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A paired case–control study of risk factors for scrapie in Irish sheep flocks

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Abstract

We did a case–control study of the association of several animal, flock and management factors with scrapie in Irish sheep flocks. The characteristics of 61 sheep flocks with at least one laboratory-confirmed case of scrapie (1990–1998) were compared to 61 flocks with no history of scrapie and matched by geographical location and attending veterinary surgeon. The 61 scrapie-affected flocks were from the database of known scrapie flocks in the Republic of Ireland at the start of the study. In conditional multiple logistic regression, factors associated with increased odds of scrapie in a sheep flock were (i) larger breeding-flock size, (ii) purchasing replacement sheep through the market, (iii) spreading sheep compost on the land and (iv) disposing of the placenta in the compost. Factors associated with decreased odds of scrapie were (i) using cattle slurry on the land and (ii) feeding proprietary concentrates to lambs.

Keywords: Scrapie sheep-microbiological disease; Case–control study; Risk factors; Ireland

1. Introduction

Scrapie is a transmissible spongiform encephalopathy (TSE) occurring naturally in sheep, goats and moufflon (Parry, 1983; Wood et al., 1992). It is difficult to estimate the true

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prevalence of the disease due to the long incubation period, lack of a practical ante mortem screening test, the finite economic worth of individual sheep and apprehension concerning admission of the presence of scrapie by flock owners. A passive surveillance system for scrapie has operated in Ireland for the past 10 years, depending upon the recognition and reporting of a clinical suspect by the flock owner and subsequent confirmation of the disease by histopathology and immunohistochemistry. On average, 10 newly infected flocks were identified annually throughout the 1990s by passive surveillance out of a population of approximately 44,000 flocks (DAFRD, 2001). In addition, the brains of approximately 8000 sheep annually (mainly cull ewes) were examined using an ELISA test (Enfer Scientific Ltd.) at slaughter between 1998 and 2001 for evidence of scrapie and 15 (from 15 different flocks) were positive (Boyle, 2002). It therefore appears, given the same difficulties in under reporting, that the national prevalence is probably less than that in the United Kingdom; 14.9% of respondents to a postal survey in the UK thought they had experienced scrapie at some time while confirmed cases were notified from 0.36% of flocks in 1998 (Hoinville et al., 1999, 2000).

Bovine spongiform encephalopathy (BSE) and its subsequent association with new-variant Creutzfeldt-Jakob Disease (nvCJD) (Will et al., 1996; Bruce et al., 1997) has refocused efforts to control scrapie even though knowledge to date has failed to demonstrate any link between scrapie and human neurological disease (Chatelain et al., 1981; Chatelain and Dautherville-Guibal, 1988; Cathala, 1990). Of concern was the knowledge that BSE could be transmitted orally to sheep (Foster et al., 1993) and that the clinical differentiation of experimental BSE in sheep and scrapie in sheep was very difficult (Foster et al., 2001). Greater understanding of the mechanisms by which flock-to-flock and intra-flock transmission occur is essential for the development of any control strategy. To date, no work has been published on the epidemiology of scrapie in sheep in Ireland.

Our aim was to identify risk factors for the introduction and/or maintenance of scrapie in sheep flocks in the Republic of Ireland.

2. Materials and methods

2.1. Selection of case flocks

All the owners of 73 flocks (Department of Agriculture and Food database) that had at least one case of scrapie confirmed at the laboratory in the period 1990–1998 were asked to participate. Sixty-eight flock owners agreed to participate in the study although data from seven of these were not used in the final model because of missing data in their matched controls.

2.2. Selection of control flocks

Control farms were matched on geographical location and referring veterinary surgeon to reduce classification bias of controls. Each veterinary surgeon was asked to provide the names of up to three flock owners for each scrapie farm for which he/she was responsible.

The veterinary surgeon was asked to select farms on the following criteria: (1) geographical proximity to the scrapie flock, (2) a flock size of at least 50 where possible and (3) on the basis of his/her own knowledge and involvement with the flock, that it was likely to be free from scrapie.

All potential control-flock owners were contacted by letter and invited to participate in the provision of data by questionnaire. In total, 90 farmers agreed to participate. Of these, four provided information that suggested that scrapie might have been present in the past and these farms were excluded. A further six were unable to provide answers for all the questions and likewise were not included in the analysis.

