Modelling scrapie transmission in a sheep flock: effect of lambing seasonality

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Abstract

We present here a mathematical model which describes the dynamics of a scrapie outbreak in a sheep flock. Unlike BSE, scrapie can widely spread in a flock, but the transmission mechanisms are still incompletely understood. A modelling approach is a flexible tool for combining epidemiological, demographic, genetic and management data, and testing through simulation several transmission hypotheses. In particular, we would like to explore the possibility of increased transmission during lambing seasons, suggested by the presence of scrapie infectivity in the placenta.

The initial model was elaborated by Woolhouse et al. [21] and has been successfully used to study several outbreaks in Scottish sheep flocks [18, 25]. It was further developed and applied to the Langlade flock (INRA Toulouse, France) in which a natural scrapie outbreak started in 1993 [7]. Extensive data are available, including pedigree, scrapie histopathological diagnoses and PrP genotypes, which determine the genetic susceptibility of an animal to the disease.

The flock is structured according to the PrP genotype, the scrapie status (susceptible or infected) and, for infected sheep only, the transmission route (horizontal or vertical). The evolution of these population densities through time, age and infection load is given by a PDE model that includes the following dynamic processes : seasonal lambings, scrapie and "natural" (all but scrapie: culling, other diseases, etc.) mortality, vertical and horizontal transmission.

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.

Contents

List of Figures

List of Tables

1 Introduction

The aim of this report is twofold: (i) to present a mathematical model which describes the dynamics of a scrapie outbreak in a sheep flock; (ii) to explore the possibility of increased horizontal transmission during lambing seasons, suggested by the presence of scrapie infectivity in the placenta [20].

The epidemiology of scrapie and particularly the transmission mechanisms are still incompletely understood [13]. 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 [16, 9, 14]. 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 [19, 11, 12, 18]. 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 [7] and for which extensive demographic, genetic and scrapie case data are available. 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 case, flock genetics and genotypes of scrapie cases.

This report starts with the description of the main features of the disease in **section 2** (p. 2), and the Langlade data on which this study is based in **section 3** (p. 3). Then **section 4** (p. 4) gives a detailed presentation of the scrapie transmission model, including the global population dynamics (4.1), and the demographic (4.2) and epidemiological (4.3) processes; **appendix A** (p. 24) contains a short summary of the mathematical model; in **appendix B** (p. 25), possible extensions to the model are mentioned. **Section 5** (p. 12) deals with parameter estimation, either directly from the Langlade data, or through the model. Simulation results are shown in **section 6** (p. 15), and discussed in **section 7** (p. 20), supporting the hypothesis of increased transmission during lambing. Finally, **references** (p. 22) are listed.

Preliminary results of this study were presented at the International Conference on TSEs in Edinburgh, September 2002 [22], and at the IIIrd INRA seminar on TSEs and prions, February 2003 [23, 6]. An paper, mostly based on these results, was submitted to *Archives of Virology* in December 2003 [24]. Moreover, a companion paper [4] has been submitted.

2 Scrapie

Scrapie is a naturally occurring *transmissible spongiform encephalopathy* (TSE) that affects sheep and goats. TSEs are slowly progressive, fatal, neuro-degenerative disorders that are characterised by the accumulation in the brain of a conformationally abnormal form of the prion protein PrP [17]. Human TSEs are rare, Creutzfeld-Jakob disease (CJD), first described in 1920, being the most common. Animal TSEs include bovine spongiform encephalopathy (BSE), which started in the UK in 1985, and chronic wasting disease (CWD), which affects cervids in the USA. Scrapie is the oldest known TSE, its first mention dates back to the 18th century. It is a common pathology in European flocks, endemic in certain regions. Scrapie is more thoroughly described [13, 5], but can be characterised by the following points.

- Unlike BSE which can contaminate humans (variant CJD), there is no evidence that scrapie constitutes a human health risk. However, experimental studies have shown that BSE can infect sheep, even if no natural contamination has been observed. Clinical signs are alike in sheep, but diagnostic tests can differentiate both diseases.

- There is no consensus that the scrapie prion constitutes the *infectious agent* of the disease, although the protein-only hypothesis (1997 Nobel price for Prusiner) is supported by recent evidence [1].

  Several "strains", defined by the incubation period and lesion profile of the brain in infected mice, have been observed.

- Unlike BSE, scrapie can widely spread in a flock, but the *transmission mechanisms* are still incompletely understood. 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 [3]. 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) [2]. However, there may be other routes of entry (e.g. scarification).

  Presence of scrapie infectivity in the placenta [20] suggests the possibility of increased transmission during lambing via the ingestion of contaminated placental material.

- Polymorphisms of the PrP gene encoding for this protein at codons 136, 154 and 171 largely control the *genetic susceptibility* and resistance of sheep to the disease [15]. Some genotypes confer partial or full resistance to scrapie. The genetics of scrapie is breed and "strain" dependent.

- Scrapie is associated with a long *incubation period* (ca. 2 years), that varies according to the scrapie strain and at an individual level, the PrP genotype (as short as 11 months in fully susceptible animals).

  Some dose-effect experiments were conducted in mice, where the incubation period appeared to depend on the inoculating dose, but not on superinfection [10].

- Scrapie is a fatal disease. *Clinical signs* usually consist in behavioural changes, tremor (hence the French name "tremblante"), pruritus (hence scrapie) and locomotor incoordination. They may vary greatly between individuals and last from a couple of weeks up to 6 months, but sheep are generally culled shortly after the onset of these signs.

- Currently there is no effective *treatment*.

- Preclinical *tests* on live animals are currently being developed (tonsil biopsies), but none are validated yet. The clinical diagnosis of scrapie is confirmed by brain histopathology or/and immunohistochemistry.

3 Langlade data

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.

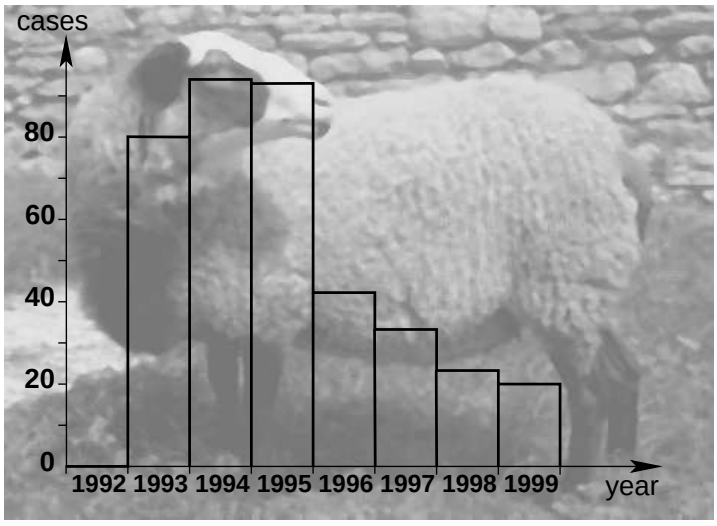

FIGURE 1 – *Scrapie outbreak in the Langlade Romanov flock.*

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].

Study flock As the flock management changed over the course of time, a first study was conducted on a shorter 4-year period, from January 1992 until December 1995. 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, and many lambs were culled under the age of 8 months, so they did not play a major role in the transmission of scrapie within the flock. 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.

4 The scrapie transmission model

The deterministic mathematical model described here is based on [21]. The main changes and developments introduced to study the possibility of increased transmission during lambing seasons, and to fit the Langlade data are: seasonality in birth and transmission, a control term on the birth rate to follow the variable population size, and an upwind integration scheme to increase the numerical stability.

The scrapie model is founded on a set of *hypotheses* and simplifications which are listed below.

