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Epidemiological investigations on the potential transmissibility of a rare disease: the case of atypical scrapie in Great Britain

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SUMMARY

Multiple cases of atypical scrapie in the same holding and co-existence 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 from: (i) random sampling and (ii) using the actual sampled population in Great Britain, being the output probability of detection of flocks with one and more cases. Cluster analysis was conducted to assess the level of geographical over- and under-sampling over the years. The probability of detecting at least two cases of atypical scrapie in the same holding is much lower in simulated random data than in simulated actual data for all scenarios. Sampling bias in the selection of sheep for testing led to multiple sampling from fewer but larger holdings, Scotland, and areas of Wales were under-sampled and the South-West and East of England oversampled. The pattern of atypical scrapie cases observed is unlikely to be explained by a multi-case event epidemiologically linked. The co-existence of classical and atypical scrapie is a rare event with 19 holdings detected in GB and does not suggest an epidemiological link between the two types of disease.

Key words: Atypical, Bayesian, multiple, scrapie, transmission.

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 [1]. The recent advancements in immunopathology and biochemistry allowed the identification in 1998 of a novel presentation of scrapie called Nor-98 or atypical scrapie [2]. Some of the features of the disease, namely, the lack of zoonotic potential [3], the low level of involvement in peripheral tissues [4, 5], the low incidence across Europe [6] and the almost null impact on the productivity and welfare of affected flocks, explain the 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, does so at a very low rate [6]. Three case-control studies, conducted in Norway [7], France [8] and the UK [9], did not find significant risk factors associated with transmission between flocks. Looking at the surveillance data in Great Britain (GB), the prevalence has not changed significantly over the years [10, 11]. However, the need to conduct further transmission and epidemiological studies to elucidate the possible spontaneous, non-contagious origin of atypical scrapie, like sporadic

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Creutzfeldt–Jakob disease in humans, has been highlighted [12].

The presentation of atypical scrapie in GB is typical of a rare disease with an overall rate in the last 10 years of 8 cases/10 000 tested sheep in both the Fallen stock (FS) and Abattoir surveys, not significantly different from the estimates reported at European level with 5·5 cases/10 000 in the Abattoir survey, and 8·1 cases/10 000 in the FS survey [6].

There have been some reports of atypical scrapie presentation inconsistent with a spontaneous origin, e.g. the co-existence of atypical and classical scrapie in an Italian sheep flock with previous cases of classical scrapie [13]. 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 three cases in two large flocks [14]. Two cases were reported from a flock with 650 sheep in the UK [15], and one report of two clinical cases born around the same time and detected in a small Irish flock [16].

Investigation of time and space clustering of disease allows the generation and testing of hypotheses about the origin of the disease and is a fundamental approach in epidemiological investigations of animal diseases. Infectious diseases usually show certain level of time–space clustering because their contagious nature [17]. 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.

In this study the presentation of multiple cases of atypical scrapie and of co-existence with classical scrapie in British sheep holdings are described and analysed 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 co-existence of atypical and classical scrapie in order to draw conclusions on the clustering of infection at the flock level, and thus determine whether there is evidence that atypical scrapie is transmissible rather than spontaneous and the impact of the surveillance on the observed pattern.

MATERIAL AND METHODS

Data

The transmissible spongiform encephalopathies (TSE) Surveillance System database (TSESS) is the GB repository for scrapie active surveillance data including test results and epidemiologically associated data at the animal level. The Scrapie Notification Database (SND) 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. Both the TSESS and SND databases are maintained at the Animal and Plant Health Agency (APHA), formerly known as the Animal Health and Veterinary Laboratories Agency (AHVLA).

Data from atypical scrapie cases and associated holdings were extracted from SND. 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 [18, 19], 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 County Parish Holding (CPH) number (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 according to 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 also had cases of classical scrapie. The initial output was then checked manually looking at whether the cases of classical scrapie appeared to be found and/or confirmed in those holdings.

