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Risk factors for herd breakdown with bovine tuberculosis in 148 cattle herds in the south west of England

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Abstract

Bovine tuberculosis (bTB) is caused by *Mycobacterium bovis.* The disease has a long latent period, heterogenous spread, can infect many species and can persist in the environment. In the UK, the rate of herd breakdowns (HBD) with bTB is increasing. A retrospective cohort study of 148 cattle herds was set up to investigate risk factors for HBD from October 2001 to November 2004. Herds were selected from farms located in the randomised badger culling trial (RBCT) and comprised holdings (24%) that were restocked with cattle after the foot and mouth disease (FMD) epidemic in 2001 and holdings (76%) that were continuously stocked throughout the FMD epidemic. Farmers were interviewed between June 2003 and February 2004. Questions on herd and farm management were asked for the period October 2001 to June 2003. Data on herd testing for bTB were sourced from the VetNet database and historic data from 1995 were used in the analysis. A discrete time survival analysis was used to examine factors associated with the risk of HBD.

By the end of the study period, November 2004, 50% of study herds had experienced a HBD with bTB at least once. Farms that were restocked for less than one year after FMD had a reduced risk of HBD (P<0.01) compared with continuously stocked farms in the same year. This reduced risk did not persist after one year of restocking. Feeding vitamin and mineral lick supplements compared with not feeding these supplements also reduced the risk of HBD. Factors associated with an increased risk of HBD were storing manure and slurry indoors or in a closed container, spreading manure all year round, herds with dairy cattle compared

with herds without dairy cattle, increasing herd size, purchase of cattle from markets, location of the farm in the proactive area of the RBCT compared with survey only and location of farms in Somerset and North Devon.

The lower risk of HBD in the first year after restocking but not the second or third year suggests that removal of all cattle might have lowered the infectious load of *M. bovis* on these premises for a period of time but that this did not persist once cattle were reintroduced. Purchase of cattle from markets suggests that there was a risk of introduction or re-introduction of bTB from these cattle. Method of storage or lack of storage of slurry might aid persistence of *M. bovis* in the environment if *M. bovis* survives in slurry in some circumstances.

Keywords: Bovine tuberculosis; Cohort study; Mycobacterium *bovis*; Farm risk factors; Survival analysis

1. Introduction

Bovine tuberculosis (bTB) is a zoonotic disease caused by *Mycobacterium bovis*, which infects a broad range of species, including wildlife (O'Reilly and Daborn, 1995). In Great Britain (GB), there has been a constant increase in the incidence of herd breakdown (HBD) with bTB since the early 1990s. In 2007 and 2008 approximately 7% of cattle herds in GB were under restriction because of a positive herd test for bTB at some point in the year (http://www.defra.gov.uk/animalh/tb/index.htm).

The current internationally accepted standard test to detect cattle infected with *M. bovis* is the intradermal tuberculin test (www.oie.int). In the United Kingdom (UK) and Eire the single intradermal comparative cervical test (SICCT) is used. This test is more than 99% specific with a reported sensitivity of 74% to 95%, (Monaghan et al., 1994; Costello et al., 1997) thus it does not detect all cattle with bTB in a herd.

Bovine tuberculin testing is regular, but not random, with up to 80% of cattle in GB not tested in their lifetime (Mitchell et al., 2006). The frequency of routine herd testing ranges from one to four years depending on the annual incidence of confirmed HBD in a parish averaged over the previous six years, as determined by the European Legislation in the 64/432/EEC Council Directive. Where this average incidence exceeds 1% (such as in most of the south west of England, West Midlands and Wales), herds are tested at one year intervals.