- The model does not discriminate by sex; since only a small number of breeding rams are kept, only the population of ewes is considered.
- A continuous and bounded age structure is introduced in the flock.
- Breeding is seasonal, random mating is assumed, and all ewes give birth to the same number of lambs.
- Culling represents all sources of mortality, scrapie excluded. It is assumed to depend on the age of the animal only.
- There is no way to identify infected sheep, so all sheep are bred and culled independently from their scrapie status. Culling here represents all sources of mortality, scrapie excluded.
- The infected sheep instantly die at the end of the incubation period, once they start showing clinical signs. Therefore, only susceptible and incubating sheep are considered.
- The vertical and horizontal transmission routes are distinguished. Vertical transmission corresponds here to in utero or perinatal maternal transmission.
- Infected sheep are also structured according to their infection load, which is a continuous and bounded variable that increases exponentially with time. It is related to the infectiousness of the sheep and is also a measure of the incubation period.
- In this study, the infectiousness is proportional to the infection load.
- The incubation period is long and variable.
- The genetic susceptibility factor is introduced.
- An age susceptibility factor can be introduced.

The flock is structured according to the PrP genotype ($g \in \{1, \ldots, N_g\}$), the scrapie status (susceptible S or infected I) and, for infected sheep only, the transmission route (horizontal H or vertical V). Time (t), age (a) and infection load (θ) are continuous variables in the model. Hence, to represent the evolution through time of the resulting population densities (e.g. $H_g(t, a, \theta)$) with respect to age and infectiousness, the model consists of a set of partial differential equations (cf. subsection 4.1).

The model incorporates the following components: seasonal breeding and routine culling for the flock demography (cf. subsection 4.2); genetic susceptibility, a long and variable incubation period preceding clinical signs and death, (seasonal) horizontal and vertical transmission for the epidemiology of scrapie (cf. subsection 4.3).

A schematic representation is given in FIGURE 2. A concise overview of the model is also given in appendix A.

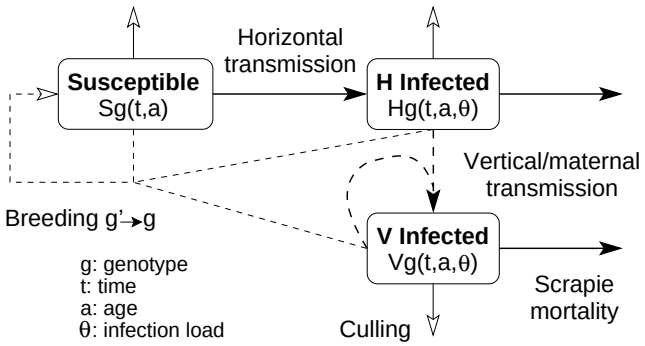

FIGURE 2 – *Representation of the flock structure and processes incorporated in the scrapie model. Vertical transmission corresponds to maternal transmission (in utero and perinatal). Culling represents all non scrapie mortality. Time, age and infection load (related to infectiousness) are continuous variables in the model. Breeding of susceptible and infected lambs (dashed lines) involves genetic recombination.*

4.1 Population dynamics

The population densities, which constitute the state variables of the model, are:

$$\begin{cases} S_g(t,a) & \text{susceptible,} \\ H_g(t,a,\theta) & \text{infected by horizontal route,} \\ V_g(t,a,\theta) & \text{infected by vertical route,} \end{cases}$$

$$\text{with: } I_g = H_g + V_g.$$

They are functions of the following continuous variables:

time $t \geqslant 0$

age $0 \leqslant a \leqslant a_{max} = A$

Time and age evolve at the same speed:

$$\frac{da}{dt} = 1. \tag{1}$$

The age variable is bounded, so that animals reaching age A are eliminated.

infection load – infectiousness $0 \leqslant \theta \leqslant \theta_{max} = 1$ (for infected groups only)

The infection load represents the development of the disease. The flow of individuals entering an infected group is given a positive initial load θ_0, set by a distribution ϕ (usually a gamma distribution). During the incubation period, it grows exponentially, with an **infection growth rate** c_g that may depend on genotype g:

$$\frac{d\theta}{dt} = c_g\theta. \tag{2}$$

This variable is also bounded, so that individuals die of scrapie once it reaches θ_{max}. Variations in the initial load θ_0 therefore lead to different incubation periods.

The infection load is also related to the infectiousness of the infected sheep (cf. pathogenesis and transmission in 4.3).

The partial differential equations representing the evolution through time of the population densities with respect to age and infection load are exposed below: equations (3,4,5) describe the *flock dynamics* and (3′,4′,5′) are the *boundary conditions*; but first the conservation laws are applied.

4.1.1 Conservation laws

Let $J(t, a, \theta)$ be the population density of the infected sheep contaminated by either the horizontal or the vertical route, and of any genotype ($J = H_g$ or $V_g, \forall g$). Conservation laws are applied to establish the evolution through time of this variable, for $a > 0$ and $\theta > 0$. In this section, we consider that there is *no mortality*.

The number of individuals in interval $[a, a + \Delta a] \times [\theta, \theta + \Delta\theta]$ is:

$$\int_a^{a+\Delta a} \int_\theta^{\theta+\Delta\theta} J(t, a, \theta) d\theta da.$$

This number only varies due to the inflow of individuals through boundaries a_- and θ_-, and to the outflow of individuals through boundaries a_+ and θ_+ (cf. FIGURE 3).

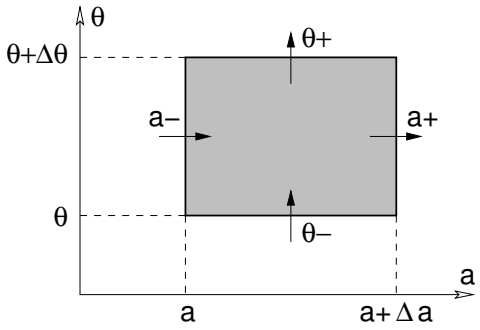

FIGURE 3 – *Conservation laws for an infected group of the population dynamics model in space* (a, θ)*, with no mortality.*

Variables a and θ verify equations (1) and (2) (here we note $c_g = c$). Therefore, the inflow through boundary θ_- is:

$$\int_a^{a+\Delta a} \frac{d\theta}{dt}(t, a, \theta)\, J(t, a, \theta)\, da = \int_a^{a+\Delta a} c\theta\, J(t, a, \theta)\, da.$$

With a similar reasoning to express the flows through the other boundaries, the following equation is obtained:

$$\begin{aligned}
\frac{d}{dt}\int_a^{a+\Delta a}\int_\theta^{\theta+\Delta\theta} J(t, a, \theta)\, d\theta\, da = &+ \int_a^{a+\Delta a} c\theta\, J(t, a, \theta)\, da && [\theta_-]\\
&- \int_a^{a+\Delta a} c(\theta + \Delta\theta)\, J(t, a, \theta + \Delta\theta)\, da && [\theta_+]\\
&+ \int_\theta^{\theta+\Delta\theta} J(t, a, \theta)\, d\theta && [a_-]\\
&- \int_\theta^{\theta+\Delta\theta} J(t, a + \Delta a, \theta)\, d\theta && [a_+]\\
= &- \int_a^{a+\Delta a}\int_\theta^{\theta+\Delta\theta} \frac{\partial[c\theta\, J(t, a, \theta)]}{\partial\theta}\, d\theta\, da && [\theta_- \,\&\, \theta_+]\\
&- \int_\theta^{\theta+\Delta\theta}\int_a^{a+\Delta a} \frac{\partial J(t, a, \theta)}{\partial a}\, da\, d\theta, && [a_- \,\&\, a_+]
\end{aligned}$$

which induces the conservation law below:

$$\frac{\partial J(t, a, \theta)}{\partial t} + \frac{\partial J(t, a, \theta)}{\partial a} + \frac{\partial[c\theta\, J(t, a, \theta)]}{\partial\theta} = 0.$$

