A Cohort Study of Bovine Tuberculosis in Cattle in South West England

by

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DECLARATION

The work presented this thesis is the result of original research carried out by the author and it has not been presented elsewhere as part of another degree.

The contents from Chapter 4 have been presented at the 4th International Conference on *Mycobacterium bovis*, Dublin, August 2005 and at the ISVEE XI International Conference, Cairns, Australia, August 2006.

The contents from Chapter 5 have been accepted for presentation at the SVEPM Conference in Liverpool, March 2008.

SUMMARY

Farm, cattle group and individual bovine risk factors for bovine tuberculosis (bTB) in cattle herds in the South West of England were explored.

A cohort study using 148 well characterised cattle herds was conducted in SW England 2001-2004. The study was set up in areas affected by foot-and-mouth disease in 2001 and all farms were taking part in the Randomised Badger Culling Trial (RBCT). The use of a standard questionnaire and national data records from the skin intradermal cervical comparative tuberculin test (SICCT) and from the British Cattle Movement Service (BCMS) databases were combined.

The two main statistical techniques used were survival analysis and multilevel logistic regression with random effects. Associations with the risk of herd breakdown with bTB were explored using survival analysis. The main factors associated with disclosure of reactor cattle were the purchase of cattle from markets and the storage of slurry and manure in close containment.

In the investigation of the risk of an individual bovine animal becoming a reactor using multilevel logistic regression with random effects analysis, explanatory variables at herd, individual cattle and test levels, were explored. The potential exposure to reactor cattle in previous tests was the most significant finding as a risk for a bovine animal reacting at a current test. Only 9/19,027 cattle became reactors if they had not been exposed to a reactor animal previously.

When the risk of an animal group having at least one reactor disclosed in the group was investigated using the location of the animal groups within the farm by monthly periods, the risk increased with the number of cattle in the groups when these were housed and with the presence of badgers in the fields when they were grazing.

This thesis has provided a deep investigation into the risk factors that can affect the introduction and persistence of infection with *M. bovis* in cattle herds, and the importance that cattle play in these factors has been highlighted.

ABBREVIATIONS AND ACRONYMS

AIDS	Adquired immune deficiency syndrome
BCMS	British Cattle Movement System
bTB	bovine tuberculosis
CI	Credibility Interval
cfu	colony forming units
CTS	Cattle Tracing System
DEFRA	Department of Environment Food and Rural Affairs
FMD	Foot-and-mouth disease
HBD	Herd Breakdown
HR	Hazard Ratio
HSe	Herd sensitivity
HSp	Herd specificity
ISG	Independent Scientific Group on Cattle Tuberculosis
NFU	National Farmers Union
OIE	Organization of International Epizooties
OR	Odds Ratio
PPD	Protein purified derivative
RBCT	Randomised Badger Culling Trial
SICCT	Single intradermal comparative cervical tuberculin test
SIT	Single intradermal tuberculin test
SE	Standard Error
SW	South West
VetNet	the data storage system from the Animal Health (former State Veterinary Service)
VLA	Veterinary Laboratory Agency

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Chapter 1 - Introduction

1.1 Overall scope of the Project SE3026

This thesis was developed as part of the project SE3026 funded by the Department of Environment Food and Rural Affairs (DEFRA). This was run in conjunction with another project funded by the Biotechnology and Biological Sciences Research Council (BBSRC), where by using the same study farms as in the SE3026 project, the objective was to carry out serological testing on individually identified serum samples collected over the period of three years. The Principal Investigators (PIs) of the projects were Prof. Laura Green and Prof. Graham Medley.

The aims of the SE3036 project were to investigate risk factors associated with the risk of bovine tuberculosis (bTB) by using the perturbation caused by the recent foot-and-mouth disease (FMD) outbreak. For that, two studies were designed: a case-control study of herd breakdowns (HBD) with bTB on restocked farms affected by FMD in England, and a longitudinal cohort study in the South West of England on restocked and continuously stocked farms within areas of the Randomised Badger Culling Trial (RBCT).

