**Epidemiological investigations on the potential transmissibility of a rare disease: the case of atypical scrapie in GB**

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**Abstract**

It is widely accepted that atypical scrapie may be a spontaneous disease and if transmissible, at a very low rate. Multiple cases of atypical scrapie in the same holding and coexistence with classical scrapie have been reported in Great Britain. A two-stage simulation tool was developed by combining a sampling algorithm and a Hierarchical Bayesian model to simulate the number of positive cases of atypical scrapie on sampled holdings from 2 different scenarios: (i) assuming random sampling and (ii) using the actual sampled population in GB, in order to estimate the probability of detection of flocks with one and more cases. In parallel cluster analysis was conducted by fitting a spatial discrete Poisson model, to assess the level of geographical over/under-sampling over the years.

The probability of detecting at least two cases of atypical scrapie in the same holding is much lower in the simulation of random data than in that of the actual data for all simulated scenarios. Sampling bias in the selection of sheep for testing led to the multiple sampling from fewer but larger holdings. Scotland and large areas of Wales were systematically under-sampled and the South-West and East of England oversampled. The pattern of atypical scrapie cases observed can be explained better by the sampling bias rather than by a multi-case event epidemiologically linked. The coexistence of classical and atypical scrapie cannot be considered a rare event in GB and does not suggest any epidemiological link between the two types of disease.

**Introduction**

Scrapie was the first prion disease described, in sheep as early as 1753 in the UK and in goats in the 1940s in France (Chelle, 1942). The recent advancements in immunopathology and biochemistry allowed the identification in 1998 of a novel presentation of scrapie called nor-98 or atypical scrapie (Benestad et al., 2003). The pathological features with low level of involvement in peripheral tissues (Andreoletti et al., 2011; Simmons et al., 2011), the low incidence across Europe (Feidaievsky et al., 2010) and the almost null impact on the productivity and welfare of affected flocks have resulted in scant efforts to elucidate the origin and epidemiological features of atypical scrapie at individual and population levels.

There is limited knowledge about the epidemiology of atypical scrapie. It is not yet known whether atypical scrapie spreads from animal to animal, although the available evidence suggests that it may be spontaneous and if transmissible, at a very low rate (Fediaevsky et al., 2010). Three case-control studies, conducted in Norway (Hopp et al., 2006), France (Fediaevsky et al., 2009) and the UK (del Rio Vilas et al., 2010), respectively, did not find significant risk factors associated with transmission between flocks. Looking at the surveillance data in GB, the prevalence has not changed significantly over the years (McIntyre et al., 2008, Ortiz-Pelaez & Arnold, 2013). However the need to conduct further transmission and epidemiological studies to elucidate the possible spontaneous, non-contagious origin of atypical scrapie, like sporadic Creutzfeldt-Jakob disease in humans, has been highlighted (Benestad et al., 2008).

There have been some reports of atypical scrapie presentation inconsistent with a spontaneous origin, like for example the coexistence of atypical and classical scrapie in an Italian sheep in a flock with previous cases of classical scrapie (Mazza et al., 2009). In an epidemiological study conducted in Germany with scrapie cases confirmed from January 2002 and March 2006, in 8% of the flocks with atypical scrapie more than one case had been confirmed with a maximum of 3 cases in two large flocks (Lühken et al., 2007). Two cases were reported from a flock with 650 sheep in the UK (Konold et al., 2007), and one report of two clinical cases born around the same time and detected in a small Irish flock (Onnasch et al., 2004).

Investigation of time and space clustering of disease allows the generation and the testing of hypotheses about the origin of the disease and is a fundamental approach of the epidemiological investigation of animal diseases. Infectious diseases usually show certain level of time-space clustering because their contagious nature (Ward and Carpenter, 2000). The presence of multiple cases in the same epidemiological unit (flock/herd) or associated with spatial proximity or contacts via live animal movements or fomites is a sign of transmissible disease.

The presentation of atypical scrapie in GB is typical of a rare disease with an overall rate in the last ten years of 8 cases case per ten thousand tested sheep in both the fallen stock and the abattoir surveys, not significantly different than the estimates reported at European level with 5.5 cases per ten thousand in the abattoir survey, and 8.1 cases per ten thousand in the fallen stock (Fediaevsky et al., 2010).

