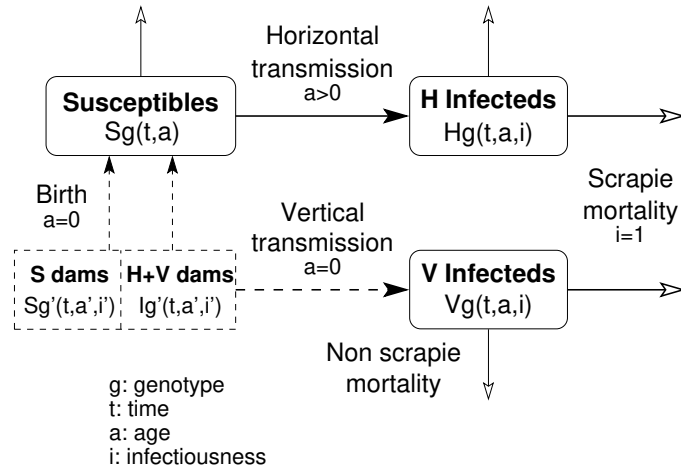


Fig. 1. Representation of the flock structure and processes included in the model

and January 1996.

Simulations were performed using a Fortran program. For numerical stability of the seasonal model, the previously used centred integration scheme (Lax-Wendroff [22, 16]) was replaced with an upwind integration scheme (Godounov) which respects the causality of the system.

Culling

Sheep are routinely culled. Replacement ewes at Langlade were selected according to various characteristics (e.g. milk production, fecundity) so as to ensure the continuation of the flock. The remaining lambs were culled prior to 8 months of age and are not represented in the model. The older and less fertile ewes were also culled; some sheep died as a result of disease, accidents etc. All these causes with the exception of scrapie are incorporated into the mortality rate which is assumed to vary only with age. This rate is based on a Weibull risk function (Fig. 2) that was fitted to the study flock data, and corresponds to a mean life expectancy in the absence of scrapie of 5.60 years with a maximum lifespan of 10 years. In the model a maximum lifespan of 13 years (the longest lifespan observed in this flock) was allowed, as some of the older animals were still alive at the end of the study period.

Seasonal breeding

Breeding at Langlade was controlled. Ewes produced replacement animals from their second mating onwards; the lambing took place once a year during a 40-day period in January-February. This lambing period is reproduced in the model and for the remainder of the year, no animals are born. The birth rate is assumed to be the same for all mature ewes. It is automatically adjusted so that the total flock size at the end of the lambing period attains a predefined target value based on the data.

The birth rate determines the number of new lambs arriving in the flock during the

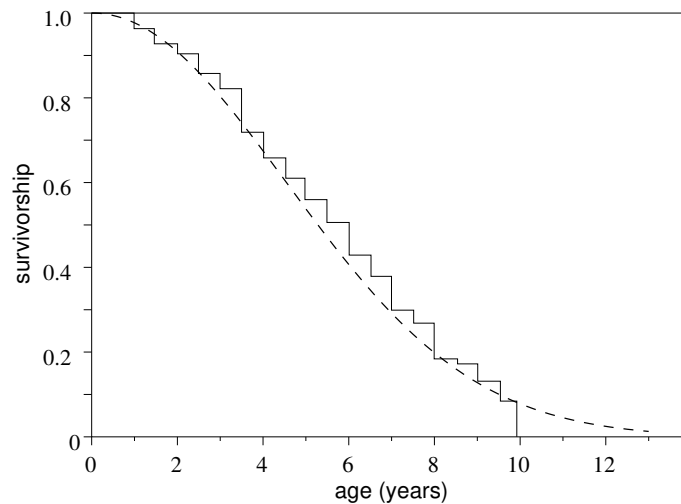


Fig. 2. Survivorship curves based on the 1986-1995 birth cohorts: Kaplan Meier curve (solid line) and Weibull fitted curve (dashed line) with parameters shape=2.04 and scale=6.32. Ewes that died before January 1, 1992 (beginning of the study period) were left truncated and ewes still alive on January 1, 1996 (end of the study period) were right censored. The raw data and fitted function correspond to mean life expectancies 5.46 and 5.60 years respectively

lambing season. As only the female population is considered in this model, the genotypes of these lambs are deduced from the dam genotypes only. They are assigned according to a random mating hypothesis, which can be summarised as follows: the allele frequencies in dams and lambs are the same at each lambing, and lamb genotypes represent a random recombination of these alleles. It corresponds to a population with no allele selection at matings and is therefore related to the Hardy-Weinberg equilibrium. However, selection is likely to occur throughout the year due to scrapie cases.