2.3. *Collection of flock data*

A detailed seven-page questionnaire was sent to the participants in both groups in advance of a farm visit. The questionnaire was checked during the visit and completed when necessary. Information was sought on the areas of farm and flock characteristics, replacement policy, breeding management, sales policy, management of lambing, disposal of organic waste, feeding and disease prevention. For scrapie flocks, details also were sought on the individual cases of scrapie. For control farms, questions were included to ascertain the level of knowledge and understanding the farmer had of scrapie so as to allow removal of control flocks where scrapie had possibly existed, although it had never been confirmed. The information provided from each scrapie farm pertained to the year in which scrapie was first confirmed in the flock. Control flocks also provided information pertaining to the same year as their scrapie-affected counterpart. Copies of the questionnaires are available from the corresponding author.

2.4. *Data entry and statistical analysis*

Data from both farm groups were entered into an Access® (Microsoft Inc., USA) database. The unit of observation in this study was the flock. The dependent variable was the flock status with regard to scrapie (confirmed or not) within the pair.

Conditional logistic regression was carried out using SAS (SAS Institute Inc., Cary, NC). Fourteen of the 61 scrapie farms had more than one control match. For those cases with two or more controls, one control was chosen at random (using SAS) to allow a 1:1 methodology to be carried out. For all variables, the observed value for the control was subtracted from the observed value of its matched case. A logistic regression (stipulating no intercept be estimated) then was carried out using these differenced data as the explanatory variables (Hosmer and Lemeshow, 1989). Because of the large number of variables (Tables 1 and 2), we decided to model separately the data from within each section of the questionnaire and then to bring forward to a final model the significant variables from these 'section' models.

Prior to model building for non-categorical data, a Wilcoxon signed-rank test was carried out to test the null hypothesis that the differenced distribution had a median centred about zero. Inclusion of continuous variables was made after demonstration of linearity of the logits. Continuous variables were transformed to hierarchical dummy variables based on the 10th, 20th, 30th, etc. percentiles. Nine dummy variables corresponded

Table 1
Summary of the distribution of categorical risk factors stratified on case ($n = 61$; diagnosed 1990–1998) or control ($n = 61$) status for scrapie in Ireland, 1990–1998

Variable	Case farms	Control farms
Farm characteristics		
Farm cattle	45	47
Cattle graze with sheep	32	41
Flock characteristics		
Breeding ewes		
Suffolks	58	53
Texel	26	15
Belclare	10	5
Charollais	9	13
Others	12	15
Pedigree breeder	9	11
Breeding policy		
Synchronisation of ewes	19	17
Rams hired	8	5
Rams monitored for service activity	41	34
Matings recorded	9	16
Lambing policy		
Housed for lambing	54	53
Cattle access to sheep housing	8	6
Disposal of placenta		
Not recovered/dumped in compost	47	36
Disposal of waste		
Cattle slurry spread on land	22	30
Compost spread on land	50	35
Replacement policy		
Source		
Home	34	29
Farm	2	6
Market	31	22
Age		
<1 year (Lamb)	35	27
1–2 years (Hogget)	30	26
>2 years (Adult ewe)	6	7
Feeding for ewes		
Concentrates	42	47
Cereals	21	19
Feeding for lambs		
Concentrates	36	46
Straights	14	7
Source		
Home grown cereals	9	3

Table 2
Summary of non-categorical variables (median and interquartile range, IQR) in a study of risk factors associated with scrapie in 61 case flocks and 61 control flocks in Ireland (cases 1990–1998)

Variable	Case farms		Control farms	
	Median	IQR	Median	IQR
Farm size (ha)	50.5	28.3–80.9	44.5	32.4–75.3
No. of breeding sheep	200	127–300	150	76–255
No. of cattle farmed	68	31–122.5	50	30–100
Lambing concentration (sheep lambed/day)	3.7	2.3–6.9	27	1.6–4.4
No. of sheep per group at lambing	35	20–50	25	16–40
No. of weeks housed	7.5	4–10	7.0	4–10
Replacement risk (%/year)	22	16–27	17	13–21

to the nine higher-level groups and the lowest-level group was used as a baseline. A conditional logistic regression analysis was carried out using these nine dummy measures as predictor variables and the lowest group as a reference. The regression coefficients then were plotted against midpoints of the deciles. Regressing the betas on the dummy terms and using Normal-regression techniques to fit a line to the plotted data provided a visual check of linearity. Failure to demonstrate significance by inclusion of higher-order terms helped demonstrate linearity of logits across the entire range of observed values. All continuous variables included in the models met the assumption of linearity.