My role as coordinator of the cohort study within the SE3026 project involved the following tasks:

1. The enrolment and maintenance of farmers in the study.
2. The design of a farmer's questionnaire.
3. To be responsible for the coordination of the project team.
4. To ensure the data were accurately collected.
5. To carry out the statistical analysis.
6. To collaborate with the PIs of the project in the production of reports.

In addition, I was responsible for the coordination of the visits for the blood sampling collection under the BBSRC project.

1.2 Scope of the thesis

The objective of this thesis was to provide a better understanding of the epidemiology of bTB in cattle by investigating associated risk factors and the role that cattle play in the transmission and maintenance of *M. bovis*, the causative agent of bTB, in cattle herds in the South West region of England by using a cohort study. The exposure to be tested over time was repopulation of herds affected by FMD in 2001.

The broad aims were:

- To provide the strengths and limitations of a field longitudinal epidemiological study by giving a detailed description of the materials and methods used in the study.
- To characterise the study farms by providing a general description based on the selection criteria and the current skin test used for the outcome variables in the analysis carried out.
- To identify, by using two different statistical techniques, risk factors associated with the risk of bovine tuberculosis at three levels: herd, individual animal and animal group within-herds.

The alternative hypothesis tested was that identified potential risk factors did have an effect (increase or decrease) in the risk of bib, being this effect not due to chance. In Chapter 4 the alternative hypothesis was that management factors identified in the Part 2 of the farmers' questionnaire would have an effect in the risk of a herd to break down with bTB. In Chapter 5 the alternative hypothesis was that factors identified with the SICCT test and movement records from the VetNet and from the BCMS databases respectively, would have an effect on the risk of an animal to becoming reactor. In Chapter 6 the alternative hypothesis was that factors identified in the Part 1 of the farmers' questionnaire would have an effect in the risk of an animal group having at least one reactor animal in the group.

The structure of the thesis is as follows. Chapter 2 provides a summary of the materials and methods used in the thesis and it describes how the study was set up. References are made to other chapters where relevant. Chapter 3 presents a

description of the study farms based on the study selection criteria, the skin tests recorded in the VetNet database that were used on the herds during the study period, with a brief description of farmers' perception of the test and the disease. In Chapter 4, the second part of the standardised farmers' questionnaire and test records were used to investigate risk factors at herd level. A Cox's proportional hazards multivariable model was developed. In Chapter 5, multilevel analysis was used to investigate risk factors at individual animal level. Data from the British Cattle Movement System (BCMS) were used. In Chapter 6, using the first part of the farmers' questionnaire, a multilevel multivariable model was developed, with the animal group within-herds as the outcome variable. In the last Chapter 7, a discussion of the main findings and conclusions from this work are presented.

An introduction to bTB in cattle is presented in this chapter. The main aim is to provide a background for the study with an overall historical perspective of bTB in cattle; a brief summary of the importance of the disease as a zoonosis and in badgers as main wildlife reservoir; a description of the main lesions and the route of transmission; the characteristics of the currently used skin test and a summary of epidemiological evidence up to the date of previous risk factor investigations and the characteristics of the statistical tools used in the thesis. Other aspects of the disease, such as the development of vaccinations as methods of control, are not included.

1.3 An historical perspective of bovine tuberculosis

Bovine tuberculosis (bTB) has been recognised as an infectious disease of cattle for many decades. It is possible that bTB existed in Northern Italy at the beginning of the Christian era with its origins being in cattle of Indian origin. During the nineteenth century, the disease spread into Europe, although infected cattle imported by the Romans could have been the source of the disease in Britain. Infection was from then on spread from Europe into many countries of the world (Francis, 1947).

