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## Dynamics of a scrapie outbreak in a flock of Romanov sheep – estimation of transmission parameters

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### SUMMARY

Knowledge of epidemiological mechanisms and parameters underlying scrapie transmission in sheep flocks remains very limited at present. Here we introduce a method for fitting stochastic transmission models to outbreak data to estimate bounds on key transmission parameters. We apply this method to data describing an outbreak of scrapie in a closed flock of Romanov sheep. The main findings are that the relative infectiousness of infected animals in this outbreak becomes appreciable early into disease incubation and that the mean incubation period is less than 1·5 years. We also find that the data are consistent with a broad range of values for the basic reproduction number  $R_0$  and describe how the boundaries of this range depend on assumptions about the mean incubation period and the contribution to transmission of a long-lived environmental reservoir of infectivity.

### INTRODUCTION

Scrapie was the first identified transmissible spongiform encephalopathy (TSE), and it appears to be one that can remain endemic in its natural host population, sheep (for a recent review see [1]). Host susceptibility is strongly influenced by genetic factors, which have been well studied [2], and depends on the strain of the scrapie agent. Many other aspects of its epidemiology are very poorly understood, however, due to limited knowledge of transmission mechanisms, scarce field data and poor past surveillance. The precise mechanisms of horizontal transmission of scrapie are unclear; faecal–oral transmission is an important candidate. There is evidence that the environment can act as a long-lived reservoir of infectivity [3, 1]. Furthermore, several studies have

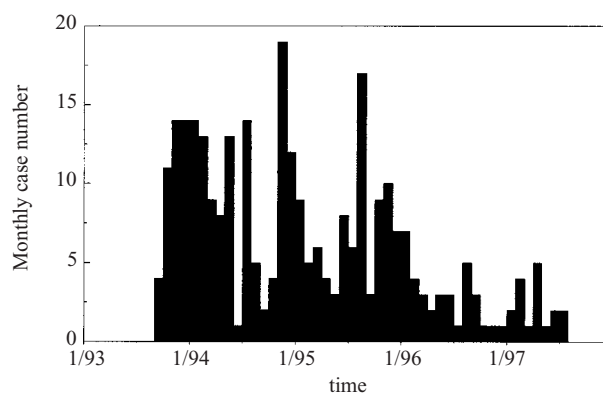
presented indirect evidence for maternal transmission, and it is argued that the observed incidence in lambs born to ewes or sires that went on to show clinical signs of scrapie, could not be explained solely by differences in inherited susceptibility [1]. Lastly, evidence suggests that apparently resistant host genotypes may sometimes be able to act as subclinical carriers of infection. In this case the infection might be pathogenic but with an incubation period that is very long, exceeding the natural life span of the host [1, 4]. As a result of the epidemic of bovine spongiform encephalopathy (BSE) and its link with new-variant Creutzfeldt–Jakob disease (vCJD) in humans, control of scrapie [5] and possibly other TSEs in mammalian species has become a priority, and epidemiological research into scrapie has been intensified. In Britain, a postal survey was conducted in 1998 [6–10], and interim results of a large-scale survey of sheep genotypes and occurrence of scrapie have been reported by Baylis et al. [11]. In 1998, a selective breeding programme for control and eventual eradication of

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scrapie has been started in The Netherlands. Similar programs have recently been launched in Britain [12] and France. A better understanding of the transmission mechanisms and dynamics of scrapie would greatly assist in achieving disease control. Mathematical models of the transmission dynamics are an important tool both for studying the transmission dynamics and for assessing the effect of different control options. Analyses of scrapie outbreaks using deterministic mathematical models have been carried out by Woolhouse et al. [13] and Matthews et al. [14]. Similar models have been used to study the within-flock transmission dynamics under a variety of hypothetical transmission scenarios [15–19]. Other recent theoretical work has considered between-flock transmission, using simple mathematical models to study the time scale of scrapie epidemics [20], estimated epidemiological parameters from a postal survey [10], and introduced a more detailed model to study the effects of alternative selective breeding policies [21].