Risk factors associated with HBD, as an indicator for the presence of bTB in cattle herds, have been investigated using case-control and case studies, the majority in Eire, Northern Ireland, New Zealand and GB. Risk factors that have been associated with bTB in cattle farms are generally related to the introduction and/or persistence of *M. bovis* on the farm. Risk factors for introduction include the presence of infected cattle in neighbouring herds and a wildlife reservoir of *M. bovis* (Denny and Wilesmith, 1999; Griffin et al., 2005), the purchase of infected cattle (Carrique-Mas et al., 2008) including through markets (Johnston et al., 2005) and direct or indirect contact with infected wildlife. Risk factors which could enhance the persistence

of *M. bovis* include larger herd sizes and reservoirs of infection in local wildlife (Pfeiffer and Morris, 1991; Griffin et al., 1996) and survival of *M. bovis* in cattle slurry (Scanlon and Quinn, 2000).

Because of the association between bTB in badgers and cattle (Clifton-Hadley et al., 1995; Martin et al., 1997), a randomised badger culling trial (RBCT) was set up to test the impact of culling badgers on the incidence of HBD in cattle herds (Krebs et al., 1997; Le Fevre et al., 2005). The RBCT started in 1998 and ended in late 2005. There were ten geographically separated triplets (A to J) in areas of the south west of England with a high incidence of bTB. In each triplet there were three randomly allocated badger treatments, giving 30 trial areas. The three treatments were proactive culling (widespread culling of badgers on all accessible farmland within the triplet, regardless of the bTB status, and repeated at intervals), reactive culling (localised culling of badgers on farmland after a HBD had occurred), and survey only (no culling of badgers). Culling did not start simultaneously in all triplets and in some, culling did not start until after the end of the foot and mouth disease (FMD) outbreak in 2001.

During the FMD outbreak in the UK in 2001 (Gibbens et al., 2001) there were 2,030 infected cattle, sheep and pig premises; 11.5% of these were cattle farms in the RBCT (http://www.defra.gov.uk/animalh/tb/index.htm). These premises were repopulated with cattle from a variety of sources (Carrique-Mas et al., 2008).

Here we present a discrete time survival analysis of herd level risks associated with HBD between 1st October 2001 and 1st November 2004 in 148 cattle herds (24% of which had been restocked after FMD in 2001) in the RBCT in south west England. The aim of this study was to investigate management factors associated with HBD with bTB in areas with a high incidence of bTB, including farms where FMD led to restocking of herds, providing information on the impact of removing all cattle.

2. Methods

2.1 Study design, source of farms and farmer recruitment

A 3 – year retrospective cohort study was designed to investigate management factors associated with time to first HBD with bTB between 1st October 2001 and 1st November 2004 in areas of England with a high incidence of bTB.

The VLA (Veterinary Laboratory Agency, UK) provided contact details for 471 farms in the RBCT. These were located in Cornwall, Devon, Somerset and Gloucestershire and Herefordshire and Worcestershire. They provided a list of all farms that had destocked and restocked. For each restocked farm three continuously stocked farms were listed that were within the same trial area of the RBCT but at least 1km from the restocked farm. No exclusion criteria were applied for herd size or type of cattle (dairy, suckler or young stock).

Farms were recruited between 25th November 2002 and 30th October 2003. An introductory letter, including an invitation to one of seven local meetings and a participation agreement form, was sent to farmers. The farmers' veterinary surgeons and members of the National Farmers Union (NFU) were also invited to the meetings. These were organised between November 2002 and February 2003: two in Gloucestershire, four in Devon and one in Somerset. Farmers were telephoned until successfully contacted, to explain the study and to encourage participation either when they had not attended a meeting or after the first contact by post. In April 2003, a reminder letter was sent to those farmers who had not enrolled. In September 2003 the VLA provided twenty five extra farm addresses and these were contacted as above.

A total of 148 farmers were recruited. With an incidence rate of HBD in the study of 50% over the three years, this sample size was sufficient to detect statistically significant factors associated with a two fold risk of HBD: the exact required number of farms was 29 and 88 exposed and non-exposed respectively (Thrusfield, 2005).

