



Institute for Animal Health

Surveillance for classical scrapie in Great Britain: analysis of future needs

SE0243: Analysis and design of scrapie surveillance strategies for Great Britain
Milestone SD01/02: Report on the extended analysis of future surveillance requirements

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Summary

Background

- The National Scrapie Plan for Great Britain (NSP) was established to increase the level of resistance to transmissible spongiform encephalopathies (TSEs) in the national flock and, ultimately, to eradicate TSEs from British sheep.
- Defra has a public service agreement (PSA9), which sets a target of reducing the prevalence of scrapie in Great Britain by 40% (from 0.33% to 0.2%) before 2010.
- It is essential that any scrapie surveillance strategy implemented is able to detect trends in the prevalence of classical scrapie.

Methods

- Changes in scrapie prevalence for 2008-2020 were predicted assuming either a compulsory ram genotyping scheme (RGS) was implemented from April 2005 or a voluntary RGS was implemented until March 2008.
- A range of surveillance strategies were simulated and assessed for their ability to identify changes in prevalence. The strategies were built around fallen stock surveys (FS), which were supplemented by surveillance at the abattoir (AS) or data on reported cases (RC).
- Each strategy was simulated using the predicted population structure and prevalence of infection obtained when assessing the impact of each RGS.

Results

- The median prevalence estimates based on each surveillance strategy captured the trend in 'true' prevalence.
- There was some gain in precision of the prevalence estimates if the sample sizes in the FS or AS were increased from 10000 to 50000.
- Incorporating data on the age of animals sampled in the FS and AS in the analysis results in more precise prevalence estimates, especially if no (data on) RC are available.
- Increasing the number of sources of surveillance data was most effective at improving the precision of the prevalence estimates: the most precise estimates were obtained when data from FS, AS and RC were integrated together.
- Estimates for the prevalence in years towards the end of the time-period under consideration (i.e. 2018-2020) are typically poor. As a consequence, there is likely to be a delay of a number of years before a reliable assessment of whether a specified level of reduction (e.g. PSA9 target) has been achieved.
- If the change in prevalence over time is small, detecting that change is likely to be difficult regardless of the sample sizes used and whether or not multiple sources of data are integrated.

Conclusions

- Integrating multiple sources of surveillance data is most important for obtaining reliable prevalence estimates.

- Increasing the sample sizes used in either the FS or AS also improves the accuracy of the prevalence estimates, especially when the prevalence is lower. There could be benefits from increasing the sample size used over time to more reliably detect changes in prevalence.
- It is important to collect data on the PrP genotypes of negative samples, as well as positive samples.
- Consideration must also be given to ensuring the relevant data on sheep demography are available to interpret the surveillance data.

1 Introduction

The National Scrapie Plan for Great Britain (NSP) was established to increase the level of resistance to transmissible spongiform encephalopathies (TSEs) in the national flock and, ultimately, to eradicate TSEs from British sheep (Defra 2006). The first strand of the NSP was a voluntary ram genotyping scheme which was launched in 2001 for registered pedigree flocks and was subsequently extended in 2002 to non-registered purebred flocks. In addition, both voluntary (applying retrospectively) and compulsory (applying to newly identified holdings) scrapie flock schemes have been implemented within affected flocks. In order to monitor the impact of these control measures, it is essential that a surveillance programme is in place which is able to detect changes in the prevalence of classical scrapie. In particular, Defra has a public service agreement (PSA9), which sets a target of reducing the prevalence of scrapie in Great Britain by 40% (from 0.33% to 0.2%) before 2010. The PSA9 target was devised following a mathematical modelling assessment of the impact of a range of compulsory ram genotyping schemes (RGS) that were to be implemented from April 2005 (Gubbins & Roden 2006; Roden *et al.* 2006).

Standard statistical methods for determining sample size for prevalence estimates (Cannon & Roe 1982; Thrusfield 1995) are not appropriate for scrapie, because they do not take into account either the heterogeneous distribution of infection within a population or the variation in sensitivity, and possibly specificity, of diagnostic tests with stage of incubation and prion protein (PrP) genotype. Moreover, it is necessary to be able to relate the prevalence of infection in each sample population (for example, the abattoir population or fallen stock) to that in the national flock. Hence, an alternative approach must be used which can allow for changes in prevalence and test sensitivity with age and PrP genotype and which can be used to scale-up from the sample to the population prevalence. Such an approach simulates a surveillance strategy (allowing for multiple sources of data on scrapie occurrence) and then determines the prevalence of infection in the population that is consistent with the simulated data.

This report describes the results of an analysis of the design of scrapie surveillance strategies to detect trends in the prevalence of classical scrapie. Future trends in the frequency of PrP genotypes were predicted using the methods on which the PSA9 target was based (Roden *et al.* 2006), though future trends in prevalence were predicted using a more recent model developed to analyse scrapie surveillance data (Gubbins 2008; Gubbins & McIntyre 2009; cf. Gubbins & Roden 2006). The strategies considered in this report focused on the use of fallen stock and abattoir surveys and reported cases. In particular, the analysis addressed the impact of sample size and integration of multiple data sources on the efficacy of surveillance. Furthermore, the report is concerned only with surveillance for classical scrapie; surveillance for atypical scrapie is not considered.

2 Predicting future trends in scrapie prevalence

Future trends in scrapie prevalence were predicted using a two-step process. First, changes over time in the frequencies of PrP genotypes in the national flock were predicted using a gene-flow model. Second, the impact of the changes in PrP genotype frequencies on the prevalence of scrapie were assessed using a simple prevalence model.

2.1 Frequency of PrP genotypes

Future trends in the frequencies of PrP genotypes in the national flock were predicted assuming that a compulsory RGS had been introduced in April 2005 under which no use or sale of VRQ-bearing rams was allowed in all flocks of high genetic merit (defined as purebred flocks whose primary business is the production of rams for further breeding). In addition, trends in PrP genotype frequencies were also considered under a second scenario in which the current voluntary RGS (Defra 2006) was implemented until March 2008, after which there was no further genotyping in member flocks (Gubbins & Roden 2007).

The effect of each strategy on the frequencies of PrP genotypes in the British sheep population (figure 2.1a,b) was predicted using a deterministic gene-flow model. The model comprised three main components: the first predicted changes in allele frequencies within purebred populations; the second predicted the dissemination of these changes throughout the crossbred population; and the third predicted the frequency of different genotypes in the British populations of breeding rams, breeding ewes and lambs entering the food chain. A detailed description of the model is presented elsewhere (Roden *et al.* 2006). The model provided predicted genotype frequencies for 2003 onwards; prior to 2003 the genotype frequencies were assumed to be static (i.e. the same as in 2003).

2.2 Prevalence of classical scrapie

The prevalence of sheep infected with classical scrapie in the GB national flock, π_t , in year t is given by,

$$\pi_t = \frac{\sum_j \sum_a p_{jat} B_{j,t-a}}{\sum_j \sum_a s_a B_{j,t-a}}, \quad (2.1)$$

where,

$$p_{jat} = s_a r_j \phi_{t-a} \int_{a-1}^a \int_v^\infty f_j(w) dw dv, \quad (2.2)$$

is the prevalence of infected sheep of genotype j in age class a (comprising animals between $a-1$ and a years of age) in year t , ϕ_t is the baseline risk of infection for animals born in year t , r_j is the relative risk of infection for genotype j , s_a is the probability of surviving to be in age class a , $B_{j,t}$ is the number of animals of genotype j in birth cohort t and f_j is the probability density function for the log-normal incubation period distribution (with genotype-specific parameters, μ_j and σ_j). The baseline risk of infection for a birth cohort was assumed to depend on the prevalence of infection that year such that $\phi_t = 1 - \exp(-\beta\pi_t)$ (cf. Gubbins & Roden 2006).