In this paper we develop a method to fit *stochastic* transmission models to outbreak data and explore its use by analysing a high-incidence scrapie epidemic. The method is based on comparing the data to samples from the distributions of outcome quantities for a broad range of hypothetical transmission scenarios. A transmission scenario is defined by a set of model parameters and assumptions. The outcome quantities are variable due to the intrinsic randomness in epidemic and demographic processes (demographic stochasticity). The distribution is obtained from stochastic realizations of each particular scenario. Each of these realizations corresponds to a model run based on a different sequence of random numbers used to generate a sequence of random events. At present, given the uncertainties surrounding the transmission characteristics of scrapie, it is important to consider a broad variety of different transmission scenarios.

This paper is the first analysis of scrapie outbreak data that uses a stochastic model to calculate outcome variability directly instead of making ad-hoc assumptions about the variability around a deterministic model result. The scrapie epidemic examined in this study occurred in a closed flock of Romanov sheep managed by the Institut National de la Recherche Agronomique (INRA) on the ‘Langlade farm’ near Toulouse, France, and has been recorded and analysed by Elsen et al. [22]. It was characterized by high incidence rates in susceptible genotypes. Figure 1 shows the observed case numbers recorded



**Fig. 1.** Monthly number of clinical scrapie cases vs. time in the INRA Romanov flock in the period 1993–1997. Data from Elsen et al. [22].

through time. The INRA Romanov flock contained approximately 600 animals in 1993, and this number dropped gradually over time to about 400 in early 1997 (Fig. 1 in Elsen et al. [22]). During this period a total of 304 scrapie cases occurred. The epidemic started in a subflock (comprising about 10% of the total flock) that had been experimentally challenged with the nematode parasite *Teladorsagia circumcincta* in 1991. As the flock was being monitored for the ongoing experiment, we will assume that no scrapie cases have been missed early on in the epidemic. PrP genotyping by Elsen and co-workers for animals in the flock found four different alleles: VRQ, ARQ, AHQ and ARR (where the three amino-acid symbols correspond to codons 136, 154 and 171 of the PrP gene, respectively). Both ARR and AHQ were dominant for nearly complete resistance to the scrapie agent (among 95 animals of ARR/ARR, ARR/AHQ or AHQ/AHQ genotype and 319 animals with a single ARR or AHQ allele, there was only 1 confirmed scrapie case). ARQ and VRQ are both associated with susceptibility, with 76% of the mature (greater than 1 year old) VRQ/VRQ animals, 52% of the mature ARQ/VRQ animals, and 42% of the mature ARQ/ARQ animals, affected by scrapie. The disease-induced mortality led to a marked decrease in the frequency of susceptible genotypes over the course of the epidemic.

## METHODS

### Mathematical model

We formulate transmission scenarios using the framework of an age-structured susceptible-infected model (SI model) as represented graphically in