Variables for inclusion in the 'section' models were made on the basis of their score-test probability using a forward-selection procedure. The criterion for entry into the model was lenient ($P < 0.25$). Initially, single-variable models were created in each section to examine the relationship between the variables by looking at the change in probability of a variable given the inclusion of another term in the model. The model-building process began with the inclusion of the variable with the most significant score-test probability. The model-building process continued until none of the remaining variables reached the $P < 0.25$ entry criterion. Variables entered into the model which were not themselves significant at the 0.1 alpha level and did not significantly increase the fit of the model (as determined by likelihood ratios) then were rejected.

Following fitting of the main-effects model, all two-way interaction terms were included in the models tested. The model fit was assessed using Pearson residuals and deviance residual plots.

All variables included in the section models were available for consideration in the final model. Again following initial univariable models to examine the interplay between variables, entry to the final model was on the basis of a score-test probability of 0.05 by forward selection. Any correlation between variables remaining in the final model and those that were not in the model was investigated using Spearman (in the case of variables with few levels) and Pearson correlations. As a final collinearity check, all of the rejected terms from each of the sections were offered individually to the final model to see if they became significant themselves or altered the significance of other variables already included.

Table 3
The final logistic-regression model of risk factors associated with scrapie in a sheep flock in Ireland: 61 case (diagnosed 1990–1998) and 61 matched control flocks

Parameter	*b*	S.E.	*P*	Odds ratio	95% CI
COMPOST	3.55	1.10	0.0012	34.6	4.02, 298
PLAC	2.82	0.96	0.0032	16.9	2.58, 110
MART	1.49	0.78	0.057	4.4	0.96, 20.5
NUMSHEEP	0.032	0.018	0.079	1.03	1.03, 1.04
SLURRY	−3.21	1.14	0.005	0.04	0.004, 0.38
LCONC	−1.75	0.85	0.039	0.17	0.033, 0.92

COMPOST, the spreading of sheep compost on grazing areas; PLAC, the disposal of placenta in the compost by direct placement or through non-retrieval from the bedding; MART, the purchase of replacements in the market; NUMSHEEP, the number of breeding females; estimate per 10 sheep; SLURRY, the spreading of cattle slurry on land; LCONC, the feeding of proprietary concentrates to lambs.

3. Results

3.1. Multivariable analysis at the flock level

The factors associated with the odds of being a case rather than control flock were (1) the number of breeding females in the flock (NUMSHEEP); (2) the purchase of replacements at a market (MART); (3) the spreading of compost (farmyard manure) (COMPOST) and (4) the disposal of the placenta in the compost (PLAC) either through failure to retrieve from the bedding or by direct placement (Table 3). Although some of the variables (NUMSHEEP and MART) are not significant at the 0.05 level (on the score test), the removal of either of the terms results in a significant reduction in fit. The factors significantly associated with the odds of being a control farm were: (1) spreading cattle slurry (SLURRY) and (2) feeding proprietary concentrates to lambs (LCONC). Correlation checks between variables in the final model and those variables not included failed to provide a satisfactory alternative explanation for the variables included. Positive correlation was demonstrated between housing and the use of compost while the feeding of concentrates was negatively correlated with the feeding of straights. A negative relationship also was demonstrated between the two major sources of replacement—market and homebred. Addition of all rejected terms individually to the final model failed to alter the significance of other variables included and no rejected terms attained significance themselves.

4. Discussion

Given its low reported prevalence, long incubation period and the practical difficulty in establishing evidence of preclinical infection, a case–control study was considered an appropriate tool by which to investigate risk factors associated with scrapie. The case group, while well defined and not requiring ‘selection’, might be considered a biased group because they mainly represent only those owned by farmers who were able to recognize and willing to report a suspect. However, the diagnosis of scrapie had been confirmed histopathologically in all case flocks—thus avoiding the criticism of inclusion of unconfirmed or ‘farmer diagnosed’ cases.

A fundamental difficulty exists in ensuring the validity of the outcome classification of the control group. The lack of a routine preclinical screening test for scrapie makes it impossible to validate absolutely the true scrapie-free status of the control farms. However, scrapie is a notifiable disease with a certain stigma attached; that the control farms that chose to participate in the study suggests that scrapie was genuinely not a problem. The involvement of the attending veterinary surgeon (who was familiar with the flock) and the inclusion of questions relating to the farmer’s knowledge and understanding of scrapie and its possible presence on the farm were further measures to preclude false controls where scrapie was experienced although never confirmed. Although selection of controls by the veterinary surgeon was directed by geographical proximity to case farms, there might have been bias in favour of those which were likely to be willing to co-operate.