The transmission parameter (β) and the relative risk of infection (r_j) were estimated by fitting the model to surveillance data on reported cases (1993-2007) and abattoir and fallen stock surveys (2002-2007) using methods described in Gubbins & McIntyre (2009; see also appendix A). Parameter estimates are presented in appendix B. The predictions for the future trends in prevalence based on the fitted model, (2.1)-(2.2), and the output from the gene-flow model (essentially the number of animals of genotype j in each birth cohort) for each selective breeding programme are presented in figure 2.1c,d.

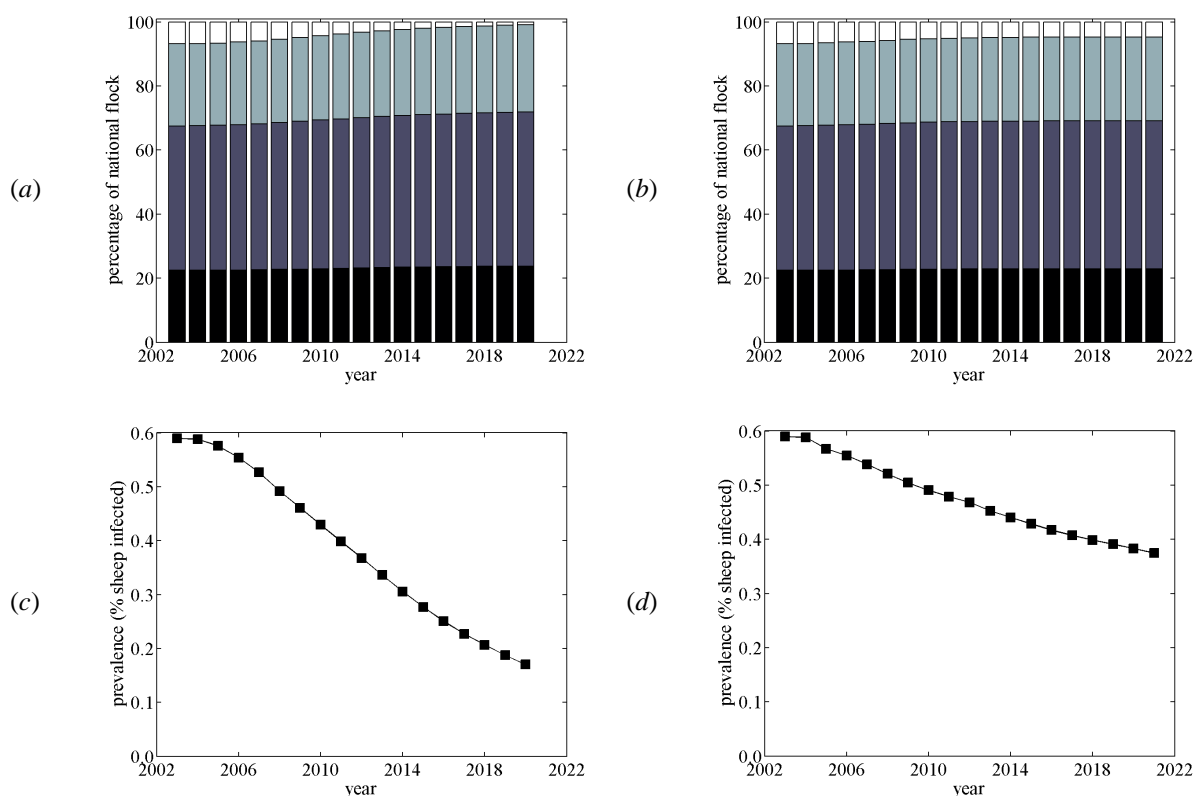


Figure 2.1. Predicted changes in (a,b) the frequency of NSP types in the national flock; and (c,d) the prevalence of infection in the national flock under: (a,c) a compulsory ram genotyping scheme implemented from 2005; or (b,d) a voluntary ram genotyping scheme implemented until 2008. In figures (a-b) different colours indicate different NSP types are: NSP type 1 (black); NSP type 2 (dark grey); NSP type 3 (light grey); and NSP types 4 and 5 (white).

3 Surveillance strategies to detect trends in prevalence

3.1 Surveillance strategies

The surveillance strategies considered in this report focus on the use of reported cases and fallen stock and abattoir surveys to detect changes in the prevalence of classical scrapie. Surveillance from 2003-2007 was implemented using the actual number of reported cases and sample sizes in the fallen stock and abattoir surveys (see Gubbins & McIntyre 2009 for details), while from 2008 onwards each strategy was defined in terms of the number of animals sampled at the abattoir or found dead on farm and the proportion of cases reported.

All the surveillance strategies were built around fallen stock surveys (FS) (with annual sample sizes of 10000 or 50000), because of the assumption that the National Fallen Stock Company (NFSCo) would provide a source of animals for testing. This was supplemented with surveillance at the abattoir (AS) (with annual sample sizes of 0, 10000 or 50000) and reported cases (RC) (with 0% or 10% of cases reported each year). Not all combinations of these sample sizes and levels of reporting were considered, however. It was assumed that: (i) only animals over 18 months of age would be sampled in the FS or AS; and (ii) the diagnostic test used in the fallen stock and abattoir surveys would detect an infected animal provided it was in the final quarter of its incubation period.

3.2 Simulating the strategies

Each strategy was simulated using the predicted population structure and prevalence of infection obtained when assessing the impact of each selective breeding programme (figure 2.1), though the focus was on the compulsory RGS implemented from 2005.

(a) Reported cases

For cases reported during 2003-2007, the number of reported cases of genotype j in age class a and year t (X_{jat}) was sampled from a multinomial distribution,

$$X_{jat} \sim \text{multinomial} \left(C_t, \frac{R_{jat}}{\sum_j \sum_a R_{jat}} \right),$$

where C_t is the total number of reported cases that year and R_{jat} is the expected number of reported cases (defined in appendix A). For 2008 onwards, the number of reported cases of genotype j in age class a and year t (X_{jat}) was sampled from a Poisson distribution with mean given by the expected number of reported cases (R_{jat}) (i.e. $X_{jat} \sim \text{Poisson}(R_{jat})$).

(b) Fallen stock and abattoir surveys

The number of animals in each age and genotype class sampled as part of survey i (fallen stock, FS, or abattoir, AS) in year t was drawn from a multinomial distribution,

$$Z_{jat}^{(i)} \sim \text{multinomial} \left(N_t^{(i)}, \frac{w_a^{(i)}(s_a - s_{a+1})B_{j,t-a}}{\sum_k \sum_{b=2}^{a_{\max}} w_b^{(i)}(s_b - s_{b+1})B_{k,t-b}} \right),$$

where $N_t^{(i)}$ is the sample size for survey i in year t , s_a is the probability of survival to age class a ,

$$w_a^{(i)} = \begin{cases} \eta_a & i = \text{FS}, \\ 1 - \eta_a & i = \text{AS}, \end{cases}$$

is the proportion of uninfected animals of age a which die and are sampled in survey i and η_a is the proportion of uninfected animals which die on farm (i.e. all animals are potentially sampled).