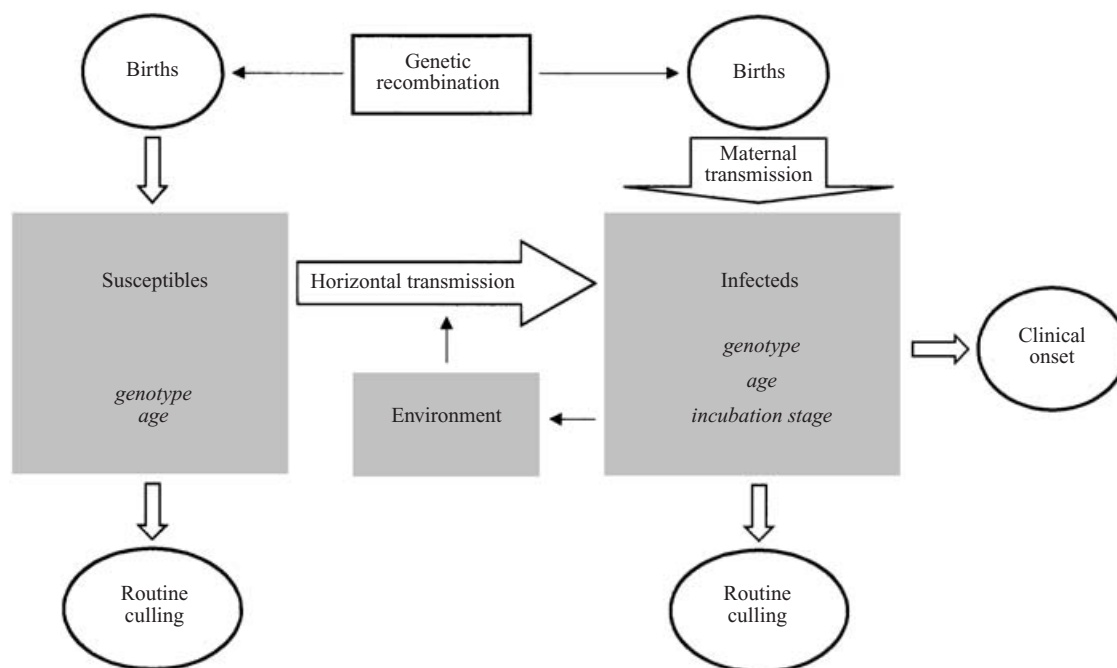


Fig. 2. Structure of the transmission model used to analyse the scrapie outbreak in the INRA Romanov flock.

Figure 2. The inclusion of age structure is needed for realistic modelling of host demography and to distinguish between animals of reproductive and of pre-reproductive age. It is also necessary to incorporate age when fitting to age-at-onset data. The infected population is further stratified by incubation stage. This allows us to study hypotheses about how infectiousness varies as a function of the expected time to clinical onset. The sheep survival curve is modelled by a truncated Weibull form as in [15]. Lambing is modelled to occur between the 5th and the 25th week of the year, with the majority of lambs being born during April [13]. Since no onsets were observed in animals aged less than 1 year, we assume a minimum incubation period of 1 year. The incubation period distribution is modelled by a gamma distribution with a one-year initial delay.

Horizontal transmission is modelled as a combination of two types of transmission: one is a direct transmission process (representing transmission through direct animal-to-animal contact or transmission via a short-lived environmental reservoir) and the other an indirect transmission process via a long-lived environmental reservoir. We assume that this long-lived reservoir accumulates and loses infectivity in such small units that these gains and losses can be described by a deterministic rate equation. Full mathematical details of the model are given in the Appendix of reference [19]. For the derivation of

expressions for the basic reproduction number,  $R_0$  (defined as the expected number of secondary infections generated by a single primary infection in an infection-naïve population) and the mean generation time of infections,  $T_g$ , we refer to [18]. The time  $T_g$  is defined as the average time (in an infection-naïve population) between the infection of a host and the transmission of the infection by this host to a second host.  $T_g$  consists of two parts (given in Eq. (32) in [18]): the first, which we denote by  $T_g^D$  here, is the mean generation time for infections that are transmitted ‘directly’ (i.e. without the agent spending an appreciable time in the environment between its shedding and the infection event), and the second measures the contribution of infections that take place via the long-lived environmental infectivity reservoir.

Host susceptibility is assumed to be independent of age and to be determined by four alleles (VRQ, ARQ, AHQ and ARR). We assume that AHQ and ARR are both dominant for resistance, leaving three susceptible genotypes (VRQ/VRQ, VRQ/ARQ and ARQ/ARQ).

### Testing scenarios

We initialize stochastic realizations with an average of 600 animals distributed over genotypes according to the frequencies observed in the INRA Romanov flock in the early part of 1993 (approximately described by

a Hardy–Weinberg equilibrium with the allele frequency of the VRQ and ARQ alleles being 0·2 and 0·5, respectively). We assume an initial infection prevalence of between 2 and 10% at the start of the simulated epidemics. This constitutes a range of assumptions about the effect of the nematode experiment in terms of generating an initial infection prevalence in the challenged subflock. The minimum of 2% initial prevalence is chosen to ensure that virtually none of the model runs describes an early extinction. The maximum of 10% initial prevalence corresponds to the complete subflock being infected.