To test this random mating assumption, χ^2 tests were used to compare at each lambing period: (i) the allele frequencies of the dams and of their female lambs that were subsequently used for replacement; (ii) the theoretical, i.e. computed from a random recombination of the allele frequencies, and observed lamb genotype frequencies; (iii) the allele frequencies of the dams which gave birth to only one replacement ewe and of those which had more than one. With the exception of one lambing season for (i), the frequencies are not significantly different ($p > 0.05$). This shows that the data are generally in accordance with the hypothesis of random mating (i) and recombination (ii). It also confirms that having the same birth rate for all mature ewes won't have a major impact on the lamb allele frequencies (iii).

The genotype information for the few rams used in the Langlade flock was available and could have been introduced as an exogenous input in the model, but the birth rate implemented homogenises the matings in the model and the random mating hypothesis is

Table 1. Relative genetic susceptibilities estimated as the relative scrapie incidence for each genotype in the Romanov study flock (replacement Romanov ewes present at Langlade between January 1992 and January 1996)

Genotype	VRQ	ARQ	ARQ	AHQ	ARR	AHQ	ARQ	AHQ	AHQ	ARR
	VRQ	VRQ	ARQ	AHQ	VRQ	ARQ	ARR	VRQ	ARR	ARR
Susceptibility	0.803	0.606	0.403	0.125	0.078	0.062	0.029	0.020	0	0

verified. Therefore, the ram information should not notably change the model results.

Genetic susceptibility

Genetic susceptibility to scrapie is determined by the PrP genotype, defined in the Romanov flock as a combination of the following four alleles: VRQ, ARQ, AHQ and ARR. As cases have been observed in all but two of the ten corresponding genotypes, the alleles AHQ and ARR were not aggregated in this study as had been done previously [22, 16]. The relative susceptibilities by genotype used in the model were estimated from the study flock data and are presented in Table 1. They correspond to the relative scrapie incidence computed within each genotype during the study period.

Seasonal or non seasonal transmission

Vertical transmission in the model corresponds to in utero or perinatal maternal transmission to lamb. By definition, it only occurs during the lambing periods, which are seasonal here. To test the hypothesis of increased horizontal transmission during lambings, we considered two model variations: one in which horizontal transmission occurs throughout the year, and a second, “seasonal model”, in which horizontal transmission is confined to the lambing period. The only difference between the two models resides in the horizontal transmission rate.

Horizontal transmission is assumed to be proportional (i) to the number of available susceptible individuals weighted by their relative susceptibilities (cf. Table 1), and (ii) to the sum of all infected animals weighted by their infectiousness. It is set to zero outside the lambing period for the seasonal model only. Vertical transmission similarly depends on the relative susceptibilities of the lambs and the number of infected breeding ewes weighted by their infectiousness. Absolute transmission rates for each route are determined by two scaling factors which need to be estimated. The horizontal scaling factor were fixed independently in each model, to allow for two very different horizontal transmission periods.

Pathogenesis

Once an individual is contaminated, the development of the disease is described in the model by means of the infectiousness. The initial infectiousness for a newly vertically or horizontally infected animal is assumed to follow a gamma distribution, which is independent of genotype. Infectiousness then increases exponentially up to a level corresponding to clinical signs, at which point sheep are removed from the flock.

The mean initial infectiousness is set at 10% of the terminal infectiousness (chosen arbitrarily to be 1), which means that an infected sheep is ten times more infectious at the end of the incubation period than at the beginning. The mean of the gamma distribution is therefore fixed (to 0.1), but the variance still needs to be estimated.

Because the initial infectiousness follows a distribution rather than being set at a fixed value, a corresponding distribution of incubation periods is obtained. We consider here a population model, so we do not draw an initial infectiousness value for each individual, but we distribute the newly infected population along the infectiousness values according to the associated probability density function; the model remains fully deterministic. The exponential rate of increase of the infectiousness determines the mean length of the incubation period and also requires estimation. This rate is assumed to be the same for all genotypes. Generally, the most susceptible PrP genotypes are associated with the shortest incubation periods [7, 4]. The susceptibility of these genotypes depends on the scrapie strain and the host breed [8, 4, 6], so it is difficult to deduct from previous studies quantitative information on incubation periods. Moreover, in natural scrapie outbreaks the infection dates are not observed, so the effect of genotypes on survival rather than on incubation can be analysed [8, 4, 6]. The age-at-death of scrapie infected animals in the Romanov study flock are fairly similar across the most susceptible genotypes. Therefore, we chose here not to introduce genetic variations in the incubation period in the model, a simplifying assumption that is not inconsistent with the data.

Model fit

The cluster of scrapie cases in the October 1991 cohort involved in the parasite experiments makes this group of animals a good candidate for the primary infections in the flock. Accordingly, a corresponding fraction of the initial population, in terms of age and genotype distributions, was assumed to be infected at the beginning of the simulation.