The number of positive samples for each age and genotype class was drawn from a binomial distribution,

$$P_{jat}^{(i)} \sim \text{binomial} \left(Z_{jat}^{(i)}, \frac{d_{jat}^{(i)} + (1 - \psi)u_{jat}^{(i)}}{p_{jat}^{(i)} + u_{jat}^{(i)}} \right),$$

where $p_{jat}^{(i)}$, $d_{jat}^{(i)}$ and $u_{jat}^{(i)}$ are the prevalence, detectable prevalence and proportion of uninfected animals for genotype j and age class a in year t and survey i , respectively (defined in appendix A), and ψ is the specificity of the diagnostic test used (assumed to be 100%). However, data on the ages of animals sampled are not necessarily available (especially for abattoir surveys), in which case the number of animals sampled and number of positive samples were aggregated across age classes, so that,

$$Y_{jt}^{(i)} = \sum_{a=2}^{a_{\max}} Z_{jat}^{(i)}, \quad D_{jt}^{(i)} = \sum_{a=2}^{a_{\max}} P_{jat}^{(i)}$$

are the number of animals over 18 months of age sampled and the number of positive samples for genotype j in survey i during year t , respectively.

3.3 Assessing the strategies

Twenty replicates of each surveillance strategy were simulated, and for each replicate trends in the prevalence were assessed using a back-calculation approach (Gubbins & McIntyre 2009; see also appendix A). Maximum likelihood methods were used to estimate: the baseline risk of infection for each birth cohort (independent parameter for each cohort); the proportion of infected animals surviving to disease onset which die on farm before clinical signs become apparent; and the proportion of cases reported each year (independent parameter for each year). The remaining parameters were assumed to be known: the relative risk of infection for each PrP genotype were taken from the estimates derived in section 2.2 (see appendix B)¹; the preclinical detection proportion was set at 25% (i.e. that used to simulate surveillance); the age-at-onset parameters, the probability of survival to each age class and the proportion of uninfected animals found dead were as described in Gubbins (2008; see also appendix B); and the frequency of animals of each PrP genotype in a birth cohort were taken from the predictions of Roden *et al.* (2006) (figure 2.1a,b).

4 Results

4.1 Simulated surveillance data

Examples of simulated surveillance data are shown in figure 4.1 for each sample size considered for the FS and AS and each level of reporting of clinical disease. Unsurprisingly, more positive samples are detected if the sample size is larger in either the FS or AS (figure 4.1a,c), though the median proportion of samples which produce a positive results is similar, regardless of sample size (figure 4.1b,d). However, the range for the proportion of positive samples tends to be narrower for a larger sample size (figure 4.1b,d). Finally, the number and proportion of positive samples in either survey decreases as the prevalence of infection decreases (figure 4.1a-d; cf. figure 2.1c).

As expected, the number of reported cases depends on the level of reporting and declines as the prevalence of infection decreases (figure 4.1e; cf. figure 2.1c). In contrast with the FS or AS, however, the variability of the number of reported cases is lower than that of the number of survey positives (figure 4.1a,c,e) (i.e. Poisson versus binomial variability).

4.2 Analysis of simulated surveillance strategies

Estimates derived from surveillance data based solely on FS with an annual sample size of 10000 animals capture the overall trend of a decline in prevalence, but they do not necessarily indicate a year on year decrease in prevalence (figure 4.2a). Moreover, there is a tendency to underestimate the actual prevalence. Including an additional source of surveillance data (either AS or RC) results in more precise estimates, though the effect is

¹ This was done principally to reduce computation time when assessing replicated surveillance data. In practice, the relative risk of infection in each genotype can be estimated directly from surveillance data without problems (Gubbins 2008; Gubbins & McIntyre 2009).

more marked for RC compared with AS (figure 4.2b,c). In particular, the tendency to underestimate the prevalence remains when using FS and AS, but this is not the case for FS and RC. The estimates based on FS and RC also tend to reflect the year on year decline in prevalence. Finally, the estimates obtained by integrating all three surveillance sources (FS, AS and RC) are the most precise and accurately capture the change in prevalence over time (figure 4.2d).

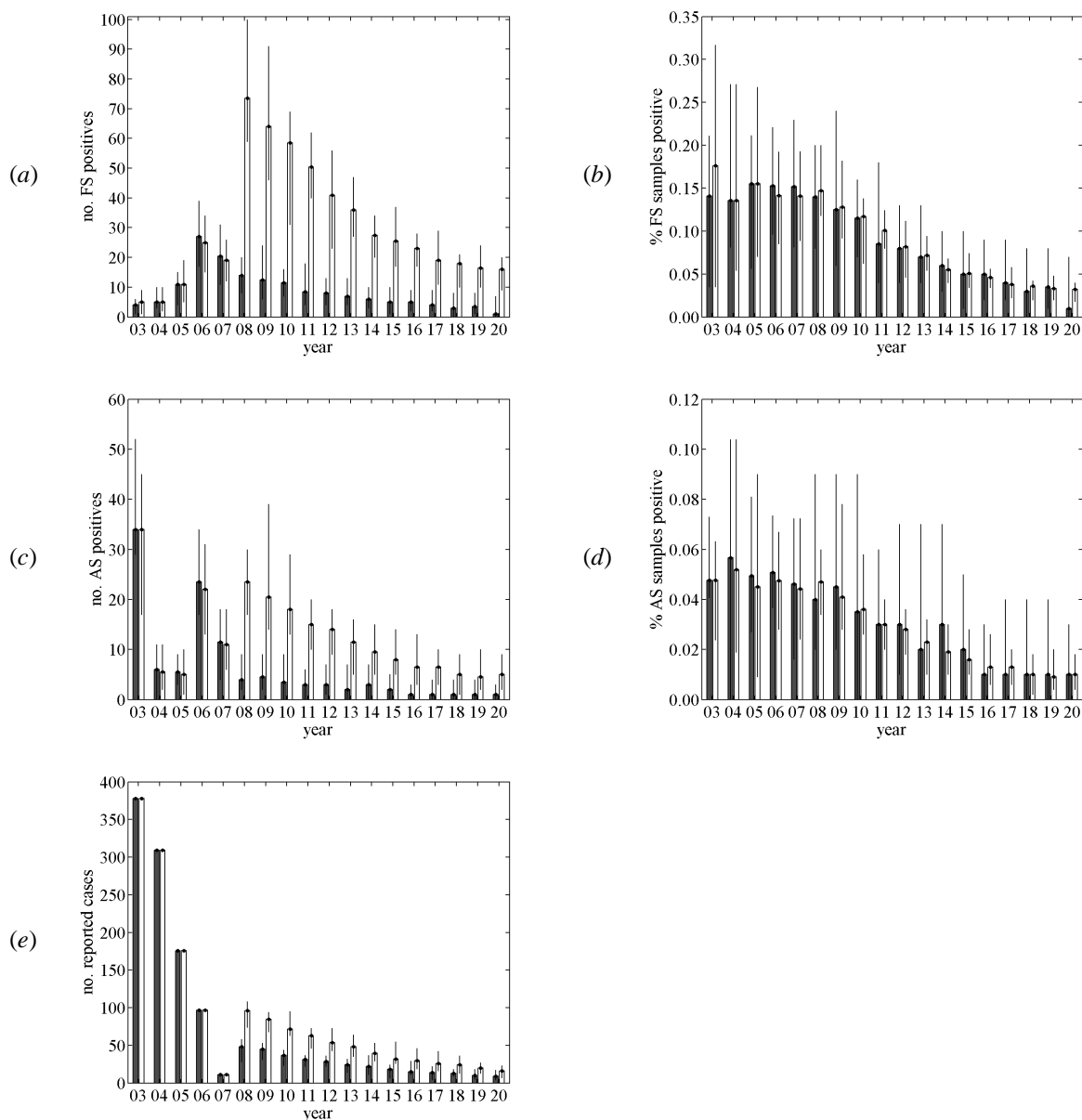


Figure 4.1. Simulated surveillance data for 2003-2020. (a,b) Fallen stock surveys (FS): (a) number of positive samples each year; and (b) percentage of positive samples. Annual sample sizes are either 10000 (grey bars) or 50000 (white bars). (c,d) Abattoir surveys (AS): (c) number of positive samples each year; and (d) percentage of positive samples. Annual sample sizes are either 10000 (grey bars) or 50000 (white bars). (e) Number of reported cases each year, when either 5% (grey bars) or 10% (white bars) of cases are reported each year. Results are shown for 20 replicates of each surveillance strategy assuming a compulsory ram genotyping scheme is implemented from April 2005 (see section 2.1). Each figure shows the median (bar) and 2.5th and 97.5th percentiles (error bars). Simulated data for 2003-2007 are based on the actual number of reported cases or sample sizes.