The ascertainment, by personal recall, of exposure to certain factors of interest might also be a source of bias. Because of the retrospective nature of this study, it is conceivable that flock owners who had experienced scrapie were more acutely aware of decisions made around that time than perhaps a control farmer who has no particular interest in the study question. Some data relating to farm size, and flock numbers were relatively easy to remember (due to the introduction of quotas in the early 1990s) and were found not to have changed much over time; details such as amounts fed in a given year or the number or breed of animals replaced in a given year were more difficult. In some instances, owners found it impossible to give what they considered to be an accurate response—resulting in a reduction in the number of cases and controls used in the regression model to 61 pairs. The small sample size also has implications for the power of the study; other factors not demonstrable by this study might nevertheless have an important role in the disease.

The risk-factor information collected from the case and control pair was for the year in which scrapie was confirmed in the case flock. We acknowledge that even if the confirmed case was indeed the first case in the flock, exposure would have occurred some years previously. Information was sought on changes in major management issues such as flock size, breeds, feed amounts and types over two time periods (1988–1992 and 1993–1998) and the data suggested that flock owners maintained similar systems from year-to-year.

The association of scrapie status with the purchase of market-sourced replacements was expected. The most likely source of scrapie infection for a flock is the introduction of a preclinically infected animal (Wineland et al., 1998). Although recent advances have made it possible to identify the abnormal form of the prion protein (PrP^{Sc}; a marker for scrapie) in tonsilar tissue, peripheral lymph nodes and lymphoid tissues of the third eyelid (O’Rourke et al., 1998; Schreuder et al., 1988; Thuring et al., 2000), these techniques are not sufficiently practical to allow use as widescale screening tests. The animal is likely to be infectious during this preclinical stage although the titre of infectivity is not always correlated with the stage of disease (Hadlow et al., 1982). Until 2001 (when scrapie was made a class-A notifiable disease), there was no restriction on sale of stock through the market from scrapie-affected farms (thereby providing a ready source of infection for other flocks). Prior to 2001, there was no individual tagging of sheep, and it was almost impossible to trace purchased sheep to their flock of origin if clinical scrapie developed. Sale of stock through the market up to that point was a relatively anonymous method of disposal of sheep. Hopp et al. (2001)

in Norway identified the purchase of female sheep from scrapie flocks as a significant risk factor for the introduction of scrapie. Other risk factors in their case–control study included the sharing of rams and sharing of pastures, two practices which appear to be relatively common in Norway but not in Ireland (Eikje, 1996a, 1996b).

A large breeding flock was also a risk factor for positive scrapie status. Although its *P* was greater than 0.05 in the final model, its removal would have significantly reduced fit. It is reasonable that the odds of disease might increase with increasing flock size because more individual animals are at risk. Given a constant replacement risk across all flock sizes, the absolute number of replacements to the flock will be higher in large flocks. As the number of breeding ewes increased, so too did the number of breeds included in the flock. Flock size and the farming of other stock were variables associated with scrapie in the UK (Hoinville et al., 2000). In the latter instance, data included both confirmed and unconfirmed scrapie flocks. Increasing flock size was also a risk factor in the maintenance of scrapie in Norwegian sheep flocks (Hopp et al., 2001).