The demographic parameters were directly estimated from the Romanov data. However, apart from the relative genetic susceptibilities, the parameters associated with scrapie transmission cannot be easily derived from the data. Therefore, a simulation-based approach was taken, whereby the incubation period parameters and transmission scaling factors were adjusted simultaneously in order to achieve the observed total number of cases and shape of epidemic curve during the study period.

To get a rough estimate of the proportion of cases occurring by maternal transmission, the population attributable risk percentage was computed in the Romanov flock, the risk factor being defined as having a scrapie affected dam [12]. The value obtained (PAR%=13.7) cannot be considered as an accurate quantitative estimate, but it gives an indication. So the vertical transmission scaling parameter was selected in order to obtain

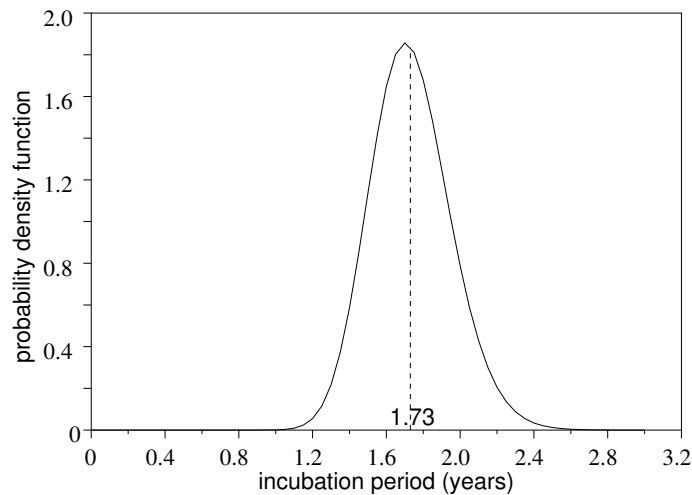


Fig. 3. Curve representing the probability density function of the incubation period distribution (mean 1.73, variance 4.7×10^{-2}), derived from the gamma distribution associated to the initial infectiousness (mean 0.1, variance 8.3×10^{-4}). The incubation distribution is deduced from the gamma distribution through a transformation depending on the exponential rate of increase of the infectiousness (1.35 years^{-1})

10-15% of cases by this route during the first years of the epidemic.

The observations in the flock are the clinical scrapie cases. Therefore, the incubation/infectiousness and transmission parameters in the model were selected as the “best fit” for the scrapie incidence data. More precisely, the transmission scaling factors were set so as to get the exact total number of cases over the whole period. Extensive numerical simulations were performed to determine the infectiousness and incubation parameters that best followed the distribution of cases over time and over genotypes; the parameter range explored (mean initial infectiousness set to 0.1; variance between 0.01 and 0.0005; exponential rate of increase between 0.9 and 2.3 years^{-1}) reflected realistic incubation periods (between 1 and 2.5 years).

The horizontal transmission scaling factor was estimated independently for the seasonal and the non seasonal transmission models (see Seasonal or non seasonal transmission). To be able to compare both models, all other parameters were given the same values. If this is expected for the data derived parameters, it is less so for the incubation period parameters: as no values could be found to get a better fit for the non seasonal model, the seasonal model estimates were kept for both. The best fit for these parameters was obtained with a narrow gamma distribution for the initial infectiousness, corresponding to a mean incubation period of 1.73 years (Fig. 3). The horizontal transmission scaling factors obtained were 0.016 for the seasonal model and 0.0018 for the non seasonal model (vertical transmission factor: 1.0).

The sensitivity of the model outputs to several key factors was examined: length of lambing season (double length up to 6 months, and the whole year); mean of incubation period (1 to 2.5 years); increased variance of initial infectiousness (twice to 6 times the initial variance) and correspondingly of incubation period; absence of vertical transmission; higher mortality rates (life expectancy of 3.6 years instead of 5.6 years); and age-dependent susceptibility. For this last factor, scrapie susceptibility is assumed to decline exponentially with age (rate: -0.5 year^{-1}); in [16], a step function was chosen (animals are susceptible up to a maximum age and then become resistant), but it seemed less appropriate in this case as scrapie deaths were observed for old animals in the Romanov flock. As these parameters were varied independently, this study does not qualify as a full sensitivity analysis. However, most factors chosen correspond to the incubation and transmission processes, about which little is known, so it allows us test the robustness of our results.

Results

Simulations were performed for the two models: the seasonal model, in which horizontal transmission is confined to the lambing periods, and the non seasonal model, in which it occurs throughout the year. They only differ in their horizontal transmission rate.

Model outputs and data are shown in Fig. 4-7. Fig. 4 shows the scrapie case data (bars), and the simulated course of time of scrapie incidence for the seasonal (solid line) and non seasonal (dashed line) model versions. Transmission rates in both models were set so that the total number of scrapie cases obtained in the simulations matched the data (247 animals). Both scenarios reproduce a time course of peaks and troughs in the data (as a result of seasonality in births) but the seasonal model produces more marked peaks which correspond far better in timing to the observed peaks in the incidence data.