In this study the presentation of multiple cases of atypical scrapie and of coexistence with classical scrapie in British sheep holdings are described and analyse in order to investigate whether the observed pattern is consistent with that of a transmissible disease. The objective of the analysis was the simulation of the occurrence of atypical scrapie and of the coexistence of atypical and classical scrapie in order to draw conclusions on the “randomness” of the presentation of atypical scrapie and the impact of the surveillance on the observed pattern.

**Material & Methods**

**Data**

Data from atypical scrapie cases and associated holdings were extracted from the Scrapie Notification Database (SND). This is a data repository that contains two types of surveillance data: a) passive surveillance since scrapie became a notifiable disease in GB in 1993: all clinical suspects and their final status, i.e. whether they were tested, confirmed, final result and some individual case data; b) all cases of scrapie confirmed in GB by all surveillance sources. By the end of 2013, some 319 cases of atypical scrapie had been confirmed. Even though all classical scrapie cases are confirmed in single or multiple holdings for statutory actions, this is not the case for atypical cases. That leaves a considerable number of cases unconfirmed and unassigned for analytical purposes. Classical scrapie is known to be acquired around birth (Lacroux et al., 2007, O’Rourke et al, 2011) hence the main target for confirmation is the natal flock. An epidemiological investigation results in the confirmation of one or more holdings based on the life history of the animal. However the confirmation of cases of atypical scrapie in specific holdings remains a challenge since it is uncertain where the infection could be acquired, if possible at all.

Since not all positive cases appeared to be confirmed officially in sheep holdings, the identification of affected CPH (case assignment) where atypical cases had most likely occurred was conducted following a three-tier procedure: a) cases officially confirmed as per official notification to flock owners (mostly after October 2011); b) recorded as confirmed in SND although no statutory action was taken (before October 2011); c) recorded as not confirmed in any holding as per SND. For the last group the ascertainment of the holding was conducted by matching the flock number (usually applied in the natal holding) as in the eartag with the CPH where found or reported. The most likely holdings were identified by cross-checking flock tag numbers of the animal and the holding in which the case was found or reported. If the matching was successful, the case was assumed to be confirmed in the CPH linked to the flock number. A second cross-check of the TSESS database was conducted to ascertain whether holdings with cases of atypical scrapie had had cases of classical scrapie as well. The initial output was then checked manually looking at whether the cases classical scrapie appeared to be found and/or confirmed in those holdings.

The number tested and positive animals in the fallen stock survey between 2006 and 2011 were extracted from the TSESS national database. This is the GB repository for scrapie active surveillance data including test results and epidemiologically associated data at animal level. Both the SND and the TSESS databases are maintained at the Animal and Plant Health Agency (APHA), formerly known as Animal Health and Veterinary Laboratories Agency (AHVLA).

**Methods**

*Investigation of spatial bias in sampling*

A cluster analysis was conducted by fitting a spatial discrete Poisson model, assuming the number of cases in each location is Poisson-distributed and the expected number of cases in each area is proportional to its population size. As case data, the list of all holdings tested by the Fallen Stock survey since the beginning of the survey in January 2002 until 31 December 2012 with the number of sheep tested in each holding was used. As population data the list of all sheep holdings with flock size as per the Sheep and Goats Inventory ([www.defra.gov.uk](http://www.defra.gov.uk)) was selected. Geo-references (XY coordinates of the British national Grid) for all sheep holdings were extracted and used to identify the location of all holdings in the cases and population datasets. The maximum cluster size was set at 20% of the population at risk and the spatial window shape circular. The analysis was conducted using SaTScanTM 9.3 (Kulldorff M. and Information Management Services, Inc. [www.satscan.org](http://www.satscan.org)). The areas with a relative risk greater than 1 significant at the 0.05 α level would correspond to areas where there has been an over-sampling in the Fallen Stock survey whereas areas with a significant at the 0.05 α level and relative risk smaller than 1 would reflect the opposite.

*Simulation of the number of cases of atypical scrapie on sampled holdings*

The objective of this analysis was to simulate the number of positive cases of atypical scrapie on sampled holdings from 2 different scenarios: (i) assuming random sampling and (ii) using the actual sampled population in GB, in order to compare the probability of detecting on or more cases in the two scenarios. If the probability of detecting flocks with 2 or more positive atypical scrapie sheep was lower in the random simulation than in the observed, assuming that within-flock prevalence depended only on flock size, this would provide evidence that the presentation arose due to sampling bias rather than within-flock transmission of atypical scrapie.