4.1.2 Susceptible sheep

The susceptible sheep $S_g(t,a)$ are exposed to scrapie contamination (β) from infected ewes I'_g and culling (μ), while newborn susceptible lambs from susceptible (b) and also a fraction $(1-\gamma)$ of infected ewes of genotype g' are introduced in the flock:

$$\begin{aligned}\frac{\partial S_g}{\partial t}+\frac{\partial S_g}{\partial a}(t,a) &= -\mu(a)S_g(t,a)\\ &\quad - S_g(t,a)\sum_{g'}\int_0^A\int_0^1 \beta_g(t,\theta',a)I_{g'}(t,a',\theta')d\theta' da', \end{aligned} \tag{3}$$

$$\begin{aligned} S_g(t,0) &= \sum_{g'} G_{gg'}(t)\int_0^A b(t,a')S_{g'}(t,a')da'\\ &\quad + \sum_{g'} G_{gg'}(t)\int_0^A\int_0^1 b(t,a')(1-\gamma_g(\theta'))I_{g'}(t,a',\theta')d\theta' da'. \end{aligned} \tag{3'}$$

4.1.3 Horizontally infected sheep

The horizontally infected sheep $H_g(t,a,\theta)$ are also exposed to culling (μ), but they only become infected after their birth, through the horizontal contamination process (β):

$$\begin{aligned}\frac{\partial H_g}{\partial t}+\frac{\partial H_g}{\partial a}+\frac{\partial c_g\theta H_g}{\partial \theta}(t,a,\theta) &= -\mu(a)H_g(t,a,\theta)\\ &\quad + \phi(\theta)S_g(t,a)\sum_{g'}\int_0^A\int_0^1 \beta_g(t,\theta',a)I_{g'}(t,a',\theta')d\theta' da', \end{aligned} \tag{4}$$

$$\begin{aligned} H_g(t,0,\theta) &= 0,\\ H_g(t,a,0) &= 0. \end{aligned} \tag{4'}$$

4.1.4 Vertically infected sheep

The vertically infected sheep $V_g(t,a,\theta)$ are culled (μ) and a proportion (γ) of the lambs born from infected ewes of genotype g' are introduced in the flock:

$$\frac{\partial V_g}{\partial t}+\frac{\partial V_g}{\partial a}+\frac{\partial c_g\theta V_g}{\partial \theta}(t,a,\theta) = -\mu(a)V_g(t,a,\theta), \tag{5}$$

$$\begin{aligned} V_g(t,0,\theta) &= \phi(\theta)\sum_{g'} G_{gg'}(t)\int_0^A\int_0^1 b(t,a')\gamma_g(\theta')I_{g'}(t,a',\theta')d\theta' da',\\ V_g(t,a,0) &= 0. \end{aligned} \tag{5'}$$

4.2 Demographic processes

4.2.1 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 **mortality rate** is based on a Weibull risk function:

$$\mu(a) = \kappa\lambda(\lambda a)^{\kappa-1} \quad \text{for: } 0<a<A. \tag{6}$$

A survival analysis on the data is performed to estimate these parameters (cf. section 5.1).

4.2.2 Seasonal breeding

Birth rate 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 $b(t, a')$ determines the number of new lambs arriving in the flock during the lambing season. It represents the mean number of offspring produced by a breeding ewe of age a' per time unit. It is assumed to be the same for all mature ewes, i.e. ewes of age $a' \in [a_1, a_2]$. So the birth rate verifies:

$$b(t, a') = \begin{cases} b(t)\, s(t) & \text{for } a_1 \leqslant a' \leqslant a_2 \text{ (mature ages)}, \\ 0 & \text{otherwise}, \end{cases} \tag{7}$$

where $s(t)$ is a **seasonal function**, the season being defined by its annual duration T and its shift t_s with respect to the initial time $t = 0$:

$$s(t) = \begin{cases} 1 & \text{if } (t \bmod 1 \text{ yr}) \in [t_s, t_s + T] \text{ (lambing period)}, \\ 0 & \text{otherwise.} \end{cases} \tag{7'}$$

The birth rate is automatically adjusted so that the total flock size at the end of the lambing period attains a predefined target value P_c based on the data. So $b(t)$ is a **control on the population size**.

Let $P(t)$ be the total population size and $P_b(t)$ the breeding population size at time t:

$$P(t) = \sum_g \int_0^A \left(S_g(t, a) + \int_0^1 I_g(t, a, \theta)\, d\theta \right) da, \tag{8}$$

$$P_b(t) = \sum_g \int_{a_1}^{a_2} \left(S_g(t, a) + \int_0^1 I_g(t, a, \theta)\, d\theta \right) da. \tag{9}$$

The total population size fluctuates as follows:

$$\frac{dP(t)}{dt} = \underbrace{\sum_g \left(S_g(t, 0) + \int_0^1 I_g(t, 0, \theta)\, d\theta \right)}_{\text{birth } u(t)} \underbrace{- \sum_g c_g \int_0^A I_g(t, a, 1)\, da}_{\text{scrapie mortality } m_s(t)}$$
$$\underbrace{- \sum_g \left(S_g(t, A) + \int_0^1 I_g(t, A, \theta)\, d\theta + \int_0^A \mu(a) \left(S_g(t, a) + \int_0^1 I_g(t, a, \theta)\, d\theta \right) da \right)}_{\text{non scrapie mortality } m_n(t)}.$$

During the lambing period, the birth corresponds to $u(t) = b(t)\, P_b(t)$. To compensate the mortality, it should be chosen so as to ensure that $dP/dt = 0$, i.e. $u(t) = m_s(t) + m_n(t)$. However, our goal is to attain a target population size P_c at the end of the lambing period. So we add a first order dynamics to the birth term:

$$u(t) = m_s(t) + m_n(t) + K[P_c - P(t)],$$

and we obtain the following birth rate:

$$b(t) = \frac{\sum_g \big[S_g(t, A) + \int_0^1 I_g(t, A, \theta)\, d\theta + \int_0^A \mu(a) \big(S_g(t, a) + \int_0^1 I_g(t, a, \theta)\, d\theta \big)\, da}{P_b(t)}$$
$$\frac{+ \int_0^A c_g I_g(t, a, 1) \big] + K[P_c - P(t)]}{P_b(t)}. \tag{7"}$$

The population size dynamics during the lambing period then becomes: $dP/dt = K[P_c - P(t)]$. Choosing $K = 3/T$, T being the length of the lambing period, at the end of this period we should have: $P \simeq P_c$.

Breeding matrix The birth rate $b(t, a')$[1] gives the global inflow of lambs in the flock, but they need to be distributed among the different genotypes. As only the female population is considered in this model, the genotypes of these lambs are deduced from the dam genotypes only. Therefore, a breeding matrix $G(t) = (G_{gg'}(t))$ has to be introduced, that gives the proportion of lambs of genotype g born from dams with genotype g' (or the probability for ewes g' to give birth to lambs g). It mainly depends on the breeding practices.

EXAMPLE – In a simple case with two alleles r and R, three genotypes are obtained: (1) rr, (2) Rr, and (3) RR, as ordered in the matrix. Defining $q(t)$ as the frequency of allele r among the sires and assuming *random mating*, the breeding matrix is given by:

$$G(t) = \begin{pmatrix} q(t) & q(t)/2 & 0 \\ 1-q(t) & 1/2 & q(t) \\ 0 & \frac{1-q(t)}{2} & 1-q(t) \end{pmatrix}.$$

Assuming that *the rams and the ewes have the same allele frequencies* in the flock, the following equation is obtained:

$$q(t) = \frac{\int_0^A \left(S_1(t,a) + S_2(t,a)/2 + \int_0^1 [I_1(t,a,\theta) + I_2(t,a,\theta)/2] d\theta \right) da}{\sum_{g=1}^3 \int_0^A \left(S_g(t,a) + \int_0^1 I_g(t,a,\theta) d\theta \right) da}.$$

Note that $\sum_g G_{gg'}(t) = 1$.