Fig. 5 shows the observed age distribution of cases (bars) and output for the seasonal model (solid line). The overall trends in the distribution are captured but the initial peak corresponding to sheep infected close to birth is underestimated.

The frequency distribution of cases by genotype produced by the seasonal model (bars) shown in Fig. 6 is in excellent agreement with the data (thick line). Fig. 7 shows the time course of the allele frequencies in the flock (seasonal model: lines, data: symbols). Overall trends are reproduced but the decline in frequency of the two most susceptible alleles, VRQ and ARQ, and consequently the increase of the more resistant alleles is less than that observed.

Further simulations were conducted to assess the sensitivity of the above results to a number of factors. The incidence patterns described above were quite robust to the length of the lambing season in the model. In contrast, increasing the variance of the incubation period tended to smooth the annual peaks observed in Fig. 4. Simulations performed without vertical transmission were qualitatively similar to those presented above, but, as might be expected, lowered the incidence peak in younger animals observed in Fig. 5. Implementing age-dependent susceptibility did not improve the overall model fit; a higher peak at younger ages in the age distribution of cases was obtained but the incidence patterns

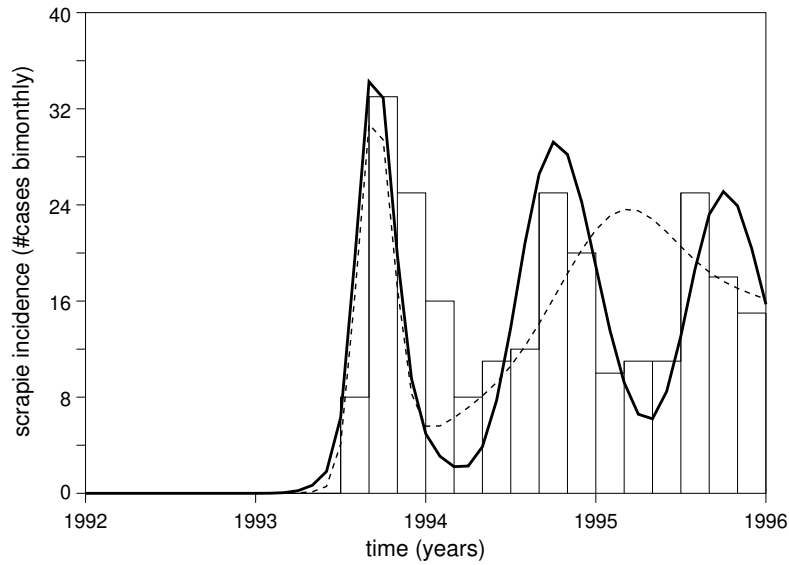


Fig. 4. Comparison between the observed (bars) and simulated (solid line) scrapie case distribution over time; the simulation was performed with the seasonal transmission hypothesis. Comparison with the non seasonal transmission case (dashed line), all parameters except the horizontal transmission scaling factor being equal (incidence in number of cases per 2-month time period)

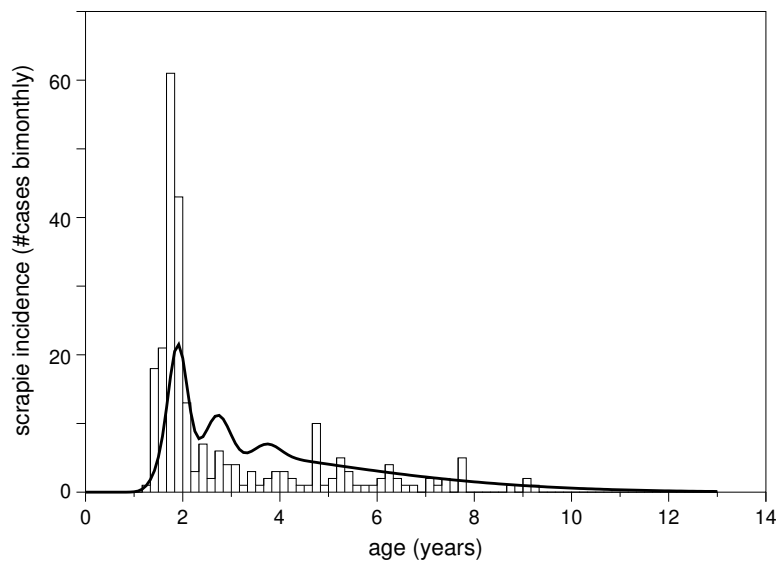


Fig. 5. Comparison between the observed (bars) and simulated (solid line) age distribution of scrapie cases; the simulation was performed with the seasonal transmission hypothesis (incidence in number of cases per 2-month age class)