*Simulation of fallen stock data*

A two-stage simulation tool was developed by combining a sampling algorithm and a Hierarchical Bayesian model to test this hypothesis. The simulation was performed in two stages. Firstly a sampling stage, which consisted in sampling sheep with replacement from the holding population. Secondly a generation of test positives stage, which took the simulated number of sheep sampled on each holding from the first stage, and used assumptions regarding within-holding prevalence and test sensitivity to estimate the number of positive sheep on each sampled farm.

For the sampling stage, the number of fallen stock atypical scrapie positives on each holding was simulated for two different scenarios: (i) the actual holdings sampled by the fallen stock survey and (ii) a random sample of sheep holdings extracted from the census (<https://www.gov.uk/sheep-and-goats-identification-registration-and-movement#sheep-and-goat-annual-inventory>) equal to the number of sampled holdings each year by the survey, using a random with replacement sampling method weighted with holding size as sampling weight

For the generation of test positives stage, a hierarchical Bayesian model implemented in Openbugs 3.2.3 was used. For each of the scenarios, the number of positives on each holding was extracted, with the following assumptions:

1. Sensitivity of the screening test (Biorad® TSEs ELISA). A beta distribution with parameters 34.166 and 1.335 was used. These parameters were obtained using the software BetaBuster (<http://www.epi.ucdavis.edu/diagnostictests/betabuster.html>*),* assuming a distribution with a mode of 99% and 95% of its values greater than 90%. A high sensitivity was assumed since similar to classical scrapie, it was expected that most positive sheep found dead on farm were old enough to have progressed sufficiently in the incubation period so as to be detected by the diagnostic test (Arnold and Ortiz-Pelaez, 2014). The specificity of the rapid test was assumed to be 100% taking into account the statutory confirmatory test used in GB for surveillance.
2. True Animal Prevalence: estimate of the prevalence of infection of atypical scrapie in the GB sheep flock using a back calculation model (see supplementary material). The true animal prevalence followed a beta distribution with parameters obtained from Betabuster assuming a mode of 0.0015 and 95% of its values less than 0.0021.

The number of positives on each flock was calculated assuming a binomial distribution, with *n* given by the number of animals tested in the flock, and *p* by the product of the test sensitivity and the true animal prevalence of scrapie.

A total of 3000 iterations with a burn-in period of 500 iterations were set, using 3 chains of initial values, where convergence was verified by use of the Gelman-Rubin plot in OpenBUGS, and a thinning value of 1, as autocorrelation plots showed that there was no correlation between iterations.

*Bayesian model outputs*

The model produced two main outputs of interest: a) number of holdings with one positive case detected, two positive cases detected and 3 or more positive cases detected; b) probability of detecting two positive cases in at least a holding.

This process was repeated for 100 times generating different random samples from the actual holding population. Six runs of the model were conducted using sampled data of the fallen stock survey from 2006 to 2011, separately. Since holdings were sampled across several years, a model with similar structure was applied to three 4-year time windows: 2006-2009, 2007-2010 and 2008-2011. We selected the actual same sample size of the fallen stock as per surveillance data for the entire study period. The animal level prevalence for atypical scrapie calculated using a back calculation model did not significantly change these years (Supplementary Material), so the same distribution for the animal prevalence as in the one-year models was used.

An extension of the multi-year model was developed by adding another stream with the classical scrapie caseload. The model assumed that each sheep sampled is tested for both types of scrapie, which is the case since 2003. The true prevalence of classical scrapie used was 0.14% (95% CI: 0.02%-0.43%), the estimation in 2011 by Arnold and Ortiz-Pelaez, (2014).

A number of extra output parameters were added to this model: a) number of holdings with 1 case of classical scrapie and at least 1 case of atypical scrapie; b) number of holdings with 2 cases of classical scrapie and at least 1 case of atypical scrapie; c) number of holdings with 3 cases of classical scrapie and at least 1 case of atypical scrapie; d) probability of each output to be greater than zero.