In this model, genotypes are also 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 allele and genotype frequencies at each lambing period. Results are shown in TABLE 1. With the exception of one lambing season (1994) for the dam and lamb alleles (a), the frequencies are not significantly different ($p > 0.05$). This shows that the data are generally in accordance with the hypothesis of random mating (a) and recombination (b). It also confirms that having the same birth rate for all mature ewes won't have a major impact on the lamb allele frequencies (c). So the breeding matrix is derived from the flock allele frequencies, as in the example above.

TABLE 1 – *Results of the χ^2 tests used to validate the random mating hypothesis, by comparing at each lambing: (a) the allele frequencies of the dams and of their female lambs that were subsequently used for replacement; (b) the allele frequencies of the dams which gave birth to only one replacement ewe and of those which had more than one; (c) the theoretical, i.e. computed from a random recombination of the allele frequencies, and observed lamb genotype frequencies.*
Data correspond to the Romanov replacement ewes at Langlade from 1992 to 1995.

χ^2 *tests (p values)*	*Lambings*			
	1992	1993	1994	1995
(a) dam/lamb alleles	0.1182	0.1735	0.0276	0.5380
(b) lamb genotype	0.9717	0.9997	0.8843	0.7721
(c) dam alleles	0.4130	0.4327	0.8487	0.0530

[1] In this subsection, a variable with a $'$ usually refers to the dam, and a variable without to the lamb.

4.3 Epidemiological processes

4.3.1 Genetic susceptibility

Genetic susceptibility to scrapie is determined by polymorphisms of the PrP genotype at codons 136, 154 and 171. Some genotypes confer partial or full resistance to scrapie. The genetics of scrapie is breed and "strain" dependent. The **relative genetic susceptibilities** σ_g are defined as the proportion of scrapie cases in each genotype and are estimated from the data: $\sigma_g \in [0, 1]$, where 0 corresponds to full resistance to scrapie.

4.3.2 (Seasonal) horizontal transmission

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, and (ii) to the sum of all infected animals weighted by their infectiousness.

Here, the **infectiousness** of an infected sheep is considered proportional to its infection load, so it increases exponentially with time during the incubation period.

Therefore, the **horizontal transmission rate** β is independent of the genotype and age of the infecting sheep, but depends on its infection load θ'.[2] It incorporates the genetic susceptibility σ_g of the infected sheep and an age-dependent susceptibility function $f(a)$ can be added. The transmission rate is set to zero outside the lambing period for the seasonal model only, in which case the seasonal function $s(t)$ defined in (7') is introduced. The absolute transmission rate is determined by a **scaling factor** k_h, which needs to be estimated and is fixed independently in each model, to allow for two very different horizontal transmission periods. The resulting horizontal transmission rate is:

$$\beta_g(t, \theta', a) = k_h \, \sigma_g \, \theta' \, \delta(t) \, [f(a)], \qquad \text{with: } \delta(t) = \begin{cases} s(t) & \text{for the seasonal model,} \\ 1 & \text{otherwise.} \end{cases} \tag{10}$$

Age-dependent susceptibility was added to examine the sensitivity of the model outputs to this factor, but is not usually retained (cf. section 6.3). f is assumed to decline exponentially with age: $f(a) = e^{-\eta a}$. In [18], a step function was chosen (animals are susceptible upto 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.

4.3.3 Vertical / maternal 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.

The **vertical transmission rate** γ defines the proportion of infected lambs born from infected ewes. As in the horizontal case, it depends on the relative genetic susceptibilities of the lambs and the infectiousness of the dams:

$$\gamma_g(\theta') = k_v \sigma_g \theta', \tag{11}$$

NOTE: As γ represents a proportion, the **scaling factor** $k_v \in [0, 1]$; it needs to be estimated.

[2] In these subsections, a variable with a $'$ usually refers to the infecting animal, and a variable without to the infected sheep.

4.3.4 Pathogenesis – incubation

Once an individual is contaminated, the development of the disease is described in the model by means of the infection load θ. The initial infection load θ_0 for a newly vertically or horizontally infected animal is assumed to follow a distribution ϕ, which is independent of genotype. The infection load then increases exponentially during the incubation period, up to a level θ_{max} corresponding to clinical signs, at which point sheep are removed from the flock (2).

The **initial load distribution** ϕ is usually chosen as a gamma distribution, with a probability density function:

$$\phi(\theta) = \frac{l(l\theta)^{k-1}e^{-l\theta}}{\Gamma(k)}, \tag{12}$$

where Γ is the gamma function ($\Gamma(k) = (k-1)!$ if k is an integer). The mean initial load is set at 10% of the terminal load θ_{max}, chosen arbitrarily to be 1. As the infectiousness is proportional to the infection load, it 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 ($k/l = 0.1$), but the variance ($v = k/l^2$) still needs to be estimated:

$$\begin{cases} l = 10\,k, \\ v = 0.01/k. \end{cases} \tag{12'}$$

The exponential rate of increase c_g of the infection load (2) determines the length τ of the incubation period corresponding to the initial infection load $\theta_0 \in [0, 1]$:

$$\tau = -\frac{\ln \theta_0}{c_g}, \qquad \text{with: } \tau \in [0, +\infty[. \tag{13}$$

Because the initial infection load θ_0 follows a distribution ϕ rather than being set at a fixed value, a corresponding distribution φ of incubation periods is obtained.

> Distribution of incubation periods – The incubation period τ can be deduced from the initial infection load θ_0:
>
> $$\theta_0 = e^{-c_g\,\tau} \text{ with: } \theta_0 \in [0,1] \;\&\; c_g > 0 \quad \Rightarrow \quad \tau = -\frac{\ln \theta_0}{c_g} \text{ with: } \tau \in [0, +\infty[.$$
>
> The initial infection load θ_0 follows a distribution with a probability density function ϕ. To establish the density probability function of the incubation period distribution, let $x \in [0, 1]$ and $y = -\frac{\ln x}{c_g} \in [0, +\infty[$.
>
> $$\begin{aligned} p(\theta_0 \geqslant x) &= p(\tau \leqslant y) \\ &= \int_x^1 \phi(u)du \\ &= \int_y^0 \phi(e^{-c_g v})(-c_g\, e^{-c_g v})dv \qquad [u = e^{-c_g v}] \\ &= \int_0^y c_g\, e^{-c_g v}\phi(e^{-c_g v})dv. \end{aligned}$$
>
> So the incubation period τ follows a distribution with a probability density function φ:
>
> $$\varphi(t) = c_g\, e^{-c_g t}\phi(e^{-c_g t}) \qquad \text{with: } t \in [0, +\infty[. \tag{13'}$$
>
> Thanks to (12'), only two of the three parameters of this distribution need to be estimated: c_g and var.

5 Parameter estimation

All parameters (and variables) in this model are non negative. They are shown in TABLE 2. The demographic parameters (5.1) and the genetic susceptibilities (5.2) are estimated directly from the data, the remaining epidemiological parameters (5.4) need to be estimated through the model. Moreover, the initial condition (5.3) is also estimated from the data.

TABLE 2 – *Parameters used in the scrapie transmission model (3,3′), (4,4′) & (5,5′).*

	Demographic parameters			
	description	function	equation(s)	value
A	maximum age	–	(6,8)	13 years
a_1	minimum breeding age	$P_b(t)$	(7,9)	22 months
a_2	maximum breeding age	$P_b(t)$	(7,9)	10.5 years
T	length of the lambing period	$s(t)$	(7')	40 days
t_s	shift of the lambing period	$s(t)$	(7')	10 days
κ	mortality rate (Weibull shape)	$\mu(a)$	(6)	2.04
λ	mortality rate (Weibull 1/scale)	$\mu(a)$	(6)	0.158 year^{-1}
	Epidemiological parameters			
	description	function	equation(s)	value
σ_g	relative genetic susceptibilities	β, γ	(10,11)	cf. TABLE 3
η	age-dependent susceptibility rate	β	(10)	usually 0
k_h	horizontal transmission scaling factor	$\beta(t,a,\theta)$	(10)	0.045 year^{-1}*†
k_v	vertical transmission scaling factor	$\gamma(t,a,\theta)$	(11)	1*
c_g	exponential growth rate of the infection load	$[\frac{d\theta}{dt}, \tau]$	(2,13)	1.35 year^{-1}*
k	initial load distribution (gamma shape)	$\phi(\theta)$	(12)	12*
l	initial load distribution (gamma 1/scale)	$\phi(\theta)$	(12)	$l = 10\,k$

**Parameters estimated through the model.*
†*Parameter estimated for the seasonal model.*

5.1 Demographic parameters

The maximum age, breeding ages and lambing season parameters are straightforward to obtain from the data.