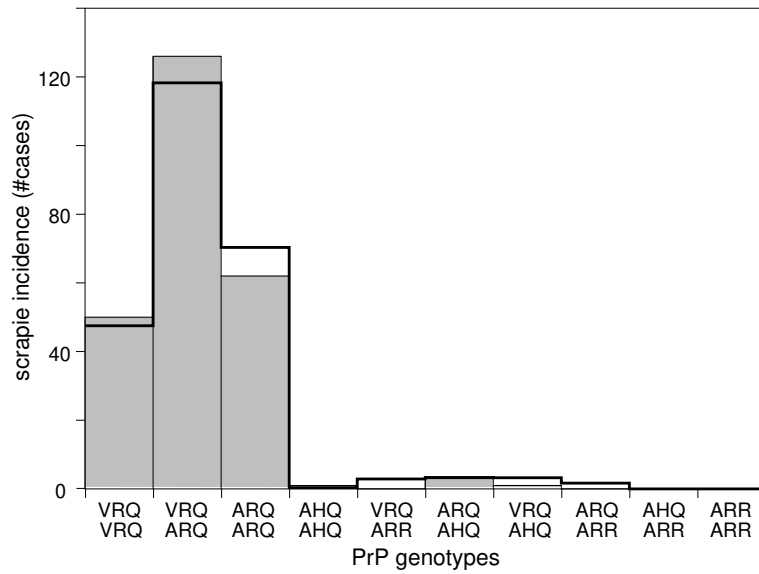


Fig. 6. Comparison between the observed (grey bars) and simulated (thick line) genotype distribution of scrapie cases; the simulation was performed with the seasonal transmission hypothesis (incidence in number of cases per genotype)

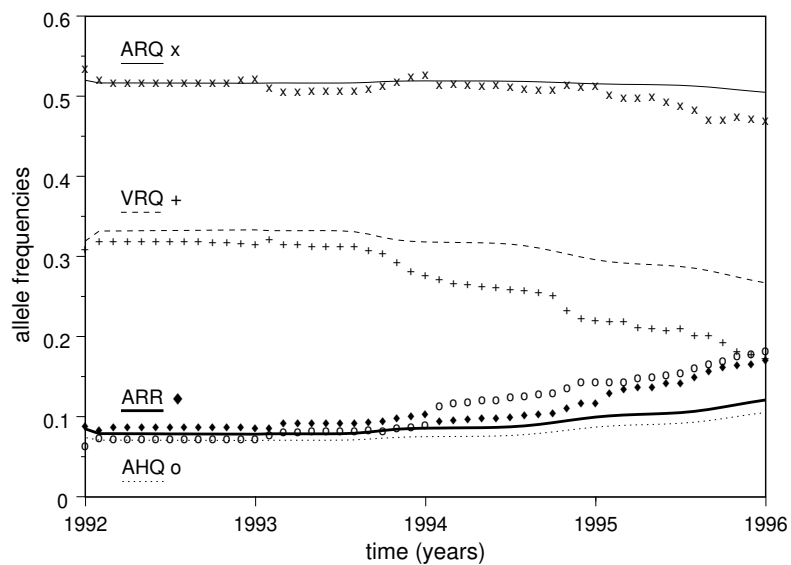


Fig. 7. Comparison between the observed (symbols) and simulated (lines) allele frequencies over time: VRQ (dashed line, + symbol), ARQ (solid line, x symbol), AHQ (dotted line, o symbol), ARR (thick line, ♦ symbol); the simulation was performed with the seasonal transmission hypothesis

Table 2. Comparison of the pattern of lambing and scrapie deaths for the Langlade Romanov flock and 3 Scottish flocks

Flock ^a	Cohorts	Lambing		Scrapie deaths		
		Distribution	GC ^b	No.	Peak in	GC
INRA Romanov	1983-95	January-February 71% in January	0.87	291	Oct.-Nov.	0.33
SAC Suffolk	1988-94	January-April 90% in January	0.90	108	Dec.-Jan.	0.34
NPU Suffolk	1956-80	January-June 53% in March	0.77	710	March	0.18
NPU Cheviot	1967-78	March-June 77% in April	0.86	137	March	0.30

^a“Flock” corresponds to the organisation in charge of the flock and the sheep breed.

^bThe Gini coefficient (GC) is an index of inequality defined as twice the area between the curve created from a plot of the cumulative percentage of births or deaths and the diagonal line (representing uniform distribution); it takes values between 0 and 1, with larger values indicating greater variability.

matched observations less well. Finally, a higher culling rate generated less scrapie cases, but a relatively higher incidence peak in younger animals.

Comparison with Scottish flocks

Data on lambing period and peak in scrapie deaths from the Langlade Romanov flock and three Scottish flocks are shown in Table 2. For each flock, the degree to which both the lambing period and the scrapie deaths are concentrated in particular months was calculated using the Gini coefficient.