2.2 Collection and collation of data

A questionnaire was designed and approved by the Census Survey Group, York, and it was pilot tested on five farmers. Both closed and open questions were included. The interview questionnaire covered the period October 2001 to June 2003. The questionnaire was divided into two main sections. The second section (used

in this analysis) covered the main aspects of farm management and practices and was divided into nine management areas (Table 1). Data from this questionnaire were used for the explanatory variables.

Table 1

A team of five researchers interviewed the farmers. All members signed a confidentiality form. Each farm visit was made by two members of the team. Farm visits were arranged two weeks in advance. At the beginning of the visits to farms, the team met regularly to discuss the phrasing of questions and responses to ensure repeatability. Farmers were interviewed between 17th June 2003 and 18th February 2004. Most interviews were complete with the first three months. Data were entered into a relational database in Microsoft Access (Microsoft Corporation, Redmond, WA, USA), as soon as possible after the visit, and by the same researcher that interviewed the farmer. Data were double-checked by one researcher who was employed for the whole duration of the study. If there was missing information the researchers telephoned farmers or revisited them to complete the data.

Data on herd test results for bTB were obtained from the national disease database (VetNet). These were used for the outcome variable and to get information on HBD before 2001. During the study and analysis, the RBCT trial area and farm were identified by codes and the nature of badger control was not disclosed to the researchers. Codes were disclosed after data analysis was complete.

One hundred and fourteen farmers (all except 33 which were only rearing beef cattle and one that left the study after completing the questionnaire), also participated in a study of five other endemic diseases, where blood samples were taken from cattle >2 years old (Woodbine et al., 2008, 2009a; 2009b). These farmers were visited once in each year of the study when blood samples were taken and once when they were interviewed. This helped maintain compliance with the study. In November 2004, a letter was sent to farmers thanking them for their collaboration in the study and for the data provided.

2.3. Statistical analysis

The outcome was time to first HBD in the period October 2001 to November 2004. A discrete time survival model with staggered entry times was used to analyse the data. This was set up as a binomial logistic regression model with random effects in MLwiN version 2.0 (Rasbash et al., 2004). The study unit was the herd year with 148 herds and up to 3 years of data per herd. The binary outcome variable was whether an

unrestricted herd experienced its first HBD during that year. A HBD was defined as the disclosure of at least one reactor on an unrestricted herd at the SICCT test, whether this was later confirmed or not. Once a herd had experienced a HBD it was censored.

There were 190 non-independent questions asked, most were binary and not exclusive. These were screened in groups with RBCT trial area and restocking status forced into sub models based on variables listed in Table 1. The variables in the final model included herd size estimated from the log average number of cattle present on the farm at the time of the SICCT tests averaged over the three years of the study. This was obtained from the VetNet database. Restocking was coded as farms restocked (yes/no) after the FMD outbreak of 2001. The risk of HBD attributable to restocking and RBCT treatment varied by calendar time and so these were coded as time dependent variables.

3. Results

3.1. Descriptive results

3.1.1. Recruitment of study farms

A total of 148 farmers were recruited into the study. The response (enrolled from the total number of eligible farms) was 40%. From a total of 471 reference farms provided by the Veterinary Laboratory Agency (VLA), 468 were successfully contacted; 19% were no longer farming. Approximately 20% of those contacted attended an evening meeting. The rest of the farmers were enrolled via telephone calls with the exception of four farmers who agreed to participate after the final reminder letter sent on 10th April 2003. Out of the total 471 farms, 87 had been depopulated during 2001, 69 of these had been restocked and 36 of these were included in the study (24% of the study farms).

The farms were located in all six counties (Table 2) where the RBCT was conducted, but in only six (A, B, C, H, I and J) of the ten RBCT triplets, and eleven of the thirty RBCT trial areas (37.2% were in reactive, 27.7% in proactive and 35.1% in survey only areas).