The mortality rate is based on a Weibull risk function that was fitted to the study flock data (cf. FIGURE 4), 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.

WEIBULL DISTRIBUTION FOR THE CULLING AGE – The age at death A_d for all causes except scrapie follows a Weibull distribution in the model.

The Weibull probability density function is: $f_W(a) = \kappa\lambda(\lambda\,a)^{\kappa-1}e^{-(\lambda\,a)^\kappa}$, with:
mean $= \frac{\Gamma((\kappa+1)/\kappa)}{\lambda}$ and variance $= \frac{\Gamma((\kappa+2)/\kappa)-[\Gamma((\kappa+1)/\kappa)]^2}{\lambda^2}$.

The survival function $S_W(a) = e^{-(\lambda a)^k}$ represents the probability to survive upto age a. It is used in FIGURE 4 to fit the data.

The hazard or risk function $\mu(a) = \kappa\lambda(\lambda a)^{\kappa-1}$ represents the risk of dying at age a, knowing that you have survived upto age a:

$$\mu(a) = \lim_{\Delta\to 0+} \frac{p(a \leqslant A_d < a+\Delta \,|\, a \leqslant A_d)}{\Delta} = \frac{f_W(a)}{p(a \leqslant A_d)} = \frac{f_W(a)}{\int_a^\infty f_W(a')da'}\,.$$

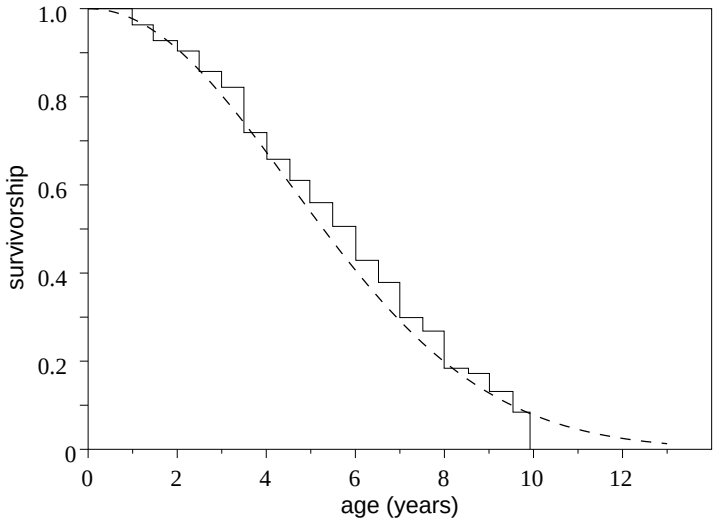

FIGURE 4 – *Survival curves based on the 1986-1995 birth cohorts: Kaplan Meier curve (solid line) and Weibull fitted curve (dashed line) with parameters* $\kappa = 2.04$ *and* $\lambda = 1/6.32$. *Ewes that died before January 1, 1992 (beginning of the study period) were left truncated and ewes still 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.*

5.2 Relative genetic susceptibilities

Genetic susceptibility to scrapie is determined by the PrP genotype, defined in the Langlade 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 [25, 18]. The relative genetic susceptibilities used in the model are estimated from the data and are presented in TABLE 3.

TABLE 3 – *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 December 1995).*

Genotype (g)	VRQ VRQ	ARQ VRQ	ARQ ARQ	AHQ AHQ	ARR VRQ	AHQ ARQ	ARQ ARR	AHQ VRQ	AHQ ARR	ARR ARR
Susceptibility (σ_g)	0.803	0.606	0.403	0.125	0.078	0.062	0.029	0.020	0	0

5.3 Initial condition

The simulation starts on the first of January 1992 with 693 sheep. Most are susceptible sheep and their age follows the Weibull survival fun.

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, is assumed to be infected at the beginning of the simulation, i.e. 18 horizontally infected sheep out of 693. The infection loads of these animals are set so as to lead to scrapie cases 20 months later (together with parameter c_g).

There are no sheep infected by the vertical route initially.

5.4 Epidemiological parameters

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 (k, l and c_g) and transmission scaling factors (k_h and k_v) were adjusted simultaneously in order to achieve the observed total number of cases and shape of epidemic curve during the study period. The exponential rate of increase of the infection load c_g is assumed to be the same for all genotypes.

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 [13]. 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 k_v was selected in order to obtain roughly 10% of cases by this route during the first years of the epidemic.

The horizontal transmission scaling factor k_h was estimated independently for the seasonal and the non seasonal transmission models. 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 infection load, corresponding to a mean incubation period of 1.73 years (cf. Table 2 and Figure 5).

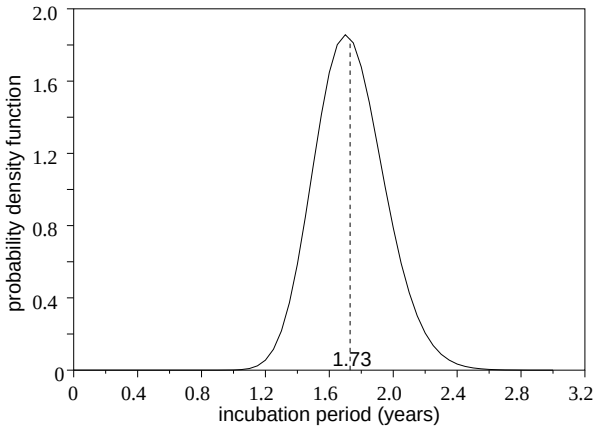

Figure 5 – *Curve representing the probability density function of the incubation period distribution (mean* 1.73*, variance* 4.7×10^{-2}*), derived from the gamma distribution associated to the initial infection load (mean* 0.1*, variance* 8.3×10^{-4}*).*

Population attributable risk percentage – The population attributable risk percentage gives a rough estimate of the maternal transmission. The exposed population is defined as the sheep that have a scrapie affected dam. N_e is the number of sheep, C_e the number of cases, and $I_e = C_e/N_e$ the incidence in the exposed group. N_o, C_o, and I_o are similarly defined in the non exposed group, and N_t, C_t, and I_t in the total population.

The *relative risk* is: $RR = \frac{I_e}{I_o}$.

The *attributable risk* is: $AR = I_e - I_o$.

The *population attributable risk* corresponds to the proportion of cases due to the risk factor in the total population. Eliminating the risk factor, the exposed population would get $C_o N_e/N_o$ cases instead of C_e. So: $PAR = \frac{C_- C_o N_e/N_o}{N_t} = AR\frac{N_e}{N_t}$.

The *population attributable risk percentage* is the proportion of cases due the risk factor among all the cases. So: $PAR\% = \frac{C_- C_o N_e/N_o}{C_e+C_o} = \frac{PAR}{I_t}$.

Note: PAR% represents both the maternal transmission, and an increased genetic susceptibility in the exposed group, as contaminated dams have more susceptible genotypes.

6 Simulation results

Simulations were performed using a Fortran program. For numerical stability of the seasonal model, the previously used centred integration scheme (Lax-Wendroff [25, 18]) was replaced with an upwind integration scheme (Godounov) which respects the causality of the system.