The number tested and positive animals and CPH numbers in the FS survey between 2002 and 2012 were extracted from TSESS.

Methods

Investigation of spatial bias in sampling

A spatial cluster analysis was conducted by fitting a spatial discrete Poisson model, assuming the number of cases in each point location is Poisson-distributed and the expected number of cases in each area is proportional to its population size. Circles of different sizes (from 0% up to 20% of the total population size) and the circular spatial window shape were applied in 999 replications. For each circle (C), a likelihood statistic $L(C)$ is computed on the basis of the number of observed and expected cases within and outside the circle and compared with the likelihood L_0 under the null hypothesis. The circles with the highest likelihood ratio values [$L(C)/L_0$] are identified as potential clusters [20]. As case data, the list of all holdings tested by the FS 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 according to the Sheep and Goats Inventory (www.defra.gov.uk) was selected. Geo-references (x,y 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 analysis was conducted using SaTScan 9.3 (M. Kulldorff and Information Management Services Inc.; www.satscan.org). The areas with a relative risk >1 significant at the 0·05 α-level would correspond to areas where there has been an oversampling in the FS survey, whereas areas with a relative risk <1 significant at the 0·05 α-level 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 two different scenarios: (i) assuming random sampling and (ii) using the actual sampled population in GB, in order to compare the probability of detecting $\geqslant 1$ cases in the two scenarios. The possibility of having within-flock transmission of atypical scrapie was assessed by comparing the probability of detecting flocks with $\geqslant 2$ positive atypical scrapie cases in sheep in the random simulation with that in the simulations of observed data. Possible explanations to explain the findings were explored.

Simulation of FS 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. First, a sampling stage, which consisted of sampling sheep with replacements from the holding population. Second, the generation of a test-positive 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 FS atypical scrapie positives on each holding was simulated for two different scenarios: (i) the actual holdings sampled by the FS 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 the test-positive stage, a hierarchical Bayesian model implemented in Openbugs 3·2·3 was used [21]. For each of the scenarios, the number of positives on each holding was extracted, with the following assumptions:

(*a*) Sensitivity of the screening test (Bio-Rad TeSeE® ELISA, USA). A beta distribution with parameters 34·166 and 1·335 was used. These parameters were obtained using BetaBuster software (http://www.epi.ucdavis.edu/diagnostictests/betabuster.html), assuming a distribution with a mode of 99% and 95% of its values >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

[22]. The specificity of the rapid test was assumed to be 100% taking into account the statutory confirmatory test used in GB for surveillance.

(*b*) True animal prevalence. Estimation 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 <0·0021.

The number of positives in 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 three chains of initial values, where convergence was verified by use of the Gelman–Rubin plot diagnostics command in the R package coda (Supplementary material), and a thinning value of 1, as autocorrelation values (Supplementary material) 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 $\geqslant 3$ positive cases detected; and (*b*) probability of detecting two positive cases in at least one holding.

This process was repeated 100 times generating different random samples from the actual holding population. Seven runs of the model were conducted using sampled data of the FS survey from 2006 to 2012, separately. Before 2006, there were few cases of atypical scrapie confirmed by the FS survey. 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 FS 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 during these years (Supplementary material), so the same distribution for the animal prevalence as in the 1-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 has been the case since 2003. The true prevalence of classical scrapie used was 0·14% [95% confidence interval (CI) 0·02–0·43], which was the estimate in 2011 [22].

A number of extra output parameters were added to this model: (*a*) number of holdings with one case of classical scrapie and at least one case of atypical scrapie; (*b*) number of holdings with two cases of classical scrapie and at least one case of atypical scrapie; (*c*) number of holdings with three cases of classical scrapie and at least one case of atypical scrapie; (*d*) probability of each output being >0.

The differences observed in the simulations of actual and random data in both the annual and the 4-year simulations were explored by evaluating the validity of the assumption of the random selection of sheep for TSE testing by the FS survey across the years. In order to assess the bias introduced by potential non-random selection of FS, 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.