After the isolation of the tubercle bacilli, *Mycobacterium tuberculosis*, by Koch in 1882, the disease was first described in the horse in 1884 and around 1888 in man, cattle and other animals. At a Congress on tuberculosis in Paris in 1889, the transmission of tuberculosis to man through the consumption of milk from tuberculous cattle was recognised (Glover, 1937). Measures such as heat treatment,

removal of carcases with lesions, the diagnosis by clinical and pathological examination and isolation of the bacilli, were recommended to reduce the risk of infection from cattle to man. Pasteurisation was enforced in the USA in 1907 and it commenced in Britain in the 1930's (Pritchard, 1988) with a hundred per cent enforcement by 1944, although human cases in rural areas remained uncontrolled. Despite the fact that the link between the disease in cattle and humans was suggested in the Royal Commission Report in 1911, an eradication programme based on meat inspection by veterinary control, did not start in Britain until 1935, whereas this measure had already been introduced in the USA in 1917. This delay in the implementation in Britain was mainly due to the controversy over the link between the human and the bovine disease (Pritchard, 1988).

Initially, there was some controversy over whether the digestive or respiratory route was the main route of transmission. From initial descriptions of lesions caused by the tuberculous bacilli in the horse, the digestive tract was thought to be the primary route after the ingestion of raw milk from infected cows (Glover, 1937). However, from several experiments and natural cases of the disease in cattle, descriptions of lesions suggested that the respiratory tract was the primary route of transmission and that the dose required for infection by the respiratory route was hundred of times lower than the dose required by the digestive route and, it would be by the swallowing of sputum containing tubercle bacilli, that lesions could develop in the mesenteric lymph nodes (Francis, 1947). From observations of the disease in humans, Langmuir suggested in 1961 that some people could be infectious by the intermittent production of aerosolised infected particles (Pritchard, 1988).

The human, avian and bovine types of tubercle bacilli were first described in the USA by Theobold Smith, and *Mycobacterium bovis* was first named by Karlson and Lessel in 1970 (Pritchard, 1988). Recent genomic analyses suggest that *M. bovis* has developed from *M. tuberculosis*, i.e. that cattle acquired tuberculosis from humans (Hewinson *et al.*, 2006).

Diagnosis - The tuberculin test was developed from the "old tuberculin" of Koch (1890), which was a concentrated sterile filtrate of autolysed heat-killed liquid cultures of tubercle bacilli (Francis, 1947; Pritchard, 1988). First tests using

tuberculin were carried out by subcutaneous injection. Although the sensitivity was high, the temperature of the animal was taken to diagnose the disease, which often resulted in low specificity, since the raise in temperature could be caused by other diseases. Koch also reported that some animals in an advanced state of the disease could fail to react to the tuberculin and that by repeating the injection twice, the sensitivity of the test could be increased. The periods observed between injection and reaction to the tuberculin, were between eight days and seven weeks (Glover, 1937).

The intradermal test and skin reaction was first assessed by Moussu and Mantoux in 1908. The purified protein derivative (PPD) was developed around 1934 and was made specific to the tubercle protein by separating impurities from bacilli grown using trichloracetic acid (Francis, 1947). The PPD tuberculin was universally used for testing cattle in Britain since 1945. In 1975, tuberculin from *M. tuberculosis* was replaced by the bovine tuberculin, which showed to have higher sensitivity and specificity.

In 1950, an eradication programme based on a test and slaughter was introduced in the UK. This involved the skin test of cattle herds, the removal of positive animals from the farm and movement controls of positive cattle herds (DEFRA, 2005a).

1.4 The importance of bovine tuberculosis

1.4.1 General overview

Bovine tuberculosis is classified as a list B disease by the Office of International Epizooties (OIE) due to the potential public health, socio-economic and animal trade implications in countries where the disease is present.

As a zoonotic disease, the problem is greater in developing countries and in countries where there is no control in place or measures are scarce. It has a major economic impact for the farming industry. In the financial year 2004/2005 in the UK, DEFRA spent £91 million on the bTB programme, £36 million of which were dedicated to cattle testing (DEFRA, 2005a). These figures are over twice as high as in the years 1999/2000, in which the government spent approximately £38 million, £17 million of which was used for cattle testing. The main cost of a herd breakdown

(HBD) involves the slaughter of the reactor cattle, and, although most of the costs to the farmer are covered by compensation (Bennett and Cooke, 2006) the disease causes major disruptions to farmers, mostly due to movement restrictions and handling of cattle for testing.