**Results**

A total of 183 holdings in which at least one case of atypical scrapie had been confirmed by the end of 2012 were identified: 75 from England (41%), 86 from Wales (47%) and 22 from Scotland (12%). Although the total number of cases confirmed between 2002 and 2012 was 302, 67% (202) were linked to an agricultural holding with a reasonable level of certainty. Most of the cases sourced by the Abattoir Survey could not be traced back to any holding.

It has been possible to identify 6 holdings in GB between 2002 and 2012 where two cases of atypical scrapie have been confirmed from multiple surveillance sources. A seventh holding had two cases of atypical scrapie confirmed by the Fallen Stock survey although they had not been born on these premises. All holdings had similar characteristics: mixed cattle-sheep holdings located in Wales, with large number of sheep and medium size cattle herds. The multiple cases occurred between 2005 and 2008.

It was possible to identify nineteen holdings that have had cases of both classical and atypical scrapie in sheep that were born or were in the farm at the time of detection. They occurred mostly between 2005 and 2008, the years where most of the cases of scrapie of any kind were detected. The holdings are in general large flocks located in Wales (11), England (6) and Scotland (2).

A total of 75449 point locations were used in the cluster analysis (few holdings appear with the same XY geo-references and were considered as one) and a total 18,676.735 sheep. The case file included a total of 129,013 sheep tested from 19,507 sheep holdings. The results showed the presence of 34 significant geographical clusters at the 0.05 α level, of variable size: 26 representing areas of oversampling, and 8 representing areas of under-sampling. The latter cover all Scotland, Cumbria, North Wales (Gwynedd, Clwyd and north Powys), western areas of neighbouring English counties (Merseyside, Cheshire and Shropshire), two small pockets in South Wales (Glamorgan and Gwent) and most of Gloucestershire. The former are concentrated in three main areas: the South West of England (Somerset, Dorset, Devon and Cornwall), a wide area covering East Anglia, central and North East of England from Hertfordshire to North Yorkshire and from Staffordshire to Norfolk. The third area includes a number of small clusters in central and South West of Wales, covering most of Dyfed and Powys, and a small area between Avon and Wiltshire. Only 13 (16.8%) of all the cases detected were in under-sampled areas which cover nearly 50% of the GB territory. Figure 1 shows both types of areas and the location of holdings with cases of atypical scrapie confirmed by the Fallen Stock between 2002 and 2012.

The six yearly models were conducted using sampled data of the fallen stock survey (summary displayed in Table 1) from 2006 to 2011, independently. The probability of detecting at least two cases of atypical scrapie in the same holding was 0.21 using actual data and 0.03 using random data for 2006, the year of the largest throughput in the fallen stock survey. Table 2 shows the median with the interquartile range (Q3- Q1), the median and the 95% credible intervals of the number of holdings with one positive case of atypical scrapie detected. It also includes the posterior probability that at least one holding with 2 or more positive cases of atypical scrapie was observed.

For the periods 2006-2009, 2007-2010 and 2008-2011 with sampling size 50728, 42783 and 42053 sheep respectively, the probabilities of finding at least one holding with two cases of atypical scrapie in the simulations of actual data were 0.83, 0.81 and 0.68, compared to 0.15, 0.1 and 0.11 in the simulations of random data. The probabilities of detecting holdings with at least 3 positive cases was 0.08 for the actual data and 0.001 for the random data in the period 2006-2009, the one with the largest sample size.

The probability of detecting one holding with one case of classical scrapie and one case of atypical scrapie was the same for the three periods 2006-2009, 2007-2010 and 2008-2011 (0.99), using the simulations of actual data, compared to 0.55, 0.44 and 0.47 using the simulations of random data, respectively. Table 3 showed the results of these four-year period simulations.

The differences observed in the simulations of actual and random data in both the annual and the four-year simulations were explored by evaluating the validity of the assumption of the random selection of sheep for TSE testing by the fallen stock across the years. In order to assess the bias introduced by potential non-random selection of the fallen stock, 100 samples from the population following the same algorithm used in the multi-year programme were generated and the distribution of the holding size of the selected holdings in each sample was calculated. The distribution of the number of tested holdings was very different between the random and the actual data (Table 4). In the actual sampled population, four sheep or less were tested on average in 75% of the holdings over the 4-year periods years. However in 75% of the randomly extracted population two or less sheep on average were tested. Multiple submissions from the same farms led to a median number of holdings tested per year of 2158, 3.6 times lower than the median of 7800 holdings expected if the selection of animals for testing had been completely at random. In terms of holding size, 50% of the sampled holdings had 616 sheep or less, whereas in the random selection 50% of the holdings had 523 or less sheep.