<u>Table 2</u>

3.1.2. Herd breakdowns (HBD) during the study period

During the risk period, 1st October 2001 to 1st November 2004, 75 herds experienced a HBD at least once. Of these, 59 (78.7%) were continuously stocked and 16 (21.3%) were restocked herds. In the first year, from 1st October 2001 to 30th September 2002, 11% (4) of restocked herds experienced a HBD compared with 24.1% (27) of continuously stocked herds. In the second year (1st October 2002-30th September 2003) there were 35 HBD, 27.7% (10) in restocked herds and 22.3% (25) in continuously stocked herds. In the last year, 9 herds experienced a HBD, 5.5% (2) were restocked and 6.2% (7) were continuously stocked herds.

A total of 283 reactors were disclosed at the 75 first HBD. There were 35% HBD with one reactor only at the disclosing test, 25% with two and 32% with more than two reactors. The median number of reactors per HBD was two, both in restocked and continuously stocked herds; however, the mean was 1.75 for the restocked and 4.32 for the continuously stocked herds. Approximately 75% of the HBD were confirmed by *post mortem* inspection and culture (14/16 HBD that occurred in restocked herds and 42/59 HBD in continuously stocked herds). Four out of the 75 HBD were detected at abattoirs, and for two HBD there were no reactors recorded in the database.

Thirteen herds were under restriction with a HBD in October 2001. They became at risk of HBD between 10th October 2001 and 21st August 2002 when they entered the study. By November 2004, seven out of these thirteen had experienced a HBD once more.

3.2. Discrete time survival model

Factors associated with a decreased risk of HBD were the use of vitamins and mineral licks (OR=0.34, 95% CI = 0.18, 0.64) compared with not using such supplements and restocked herds (OR=0.36, 95%CI = 0.10, 1.24) compared with continuously stocked herds in 2002, the first year after they restocked compared with continuously stocked herds. Herds that were in their second or third year since restocking were not at lower risk of HBD than continuously stocked herds. Factors associated with an increased risk of HBD were increasing log herd size (OR=2.11, 95%CI = 1.47, 3.04), presence of dairy cattle (OR=2.18, 95%CI = 1.12,

4.24), slurry stored indoors in a closed container (OR=2.49, 95%CI = 1.30, 4.76), slurry or manure spread all year round (OR=2.23, 95%CI = 1.10, 4.51), the purchase of cattle from markets (OR=1.95, 95%CI = 1.05, 3.63) and location of farms in the counties of Somerset or North Devon (OR=4.21, 95%CI = 1.60, 11.07). Farms located in the proactive treatment of the RBCT were at an increased risk of HBD in year two (OR=2.92, 95%CI = 1.16, 7.38) and non- significant decreased risk of HBD in year one (OR=0.42, 95%CI = 0.13, 1.39). A HBD on the farm before 2001 was not significant in the final model.

Table 3

4. Discussion

Study design

The aim of this study was to investigate herd management factors associated with HBD with bTB in areas with a high incidence of bTB, including farms where FMD led to restocking of herds, providing information on the impact of removing all cattle. The study population were farms located in the RBCT where bTB is endemic. There was no difference in the proportion of farmers who participated in the cohort study when categorised by restocked and continuously stocked farms. The recruitment of restocked farms was limited by the number of farms that were destocked and subsequently restocked and were in the RBCT. Apart from being in the RBCT no criteria (i.e. history of bTB on farms where farms restocked from) were applied for the selection of restocked farms in the study. All wholly restocked farms in the RBCT were offered to the study as restocked farms (VLA, personal communication).

Over 80% of the herds in the study had been tested for bTB in the 18 months before October 2001, with 50% tested between October 2000 and October 2001. Farms that were restricted during FMD were not tested with follow up tests until the FMD epidemic was over and so undisclosed bTB infection might have been spreading within these herds. We included these herds only after they were unrestricted giving staggered entry time to the analysis. We tested whether a HBD before 2001 was a significant risk for HBD after 2001: it was not when other variables were included on the model, indicating that previous HBD is probably a correlate of other management practices that we investigated.