The brush-tailed possum (*Trichosurus vulpecula*) in New Zealand, the Eurasian badger (*Meles meles*) in Ireland and in the UK (in particular the South West region) and more recently free-ranging white tailed deer in Michigan (USA) as well as in other European countries such as Spain, have been identified as wildlife reservoirs for *M. bovis* (Griffin and Mackintosh, 2000; Delahay *et al.*, 2002; Aranaz *et al.*, 2004). The persistence of the disease in these countries has been associated with the presence of these wildlife reservoirs. In the same countries, the transmission of *M. bovis* between cattle as the source of infection may be more difficult to elucidate compared to countries where cattle are the only recognised source of infection.

1.4.2 Bovine tuberculosis as zoonosis

Overall, one third of the world population is infected with the tubercle bacillus but the number of cases attributed to *M. bovis* has been difficult to demonstrate. Tuberculosis in humans can be caused by both *M. tuberculosis* and *M. bovis* and the differentiation of these two species are clinically, radiologically and pathologically indistinguishable. The lack of clinical signs in most human cases (only 5% of human cases develop disease and the other 95% remain latent) and the lack of diagnostic laboratory tests in both industrialised and developing countries make the differentiation between the two species difficult (Cosivi *et al.*, 1998; Thoen *et al.*, 2006; de la Rua-Domenech, 2006a).

Human tuberculosis caused by *M. bovis* was traditionally due to the ingestion of contaminated milk, causing cervical lymphadenitis, abdominal tuberculosis and other non-pulmonary lesions (Grange and Yates, 1994). However, agricultural workers may acquire the disease by inhaling cough spray from infected cattle, developing typical pulmonary tuberculosis. Two cases of pulmonary tuberculosis infection *by M. bovis* from a farming family were reported recently in the UK, suggesting that there is a risk to humans from *M. bovis* sourced in the respiratory tract of cattle (Smith *et*

al., 2004). Cutaneous exposure by contact with mucous membranes and skin abrasions is very rare nowadays, however, it was known to occur in the past, especially in workers handling infected carcases.

In developed countries, the risk of human infection due to *M. bovis* is low, mainly because of the introduction of pasteurization of milk and the inspection and removal of infected cattle after the implementation of the test-slaughter policy. In 2004, in Great Britain (GB), *M. bovis* was confirmed by bacteriology in 21 human cases, a number that has decreased since 1993 and before, when approximately 50 were confirmed per year (Dean *et al.*, 2005). The main concerns in developed countries now are related to the re-emergence of infections due to the introduction of *M. bovis* by immigrants from countries with high tuberculosis prevalence, the reactivation of the disease in HIV-infected patients and the risk of transmission from wildlife and from airborne transmission for farmers and slaughterhouse personnel (Pavlik *et al.*, 2003; Thoen *et al.*, 2006).

The disease in developing countries is a major problem. Due to the widespread of immunodeficiency with AIDS, the low standards on hygiene conditions and the lack of information, bTB is one of the priorities of the World Health Organization (Etter *et al.*, 2006). There are no data available in some countries in Asia, Africa and Latin America, but it is estimated that a high proportion (approximately between 60% and 90%) of the cattle and human population live in areas where bTB is only partially controlled or no control measures are in place. Between 1954 and 1970, 3.1% of cases of human tuberculosis were attributed to *M. bovis* (Cosivi *et al.*, 1998).