**Discussion**

The objective of this study was to provide an epidemiological description of the occurrence of multiple cases of atypical scrapie in GB, with the view to formulate further hypotheses on the potential transmissibility of the disease in natural conditions. This effort has been hampered at certain extent for three main reasons: the difficulty to assign many of the cases confirmed to a particular holding, the lack of already available epidemiological data from these holdings and the lack of holding of origin in sheep tested by the Abattoir survey, precluding their inclusion in the analyses. Moreover, the epidemiological criteria applied to officially confirm a case of classical scrapie in a holding cannot be used for atypical scrapie. It is assumed that in atypical scrapie and due to the long incubation period, infected sheep are as likely to be detected by the fallen stock as by the abattoir survey since they do not develop clinical disease during their productive life. However classical scrapie cases are more likely to die on farm before they are sent to the abattoir for slaughter.

Despite these constraints and the fact that more than 40% of all cases cannot be linked with certainty to any holding, seven holdings have been identified where two cases of atypical scrapie have been confirmed officially or epidemiologically-assigned to, between 2002 and 2012. The presentation of cases of both atypical and classical in the same holding cannot be considered a very rare event in GB with 19 holdings showing this feature in the 10-year study period.

This descriptive analysis was designed as a preliminary step towards the analytical component.. There is no evidence to reject the null hypothesis that the disease can be a spontaneous event that occurred at certain low rate in the general population. The simulation of actual data rendered a small chance of detecting holdings with 2 positive cases of atypical scrapie in the fallen stock survey between 2006 and 2011, but not negligible, with potential explanations for that either the transmission of the disease within flocks or the artefacts of the sampling. On the other side, the detection probability of holdings with multiple cases in the simulation of random data was much lower.

The assessment of the sampling bias revealed big differences between the random and the actual selection, confirming the historic biased selection of sheep for TSE testing in the fallen stock survey favouring multiple sampling from fewer holdings of larger size than would be expected. The seven holdings with more than one detected case of atypical scrapie in GB were all holdings with large flock sizes and over sampled by the fallen stock survey. An exceptional case is among them is the holding where the two cases of atypical scrapie were detected by the Fallen Stock in 2007. This holding had 84 sheep tested by this surveillance stream in 2007 and a total of 564 sheep tested between 2004 and 2011, with an average of 70.5 sheep per year.

When looking at the probability of having cases of atypical and classical scrapie in the same holding, the results of this analysis showed that even in the random simulation of the survey, the detection of a holding with one case of classical scrapie and one case of atypical scrapie, is nor rare, especially in the period 2006-2009 when the detection levels were high and the TSE testing of infected flocks and the consequent enhanced surveillance increased substantially the throughput.

In the case of a spontaneous disease with a very low prevalence, testing more animals from fewer holdings increases the probability of detecting multiple cases. It has been shown that the actual selection of sheep for testing in the fallen stock survey is consistent with this pattern with the added factor that sampled holdings were of larger size than the random selection.

The total number of sheep tested by the Fallen Stock survey s is driven by the quota set by the EU. The EU legislation (EC, 2007) Commission Regulation (EC) 727/2007 of 26 June 2007 established sampling rules to the animals selected for TSE testing for monitoring the slaughtered for human consumption (abattoir survey) and the not slaughtered for human consumption (fallen stock), as follows: *“Multiple sampling in the same flock shall be avoided, wherever possible. Member States shall aim their monitoring programmes to achieve, wherever possible, that in successive sampling years all officially registered holdings with more than 100 animals and where TSE cases have never been detected are subject to TSE testing.”*

Despite these rules, due to logistic and financial reasons, it is recognised the difficulty to achieve a representative selection (EFSA, 2014), avoiding multiple sampling from the same flock and other types of bias. The cluster analysis has also revealed the geographical bias of the sampling in the FS whereby areas over sampled where those in which most of the cases of atypical scrapie had been detected.