For all four flocks the peak in scrapie incidence occurs shortly before the lambing season, consistent with a mean incidence period of just below 2 years. As can be readily observed in Table 2, greater Gini coefficients for lambing correspond to greater Gini coefficients for scrapie deaths. A Spearman rank correlation was used to look at the strength of the association between the two coefficients and demonstrated that the Gini coefficients are perfectly correlated ($\rho = 1$, one-tail, $p < 0.001$).

Discussion

In this paper we have presented a detailed model of scrapie transmission dynamics within a flock of Romanov sheep. The model successfully reproduces key features of the outbreak including seasonal variation in incidence, age-related patterns of incidence, the distribution of genotypes amongst scrapie cases and the decline in susceptible genotypes during the course of the outbreak.

Specifically, we have used this model to explore the hypothesis of increased trans-

mission during lambing which might result from ingestion of scrapie-infective placental material. The model was used to consider two extreme scenarios, one in which horizontal transmission occurs throughout the year and a second, seasonal model, in which horizontal transmission occurs only during the lambing period.

Our results clearly demonstrate that the assumption of restricted horizontal transmission in the model produces a time course of incidence which matches far better the seasonal patterns in the incidence data. However, our results cannot exclude the possibility of some small amount of transmission occurring outside the lambing period which would be difficult to observe. Further simulations have shown that these results are quite robust to the length of the lambing season, but that the seasonal pattern of scrapie cases is lost as the incubation period distribution becomes more variable.

Although no age-dependent susceptibility was incorporated in the model, the age distribution of scrapie cases displays a strong peak at young ages due to high levels of exposure from birth. However, the height of peak is underestimated by the model, possibly due to increased susceptibility in younger animals (though simulations were not conclusive). The use of a continuous age distribution in the model, which does not reproduce the population cohort structure exactly, might also have the effect of smoothing the curve. Finally, due to some difficulties in simulating year-to-year variations in flock management, the number of newborns was globally underestimated by the model which therefore reduced the potential number of young sheep exposed to scrapie in the flock. In support of this hypothesis, simulations that were run with a higher mortality rate, which is compensated in the model by a higher birth rate to maintain flock size, did generate a correspondingly higher incidence age peak.

The number of scrapie cases per genotype is extremely well reproduced by the model. It can a priori be expected, as the relative genetic susceptibilities were derived from the same data set the model is fitted to, and provided that the incubation period does not vary much with the genotype. However, the simulated genotype frequencies in the flock do not follow the data very closely, so this result is particularly robust.

Therefore, it seems likely that the discrepancy between model and data in the time evolution of the allele frequencies is due to sheep that did not show clinical signs of scrapie. Since the mortality rate of these animals is assumed to depend neither on their genotype, nor on their scrapie status, one possible explanation is that the selection of replacement ewes was not entirely independent from genotype. The discrepancy could also be due to the ram selection, which could have favoured the susceptible alleles. Both these arguments are not entirely satisfying though, because the hypothesis of Hardy-Weinberg equilibrium between dams and lambs was globally verified on the data. However in this case, the use of ram genotypes might have reduced the distance between model and data.

Another interesting possibility is that the decline in the frequencies of the most susceptible alleles is underestimated as a consequence of additional mortality due to pre-clinical scrapie in these individuals. Further evidence supporting this claim can be found in Chase-Topping et al. [5].

The incubation period of 1.73 years estimated for the Romanov outbreak is slightly shorter than previous estimates, for example the 1.9 year incubation period estimated for

the NPU Cheviot flock [22]. This could be explained by the particularly high force of infection in the early stages of the outbreak or by genotype dependency in the incubation period. Evidence supporting such differences in the incubation period has come from both experimental [8] and recent modelling work [16]. In the Romanov data, we found differences in the observed distributions of age at scrapie death amongst the three most susceptible genotypes, which would also tend to support the existence of genotype dependency. Consequently, the time evolution of the flock genotype frequencies could change the observed mean incubation, and a longer study period might have given rise to a higher mean incubation period.

The detailed analysis of the Romanov outbreak has provided evidence to support the hypothesis of increased scrapie transmission during the lambing period. Furthermore, analyses of summary data from three additional Scottish outbreaks allowed us to identify patterns of incidence common to the four flocks, which also support this conclusion: seasonality in scrapie incidence; an incidence peak occurring shortly before the lambing peak; and a strong correspondence between clustering of the lambing period into a short time period and clustering of scrapie deaths. Let us note that the peak of scrapie cases happens outside the lambing season in Langlade (cf. Table 2): lambs are born in January-February, while clinical signs are mostly detected in October-November. Therefore, the seasonal patterns observed in the onset of scrapie cannot be due to stress associated to lambing or to closer observation at that period in the Romanov flock.