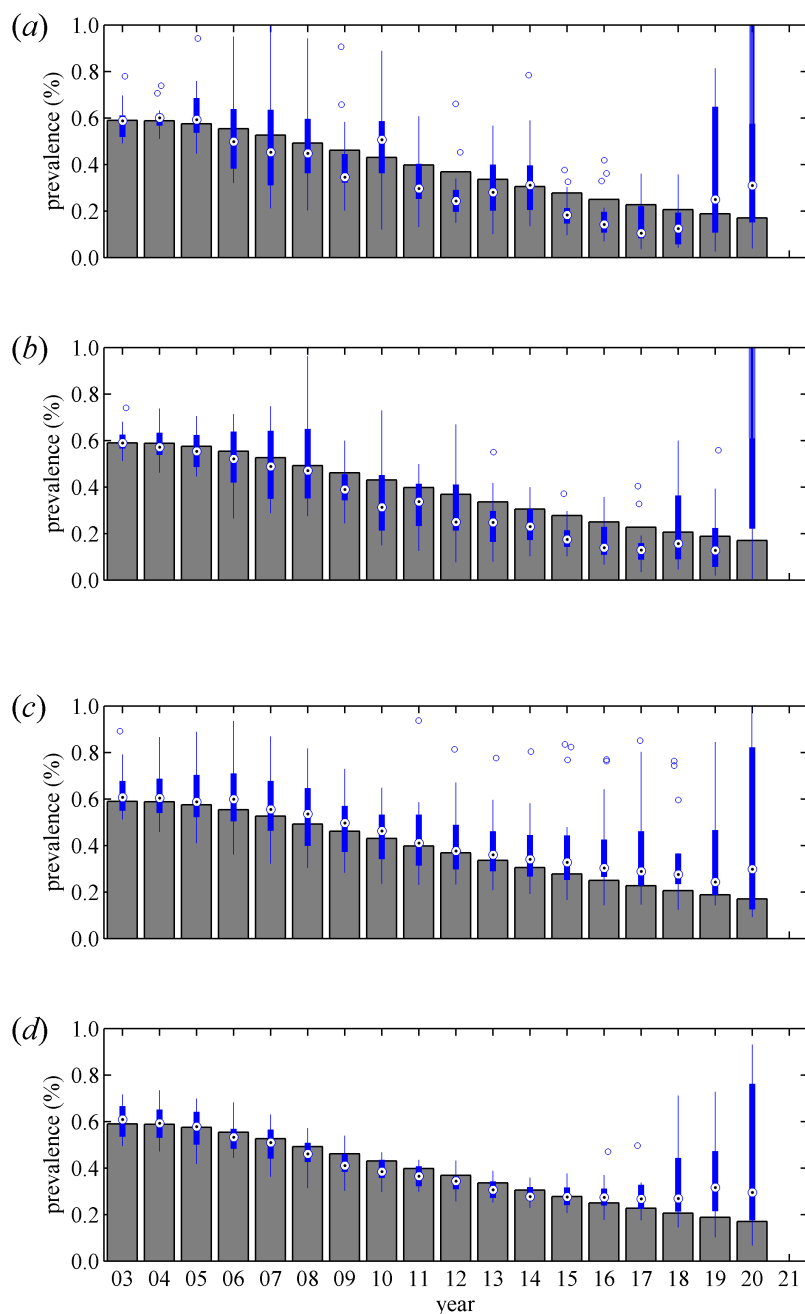


Figure 4.2. Comparison of prevalence estimates obtained from simulated scrapie surveillance strategies based on fallen stock surveys (FS), abattoir surveys (AS) and reported cases (RC), assuming a compulsory ram genotyping scheme is implemented from April 2005 (see section 2.1). Annual sample sizes in each survey and levels of reporting (% cases reported) (FS, AS, RC) were: (a) 10000, 0, 0%; (b) 10000, 10000, 0%; (c) 10000, 0, 10%; and (d) 10000, 10000, 10%. Each figure shows the true prevalence (bars) and maximum-likelihood estimates (MLEs) for each replicate of the surveillance strategy (boxplots: the dotted circles show the median; the boxes show the lower and upper quartiles; the whiskers indicate 1.5 times the interquartile ranges; and circles indicate any outlying values). Results are shown for 20 replicates of each surveillance strategy; simulated data for 2003-2007 were based on the actual number of reported cases or sample sizes.

Increasing the sample size in the FS or AS from 10000 to 50000 improves the precision of the prevalence estimates, particularly in later years (after 2012) when the prevalence is lower (figure 4.3; cf. figure 4.2). This improvement is most noticeable when surveillance is based only on these two sources. Similarly, collecting data on the age of animals sampled in the FS and AS and incorporating them in the analysis results in more precise

prevalence estimates, especially if there are no data on RC collected (which would otherwise be the only source of age-related surveillance data) (figure 4.4). The effect is more marked for the ages in the FS than the AS (cf. figures 4.2b, 4.4b & 4.4d).

For all surveillance strategies the prevalence estimates become increasingly poor for years towards the end of the time-period covered by surveillance (i.e. 2018-2020) (figures 4.2-4.4). This reflects a lack of data relating to infected animals in cohorts born in those years, largely because they will be at too early a stage of incubation to be detected in AS or FS and are unlikely to have developed clinical disease.

If the change in prevalence over time is smaller (i.e. assuming a voluntary RGS until March 2008), detecting that change is likely to be difficult regardless of the sample sizes used and whether or not multiple data sources are integrated (figure 4.5). However, the broad conclusions regarding sample sizes and integrating data still hold; in particular, the most precise estimates are obtained from the surveillance strategy which integrates FS, AS and RC (figure 4.5d).

5 Discussion

The back-calculation approach developed previously (Gubbins 2008; Gubbins & McIntyre 2009) provides a framework in which to estimate the prevalence of classical scrapie and, in particular, to detect any trends in prevalence. However, it is essential that appropriate data are collected, and the principal objective of this report has been to investigate the requirements for surveillance data. The methods used to interpret surveillance data also require a large amount of data on the demography of the sheep population, and, hence, it is essential to consider collection of these data in some detail too.

5.1 Surveillance data

The results of the analysis of simulated surveillance data indicate that integrating multiple sources of surveillance data is most important for obtaining reliable prevalence estimates (figures 4.2 & 4.3). In particular, those strategies which include both FS and RC (with or without AS) capture the change in prevalence most accurately. Increasing the sample sizes used in either the FS or AS also improves the accuracy of the prevalence estimates, though not to the same degree as integrating multiple sources of data. As the prevalence declines, the larger sample sizes do have more of an impact (figure 4.3), such that there could be benefits from increasing the sample size used over time to more reliably detect changes in prevalence.