We test scenarios for consistency with the observed epidemic by means of what could be called a parametric bootstrap technique. The rationale of our procedure is to judge whether the data could have arisen from the model by evaluating its deviation from the mean model outcome and comparing this deviation with the typical deviation of a single model realization. For each set of parameters and assumptions we obtain summary statistics from 20 stochastic realizations and compare their values with those from the epidemic in the INRA Romanov flock as observed by Elsen et al. [22]. These summary parameters are: the numbers of cases in the first 8 months of the epidemic, and in the subsequent three 6-month periods, the proportion of cases made up by each of the three different susceptible genotypes, and the mean ages at onset for each of these genotypes. Their numerical values are listed in Table 1. We assemble the summary statistics into one measure  $X_\rho^2$  for each realization:

$$\begin{aligned}
 X_\rho^2 &= x_{\rho C}^2 + x_{\rho F}^2 + x_{\rho A}^2, \\
 x_{\rho C}^2 &= \sum_{j=1}^4 \frac{(C_{\rho j} - \underline{C}_j)^2}{s_{C_j}^2}, \\
 x_{\rho F}^2 &= \sum_{\gamma=1}^2 \frac{(F_{\rho\gamma} - \underline{F}_\gamma)^2}{s_{F_\gamma}^2}, \\
 x_{\rho A}^2 &= \sum_{\gamma=1}^3 \frac{(A_{\rho\gamma} - \underline{A}_\gamma)^2}{s_{A_\gamma}^2}. \tag{1}
 \end{aligned}$$

Where  $C_{\rho j}$  is the total number of cases in the  $j$ -th monitoring subperiod,  $F_{\rho\gamma}$  is the fraction of the total number of genotype  $\gamma$  cases in the full monitoring period, and  $A_{\rho\gamma}$  is the mean age at onset of genotype  $\gamma$  cases in the full monitoring period. The  $\underline{C}_j$ ,  $\underline{F}_\gamma$ ,  $\underline{A}_\gamma$  are means over the 20 realizations, and  $s_{C_j}^2$ ,  $s_{F_\gamma}^2$  and  $s_{A_\gamma}^2$  are the corresponding variances. The target function (1) is very similar to Hotelling's  $T^2$ -statistic and an heuristic justification is as follows: quadratic addition

Table 1. *Summary statistics. Values of the summary statistics as observed in the epidemic in the INRA Romanov flock*

Statistic	Value
Fraction of cases of ARQ/ARQ genotype	0·280
Fraction of cases of ARQ/VRQ genotype	0·516
Fraction of cases of VRQ/VRQ genotype	0·204
Mean age at onset for ARQ/ARQ genotype	2·17 years
Mean age at onset for ARQ/VRQ genotype	2·42 years
Mean age at onset for VRQ/VRQ genotype	2·55 years
First 4 subperiod case numbers	79, 43, 55, 41

ensures that deviations of the various independent statistics cannot cancel out, and the weighting of terms by variances makes all contributing statistics equally important.

We compare the  $X_\rho^2$  values for  $\rho = 1, \dots, 20$  to the value  $X_*^2$  obtained by substituting the values of the data observed by Elsen et al. [22] on the position of the  $C_{\rho j}$ ,  $F_{\rho\gamma}$ , and  $A_{\rho\gamma}$  in the above expressions. A given parameter set is accepted when  $X_*^2$  lies within the 5th and 95th percentiles of the results for the  $X_\rho^2$  and rejected otherwise. Clearly, based on 20 realizations this procedure will not result in a very accurate estimate of the precise 90% confidence bounds. However, since using a very large number of realizations would be very computationally intensive when done concomitantly with sampling over a broad range of transmission scenarios, we settle for a crude estimate of the variation in  $X_\rho^2$ . In a subregion of the full sampling space of Table 2, we have also tested transmission scenarios on the basis of as many as 60 realizations and found no difference in the parameter ranges spanned by consistent scenarios in this subregion. This clearly indicates that calculations based on 20 realizations are sufficient for the purpose of obtaining insight into the ranges of parameter values consistent with the observed epidemic. However, we note that the limitation in the number of realizations that is computationally feasible prevents us from assessing the goodness-of-fit for accepted scenarios, since this would require a much larger number of realizations.