The two models were simulated: 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 k_h. Both scenarios are compared in 6.1. Further outputs *for the seasonal model only* are shown in 6.2. Finally, the sensitivity of these outputs to various factors is explored in 6.3.

6.1 Comparison of the seasonal and non seasonal models

In FIGURE 6, both scenarios reproduce a time course of peaks and troughs in the scrapie incidence 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.

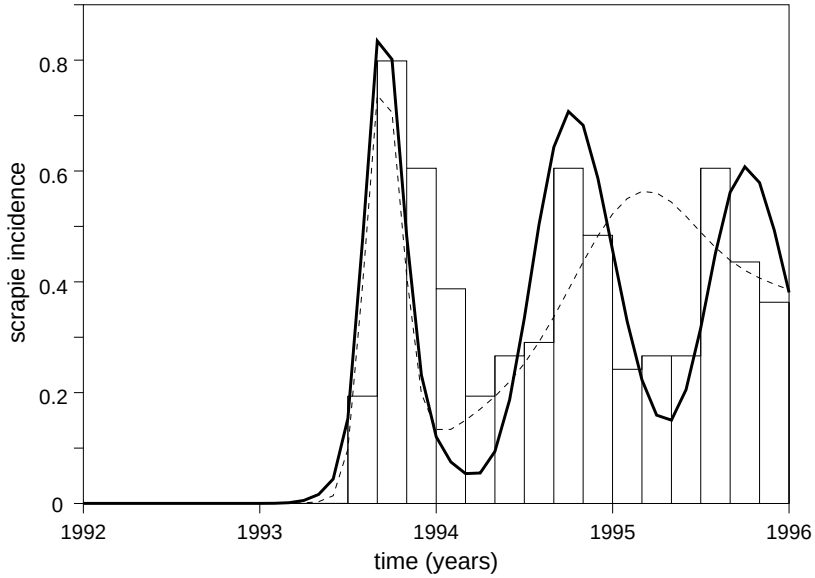

FIGURE 6 – *Comparison between the observed (bars) and simulated (solid line) scrapie case distribution over time; the simulation was performed with the seasonal transmission hypothesis. Comparison with the non seasonal transmission case (dashed line), all parameters except the horizontal transmission scaling factor k_h being equal.*

6.2 Outputs for the seasonal model

- In FIGURE 7, the overall trends in the age distribution of scrapie cases are captured but the initial peak corresponding to sheep infected close to birth is underestimated.
- In FIGURE 8, the fluctuations of the flock size (due to the seasonality in births) are well reproduced, except in the second half of 1993, when the mortality is underestimated.
- The frequency distribution of cases by genotype in FIGURE 9 is in excellent agreement with the data.
- FIGURE 10 shows the time course of the allele frequencies: overall trends are reproduced but the decline in frequency of the two most susceptible alleles, VRQ and ARQ, is less than that observed.

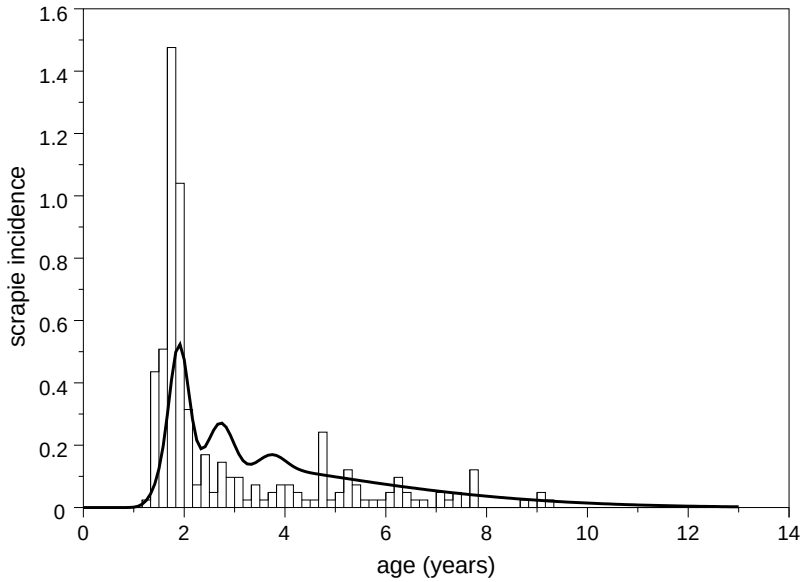

FIGURE 7 – *Comparison between the observed (bars) and simulated (solid line) age distribution of scrapie cases; the simulation was performed with the seasonal transmission hypothesis.*

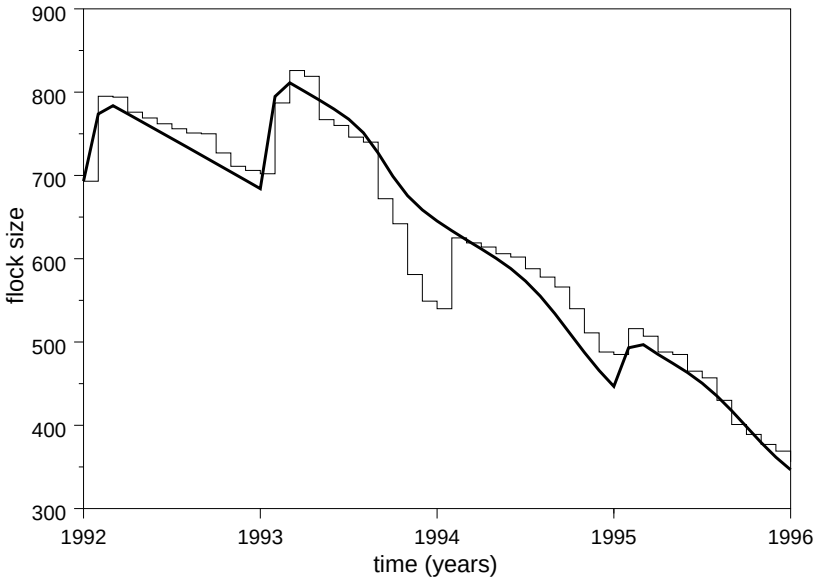

FIGURE 8 – *Comparison between the observed (thin line) and simulated (thick line) flock size over time; the simulation was performed with the seasonal transmission hypothesis.*

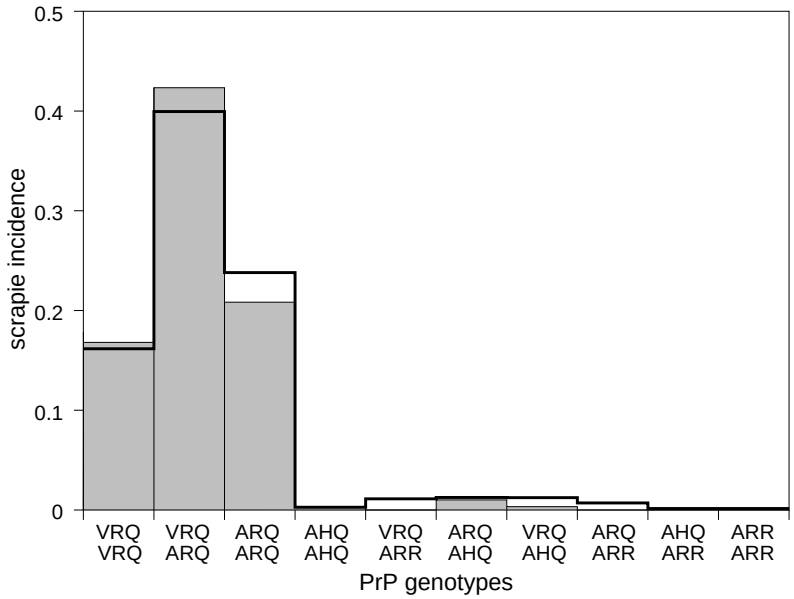

FIGURE 9 – *Comparison between the observed (grey bars) and simulated (thick line) genotype distribution of scrapie cases; the simulation was performed with the seasonal transmission hypothesis.*