Previous analyses of scrapie surveillance and, in particular, of sample sizes necessary to estimate prevalence with a particular level of accuracy have suggested that very large sample sizes (>50000) are necessary to be able to detect a change in prevalence (Webb *et al.* 2001; Hopp *et al.* 2003; Gubbins 2006), especially when relying on abattoir-based surveillance. However, these studies were all based on snapshots using data from a single year, often utilising only one surveillance source. Using data accumulated over a number of years is more efficient, because data from a single year provides information on the prevalence of infection in a number of birth cohorts.

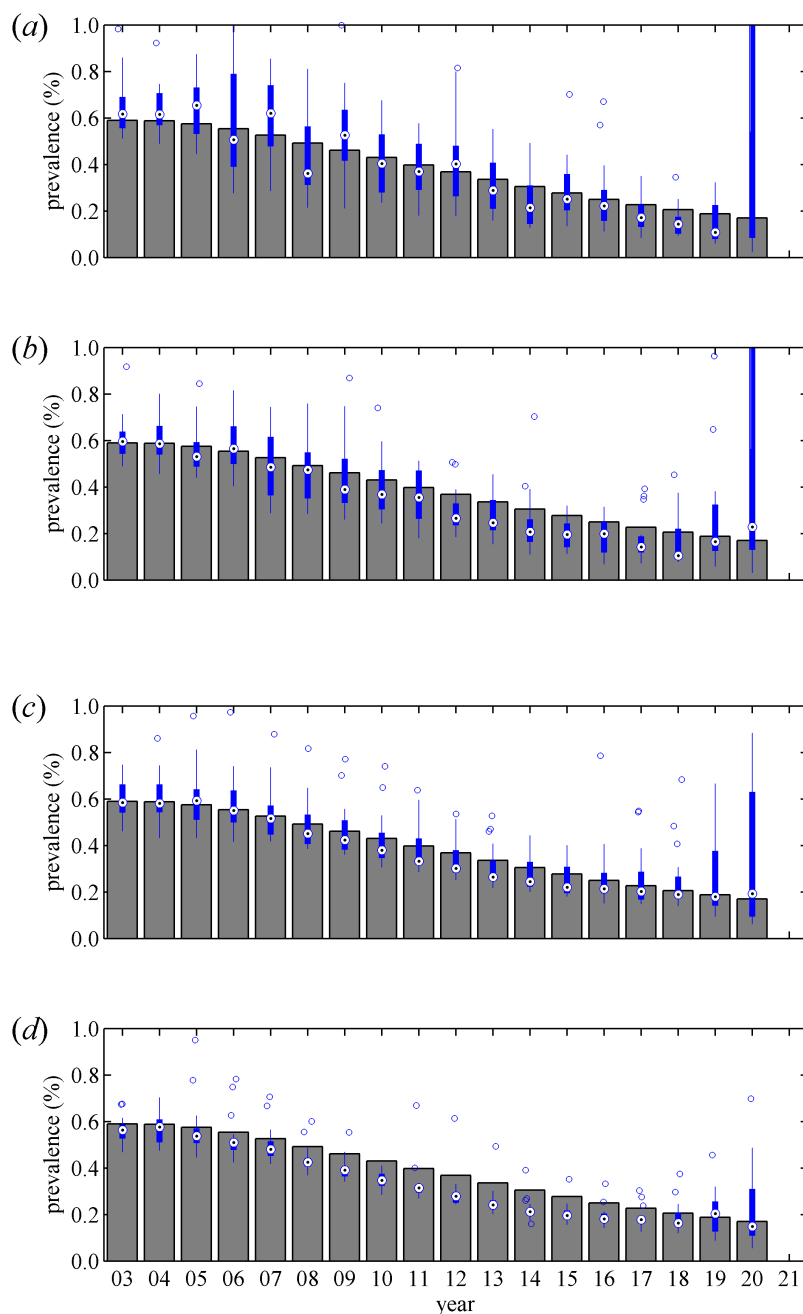


Figure 4.3. Comparison of prevalence estimates obtained from simulated scrapie surveillance strategies based on fallen stock surveys (FS), abattoir surveys (AS) and reported cases (RC), assuming a compulsory ram genotyping scheme is implemented from April 2005 (see section 2.1). Annual sample sizes in each survey and levels of reporting (% cases reported) (FS, AS, RC) were: (a) 50000, 0, 0%; (b) 50000, 50000, 0%; (c) 50000, 0, 10%; and (d) 50000, 50000, 10%. Each figure shows the true prevalence (bars) and maximum-likelihood estimates (MLEs) for each replicate of the surveillance strategy (boxplots: the dotted circles show the median; the boxes show the lower and upper quartiles; the whiskers indicate 1.5 times the interquartile ranges; and circles indicate any outlying values). Results are shown for 20 replicates of each surveillance strategy; simulated data for 2003-2007 were based on the actual number of reported cases or sample sizes.