1.4.3 Bovine tuberculosis in cattle

Bovine tuberculosis has a great impact on animal health and the farming industry in many countries. In England, in the 1930s, approximately 40% of slaughtered cattle showed tuberculous lesions at post-mortem (Collins and Grange, 1983). Due to the implementation of the eradication programme in England in 1950, which was extended to Great Britain by 1960, by the end of 1970s the herd incidence reached the historical minimum: from 3.5% in 1961 to 0.49% in 1979 (de la Rua-Domenech *et al.*, 2006b). However, despite the control measures, the incidence in cattle has

been increasing over the last fifteen years by an average of 14% per year (DEFRA, 2005a). Since 1990, the number of confirmed herd incidents was increasing by 18% per annum (DEFRA, 2004b). It has only been recently that a reduction in the incidence has been observed. The reasons for this decline are being investigated and the incidence needs to be observed for a longer period of time before reaching any conclusions (Figure 1.1) (DEFRA, 2006).

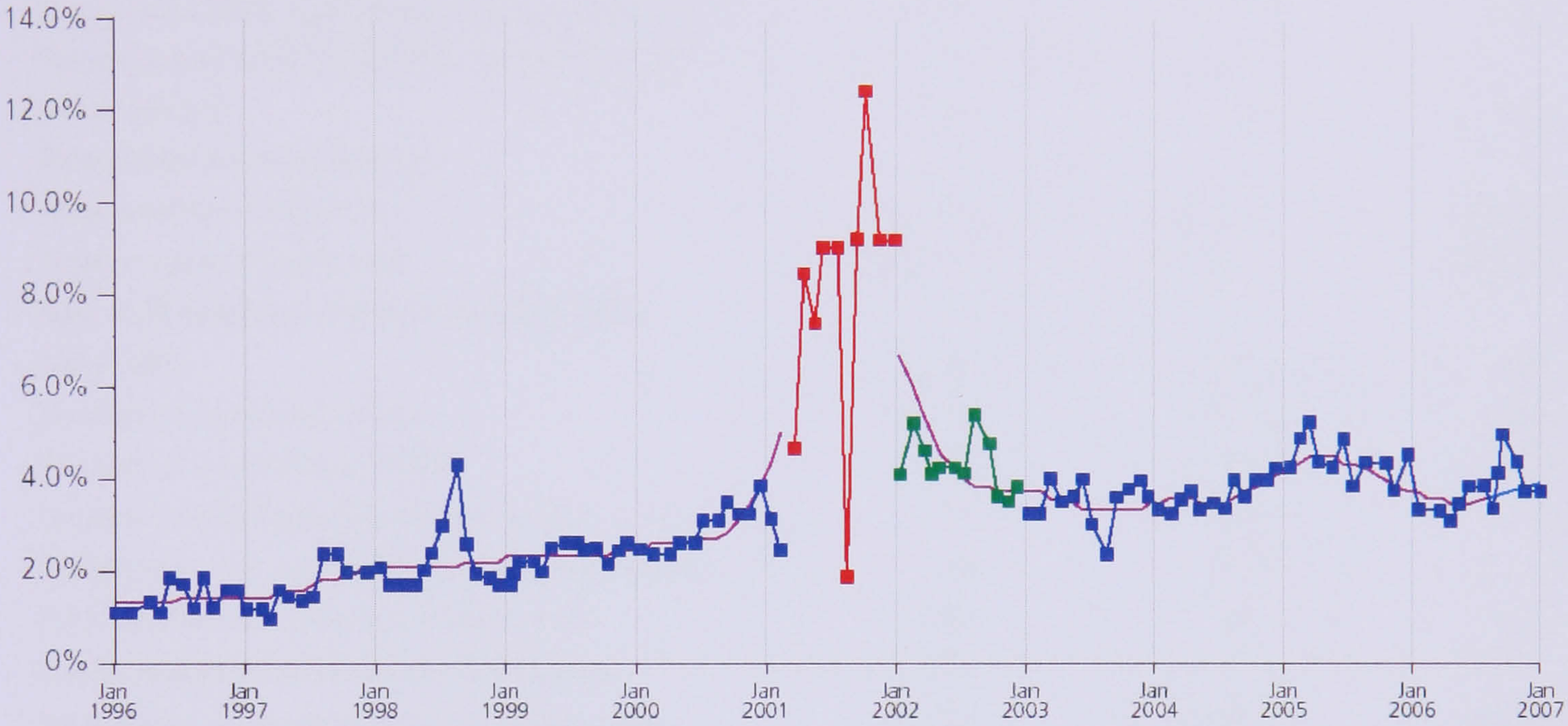


Figure 1.1 - Percentage of tests on unrestricted herds that triggered confirmed new incidents.