As demonstrated in this study, sheep selected by the fallen stock survey have not been representative of the national sheep flock in terms of number and size of the tested holdings and geographical areas. The bias in the fallen stock sampling has been previously reported (Ortiz-Pelaez &. Arnold, 2013). Changes to the operation of the FS survey introduced in 2011 in GB including the elimination of the free collection of carcasses and the increase in the number of sampling sites contributed to the reduction of the observed sampling bias. For example, two years (2008 and 2013) with similar throughputs in the fallen stock survey, 12377 and 12246, respectively, presented very different profiles. In 2008 there were 282 holdings (10% of tested holdings) in which more than 10 sheep were tested in each, accounting for more than 44.5% of all samples. In 2011 the number of tested holdings increased to 4965, the number of holdings that had more than 10 sheep tested decreased to 110 (2.2%) accounting for 15.7% of all samples.

A proper assessment of the impact of such sampling distribution on the prevalence estimates of both classical and atypical scrapie has become pertinent in the light of these observations and a revision of the implementation of the Fallen Stock survey merits consideration. Future surveillance strategies aimed at detecting new cases rather than monitoring trends should follow risk-based approaches, for example by prioritizing specific characteristics based on known risk factors (age) or specific holdings/areas at higher risk. Similar approaches have been suggested by calculating the sample size using holding as the unit of interest, and not total sheep population (Del Rio Vilas & Pfeiffer, 2010). Alternatively larger number of holdings within the quotas could be targeted by the fallen stock survey, or replaced altogether by a two-stage sampling adequate to the GB situation considering both the holding and animal levels.

**Conclusions**

Surveillance data in GB has shown the presentation of atypical scrapie alone or in conjunction with classical scrapie in time and space. This study contributes to the body of evidence showing that the presence of multiple cases of atypical cases in a holding does not preclude the possibility of atypical scrapie being a sporadic disease. The pattern of cases observed can be explained better by the sampling bias rather than by a multi-case event epidemiologically linked. The coexistence of classical and atypical scrapie cannot be considered a rare event in GB and yet again does not suggest any epidemiological link between the two types of disease.

A revision of the implementation of the Fallen Stock survey merits consideration, aiming at targeting larger number of holdings within the quotas, or its replacement by a two-stage sampling adequate to the GB situation considering both the holding and animal levels. If the objective of this survey is to detect new cases following a risk-based approach, older animals or specific holdings/areas at higher risk should be prioritized.

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**Tables**

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| ***Table 1 Number of sheep tested and detected cases of scrapie types by the fallen stock survey between 2006 and 2011*** |
| **Year** | **2006** | **2007** | **2008** | **2009** | **2010** | **2011** |
| ***Tested*** | 17989 | 12670 | 10128 | 9941 | 10044 | 11940 |
| ***Classical*** | 31 | 17 | 4 | 2 | 0 | 3 |
| ***Atypical*** | 11 | 10 | 4 | 8 | 6 | 11 |

|  |
| --- |
| ***Table 2 Median with interquartile range (IQR) and maximum number of holdings with at least one case of atypical scrapie and probability of finding at least one holding with 2 cases of atypical scrapie (AS) using simulation of the actual Fallen Stock survey data and randomly extracted date from the census for the years 2006 to 2011*** |
|  | **Actual** | **Random** |
|  | **Probability of detecting at least one holding with 2 cases of AS** | **Median # holdings with 1 case of AS (IQR)** | **Maximum # holdings with 1 case of AS** | **Probability of detecting at least one holding with 2 cases of AS** | **Median # holdings with 1 case of AS (IQR)** | **Maximum # holdings with 1 case of AS** |
| **2006** | 0.21 | 26 (30-22) | 48 | 0.03 | 27 (30-23) | 57 |
| **2007** | 0.13 | 19 (22-16) | 48 | 0.01 | 19 (22-17) | 42 |
| **2008** | 0.12 | 15 (17-12) | 30 | 0.008 | 15 (18-12) | 35 |
| **2009** | 0.16 | 14 (17-12) | 32 | 0.008 | 15 (17-12) | 36 |
| **2010** | 0.17 | 15 (17-12)  | 48 | 0.008 | 15 (18-12) | 37 |
| **2011** | 0.16 | 16 (19-13) | 32 | 0.013 | 18 (21-15) | 40 |