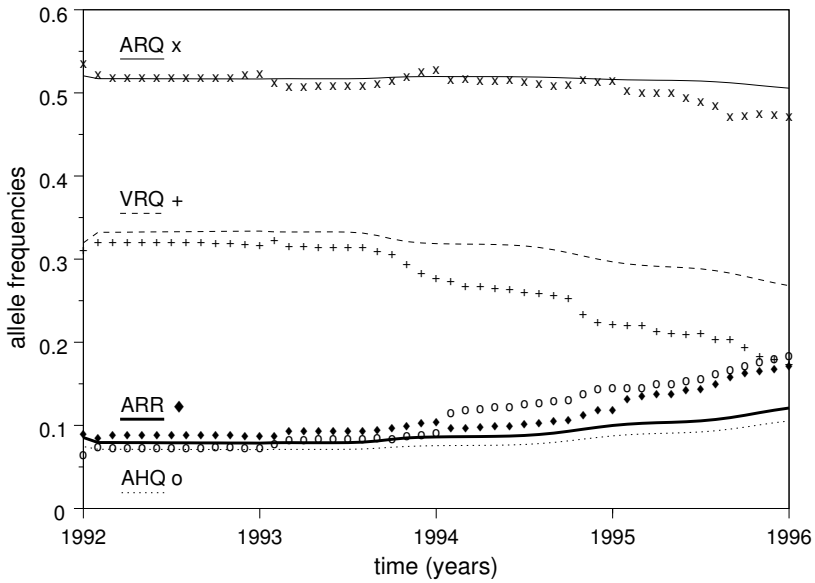

FIGURE 10 – *Comparison between the observed (symbols) and simulated (lines) allele frequencies over time: VRQ (dashed line, + symbol), ARQ (solid line, × symbol), AHQ (dotted line, o symbol), ARR (thick line, ♦ symbol); the simulation was performed with the seasonal transmission hypothesis.*

6.3 Sensitivity of the results

Further simulations were conducted on a longer time period to assess the sensitivity of the above results to a number of factors.

- The incidence patterns described above are quite robust to the length of the lambing season in the model, as shown in FIGURE 11. Taking twice its usual value barely changed the total incidence, and even with a 6 months period, the seasonality is conserved. The peaks observed when there is no seasonality are due to the cluster of initial cases.

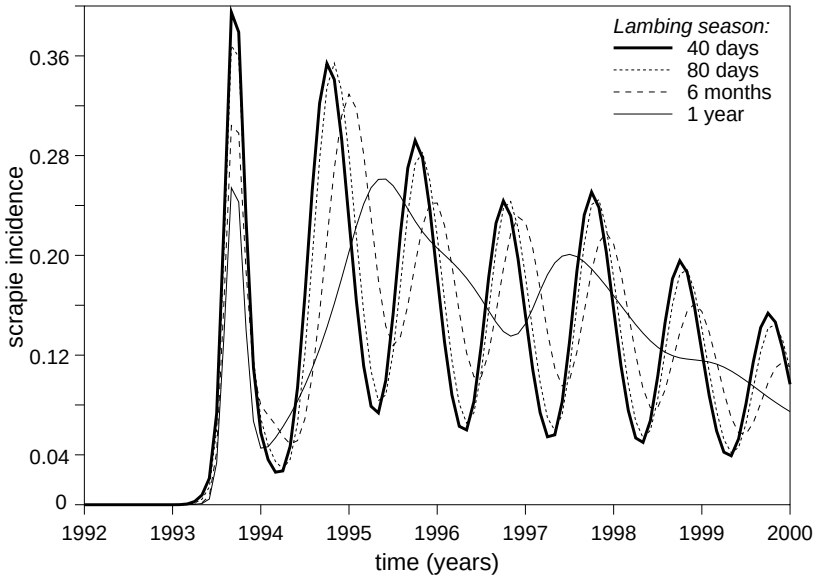

FIGURE 11 – *Influence of the length of the lambing season T on the total incidence. The curves correspond to a period of 40 days (thik line, usual period), 80 days (dotted line), 6 months (dashed line), and the whole year (solid line).*

- FIGURE 12 shows the total incidence for three incubation period distributions φ plotted in FIGURE 13. Each φ is deduced from the initial load distribution ϕ, as shown in equations (12), (13), and (13'). As its mean is set to 0.1, changing ϕ consists in changing its shape parameter k, a higher k corresponding to a higher variance (12'). It also leads to higher values of the mean and variance of the incubation period ditributionφ, as indicated in FIGURE 13. Increasing the variance of the incubation period tends to smooth the annual peaks observed in FIGURE 6.

- Simulations performed without vertical transmission are qualitatively similar to those presented above, but, as might be expected, slightly lower the incidence peak in younger animals observed in FIGURE 7 (very few cases by the vertical route were obtained, less than 10%).

- Implementing age-dependent susceptibility does not improve the overall model fit; a higher peak at younger ages in the age distribution of cases is obtained but the incidence patterns match observations less well.

- Finally, a higher culling rate generates less scrapie cases, but a relatively higher incidence peak in younger animals.

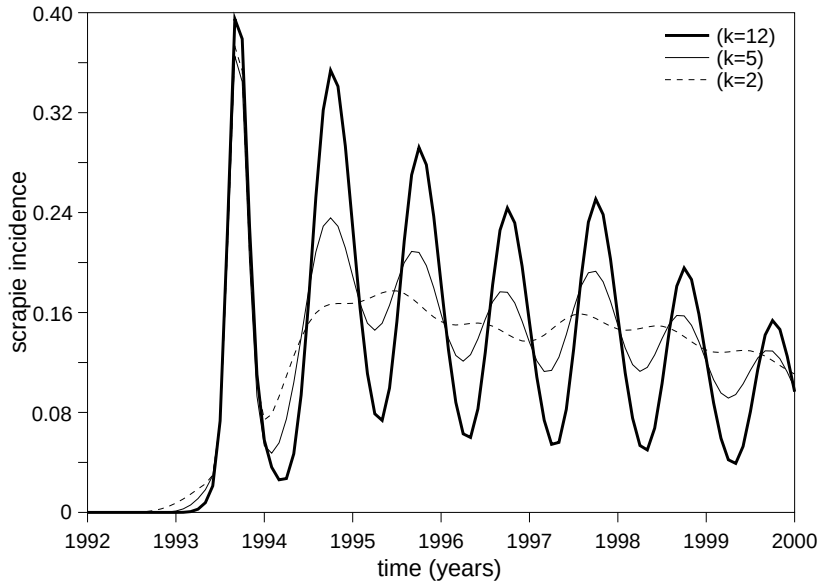

FIGURE 12 – *Influence of the incubation period distribution on the total incidence. The curves were plotted for various values of the shape parameter k of the gamma distribution used for the initial infection load: 12 (thick line, usual shape), 5 (solid line), and 2 (dashed line). The corresponding incubation period distributions are shown in* FIGURE 13.