Source: Animal Health 2006. The Report of the Chief Veterinary Officer (www.defra.gov.uk)

Keys:

- Percentage of tests on unrestricted herds resulting in a confirmed new incident
- Trend (23 term henderson moving average of seasonally adjusted data)
- Provisional trend-line
- bTB testing significantly reduced due to the FMD outbreak and targeted to higher risk areas
- bTB testing resumed in 2002 and was initially concentrated on clearing the backlog of overdue tests

The disease has been concentrated in the South West region in the last 25 years (Krebs *et al.*, 1997). About 5.6% of cattle herds in Great Britain were affected by movement restrictions due to bTB at some point in 2004 and 6.2% in 2005, 4% of which were within the South West (Table 1.1). Table 1.1 shows surveillance results from 2006 for the South West region and from 2006 and 2005 for Great Britain.

Table 1.1 - Surveillance statistics for South West region for 2006 and for Great Britain for 2006 and 2005

Surveillance statistics	**South West in 2006**	**Total in Great Britain in 2006**	**Total in Great Britain in 2005**
Total number of cattle herds registered in Vetnet	22,996	89,461	91,103
Total number of herds under restriction due to a bTB incident some time during the year	3,734	5,848	5,682
Herds under bTB restriction at the end of the year	3,070	6,856	5,748
Percentage of herds under bTB restriction at the end of the year	13.4	7.7	16.3
Tuberculin tests carried out			
Total number of herd tests	22,183	50,327	43,627
Total number of cattle tests	2,862,471	5,475,466	4,849,206
New bTB incidents (HBD) started in 2005			
Total HBD	2,163	3,567	3,673
Number of confirmed HBD	1,308	1,993	2,086
Number of unconfirmed HBD	802	1,578	1,578
Number of HBD pending culture results	53	119	9
Percentage of all new HBD that were confirmed	60	56.8	57
Total number of confirmed HBD in 2005	1,440	n/a	2,086
Cattle slaughtered under the TB orders			
As reactors, inconclusive reactors (three times)	12,520	19,963	25,769
As inconclusive reactors	290	467	57
As Direct Contacts	786	1,812	3,744
Total number of cattle slaughtered for bTB control	13,596	22,242	30,081
Slaughterhouse reported cases (confirmed)	489 (295)	790 (450)	591 (390)

Source: Animal Health 2006. The Report of the Chief Veterinary Officer (www.defra.gov.uk)

1.4.4 *Mycobacterium bovis* infection in badgers

The Eurasian badger (*Meles meles*) is one of the wildlife species susceptible to *M. bovis*. Tuberculosis was first diagnosed in badgers in Switzerland in 1956 (Bouvier *et al.,* 1957, reviewed by Morris *et al.*, 1994).

After the control programme of bTB was initiated in the UK in 1950-1960, the incidence in positive herds decreased dramatically in the UK with exception of the South West of England, where the incidence remained three times higher than the rest of the UK (Krebs *et al.*, 1997). In 1971, *M. bovis* was isolated from a badger carcase in the South West of England (O'Reilly and Daborn, 1995). Badgers were first linked to bTB in cattle in 1973. Since then, different strategies to remove badgers from farmland have been adopted. In 1986 culling was limited to those

badgers whose setts were found on land used by cattle from which *M. bovis* had been isolated. Until then, the strategies used to control badgers were not comparable to each other, therefore the effectiveness of these control measures on decreasing the incidence of bTB in cattle was unknown (Donnelly *et al.*, 2003).