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| ***Table 3 Median with interquartile range (IQR), maximum number of holdings and probability of detection for the different combinations of classical (CS) and atypical scrapie (AS) cases using the simulation of actual Fallen Stock survey data and randomly extracted date from the census for three four-year periods*** |
|  | **Actual** | **Random** |
|  | **Detection probability** | **Median # holdings (IQR)** | **Maximum # holdings** | **Detection probability** | **Median # holdings (IQR)** | **Maximum # holdings** |
| **2006-2009** |  |  |  |  |  |  |
| **# holdings with 1 AS case** | 1 | 72 (79-67) | 104 | 1 | 76 (82-70) | 123 |
| **# holdings with 2 AS cases** | 0.83 | 2 (3-1) | 9 | 0.15 | 0 (0-0) | 5 |
| **# holdings with 3 AS cases** | 0.82 | 0 (0-0) | 3 | 0.0001 | 0 (0-0) | 1 |
| **# holdings ≥1 AS case1 CS case** | 0.99 | 6 (8-5) | 18 | 0.5 | 1 (1-0) | 8 |
| **# holdings ≥1 AS case 2 CS cases** | 0.4 | 0 (1-0) | 7 | 0.0009 | 0 (0-0) | 2 |
| **# holdings ≥1 AS case 3 CS cases** | 0.06 | 0 (0-0) | 2 | 0.00004 | 0 (0-0) | 1 |
| **2007-2010** |  |  |  |  |  |  |
| **# holdings with 1 AS case** | 1 | 61 (66-55) | 88 | 1 | 64 (69-58) | 94 |
| **# holdings with 2 AS cases** | 0.81 | 1 (2-1) | 8 | 0.11 | 0 (0-0) | 3 |
| **# holdings with 3 AS cases** | 0.08 | 0 (0-0) | 3 | 0.0004 | 0 (0-0) | 1 |
| **# holdings ≥1 AS case1 CS case** | 0.99 | 6 (8-4) | 17 | 0.44 | 0 (1-0) | 8 |
| **# holdings ≥1 AS case 2 CS cases** | 0.38 | 0 (1-0) | 5 | 0.003 | 0 (0-0) | 1 |
| **# holdings ≥1 AS case 3 CS cases** | 0.05 | 0 (0-0) | 2 | 0 | 0 (0-0) | 0 |
| **2008-2011** |  |  |  |  |  |  |
| **# holdings with 1 AS case** | 1 | 59 (65-54) | 123 | 1 | 64 (69-58) | 93 |
| **# holdings with 2 AS cases** | 0.68 | 1 (2-0) | 7 | 0.11 | 0 (0-0) | 3 |
| **# holdings with 3 AS cases** | 0.04 | 0 (0-0) | 4 | 0.004 | 0 (0-0) | 1 |
| **# holdings ≥1 AS case1 CS case** | 0.98 | 4 (6-3) | 14 | 0.47 | 0 (1-0) | 5 |
| **# holdings ≥1 AS case 2 CS cases** | 0.26 | 0 (1-0) | 4 | 0.0006 | 0 (0-0) | 1 |
| **# holdings ≥1 AS case 3 CS cases** | 0.03 | 0 (0-0) | 2 | 0 | 0 (0-0) | 0 |
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| ***Table 4 Distribution of holding size, number of tested holdings and sheep per holding in the random simulation of the Fallen Stock survey data and in the actual samples of holdings by the Fallen Stock survey between 2006 and 2011*** |
|  **75% 95%** | **5%** | **25%** | **Median** | **Mean** |  **75%** | **95%** |
| ***Random data*** | ***Average holding size*** | 63 | 247 | 523 | 740 | 969 | 2096  |
|  | ***Average number of tested holdings per year*** | 7675 | 7721 | 7800 | 8019 | 8128 | 8812 |
|  | ***Average number of tested sheep per holding in the 4-year periods*** | 1 | 1 | 1 | 1.90 | 2 | 5 |
| **Actual data** | ***Average holding size*** | 72 | 306 | 616 | 586 | 1139 | 2275 |
| ***Average number of tested holdings per year*** | 1748 | 1778 | 2158 | 2936 | 3316 | 5679 |
| ***Average number of tested sheep per holding in the 4-year periods*** | 1 | 1 | 2 | 4 | 4.55 | 17 |

**Figures**

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| ***Figure 1. Areas of statistically significant over (red) and under (blue) sampling in the Fallen Stock between 2002 and 2012 and location of holdings with cases of atypical scrapie confirmed by this survey during the same period.*** |

**Supplementary material: Estimation of atypical scrapie prevalence of infection in GB at animal level**

**Methods**

The basic approach used followed that described in Gubbins (2008), where abattoir survey and fallen stock data were integrated using a back-calculation approach to determine the prevalence of infection in the national flock.