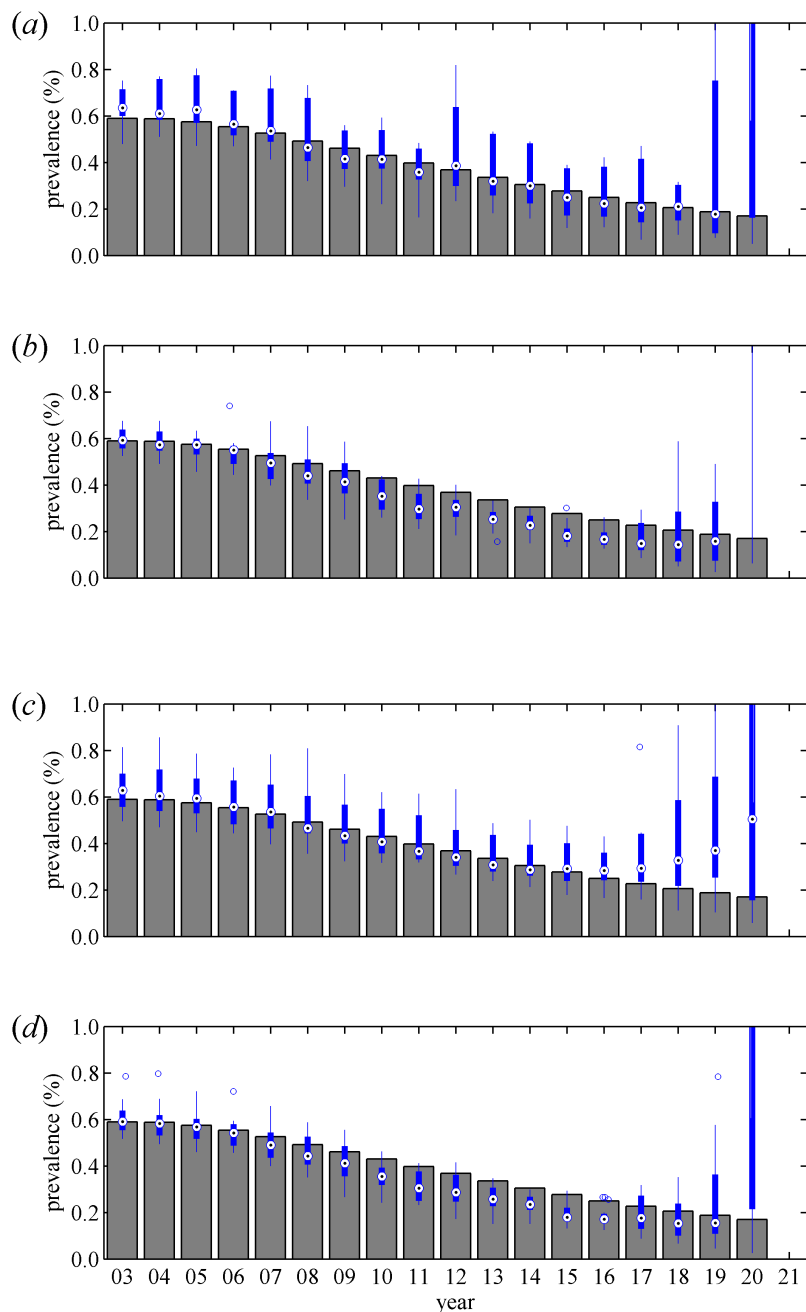


Figure 4.4. Comparison of prevalence estimates obtained from simulated scrapie surveillance strategies based on fallen stock surveys (FS), abattoir surveys (AS) and reported cases (RC), assuming a compulsory ram genotyping scheme is implemented from April 2005 (see section 2.1). Annual sample sizes in each survey and levels of reporting (% cases reported) (FS, AS, RC) were: (a) 10000, 0, 0%; (b) 10000, 10000, 0%; (c) 10000, 10000, 10%; and (d) 10000, 10000, 0%. Furthermore, data on the age of animals sampled were assumed to be available for: (a-c) FS only; and (d) FS and AS. Each figure shows the true prevalence (bars) and maximum-likelihood estimates (MLEs) for each replicate of the surveillance strategy (boxplots: the dotted circles show the median; the boxes show the lower and upper quartiles; the whiskers indicate 1.5 times the interquartile ranges; and circles indicate any outlying values). Results are shown for 20 replicates of each surveillance strategy; simulated data for 2003-2007 were based on the actual number of reported cases or sample sizes.

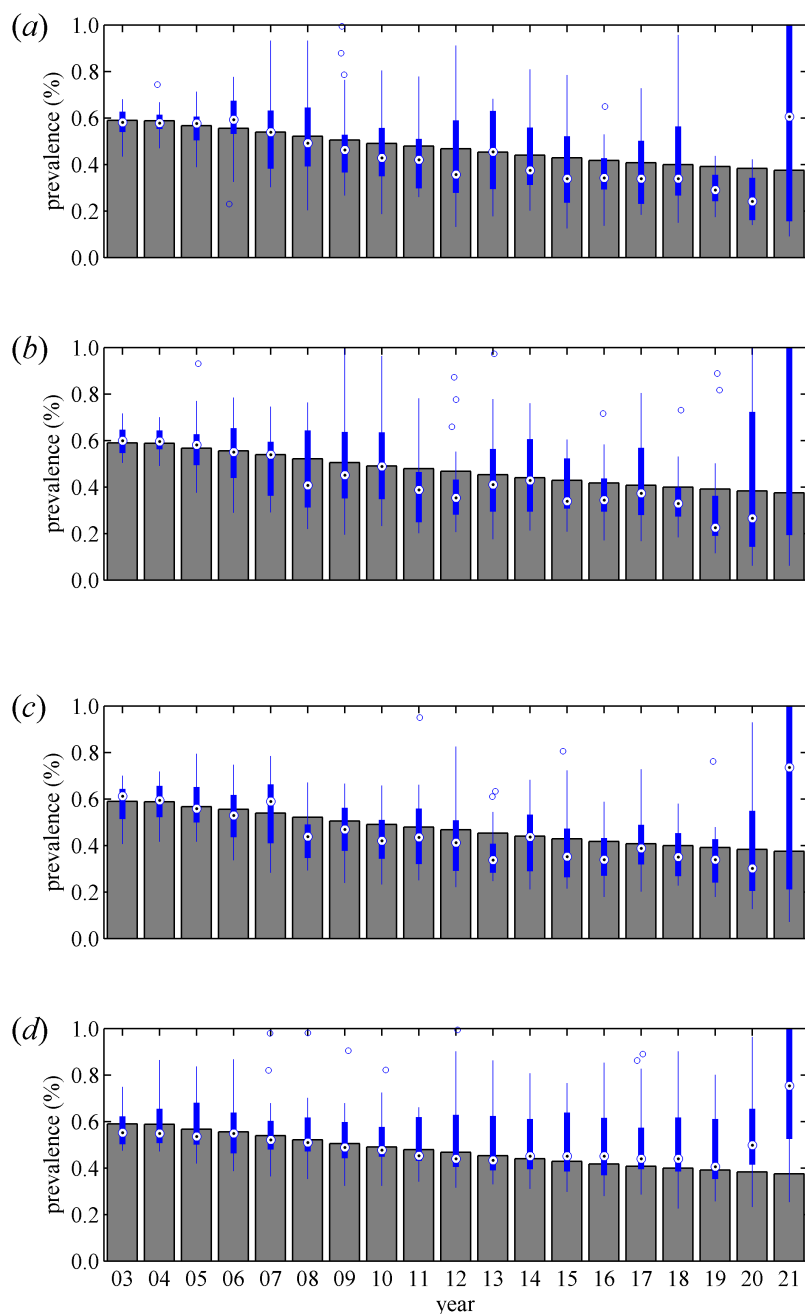


Figure 4.5. Comparison of prevalence estimates obtained from simulated scrapie surveillance strategies based on fallen stock surveys (FS), abattoir surveys (AS) and reported cases (RC), assuming a voluntary ram genotyping scheme is implemented until March 2008 (see section 2.1). Annual sample sizes in each survey and levels of reporting (% cases reported) (FS, AS, RC) were: (a) 10000, 0, 0%; (b) 10000, 10000, 0%; (c) 50000, 50000, 0%; and (d) 10000, 10000, 10%. Each figure shows the true prevalence (bars) and maximum-likelihood estimates (MLEs) for each replicate of the surveillance strategy (boxplots: the dotted circles show the median; the boxes show the lower and upper quartiles; the whiskers indicate 1.5 times the interquartile ranges; and circles indicate any outlying values). Results are shown for 20 replicates of each surveillance strategy; simulated data for 2003-2007 were based on the actual number of reported cases or sample sizes.

Irrespective of the surveillance strategy implemented, there is likely to be a delay of a number of years before a reliable assessment of whether a specified level of reduction (e.g. PSA9 target) has been achieved (figures 4.2-4.4). This reflects the fact that several years' data are required to provide a reliable estimate for the baseline risk of infection for a birth cohort, a consequence of the long incubation period for scrapie. Alternatively, a model which relates the baseline risk to the current level of infection can be used, which does result in narrower confidence limits for the prevalence (Gubbins & McIntyre 2009), but this assumes that the model used provides a reasonable description the relationship between prevalence and risk.

The analyses also examined the impact on the prevalence estimates of incorporating data on the ages of the animals sampled. If there were no other source of data on the age distribution of infected animals (for example, if there were no reported cases), collecting this information does help improve the prevalence estimates, though the impact is greater for FS than for AS. This suggests that from the perspective of estimating prevalence it is worth collecting data on the ages of animals sampled as fallen stock, but not those sampled at the abattoir. However, collecting data on the ages of animals sampled (i.e. not just those which are infected) will provide important demographic information needed when interpreting the surveillance data (see below).