This study has highlighted two points of general importance when conducting studies of this type of outbreak: first, the need to incorporate management and demographic data into epidemiological models, without which transmission dynamics cannot be fully understood; and second, the value of mathematical models as a flexible tool for combining complex sets of data inputs and exploring the consequences of alternative biological scenarios.

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APPENDIX

Overview of the mathematical model

The dynamics at time t of susceptible sheep $S_g(t, a)$ of age $a > 0$ and genotype g is given by equation (1), where $\mu(a)$ represents the non scrapie mortality rate (estimated from data), and $\beta_g(t, i')$ the horizontal transmission rate corresponding to infected sheep $I_{g'}(t, a', i')$ of age a' , infectiousness i' and genotype g' . Equation (1') describes the birth of susceptible lambs, where $G_{gg'}(t)$ represents the probability for a ewe of genotype g' to get a lamb of genotype g , $b(t, a')$ the birth rate for ewes of age a' , and $\gamma_g(i')$ the proportion of infected lambs born from infected ewes

$I_{g'}(t, a', i')$.

$$\frac{\partial S_g}{\partial t} + \frac{\partial S_g}{\partial a}(t, a) = -\mu(a)S_g(t, a) - S_g(t, a) \sum_{g'} \int_0^A \int_0^1 \beta_g(t, i') I_{g'}(t, a', i') di' da' \quad (1)$$

$$S_g(t, 0) = \sum_{g'} G_{gg'}(t) \int_0^A b(t, a') S_{g'}(t, a') da' + \sum_{g'} G_{gg'}(t) \int_0^A b(t, a') \int_0^1 (1 - \gamma_g(i')) I_{g'}(t, a', i') di' da' \quad (1')$$

If a' is a mature age (i.e. $a' \in [a_1, a_2]$ reproductive age range), $b(t, a') = b(t)s(t)$, where $s(t)$ is a seasonal function (1 during lambing seasons, 0 outside), and $b(t)$ is a control term that determines the population size. $G_{gg'}(t)$ depends on the dam allele frequencies at time t . $\gamma_g(i') = k_v \sigma_g i'$, where σ_g represents the relative susceptibility of genotype g (estimated from data) and k_v the vertical transmission scaling factor. Similarly $\beta_g(t, i') = k_h \sigma_g i' [s(t)]$, $s(t)$ being there only for the seasonal transmission hypothesis.

The infected sheep $I_g(t, a, i)$ are split according to their contamination route: $H_g(t, a, i)$ for the horizontal route and $V_g(t, a, i)$ for the ‘‘vertical’’ one (in utero or perinatal). Their dynamics are given by equations (2) and (3), where $\Phi(i)$ represents the initial infectiousness distribution (gamma distribution), and c the exponential rate of increase of the infectiousness ($\frac{di}{dt} = ci$). Equation (3') describes the birth of infected lambs, which by definition only occurs by vertical transmission. Moreover, infected sheep have a positive infectiousness, hence boundary conditions (2', 2'', 3'').

$$\frac{\partial H_g}{\partial t} + \frac{\partial H_g}{\partial a} + \frac{\partial ciH_g}{\partial i}(t, a, i) = -\mu(a)H_g(t, a, i) + \Phi(i)S_g(t, a) \sum_{g'} \int_0^A \int_0^1 \beta_g(t, i') I_{g'}(t, a', i') di' da' \quad (2)$$

$$H_g(t, 0, i) = 0 \quad H_g(t, a, 0) = 0 \quad (2', 2'')$$

$$\frac{\partial V_g}{\partial t} + \frac{\partial V_g}{\partial a} + \frac{\partial ciV_g}{\partial i}(t, a, i) = -\mu(a)V_g(t, a, i) \quad (3)$$

$$V_g(t, 0, i) = \Phi(i) \sum_{g'} G_{gg'}(t) \int_0^A \int_0^1 b(t, a') \gamma_g(i') I_{g'}(t, a', i') di' da' \quad (3')$$

$$V_g(t, a, 0) = 0 \quad (3'')$$

References

- [1] Andréoletti O, Berthon P, Marc D, Sarradin P, Grosclaude J, van Keulen L, Schelcher F, Elsen JM, Lantier F (2000) Early accumulation of PrP^{Sc} in gut-associated lymphoid and nervous tissues of susceptible sheep from a Romanov flock with natural scrapie. *J Gen Virol.* 81: 3115–3126
- [2] Andréoletti O, Lacroux C, Chabert A, Monnereau L, Tabouret G, Lantier F, Berthon P, Eychenne F, Lafond-Benestad S, Elsen JM, Schelcher F (2002) PrP^{Sc} accumulation in placentas