### Ranges of transmission scenarios

Table 2 describes the range of transmission scenarios examined. 75 000 parameter combinations were sampled using Latin Hypercube sampling [23]. The relative infectiousness of animals is assumed to be of a genotype-independent form consisting two terms: a

Table 2. *Parameter ranges. Model parameter values were sampled (using Latin hypercube sampling) from the ranges defined here. These ranges were examined both without maternal transmission and with a maternal transmission probability of 0.8 in the last incubation stage before onset*

Parameter	Min	Max
Mean life span	2.5 years	4.0 years
Initial prevalence of new infections	0.02	0.10
Relative susceptibility of ARQ/ARQ and VRQ/ARQ animals	0.0	1.0
Baseline relative infectiousness level	0.01	0.99
Fraction of mean incubation period elapsed when exponential increase in infectiousness starts	0.05	0.95
Mean incubation period	58 weeks	90 weeks
Number of post 1-year incubation stages	2	6
Half-life of environmental infection reservoir	50 weeks	200 weeks
Fraction of $R_0$ due to transmission via environmental reservoir	0.0	0.98
$R_0$	1.0	20

baseline level between 1 and 99% of maximal infectiousness and, starting when between 5 and 95% of the mean incubation period has elapsed, a term that grows exponentially towards onset. Scenarios with and without maternal transmission were examined.

The environmental reservoir is characterized by an infectivity half-life of between 50 and 200 weeks, and is responsible for up to 98% percent of the basic reproduction number  $R_0$ . Table 2 also lists the sampling ranges for the mean incubation period, the variance of the incubation period distribution (as determined by the number of incubation stage) and the relative susceptibilities of ARQ/VRQ and ARQ/ARQ animals. The overall horizontal transmission coefficient is the only parameter which sampling range depends on the values of the other parameters. This range is chosen such that the corresponding  $R_0$  values fall between 1 and 20.

## RESULTS

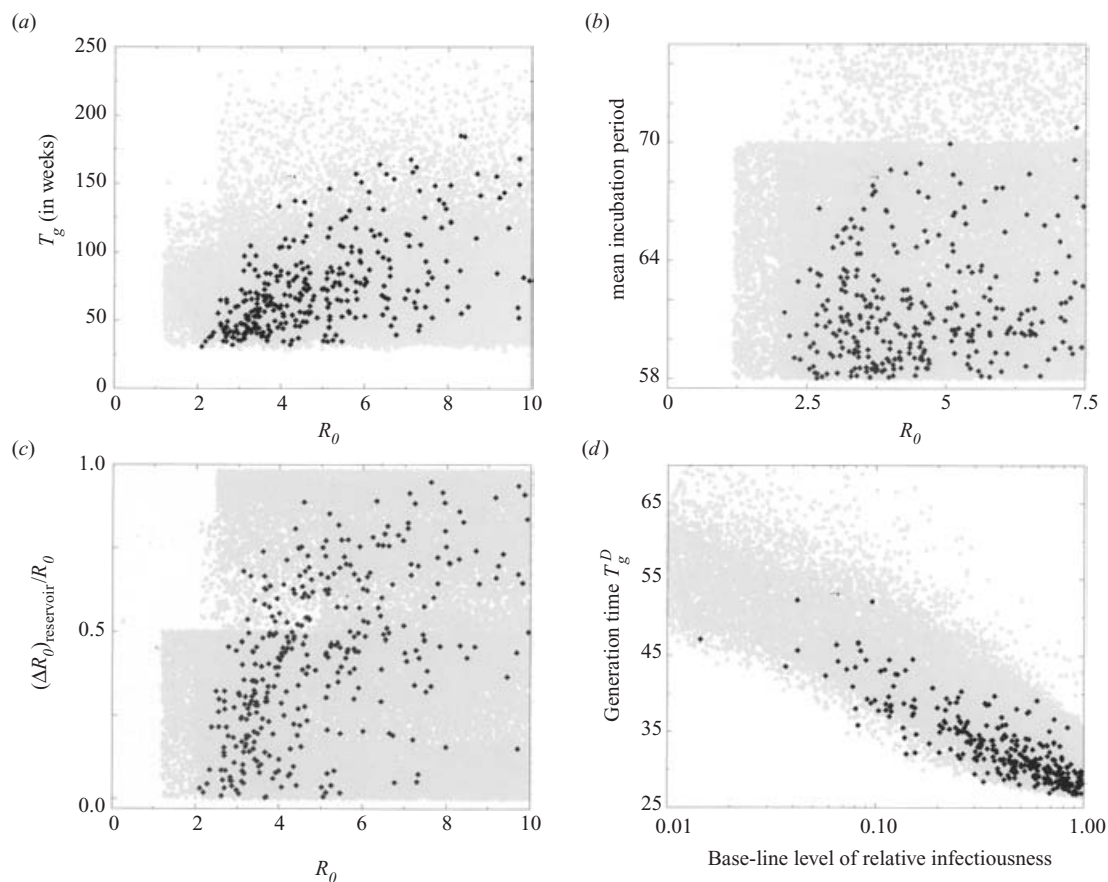
Figure 3 shows the regions from which parameter combinations were sampled as well as the subregions where consistent parameter combinations are located. Each point represents a transmission scenario. Parameter combinations consistent with the epidemic in the INRA Romanov flock, at the approximate 90% level of confidence, are indicated in black. We are mainly interested in the (approximate) boundaries of the parameter regions spanned by consistent scenarios. We avoid interpreting differences in relative