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 (41%) from England, 86 (47%) from Wales and 22 (12%) from Scotland. 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 six 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 FS 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 19 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

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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 75 449 point locations were used in the cluster analysis (few holdings appear with the same x,y 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 eight 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 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 FS survey between 2002 and 2012.

The seven yearly models were conducted using sampled data of the FS survey (summary displayed in Table 1) from 2006 to 2012, 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 FS survey. Table 2 shows the median number of holdings with one case of atypical scrapie with interquartile range, the maximum number of holdings with at least one case of atypical scrapie and probability of detecting at least one holding with two cases of atypical scrapie.

For the periods 2006–2009, 2007–2010 and 2008–2011 with sampling size 50 728, 42 783 and 42 053 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, 0·11 and 0·11, respectively, in the simulations of random data. The probabilities of detecting holdings with at least three positive cases was 0·08 for the actual data and 0·0001 for the random data in the period 2006–2009, the period 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 and was very similar (0·99, 0·99 and 0·98, respectively), using the simulations of actual data, compared to 0·55, 0·44 and 0·47 using the simulations of random data, respectively. Table 3 shows the results of these 4-year period simulations. When adjusting for the sampling bias, the simulation was able to reproduce the number of farms with multiple scrapie cases (Table 3), as the maximum number of simulated farms with multiple cases was sometimes greater than that observed.

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, $\leqslant 4$ sheep were tested on average in 75% of the holdings over the 4-year period. However, in 75% of the randomly extracted population an average of $\leqslant 2$ sheep 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 $\leqslant 616$, whereas in the random selection 50% of the holdings had $\leqslant 523$ 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

Table 1. *Number of sheep tested and detected cases of scrapie types by the Fallen stock survey between 2006 and 2012*

Year	2006	2007	2008	2009	2010	2011	2012
Tested	17 989	12 670	10 128	9941	10 044	11 94	12 87
Classical	31	17	4	2	0	3	2
Atypical	11	10	4	8	6	11	17

Table 2. *Median with interquartile range (IQR) and maximum number of holdings with at least one case of atypical scrapie (AS) and probability of finding at least one holding with two cases of AS using simulation of the actual Fallen stock survey data and randomly extracted data from the census for the years 2006–2012*

	Actual			Random		
	Probability of detecting at least one holding with two cases of AS	Median no. holdings with one case of AS (IQR)	Maximum no. holdings with one case of AS	Probability of detecting at least one holding with two cases of AS	Median no. holdings with one case of AS (IQR)	Maximum no. holdings with one 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
2012	0·07	19 (22–16)	35	0·014	19 (22–16)	43

incubation period, infected sheep are as likely to be detected by the FS survey 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 >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, between 2002 and 2012. The presentation of cases of both atypical and classical scrapie in the same holding is a rare event in GB with 19 holdings showing this feature in the 10-year study period.

Since the simulation model was able to reproduce the observed pattern of atypical scrapie cases in flocks once the sampling bias was included, 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. However, the possibility of a low level of within-flock transmission, consistent with the data, cannot be excluded. To explore this further would require models that explicitly represent transmission to enable direct comparison of the fit of models with and without transmission. However, given the relatively small number of cases the power of such a study to detect very low levels of within-flock transmission could be insufficient.

The assessment of the sampling bias revealed big differences between random and actual selection, confirming the historically biased selection of sheep for TSE testing in the FS survey favouring multiple sampling from fewer holdings of larger size than would be expected. The seven holdings with >1 detected case of atypical scrapie in GB were all holdings with large flock sizes and oversampled by the FS survey. An exceptional case among them is the holding where two cases of atypical scrapie were detected by the FS survey 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 tested.