Given that the sensitivity of the skin test is approximately 70% (Monaghan et al., 1994), the true bTB infection status of the herds is impossible to assess accurately. We therefore assessed survival to HBD in this analysis, rather than infection with bTB. All test types were used to identify HBD and all HBD were included in the analysis whether reactors were confirmed by *post-mortem* examination and or culture or not. Although only 75% of the HBD were confirmed, we believed it appropriate to include unconfirmed HBD in the analysis because the characteristics of the SICCT (low sensitivity and high specificity) and the herd sizes in this study are such that few, if any, tests would be false positives.

Variables were recorded from October 2001 to June 2003, and the outcome from October 2001 to November 2004. It could be argued that farm management and practices could have changed from June 2003 however, given that testing for bTB is annual, and we do not know when these cattle become infected, the managements in 2003 seem temporally appropriate for a test in 2004.

There are still relatively few studies of risk for HBD in GB and this study was the first cohort study and the first to investigate risks of restocking in an area of the country considered endemic with bTB. The variables investigated were not independent and were typically a series of questions that overlapped (e.g. to identify the type of manure produced, stored and spread was approximately 15 questions to ascertain 3 variables). All the questions were possible explanations for the occurrence of bTB from cattle, the environment and from other animal species. There were fewer less correlated variables in the sub-models and the variables in the final model were not correlated. We therefore consider that the risk of false positive associations whilst present is low; unfortunately because of the numbers of farms available the power of the study is also quite low. The results from this study will be useful to test hypotheses that arise from it using other data and could be used to initiate intervention studies. Where results agree with other case control and case studies they contribute to the evidence base for a risk; for example, the purchase of cattle from markets is repeatedly identified as a risk for HBD.

Study results

The purchase of cattle into a herd can introduce cattle infected with bTB, even in an area endemic with bTB; this new introduction is an important cause of persistence because herds are re-exposed and possibly re

infected each time infected cattle are purchased. Purchase of cattle from markets, in particular, was a risk for HBD in this study and has been reported as a risk for HBD in other studies (Pfeiffer and Morris, 1991; Griffin et al., 1992; Griffin et al., 1996; Marangon et al., 1998; Johnston et al., 2005). At the time of the study, there was no statutory requirement for pre-movement testing of cattle, so cattle from all unrestricted herds were freely bought and sold. In addition, young stock (which were statistically significantly associated with HBD in the univariable analysis) were sometimes sold before a routine bTB test, to avoid the risk of having to keep these young stock if the herd became restricted to avoid overcrowding. Pre-movement testing will have changed the pattern of sales of cattle and prevented some infected cattle being sold.

In addition to movements of a group of cattle onto the farm, replacement of all the cattle (destocking) altered the risk of HBD. Restocking reduced the risk of HBD for one year suggesting a temporary reduction in risk of HBD from restocking. The number of restocked farms was small and the association was not precise, perhaps because of low power, however it was present. One explanation for this could be that these farms had a period of time without any cattle and that the infectious load on the farm, both from slurry (less produced and so less spread) and excretion of *M. bovis* by cattle, decreased temporarily. It is also possible that cattle in these newly formed herds had less *M. bovis* infection initially (from undetected infected cattle) than cattle in continuously stocked herds in this endemic area if they were, on average, sourced from herds with a lower prevalence of bTB; this has been reported for IBR (Woodbine et al., 2009a) and Johnes disease (Woodbine et al., 2009b). It is also possible (given the test sensitivity and cessation of testing for bTB in 2001) that continuously stocked herds had undetected infected cattle that were restricted on the farm and untested because of FMD and so transmission of bTB was greater that usual in continuously stocked herds. Both these processes indicate that perturbation of cattle (removal or stand-still) is associated with the risk of HBD in these endemic areas. It is not possible from this study to determine whether HBD in restocked herds was due to imported infection or residual/persistent infection on the farm, although this has been examined using a different study (Carrique-Mas et al., 2008).