densities of consistent scenarios within such boundaries, as these relate at least in part to non-uniform densities of sampled parameter combinations.

Here we consider scenarios with horizontal transmission only; the inclusion of maternal transmission is discussed below. In Figure 3*a* we focus on the parameters  $R_0$  and  $T_g$ . We find that a broad range of  $(R_0, T_g)$  values are consistent with the Langlade epidemic. The lower bound on  $R_0$  is approximately 2.2, for a mean generation time  $T_g$  of approximately 0.6 years. As a function of an increasing mean generation time, the lower bound on  $R_0$  is observed to increase. This is as expected, since in order to obtain a given rate of epidemic growth, an increase in the average time between two generations of infections needs to be accompanied by an increase in  $R_0$ .

We have considered  $R_0$  values larger than the maximum of 10 shown in Figure 3*a*, and find consistent parameter combinations with  $R_0$  values at least as large as 20. In order to obtain a meaningful upper bound on  $R_0$ , more details of the observed initial rise in case numbers need to be included in our acceptance criterion. This can be achieved by distinguishing more (and shorter) time periods at the beginning of the epidemic. When splitting up the initial 8-month subperiod into three subperiods of 2, 2 and 4 months, Latin hypercube sampling of 10 000 scenarios yielded an upper bound of about 14 for  $R_0$ . At the same time the lower bound on  $R_0$  is goes up slightly to 2.5.

In contrast to the broad range of consistent  $R_0$  values, we find a very narrow range of values for the



**Fig. 3.** Transmission scenarios consistent (black symbols) and inconsistent (grey symbols) with the outbreak in the INRA Romanov flock. Time scales are measured in weeks. Only a subset of all inconsistent scenarios obtained are included to avoid capacity problems of the viewing software used. (a) Scenarios represented as points in the  $(R_0, T_g)$  plane. (b) Scenarios plotted as the mean incubation period vs.  $R_0$ . (c) Epidemic scenarios plotted as  $(\Delta R_0)_{\text{reservoir}}/R_0$ , the fraction of  $R_0$  due to transmission via the environmental reservoir, vs.  $R_0$ . (d) Epidemic scenarios plotted as the mean generation time  $T_g^D$  vs. the baseline level of the relative infectiousness.

mean incubation period. This is shown in Figure 3*b*, where transmission scenarios are labelled by their  $R_0$  and mean incubation period. The range of consistent mean incubation periods is bounded between 58 weeks, the assumed lower limit, and 75 weeks. The fact that the consistent values found for  $T_g$  (see Fig. 3*a*) can be well in excess of 75 weeks is due to the contribution of transmission via a long-lived environmental reservoir to  $T_g$ . This environmental contribution, measured as a fraction of the reproduction number  $R_0$ , is plotted against  $R_0$  in Figure 3*c*. We find a wide range of values spanned by consistent scenarios, with the lower bound on  $R_0$  weakly increasing with increasing environmental contribution.