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 not rare, especially in the

Table 3. *Median with interquartile range (IQR), maximum number of holdings and probability of detection for the different combinations of classical scrapie (CS) and atypical scrapie (AS) cases using the simulation of actual Fallen stock survey data and randomly extracted data from the census for three 4-year periods*

	Actual			Random		
No. of holdings with cases	Detection probability	Median no. holdings (IQR)	Maximum no. holdings	Detection probability	Median no. holdings (IQR)	Maximum no. holdings
2006–2009						
1 AS case	1	72 (79–67)	104	1	76 (82–70)	123
2 AS cases	0·83	2 (3–1)	9	0·15	0 (0–0)	5
3 AS cases	0·08	0 (0–0)	3	0·0001	0 (0–0)	1
⩾1 AS case, 1 CS case	0·99	6 (8–5)	18	0·55	1 (1–0)	8
⩾1 AS case, 2 CS cases	0·4	0 (1–0)	7	0·0009	0 (0–0)	2
⩾1 AS case, 3 CS cases	0·06	0 (0–0)	2	0·00004	0 (0–0)	1
2007–2010						
1 AS case	1	61 (66–55)	88	1	64 (69–58)	94
2 AS cases	0·81	1 (2–1)	8	0·11	0 (0–0)	3
3 AS cases	0·08	0 (0–0)	3	0·0004	0 (0–0)	1
⩾1 AS case, 1 CS case	0·99	6 (8–4)	17	0·44	0 (1–0)	8
⩾1 AS case, 2 CS cases	0·38	0 (1–0)	5	0·003	0 (0–0)	1
⩾1 AS case, 3 CS cases	0·05	0 (0–0)	2	0	0 (0–0)	0
2008–2011						
1 AS case	1	59 (65–54)	123	1	64 (69–58)	93
2 AS cases	0·68	1 (2–0)	7	0·11	0 (0–0)	3
3 AS cases	0·04	0 (0–0)	4	0·004	0 (0–0)	1
⩾1 AS case, 1 CS case	0·98	4 (6–3)	14	0·47	0 (1–0)	5
⩾1 AS case, 2 CS cases	0·26	0 (1–0)	4	0·0006	0 (0–0)	1
⩾1 AS case, 3 CS cases	0·03	0 (0–0)	2	0	0 (0–0)	0

Table 4. *Distribution of holding size, number of tested holdings and sheep per holding in the random simulation of the Fallen stock (FS) survey data and in the actual samples of holdings by the FS survey between 2006 and 2011*

		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

period 2006–2009 when detection levels were high and the TSE testing of infected flocks and the consequent enhanced surveillance increased throughput substantially.