- of ewes exposed to natural scrapie: influence of foetal PrP genotype and effect on ewe-to-lamb transmission. *J Gen Virol* 83: 2607–2616
- [3] Andréoletti O, Simon S, Lacroux C, Morel N, Tabouret G, Chabert A, Lugan S, Corbière F, Ferré P, Foucras G, Laude H, Eychenne F, Grassi J, Schelcher F (2004) PrPSc accumulation in myocytes from sheep incubating natural scrapie. *Nature Medicine*. Published online: 23 May 2004
- [4] Baylis M, Goldmann W, Houston F, Cairns D, Chong A, Ross A, Smith A, Hunter N, McLean AR (2002) Scrapie epidemic in a fully PrP-genotyped sheep flock. *J Gen Virol* 83: 2907–2914
- [5] Chase-Topping ME, Kruuk LEB, Lajous D, Touzeau S, Matthews L, Simm G, Foster JD, Rupp R, Eychenne F, Hunter N, Elsen JM, Woolhouse MEJ (2005) Genotype-level variation in lifetime breeding success, litter size and survival of sheep in scrapie affected flocks. *J Gen Virol* 86: 1229–1238
- [6] Díaz C, Vitezica ZG, Rupp R, Andréoletti O, Elsen JM (2005) Polygenic variation and transmission factors involved in the resistance/susceptibility to scrapie in a Romanov flock. *J Gen Virol* 86: 849–857
- [7] Elsen JM, Amigues Y, Schelcher F, Ducrocq V, Andréoletti O, Eychenne F, Tien Khang JV, Polvey JP, Laplanche JL (1999) Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov. *Arch Virol* 144: 431–445
- [8] Goldmann W, Hunter N, Smith G, Foster J, Hope J (1994) PrP genotype and agent effects in scrapie: change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. *J Gen Virol* 75: 989–995
- [9] Gravenor MB, Cox DR, Hoinville LJ, Hoek A, McLean AR (2001) The flock-to-flock force of infection for scrapie in Britain. *Proc R Soc Lond B* 268: 587–592
- [10] Hagenaars TJ, Donnelly CA, Ferguson NM, Anderson RM (2000) The transmission dynamics of the aetiological agent of scrapie in a sheep flock. *Math Biosci* 168: 117–135
- [11] Hagenaars TJ, Ferguson NM, Donnelly CA, Anderson RM (2001) Persistence patterns of scrapie in a sheep flock. *Epidemiol Infect* 127: 157–167
- [12] Hoinville LJ (1996) A review of the epidemiology of scrapie in sheep. *Rev Sci Tech Off Int Epiz* 15: 827
- [13] Hopp P, Webb CR, Jarp J (2003) Monte Carlo simulation of surveillance strategies for scrapie in Norwegian sheep. *Prev Vet Med* 61: 103–125
- [14] Hunter N (1997) PrP genetics in sheep and the implications for scrapie and BSE. *Trends Microbiol* 5: 331–334
- [15] Kao RR, Gravenor MB, McLean AR (2001) Modelling the national scrapie eradication programme in the UK. *Math Biosci* 174: 61–76
- [16] Matthews L, Coen PG, Foster JD, Hunter N, Woolhouse MEJ (2001) Population dynamics of a scrapie outbreak. *Arch Virol* 146: 1173–1186

- [17] Matthews L, Woolhouse MEJ, Hunter N (1999) The basic reproduction number for scrapie. *Proc R Soc Lond B* 266: 1085–1090
- [18] Moreno CR, Cosseddu G, Andreoletti O, Schibler L, Roig A, Moazami-Goudarzi K, Ey-chenne F, Lajous D, Schelcher F, Cribiu EP, Vaiman D, Elsen JM. Identification of quantitative trait loci (QTL) modulating prion incubation period in sheep. In *Proceedings of the International Workshop on Major Genes and QTL in Sheep and Goat* Toulouse, France (2003). Communication 2-27
- [19] Pattison IH, Hoare MN, Jebbett JN, Watson WA (1972) Spread of scrapie to sheep and goats by oral dosing with foetal membranes from scrapie-affected sheep. *Vet Rec* 90: 465–468
- [20] Redman CA, Coen PG, Matthews L, Lewis RM, Dingwall WS, Foster JD, Chase-Topping ME, Hunter N, Woolhouse MEJ (2002) Comparative epidemiology of scrapie outbreaks in individual sheep flocks. *Epidemiol Infect* 128: 513–521
- [21] Stringer SM, Hunter N, Woolhouse MEJ (1998) A mathematical model of the dynamics of scrapie in a sheep flock. *Math Biosci* 153: 79–98
- [22] Woolhouse MEJ, Matthews L, Coen P, Stringer SM, Foster JD, Hunter N (1999) Population dynamics of scrapie in a sheep flock. *Phil Trans R Soc Lond B* 354: 751–756