In Figure 3*d* we present the consistent ranges of assumptions about how the infectiousness develops during incubation by showing the generation time  $T_g^D$  vs. the baseline level of infectiousness. Despite

intensive sampling few consistent scenarios were found with a baseline level of relative infectiousness below 0.05; the few that were found tend to have generation times at the lower end of the available spectrum. This suggests that the onset of the outbreak could not have been as rapid as observed if the infectiousness were to stay smaller than one-tenth of the maximum infectiousness until most of the incubation time has elapsed. This relative infectiousness has to be viewed in the context of tissue infectivity titres (bio-assayed in mice) in brain and CNS that were found to rise over at least 2–3  $\log_{10}$ s in the second half of the incubation period by Hadlow et al. [24]. Our result is consistent with the observation by Andreoletti et al. [25] of an early accumulation of the abnormal prion protein PrP<sup>Sc</sup> in gut-associated lymphoid and nervous tissues of sheep from this flock.

### Maternal transmission

In the above we have focussed on scenarios with horizontal transmission only. When maternal transmission is included, we find consistent scenarios up to very high maternal transmission probabilities. For instance, for scenarios with a maternal transmission probability of 0.8 in the last incubation stage before onset of disease, we find essentially no difference (from the results for horizontal transmission only) in the ranges of values for the remaining parameters spanned by accepted scenarios.

### DISCUSSION

In this paper we have used a stochastic transmission model to estimate transmission parameters from detailed data on a severe scrapie epidemic in France. The use of stochastic models allows us to estimate the typical variability in epidemic outcome (due to demographic stochasticity) and determine if an assumed underlying transmission scenario is consistent with the observed outbreak. To account for the current uncertainties surrounding the detailed transmission mechanisms of scrapie in sheep, we examined a large variety of transmission scenarios by sampling model parameter values from broad ranges. Perhaps the most interesting result from the present model analysis is the finding that the relative infectiousness of infected animals becomes appreciable early into disease incubation, in agreement with immunostaining results for this outbreak [25]. We note that this result could well be specific to the outbreak, in which the experimental challenge with nematodes might have altered the scrapie pathogenesis. We also find that the upper bound for mean incubation period is approximately 1.5 years. Thus the incubation periods in this outbreak are shorter than in most other outbreaks studied, where the mean incubation period is typically estimated to be around 2 years [13–16]. We estimate that the basic reproduction number  $R_0$  has a lower bound of 2.5 if one assumes a mean incubation period of at least 58 weeks. For longer mean incubation periods the lower bound on  $R_0$  increases rapidly. The lower bound on  $R_0$  also increases in the presence of a long-lived environmental reservoir of infectivity.

Although we are able to eliminate many transmission scenarios for this particular outbreak, the range of consistent values is still broad for many parameters. This highlights the need for more research

into the transmission dynamics of scrapie and the basic determinants of scrapie transmission. In future research, similar model analyses of data on less severe scrapie epidemics should provide more detailed information. For example, such studies may confirm the deterministic model analysis in [14] that suggested that older animals have reduced scrapie susceptibility, and homozygous and heterozygous susceptibles have different incubation periods. Parameter uncertainty is likely to be reduced in the future when more scrapie outbreaks have been followed in detail, especially when preclinical tests for scrapie infection (ideally with well characterized sensitivity and specificity at different stages of the incubation period) have been carried out during such an outbreak.

The estimation of transmission parameters for infectious diseases is an area that can benefit significantly from the increasing speed of computer processors. Here we selected consistent parameter combinations from a very broad range for a fairly detailed transmission model for ovine scrapie. However, it turned out to be computationally unfeasible to assess the goodness-of-fit for accepted scenarios, as this requires a much larger number of realizations per transmission scenario. Alternative estimation methods that might prove to be more powerful and/or efficient in estimating transmission parameters include Bayesian techniques that have recently begun to be applied to inference in infectious-disease epidemiology [26].

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