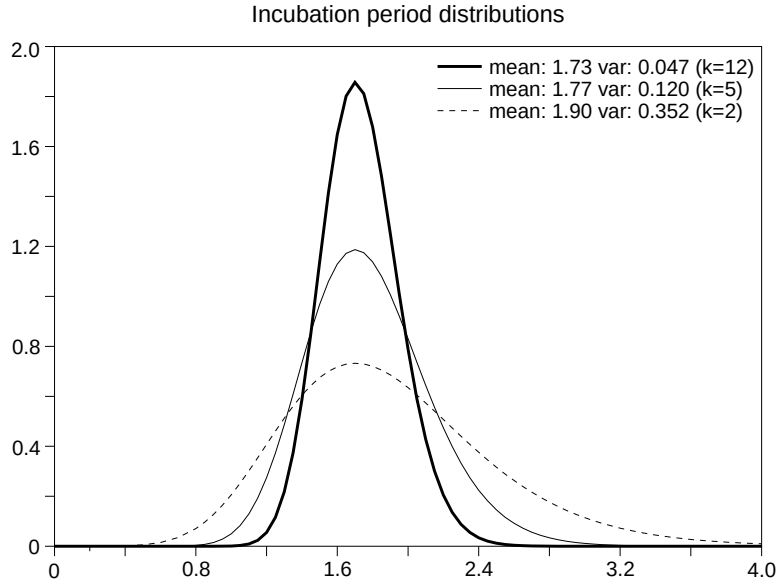

FIGURE 13 – *Incubation period distribuations used in* FIGURE 12. *They correspond to a log-like transformation of the gamma distribution used for the initial infection load, as shown in equations* (12), (13) *and* (13'), *for various values of the shape parameter k: 12 (thick line, usual shape), 5 (solid line), and 2 (dashed line).*

7 Discussion

We have presented a detailed model of scrapie transmission dynamics within a sheep flock. *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.

Figures 6, 11 & 12 Specifically, we have used this model to explore the hypothesis of increased transmission during lambing which might result from ingestion of scrapie-infective placental material. Two extreme scenarios were considered, 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.

Figures 7 & 8 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.

Figures 9 & 10 *The number of scrapie cases per genotype is extremely well reproduced by the model.* 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, although the hypothesis of Hardy-Weinberg equilibrium was globally verified on the 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 [4].

Incubation period The incubation period of 1.73 years estimated for the Langlade Romanov outbreak is slightly shorter than previous estimates, for example the 1.9 year incubation period estimated for the NPU Cheviot flock [25]. 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 [18]. 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.

Comparison with other outbreaks The detailed analysis of the Langlade outbreak has provided evidence to support the hypothesis of increased scrapie transmission during the lambing period. Further analyses of summary data from three additional Scottish outbreaks [24] 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.

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 flexible tools* for combining complex sets of data inputs and exploring the consequences of alternative biological scenarios.

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APPENDIX

A Overview of the mathematical model

Note: In the equations below corresponding to a "simplified" version of the mathematical model, the infection load θ is replaced by the infectiousness i, $c_g = c$, and $\beta_g(t, \theta', a) = \beta_g(t, i')$.

The dynamics at time t of susceptible sheep $S_g(t, a)$ of age $a > 0$ and genotype g is given by equation (14), 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 (14′) 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 $I_{g'}(t, a', i')$.

$$\begin{aligned}\frac{\partial S_g}{\partial t} + \frac{\partial S_g}{\partial a}(t,a) &= -\mu(a) S_g(t,a) \\ &\quad - S_g(t,a) \sum_{g'} \int_0^A \int_0^1 \beta_g(t,i') I_{g'}(t,a',i') di' da' \end{aligned} \tag{14}$$

$$\begin{aligned} S_g(t,0) &= \sum_{g'} G_{gg'}(t) \int_0^A b(t,a') S_{g'}(t,a') da' \\ &\quad + \sum_{g'} G_{gg'}(t) \int_0^A b(t,a') \int_0^1 (1-\gamma_g(i')) I_{g'}(t,a',i') di' da' \end{aligned} \tag{14'}$$

If a' is a mature age, $b(t, a') = b(t)s(t)$, 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 (15) and (16), where $\phi(i)$ represents the initial infectiousness distribution (gamma distribution), and c the exponential rate of increase of the infectiousness ($\frac{di}{dt} = c\, i$). Equation (16′) describes the birth of infected lambs, which by definition only occurs by vertical transmission, hence (15′). Moreover, infected sheep have a positive infectiousness, hence boundary conditions (15″,16″).

$$\begin{aligned}\frac{\partial H_g}{\partial t} + \frac{\partial H_g}{\partial a} + \frac{\partial c\, i H_g}{\partial i}(t,a,i) &= -\mu(a) H_g(t,a,i) \\ &\quad + \phi(i) S_g(t,a) \sum_{g'} \int_0^A \int_0^1 \beta_g(t,i') I_{g'}(t,a',i') di' da' \end{aligned} \tag{15}$$

$$H_g(t,0,i) = 0 \tag{15'}$$

$$H_g(t,a,0) = 0 \tag{15''}$$

$$\frac{\partial V_g}{\partial t} + \frac{\partial V_g}{\partial a} + \frac{\partial c\, i V_g}{\partial i}(t,a,i) = -\mu(a) V_g(t,a,i) \tag{16}$$

$$V_g(t,0,i) = \phi(i) \sum_{g'} G_{gg'}(t) \int_0^A \int_0^1 b(t,a') \gamma_g(i') I_{g'}(t,a',i') di' da' \tag{16'}$$

$$V_g(t,a,0) = 0 \tag{16''}$$

B Possible extensions to the model

Birth rate Possible simplifications for the calculation of the birth rate $b(t) = u(t)\,P_b(t)$, where $P_b(t)$ represents the breeding population size and $u(t)$ a control term – cf. equations (7,7',7") page 8, are listed below.

- With $b(t)$ chosen so as to compensate the mortality and attain the target population size P_c at the end of the lambing period, birth equations (3′) and (5′) become:

$$S_g(t,0) = u(t)\,\frac{\overbrace{\sum_{g'} G_{gg'}(t) \int_{a_1}^{a_2} \big(S_{g'}(t,a') + \int_0^1 (1-\gamma_g(\theta'))I_{g'}(t,a',\theta')\,d\theta'\big)da'}^{B_S(t)}}{P_b(t)},$$

$$V_g(t,0,\theta) = u(t)\,\phi(\theta)\,\frac{\overbrace{\sum_{g'} G_{gg'}(t) \int_{a_1}^{a_2}\int_0^1 \gamma_g(\theta')I_{g'}(t,a',\theta')\,d\theta'\,da'}^{B_I(t)}}{P_b(t)}.$$

  We could fix $B_S(t)$ and $B_I(t)$ to their values at the beginning of the lambing period; the first year it would be: $B_S(t) = B_S(t_s)$ and $B_I(t) = B_I(t_s)$, $\forall t \in [t_s, t_s + T]$.

- We could choose a control $u(t) = K[P_c - P(t)]$ with K very big (so the mortality could be neglected).

Infectiousness The infectiousness used in the transmission rates (10) and (11) is proportional to the infection load θ', which increases exponentially during the incubation period (rate c_g). This assumption is not easy to verify. We could instead use a non decreasing function $g(\theta')$ for the infectiousness. A possible scenario would be to assume that all infected animals have the same infectiousness, i.e. $g(\theta') = 1,\ \forall\theta'$.

Horizontal transmission To emphasize the seasonal transmission hypothesis – for which horizontal transmission mostly occurs from ingestion of scrapie-infective placental material – horizontal transmission could be chosen proportional to the sum of all infected *breeding* animals instead of the total infected population (still weighted by their infectiousness). So the horizontal transmission rate (10) would also depend on the age a' of the infecting sheep:

$$\beta_g(t,a',\theta',a) = \begin{cases} k_h\,\sigma_g\,\theta'\,\delta(t)\,[f(a)] & \text{if } a_1 \leqslant a' \leqslant a_2, \\ 0 & \text{otherwise.} \end{cases}$$

Seasonality Two seasonal functions could be introduced instead of $s(t)$ defined in (7'): the first one $s_l(t) = s(t)$ for the lambing period; and the second one $s_h(t)$ for the horizontal transmission period, which could last longer than the lambing season, or be shifted... For instance, instead of having no transmission outside the lambing period, it could be set at a very low level ε for the seasonal model:

$$s_h(t) = \begin{cases} 1 & \text{if } (t \bmod 1\text{ yr}) \in [t_s, t_s + T] \text{ (lambing period)}, \\ \varepsilon & \text{otherwise.} \end{cases}$$