In the analyses we have assumed that data on the PrP genotypes of negative samples in the fallen or abattoir surveys would also be available, though, in practice, these data have not been collected routinely, except for those animals sampled between January 2002 and March 2003. It is difficult to assess the impact of not having these data in a theoretical study, because the model used to generate the surveillance data is also that which would be used to impute the PrP genotypes of negative samples. However, some evidence for the impact of having to impute the data comes from the analysis of actual surveillance data for 1993-2007 (Gubbins & McIntyre 2009). In this case, the fit of the model to data from years for which PrP genotype data were not available (2004-2007) was noticeably poorer. Moreover, comparing the observed and imputed PrP genotypes for animals sampled in abattoir surveys in 2002 and 2003 showed that they differed significantly. In particular, the imputed PrP genotypes tended to overestimate the frequency of ARR- and ARH-bearing animals and underestimate the frequency of VRQ-bearing animals. Differences between the observed and imputed PrP genotype frequencies will reflect, in part, biases in the NSP data used to estimate the frequency of PrP genotypes in the national flock. It could also be a consequence of the estimates used for the survival probabilities or the proportion of uninfected animals found dead on farm. In principle, this problem could be rectified by collecting data on the PrP genotypes of the negative samples for a large enough proportion of the negative animals. For example, to estimate a genotype prevalence of $0.1\% \pm 0.1\%$ with 95% confidence would require 3837 animals to be genotyped.

5.2 Demographic data

To interpret the surveillance data it essential to have reliable data on the demography and, in particular, the age and PrP genotype structure of the relevant sheep populations: abattoir, fallen stock and national flock. Importantly, in the analyses of simulated surveillance data presented in this report, and in previous analyses of observed data, we have assumed that reliable demographic data are available.

The back-calculation model used to estimate the prevalence of infection (see appendix A; Gubbins & McIntyre 2009) includes a number of demographic parameters: (i) probability of survival to each age class; (ii) proportion of uninfected animals which die on farm in each age class; (iii) number of lambs born each year; and (iv) proportion of lambs of each PrP genotype in a birth cohort. These have been estimated from a range of data sources (discussed below), but only the number of lambs born each year is collected routinely (as part of the June agricultural survey). This contrasts with the situation for cattle where detailed demographic data are available via the Cattle Tracing System (see, for example, Ferguson & Donnelly (2003) in the context of interpreting BSE surveillance data).

The probability of survival to each age class was estimated from data collected as part of an anonymous postal survey conducted in 2002 (Sivam *et al.* 2003, 2006). The proportion of uninfected animals in each age class found dead on farm was estimated from data collected between 2004 and 2006 as part of a study investigating possible associations between PrP genotype and survival and production traits (Defra-funded project SE0236). Similar approaches could be used to collect updated data to investigate whether these parameters change over time, as could be the case if the age structure of the sheep population were to change, for example, in response to changes in policy or subsidies. Alternatively, these parameters could be inferred directly from surveillance data, if the ages of the animals sampled were recorded. Without more detailed recording of sheep, however, it is unlikely that age data could be obtained to the level of accuracy required (age in years). For example, aging by dentition is problematic and can only be used to distinguish between animals under four years of age (Cocquyt *et al.* 2005).

The proportion of lambs of each PrP genotype in a birth cohort was estimated from the results of the NSP RGS. When analysing actual data the observed RGS genotypes were used (Gubbins 2008; Gubbins & McIntyre 2009), while in this study genotypes were predicted using a gene-flow model using the RGS results as an input. However, the RGS formally closes at the end of March 2009, which means that this source of PrP genotype data will no longer be available. It is still possible to use the back calculation methods in the absence of data on PrP genotype. Indeed, for the same preclinical detection proportion, the estimates for prevalence in the national flock between 1993 and 2007 are comparable, though not identical, with those which include PrP genotype. Consequently, it should be possible to detect a trend in prevalence even in the absence of PrP genotype data. However, the strong association between PrP genotype and the risk of infection and incubation period (Baylis *et al.* 2004; Tongue *et al.* 2006; Gubbins 2008; Gubbins & McIntyre 2009) mean that such a simplified approach will miss important aspects of the epidemiology of scrapie, which may influence the prevalence estimates. In particular, it will not be able to distinguish between negative samples in resistant genotypes (i.e. unlikely to be infected) and those in at-risk genotypes (which may be infected, but at too early a stage of incubation to be detected).

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Appendix A Back-calculation methods

This appendix presents in detail the back-calculation approach used to estimate the prevalence of classical scrapie in the GB national flock from either actual or simulated surveillance data (Gubbins 2008; Gubbins & McIntyre 2009).

A.1 Modelling approach

Because the risk of infection is greatest during the perinatal period (Foster & Dickinson 1989; Hunter & Cairns 1998) and there is evidence for a decrease in susceptibility with age (St Rose *et al.* 2006), animals were assumed to become infected at, or close to birth. When calculating the probability that an animal will develop clinical disease, it is essential to include survivorship because the incubation period for scrapie is long relative to the life expectancy of a sheep. Moreover, to allow for an increased risk of mortality in animals close to the onset of disease, a proportion (K) of infected animals which survive to disease onset were assumed die on farm prior to clinical signs becoming apparent (Donnelly *et al.* 2002; Gubbins 2008). Finally, there is under-reporting of cases (Hoinville *et al.* 2000; Sivam *et al.* 2003) and, hence, it is necessary to consider the probability of a case being reported.

Consequently, the expected number of reported cases in genotype j and age class a (comprising animals between $a-1$ and a years of age), is given by,

$$R_{jat} = \rho_t(1-K)s_a B_{j,t-a} r_j \phi_{t-a} \int_{a-1}^a f_j(v) dv, \quad (\text{A.1})$$

where ϕ_t is the baseline risk of infection for animals born in year t , r_j is the relative risk of infection for genotype j , ρ_t is the probability of reporting in year t (assumed to be independent of age and PrP genotype), K is the proportion of infected animals surviving to disease onset, which die on farm before clinical signs become apparent, s_a is the probability of surviving to be in age class a , $B_{j,t}$ is the number of animals of genotype j in birth cohort t and f_j is the probability density function for the log-normal incubation period distribution (with genotype-specific parameters, μ_j and σ_j).

The prevalence of infection in the fallen stock survey (FS) has two components: infected animals found dead prior to the onset of clinical disease; and infected animals that survive to disease onset, but which die on farm before clinical signs become apparent. The prevalence of infection in animals of genotype j and age class a found dead on farm in year t is,

$$P_{jat}^{(FS)} = r_j \phi_{t-a} \left\{ \eta_a (s_a - s_{a+1}) \int_{a-1}^a \int_v^\infty f_j(w) dw dv + s_a K \int_{a-1}^a f_j(v) dv \right\}. \quad (\text{A.2})$$

where η_a is the proportion of uninfected animals which are found dead on farm in age class a . By contrast, the prevalence of infection in animals sent to slaughter (i.e. in the abattoir survey; AS) comprises only infected animals sent to slaughter prior to the onset of clinical disease, which is given by,

$$P_{jat}^{(AS)} = (1 - \eta_a)(s_a - s_{a+1}) r_j \phi_{t-a} \int_{a-1}^a \int_v^\infty f_j(w) dw dv. \quad (\text{A.3})$$