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

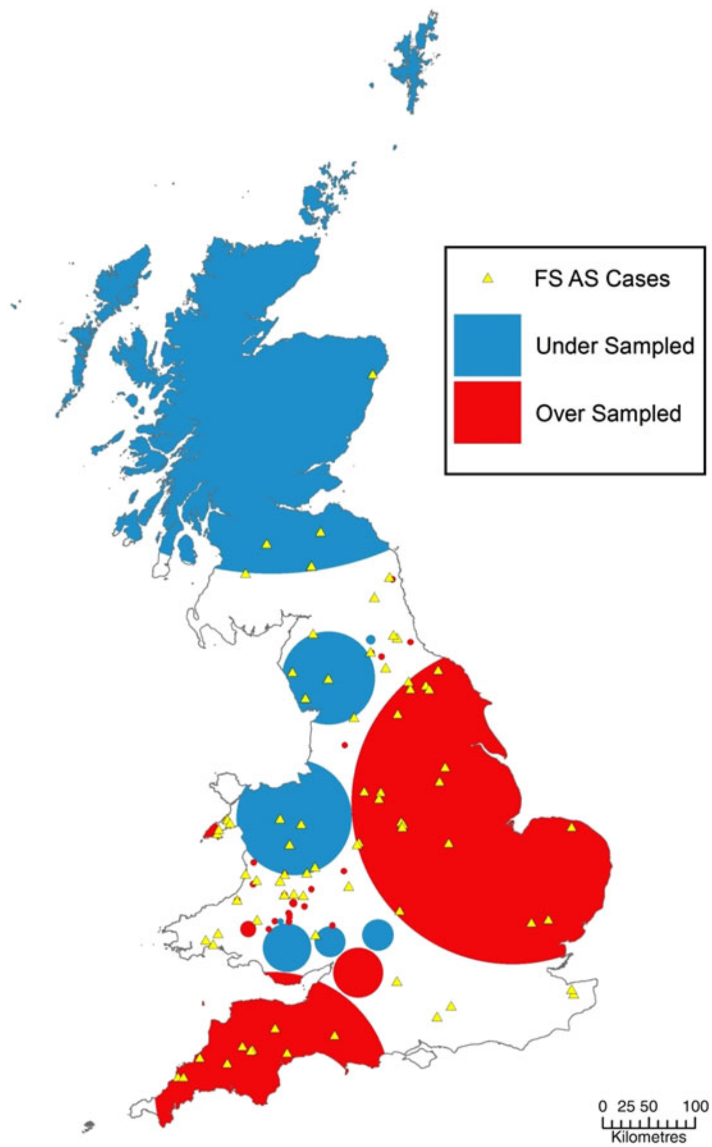

Fig. 1. Areas of statistically significant over- (red) and under- (blue) sampling in the Fallen stock (FS) survey between 2002 and 2012 and location of holdings with cases of atypical scrapie (AS) confirmed by this survey during the same period.

testing in the FS survey is consistent with this pattern with the added factor that sampled holdings were of larger size than the random selection. However, it would be speculative to suggest that flock size is a risk factor for atypical scrapie unless large flocks had higher prevalence of alleles susceptible to atypical scrapie. There is no evidence of this.

The total number of sheep tested by the FS survey is driven by the quota set by the EU. The EU legislation (EC, 2007) Commission Regulation (EC) 727/2007 of 26 June 2007 [23] established sampling rules for animals selected for TSE testing, monitoring those slaughtered for human consumption (Abattoir survey) and those not slaughtered for human consumption (FS survey), 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, the difficulty in achieving a representative selection is recognized [24], which would require avoiding oversampling from the same flock and other types of bias. The cluster analysis has also revealed the geographical bias of sampling in the FS study whereby areas oversampled were those in which most of the cases of atypical scrapie had been detected.

As demonstrated in this study, sheep selected by the FS 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 FS sampling has been reported previously [11]. 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 FS survey, 12 377 and 12 246, respectively, presented very different profiles. In 2008 there were 282 holdings (10% of tested holdings) in which >10 sheep were tested in each, accounting for > 44·5% of all samples. In 2011 the number of tested holdings increased to 4965, the number of holdings that had >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 FS survey merits consideration. Alternatively, and following the recent recommendations by EFSA [24], 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 [25]. Larger numbers of holdings within the quotas could be targeted by the FS survey, or replaced altogether by two-stage sampling adequate for the GB situation considering both holding and animal levels.

CONCLUSIONS

Surveillance data from the FS survey 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 in the FS survey can be explained better by the sampling bias rather than by a multi-case event epidemiologically linked. The coexistence of classical and atypical scrapie is a rare event in GB and yet again does not suggest any epidemiological link between the two types of disease.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268816000303.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Chelle PLP.** A case of scrapie in goats [in French]. *Bulletin de l'Académie Vétérinaire de France* 1942; **15**, 294–295.
2. **Benestad SL, *et al.*** Cases of scrapie with unusual features in Norway and designation of a new type, Nor98. *Veterinary Record* 2003; **153**, 202–208.
3. **EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards).** Scientific Opinion on a request for a review of a scientific publication concerning the zoonotic potential of ovine scrapie prions. *EFSA Journal* 2015; **13**: 4197 (58 pp).
4. **Andreoletti O, *et al.*** Atypical/Nor98 scrapie infectivity in sheep peripheral tissues. *PLoS Pathogens* 2011; **7**, e1001285.
5. **Simmons MM, *et al.*** Experimental oral transmission of atypical scrapie to sheep. *Emerging Infectious Diseases* 2011; **17**, 848–854.
6. **Fediaevsky A, *et al.*** The prevalence of atypical scrapie in sheep from positive flocks is not higher than in the general sheep population in 11 European countries. *BMC Veterinary Research* 2010; **6**: 9.
7. **Hopp P, Omer MK, Heier BT.** A case-control study of scrapie Nor98 in Norwegian sheep flocks. *Journal General of Virology* 2006; **87**: 3729–3736.
8. **Fediaevsky A, *et al.*** A case-control study on the origin of atypical scrapie in sheep, France. *Emerging Infectious Diseases* **15**: 2009; **710–718**.