The increased risks associated with purchase of cattle and continuously stocked (vs restocked) farms are also consistent with the presence of undetected infection in cattle which pose a risk to other cattle. There are several options for increasing the detection of these infected cattle. These include ensuring that testing is

done to a high standard and interpreted objectively (Enticott, 2009), increasing the rate of testing to overcome the low sensitivity of the test (assuming that false negative results are randomly distributed between individual cattle), and using an alternative or additional test such as gamma interferon (DEFRA, 2007). In the UK, pre-movement testing of cattle from herds tested at 1 or 2 year intervals is now compulsory. This might help to reduce the number of undetected infected cattle movements, but will not detect all infected cattle because of the sensitivity of the test.

The disruption of the RBCT during the FMD outbreak could have affected the impact of the trial on HBD and might explain the contrasting results from the RBCT proactive trial areas for the first and second year. It is important to appreciate that the current study was a subset of herds in the RBCT and that we had an uneven number of herds from the three treatment trials and could not analyse the data by triplet. Consequently, the results from the RBCT in our study are unlikely to reflect those of the whole RBCT study. In the overall trial, proactive culling of badgers in the RBCT was associated with a lower risk of HBD, although farms at the edges of proactive areas were less protected (Donnelly et al., 2007). The impact of culling by treatment and area within the RBCT has not yet been analysed fully.

In our study, spreading manure all year round and storing slurry in a closed container both increased the risk of HBD. Heat and ultraviolet light from sunlight destroy microbes and these two factors might explain why not storing waste (spreading manure all year round) or storing in a closed container were associated with an increased risk of HBD; there was insufficient time or opportunity for light to destroy the bacteria before they were spread on pasture. In both situations, *M. bovis* organisms are likely to have survived and been put onto the pasture whist alive, suggesting a process by which *M. bovis* can be transmitted via pasture. This is important because the public health recommendations for storage and spreading of slurry (the most common form of faeces from dairy cattle farms) reduce exposure to sunlight and may enhance pathogen survival (Menzies and Neill, 2000; Scanlon and Quinn, 2000).The storage of manure for 6 months or more was reported as a risk for transient HBD as opposed to persistent HBD with farm restrictions for more than 6 months by Reilly and Courtenay (2007).

Larger herd sizes have been reported as a risk for HBD in previous studies (Pfeiffer and Morris, 1991; Griffin et al., 1996; Porphyre et al., 2008). This fits with known patterns of infectious diseases where the

larger the population, the greater the probability that an infectious agent is introduced and persists. Since HBD is defined as at least one positive bovine positive to the SICCT, and the animal test sensitivity is approximately 70%, the probability of detection of at least one reactor will increase with herd size, assuming that the number truly infected also increases with herd size. Dairy herds also have the potential to be at higher risk of HBD, one explanation for this is that dairy cattle reach an older age than cattle intended for meat and so have a longer time to be exposed and infected with bTB, and longer to incubate infection. They also have many other different managements from beef cattle; e.g. milked twice a day, housed for large parts of the year, and a different genetic background that might make them at increased risk of bTB or HBD.

Some studies have suggested that metabolic factors (Dubos, 1955) and micro-nutrient levels in cattle (Downs et al., 2008) might affect susceptibility to *M. bovis*, however, these studies have not been very conclusive. The use of vitamins and mineral licks was associated with a decreased risk of HBD in the current study in contrast to the findings by Griffin et al. (1993). It seems more biologically plausible that cattle fed such supplements are in better health and therefore less prone to bTB than cattle not fed supplements. Other potential risks for HBD, such as the observation of wildlife in bedding and feeding stores, the use of different types of bedding and of hired equipment were investigated, but none were associated with a significant risk of HBD in the current study.