Our study demonstrated that either failure to recover the placenta from the pen or its disposal in the compost was associated with increased odds of scrapie-positive status. Because transmission of the disease can occur at lambing under natural conditions, the questionnaire focused on the management of lambing (particularly, the potential for contact between sheep and the method of disposal of the placenta). Pattison et al. (1972, 1974) demonstrated infectivity in the placenta using a suspension of foetal membranes from scrapie-affected sheep to induce disease in recipient sheep and goats. Subsequently, PrP^{Sc} was demonstrated in the placentae of some scrapie-infected sheep although the finding has not been consistent (Hadlow et al., 1982; Race et al., 1998; Tuo et al., 2001). Race et al. (1998) in two instances demonstrated PrP^{Sc} and infectivity in the placentae of two preclinical sheep while placentae recovered at a subsequent pregnancy were negative. Further work by Tuo et al. (2002) demonstrated that the accumulation of PrP^{Sc} was eliminated (or at least reduced to undetectable levels) in reproductive and placental tissues if the infected ewe was either non-pregnant or was pregnant with a conceptus of a resistant genotype. These findings help explain the apparent contradictory findings in some of the previous transmission studies (Hadlow et al., 1982; Race et al., 1998). If the placenta was the only source for lateral transmission of scrapie, measures to ensure immediate destruction of the placenta might reduce the incidence of disease. Why management of the placenta is different on scrapie farms compared to control farms is not clear. The tradition exists in some areas where the placenta is recovered and put hanging up on a bush. Failure to collect the placenta or its deposition in the compost conceivably could increase the persistence of infection within the flock by increasing exposure at the pen level or through the dissemination of contaminated compost. Irrespective of the method of placental disposal, there was a strong association with the spreading of sheep compost and scrapie. Assuming that similar waste management was carried out in the years preceding the confirmation of scrapie might indicate that spreading of compost is somewhere in the causal path.

There is a strong negative association between spreading of cattle slurry and scrapie. Both groups kept cattle, although the tendency was for scrapie farms to have more cattle. This waste-management decision might be based on other farm enterprises (information was not sought on other land uses). The more frequent use of cereals as feed on case farms might imply arable farming on these farms. However, it is more likely that compost would be ploughed into this ground rather than slurry being land-spread. The biological process whereby micro-organisms degrade organic material leaving a bulk-reduced, stabilized residue known as "compost" is unlikely to alter the infectivity of any prion-infected material present (although there is no published work in this area). Temperatures within the compost rarely exceed 60–70 °C and alterations in pH are small (Taiganides, 1977). $\mathrm{PrP^{Sc}}$ is highly stable; it survives the effects of irradiation, chemical treatment with alcohols and aldehydes and dry heat (200 °C for 1 h). In fact, procedures that rapidly fix protein enhance the thermostability of TSE agents (Taylor, 2000). Little inactivation occurs over pH ranges of 2–10 (Mould et al., 1965), although treatment of fixed tissues with concentrated formic acid reduces infectivity (Taylor et al., 1997).

The role of environmental contamination in the persistence of infection on farm is of particular interest, given the recent decision in the Republic of Ireland to depopulate scrapie-affected flocks. Brown and Gajdeusk (1991) demonstrated residual infectivity in a scrapie-infected hamster brain that had been buried in soil for 3 years. Some earlier studies suggested that the incidence of disease is higher, the longer animals are exposed to a potentially contaminated environment (Dickinson et al., 1966; Hourrigan et al., 1979). In Iceland, control of scrapie has been primarily through a depopulation policy. The recurrence of scrapie on some farms after restocking after intervals of up to 3 years has been attributed to residual environmental infection (although the initial disease status of the stock used in repopulation of these farms could not be verified) (Pálsson and Sigurdsson, 1958). However, following the introduction of more stringent disinfection procedures, the removal of topsoil in front of sheep houses and other heavily exposed areas and the destruction of home-conserved forages, the recurrence of scrapie on restocked farms is now rare (Sigurdarson, 1991; Sigurdarson, personal communication).

Although it is probably acceptable to consider the risk of BSE in sheep to be related to the geographical risk of BSE in cattle, the risk assessment for BSE in sheep is complicated by the diverse management systems for various flocks and breeds as well as the possible recycling of infection in sheep populations (Schreuder and Somerville, 2003). That feeding proprietary concentrates to lambs was associated with decreased odds of scrapie-positive status is interesting considering the possible transmission of BSE naturally to sheep through infected meat-and-bone meal (MBM). It suggests that BSE has not been transmitted by this route or at least was not recognized as a TSE in sheep in the Republic of Ireland. Unfortunately, there were not sufficient number of case farms to allow a comparison of this effect for farms that experienced scrapie before, versus those with scrapie after the imposition of the ban on MBM in ruminant feeds. Sheep seem to find MBM less palatable than cattle and there is less demand for undegradable dietary protein for sheep rations compared to dairy cattle (Schreuder and Somerville, 2003); both factors might reduce the level of exposure to infected MBM for sheep.

5. Conclusion

In conclusion, scrapie was associated with a large breeding flock size and the purchase of market-sourced replacements. Within-flock management practices that might lead to increased odds of scrapie within a flock were spreading of sheep compost on grazing areas and the disposal of the placenta in the compost or failure to retrieve the placenta from the lambing pen.

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