9. **Del Rio Vilas VJ, Vink WD, Hubbard R.** A case-control study of atypical scrapie in GB sheep flocks. *Preventive Veterinary Medicine* 2010; **96**: 241–251.
10. **McIntyre KM, Gubbins S, Del Rio Vilas VJ.** No temporal trends in the prevalence of atypical scrapie in British sheep, 2002–2006. *BMC Veterinary Research* 2008; **4**, 13.
11. **Ortiz-Pelaez A, Arnold ME.** Sheep and goats scrapie surveillance 2012. Joint Descriptive Report for Great Britain (http://www.defra.gov.uk/ahvla-en/files/pub-tse-stats-scrapie.pdf), 2013.
12. **Benestad SL, *et al*.** Atypical/Nor98 scrapie: properties of the agent, genetics, and epidemiology. *Veterinary Research* 2008; **39**: 19.
13. **Mazza M, *et al*.** Co-existence of classical scrapie and Nor98 in a sheep from an Italian outbreak. *Research in Veterinary Science* 2010; **88**: 478–485.
14. **Lühken G, *et al*.** Epidemiological and genetical differences between classical and atypical scrapie cases. *Veterinary Research* 2007; **38**: 65–80.
15. **Konold T, *et al*.** Clinical findings in two cases of atypical scrapie in sheep: a case report. *BMC Veterinary Research* 2007; **3**: 2.
16. **Onnasch H, *et al*.** Two Irish cases of scrapie resembling Nor98. *Veterinary Record* 2004; **155**: 636–637.
17. **Ward MP, Carpenter TE.** Analysis of time-space clustering in veterinary epidemiology. *Preventive Veterinary Medicine* 2000; **43**: 225–237.
18. **Lacroux C, *et al*.** Dynamics and genetics of PrPSc placental accumulation in sheep. *Journal of General Virology* 2007; **88**: 1056–1061.
19. **O'Rourke KI, *et al*.** Sparse PrPSc accumulation in the placentas of goats with naturally acquired scrapie. *BMC Veterinary Research* 2011; **7**.
20. **Aamodt D, *et al*.** Spatiotemporal trends and age-period-cohort modeling of the incidence of type 1 diabetes among children aged <15 years in Norway 1973–1982 and 1989–2003. *Diabetes Care* 2007; **30**: 884–889.
21. **Lunn D, *et al*.** The BUGS project: evolution, critique and future directions (with discussion). *Statistics in Medicine* 2009; **28**: 3049–3082.
22. **Arnold M, Ortiz-Pelaez A.** The evolution of the prevalence of classical scrapie in sheep in Great Britain using surveillance data between 2005 and 2012. *Preventive Veterinary Medicine* 2014; **117**: 242–250.
23. **EC** (2007). Commission Regulation (EC) No. 727/2007 of the European Parliament and of the Council 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. O.J. No. L 165, 2007, pp. 8–20 and subsequent amendments.
24. **EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards).** Scientific Opinion on the scrapie situation in the EU after 10 years of monitoring and control in sheep and goats. *EFSA Journal* 2014; **12**: 3781 (155 pp).
25. **Del Rio Vilas J, Pfeiffer DU.** The evaluation of bias in scrapie surveillance: a review. *Veterinary Journal* 2010; **185**: 259–264.