However, not every infected animal will be detected by the diagnostic tests used in the surveys. Assuming that test sensitivity depends on the time to the onset of clinical disease, the detectable prevalence for genotype j and age class a in animals found dead on farm in year t is,

$$d_{jat}^{(FS)} = r_j \phi_{t-a} \left\{ \eta_a (s_a - s_{a+1}) \int_{a-1}^a \int_v^\infty \zeta(v, w) f_j(w) dw dv + s_a K \int_{a-1}^a \zeta(v, v) f_j(v) dv \right\}, \quad (\text{A.4})$$

while for animals sent to slaughter it is,

$$d_{jat}^{(AS)} = (1 - \eta_a) (s_a - s_{a+1}) r_j \phi_{t-a} \int_{a-1}^a \int_v^\infty \zeta(v, w) f_j(w) dw dv, \quad (\text{A.5})$$

where $\zeta(v, w)$ is the probability of detecting an infected animal tested at age v given that it would have developed clinical disease at age w . It was assumed that an infected animal would be detected provided it was in the final proportion of the incubation period, so that the probability of detection, $\zeta(v, w)$, is given by,

$$\zeta(v, w) = \begin{cases} 0 & v \leq (1 - \delta)w, \\ 1 & v > (1 - \delta)w. \end{cases} \quad (\text{A.6})$$

where δ is the preclinical detection proportion (cf. Donnelly *et al.* 2002; Clarke & Ghani 2005; Gubbins 2008). Finally, the proportion of uninfected animals of genotype j in age class a found dead on farm in year t is,

$$u_{jat}^{(FS)} = (1 - r_j \phi_{t-a}) \eta_a (s_a - s_{a+1}), \quad (\text{A.7})$$

while that for animals sent to slaughter is,

$$u_{jat}^{(AS)} = (1 - r_j \phi_{t-a}) (1 - \eta_a) (s_a - s_{a+1}). \quad (\text{A.8})$$

A.2 Maximum-likelihood methods

Scrapie notifications data provide the number of cases, X_{jat} , of genotype j in age class a reported in year t . The number of reported cases was assumed to follow a Poisson distribution with a mean given by the expected number of reported cases, so that the log-likelihood for reported cases (l_{RC}) is given by,

$$l_{RC} = \sum_t \sum_j \sum_a \left\{ -R_{jat} + X_{jat} \log(R_{jat}) - \log(X_{jat}!) \right\}, \quad (\text{A.9})$$

where R_{jat} is the expected number of reported cases of genotype j and age class a in year t .

FS and AS data provide the number of animals over 18 months of age sampled and the number of positive samples. The number of positive samples is drawn from a binomial distribution with the number of animals tested and the probability that a tested animal produces a positive result as parameters. If data on the age of animals sampled are available, the log-likelihood for the survey (l_i) ($i=FS, AS$) is given by,

$$l_i = \sum_t \sum_j \sum_a \left\{ \log \left(\frac{Z_{jat}^{(i)}!}{P_{jat}^{(i)}!(Z_{jat}^{(i)} - P_{jat}^{(i)})!} \right) + P_{jat}^{(i)} \log(q_{jat}^{(i)}) + (Z_{jat}^{(i)} - P_{jat}^{(i)}) \log(1 - q_{jat}^{(i)}) \right\}, \quad (\text{A.10})$$

where,

$$q_{jat}^{(i)} = \frac{d_{jat}^{(i)} + (1 - \psi)u_{jat}^{(i)}}{p_{jat}^{(i)} + u_{jat}^{(i)}}, \quad (\text{A.11})$$

is the probability that an animal of genotype j in age class a tested during year t produces a positive result in survey i , a_{\max} is the last age class and ψ is the specificity of the diagnostic test. If, however, data on the age of animals sampled are not available, the log-likelihood for each survey (l_i) is given by,

$$l_i = \sum_t \sum_j \left\{ \log \left(\frac{Y_{jt}^{(i)}!}{D_{jt}^{(i)}!(Y_{jt}^{(i)} - D_{jt}^{(i)})!} \right) + D_{jt}^{(i)} \log(q_{jt}^{(i)}) + (Y_{jt}^{(i)} - D_{jt}^{(i)}) \log(1 - q_{jt}^{(i)}) \right\}, \quad (\text{A.12})$$

where,

$$q_{jt}^{(i)} = \frac{\sum_{a=2}^{a_{\max}} d_{jat}^{(i)} B_{j,t-a} + (1-\psi) u_{jat}^{(i)} B_{j,t-a}}{\sum_{a=2}^{a_{\max}} p_{jat}^{(i)} B_{j,t-a} + u_{jat}^{(i)} B_{j,t-a}}, \quad (\text{A.13})$$

is the probability that an animal of genotype j tested in year t produces a positive result in survey i , a_{\max} is the last age class and ψ is the specificity of the diagnostic test.

Because the surveillance streams are independent, the log-likelihood for the overall results (l) is found by adding the log-likelihoods for each surveillance source. The maximum-likelihood estimates (MLEs) were found by determining the values of the parameters which maximise the log-likelihood (l).

Appendix B Model parameters

This appendix presents the parameter estimates used in the models to predict prevalence trends and simulate surveillance strategies. Tables B.1 and Tables B.2 contain previously published estimates for demographic and age-at-onset parameters (Gubbins 2008), while Table B.3 presents those obtained in the current study by fitting to scrapie surveillance data collected between 1993 and 2007.

Table B.1. *Estimates for age-related parameters in the back-calculation model.*

age class	1	2	3	4	5	6	7	8	9
age (years)	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	>8
probability of survival (s_a)	1.000	0.435	0.350	0.258	0.163	0.077	0.026	0.008	0.003
proportion of uninfected animals found dead on farm (η_a)	- [†]	0.726	0.335	0.216	0.199	0.147	0.147	0.106	0.122

[†] only animals over 18 months of age were (assumed to be) sampled in the fallen stock and abattoir surveys

Table B.2. *Maximum likelihood estimates for the age-at-onset distribution parameters for each PrP genotype.*

PrP genotype [†]	age-at-onset parameter	
	μ_j	σ_j
ARR/ARR ^{a‡}	2.21	0.40
ARR/AHQ ^a	2.21	0.40
ARR/ARH ^{a‡}	2.21	0.40
ARR/ARQ ^a	2.21	0.40
AHQ/AHQ ^b	2.65	0.50
AHQ/ARH ^{c‡}	1.16	0.60
AHQ/ARQ ^b	2.65	0.50
ARH/ARH ^c	1.16	0.60
ARH/ARQ ^c	1.16	0.60
ARQ/ARQ	2.58	0.80
ARR/VRQ	2.46	0.37
AHQ/VRQ ^a	2.21	0.40
ARH/VRQ	1.43	0.35
ARQ/VRQ	1.89	0.47
VRQ/VRQ	1.65	0.53

[†] data for PrP genotypes with a common superscripted letter were combined when estimating the age-at-onset parameters

[‡] there were no reported cases for these PrP genotypes

Table B.3. Maximum likelihood estimates for model parameters obtained by fitting the back-calculation model to surveillance data for 1993-2007, assuming PrP genotype frequencies computed by Roden et al. (2006).

parameter		estimate
transmission parameter (β)		41.88
relative risk of infection (r_i)	ARR/ARR [†]	0
	ARR/AHQ	0.0001
	ARR/ARH [†]	0
	ARR/ARQ	0.0003
	AHQ/AHQ	0.036
	AHQ/ARH [†]	0
	AHQ/ARQ	0.042
	ARH/ARH	0.008
	ARH/ARQ	0.006
	ARQ/ARQ	0.041
	ARR/VRQ	0.241
	AHQ/VRQ	0.004
	ARH/VRQ	0.302
	ARQ/VRQ	0.308
VRQ/VRQ [‡]	1	
proportion (%) of infected animals surviving to disease onset, which die on farm before clinical signs become apparent (K)		74.93
proportion (%) of cases reported (ρ_i) each year (estimated for 1993-2007, but only those for 2003-2007 are presented)	2003	37.43
	2004	30.63
	2005	17.44
	2006	9.73
	2007	1.15

[†] no cases reported or fallen stock or abattoir positives found in these PrP genotypes

[‡] baseline PrP genotype

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