*Back-calculation model*

There are two possible ways that an animal could end up as a fallen stock positive at age *a*: (i) it reached clinical onset without being identified by the farmer and died of scrapie (we assume that there are no reported cases of scrapie so animals reaching clinical onset will die and become fallen stock) (ii) it died on farm (not of scrapie) and happened to have scrapie. Probability (i) is the product of the risk of infection (denoted *r*), the probability of surviving to age *a (*denoted *S(a))*, and the probability density of the incubation period at age *a (*denoted *f(a))*. Probability (ii) is the product of the risk of infection, the proportion of animals of age *a* that are found dead on farm(denoted (S(*a*)-S(*a*+1)), and the likelihood that an infected animal of age *a* will be detected by the diagnostic test. We also allow the potential for differential slaughter of subclinically affected sheep, where subclinical sheep may be at higher risk of slaughter due to an effect of scrapie on their production traits, so that sheep end up in the fallen stock/clinical stream with probability 1-K, and in the healthy slaughter stream with probability K. Therefore the probability of being detected as a fallen stock positive at age *a,* denoted, is given by:



Where is the probability that the diagnostic test will detect an infected animal *t* months before clinical onset.

The likelihood that an animal would end up as an abattoir survey positive was given by the product of the risk of infection, the proportion of animals of age a that are sent for slaughter (i.e. do not die on farm) (1- (S(*a*)-S(*a*+1)), and the likelihood that an infected animal of age *a* will be detected by the diagnostic test. Therefore the probability of being detected as an abattoir survey positive at age *a* is given by:



The total likelihood of an animal ending up in the fallen stock or abattoir survey streams is the sum of the respective probabilities over all ages. The final log-likelihood is then the sum of the log-likelihoods of the binomial probabilities of the observed number of fallen stock/abattoir survey positives given the number of fallen stock/abattoir survey tested.

*Estimation of the age of onset distribution*

The age of onset distribution was estimated by fitting a lognormal incubation period to the observed age at onset of clinical cases in the Scrapie Notifications Database (SND) between 1993 and 2011, accounting for the age distribution of the population as applied by Gubbins et al. (2003). However due to a low number of passive surveillance cases with known ages (n=8), the positive cases confirmed by the Fallen Stock survey (2003-2012) were also included in the calculation, making the assumption that the majority of fallen stock atypical scrapie positives died due to scrapie. When the fallen stock positives were included, the total number of positive animals increased to 50 (Figure S1).

Although there was no data with which to estimate the sensitivity of the rapid test to detect atypical scrapie, it can be assumed it has the same sensitivity as the rapid test to detect classical scrapie (Arnold and Ortiz-Pelaez, 2014), where it was estimated that it followed a logistic regression curve, with a sensitivity of 50% at approximately 70% of the incubation period completed, increasing to 100% at clinical onset

**Results**

*Estimation of the age of onset distribution*

The estimated prevalence of atypical scrapie in GB varied between years (Fig. S2) but a linear regression applied to the data showed no statistically significant trend in the prevalence of infection over time (p=0.79). The estimated prevalence of atypical scrapie, using the test sensitivity estimate from classical scrapie data was 0.10% (95% CI: 0.08-0.11%)

**Figures (Supplementary material)**

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| *ageonsetplot* |
| Figure S1. Comparison of the age distribution of reported cases/fallen stock of atypical scrapie in the Scrapie Notifications Database compared with that predicted by the lognormal incubation period distribution used in the back-calculation model of atypical scrapie infection prevalence. |

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Figure S2. The estimated prevalence of atypical scrapie each year 2005-2012 (black dots) and the mean prevalence across all years (dotted line) estimated using a back-calculation model applied to the number of atypical scrapie cases in GB.