Conclusions

The main results from this study emphasise the importance of introduction of cattle in the introduction or re-introduction of *M. bovis*, and the approach to storage and management of manure / slurry in the persistence of *M. bovis*. A break in presence of cattle on the farm reduced the risk of HBD for one year suggesting that cattle are contributing to the persistence of *M. bovis*.

Authors' contributions

ARV coordinated the study, contacted farmers, contributed to the design of the questionnaire, helped to collect the data, performed the statistical analysis and drafted the manuscript. LEG and GFM were co-

principal investigators on this project. LEG participated in the design of the study and questionnaire, the pilot study, assisted with statistical analysis and helped to draft the manuscript. GFM participated in the design of the study, analysis and drafting of the paper. SM set up and maintained the project database and compiled data for analysis.

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Tables

Table 1 - The main areas of farm management and practices asked in the farmer's questionnaire

Risk factor group	General description
General	Herd type, herd size, restocking status, ownership
Manure/slurry management	animal origin, where produced, type, storage, use of spreader and whether shared, spreading time and time of storage
Bedding practices	type, where produced, where stored, wildlife presence in stores
Feeding practices	type, feeding method, where produced, where stored, wildlife presence in stores
Contacts with other cattle	bulls hired in/out, breaks in/out the farm land, cattle walking through farm, returns from markets, shows and abattoirs
Presence of diseases	persistence of disease, presence of BVDV, IBR, Johne's disease, Neosporosis or Leptospirosis, and other clinical signs since January 2000
Vaccination programme	reasons, type of vaccines given since January 2000
Purchase of cattle	number and type and sources of cattle bought since January 2000
People and equipment	number of staff working with herd, number of vet visits per year and having or not visitors, contract of different types of farming equipment

Table 2 - The 148 study farms by geographical location, intervention treatment of the randomised badger culling trial (RBCT) and restocking status*

County	RBCT survey		RBCT reactive		RBCT proactive	
	restocked		restocked		restocked	
	yes	no	yes	no	yes	no
Cornwall	0	2	1	2	0	6
Devon	10	30	2	10	11	21
Gloucestershire	2	4	8	30	0	0
Hereford & Worcester	0	0	0	2	1	2
Somerset	1	3	0	0	0	0
Total	**13**	**39**	**11**	**44**	**12**	**29**

*** Farms were located in one of the three treatments of the RBCT (survey, reactive or proactive). In each group, farms were or not restocked after FMD 2001.**

Table 3 – Discrete time survival model of study herds from1st October 2001 to 1st November 2004

Variable	Covariate		coef	S.E.	OR	95% CI
Herd size	Ln	148	0.75	0.19	2.11	1.47, 3.04
Dairy cattle	No	91			ref	
	Yes	57	0.78	0.34	2.18	1.12, 4.24
Manure stored in closed container	No	46			ref	
	Yes	71	0.91	0.33	2.49	1.30, 4.76
Manure spread all year round	No	111			ref	
	Yes	37	0.8	0.36	2.23	1.10, 4.51
Purchase from market	No	67			ref	
	Yes	50	0.67	0.32	1.95	1.05, 3.63
Feed vitamins / minerals	No	76			ref	
	Yes	72	-1.07	0.32	0.34	0.18, 0.64
Farm located in Somerset / North Devon	No	138			ref	
	Yes	18	1.44	0.49	4.21	1.60, 11.07
Years since restocked	>2	22			ref	
	>1 – 2	32	0.18	0.48	1.2	0.47, 3.05
	≤ 1	34	-1.03	0.63	0.36	0.10, 1.24
RBCT treatment	Survey	29			ref	
	Reactive ~ year 1	52	0.26	0.44	1.3	0.55, 3.10
	Proactive ~ year 1	41	-0.86	0.61	0.42	0.13, 1.39
	Reactive ~ year 2	42	0.62	0.48	1.86	0.73, 4.73
	Proactive ~ year 2	36	1.07	0.47	2.92	1.16, 7.38