The Randomised Badger Culling Trial (RBCT) (the Kreb's trial) - In 1997, the Independent Scientific Group (ISG) advising on bTB proposed a trial with the aim to test the impact of two different badger control strategies on the incidence of bTB in cattle herds. The trial was initiated in 1998 and it was finished in 2006. Three experimental treatments were designed: reactive, proactive and survey only. The reactive treatment involved the removal of badgers from small areas in response to bTB outbreaks in cattle; the proactive culling carried out approximately annually, aimed to reduce badger densities to low levels in all trial areas; and the survey only, involved no culling of badgers. Thirty trial areas, each covering approximately 100 Km^2 were selected within areas of high incidence of bTB in the South West region, Staffordshire and Derbyshire. After a survey of badger activity, areas were randomly allocated a treatment and each treatment was replicated ten times, creating ten triplets (from A to J) (MAFF, 1999; Donnelly *et al.*, 2003).

The trial was affected by the foot-and-mouth disease (FMD) epidemic in 2001. Field activity was suspended between the end of February and the end of November 2001 to avoid any risk of spreading FMD. No culling was carried out between February 2001 and April 2002 (Le Fevre *et al.*, 2005).

The first results published from the RBCT referred to the reactive trial areas compared to survey only. The results showed that reactive treatment was associated with a 27% increase in the herd incidence (HBD) when compared to survey only areas (no culling). Given these results, it was concluded that the removal of badgers from land associated with herds with HBD did not contribute to the control of bTB in cattle herds and the reactive treatment was discontinued (Donnelly *et al.*, 2003). Disruption of the territorial organization of badgers, resulting in an increase in the contact rate between badgers and hence an increase in the spread of infection was suggested as a reason for this increase in HBD incidence (Woodroffe *et al.*, 2006). Further results from the RBCT suggested that the proactive treatment reduced the

incidence of HBD in cattle herds compared to survey treatment by 19% in areas where badgers were culled, but herds on land bordering the proactive treatment areas, experienced an increase in incidence of 29% compared to those on land bordering survey only areas (Donnelly *et al.*, 2006).

From 1997 to August 2002, another badger trial in Ireland (the Four Area Trial) was carried out. This involved four different counties and the treatments were removal and reference (equivalent to proactive and reactive treatment areas of the RBCT respectively). Griffin *et al.* (2005) reported a decrease in the odds of a confirmed herd restriction in areas of removal compared to reference in the four areas of the study. These results would be in accordance with those reported by Donnelly *et al.*(2006) from the RBCT. However, the non-existence of a survey only treatment in the Irish trial makes comparison between the two trials more difficult.

Other studies have reported the association between the presence of badgers on farm land used by cattle and bTB in cattle herds in Ireland (Martin *et al.*, 1997), and in Great Britain (Clifton-Hadley *et al.*, 1995). Several case-control studies have associated HBD with cattle exposure to badgers (Griffin *et al.*, 1993; Piran *et al.*, 2004). Despite the associations found, the mechanism by which the infection is transmitted from badgers to cattle is not well understood (Garnett *et al.*, 2003; More and Good, 2006). Using epidemiological data from the RBCT, (Woodroffe *et al.*, 2005) recently reported a spatial association between infection in badgers and cattle, as they were both found to be infected with the same strain type. However, the significance of this finding to the transmission of bTB from badgers to cattle and *vice-versa* could not be assessed.

Some authors have suggested that pasture contaminated with infected badger urine or faeces could serve as a source of infection to cattle (Morris *et al.*, 1994) whilst others have reported that cattle will avoid grazing contaminated swards (Morris *et al.*, 1994 ; Garnett *et al.*, 2003). Feeding troughs and buildings within farms could be also contaminated by *M. bovis* excreted by badgers (Garnett *et al.*, 2002; Garnett *et al.*, 2003). The exposure of cattle to badgers, based on the number of badger setts, badgers or tuberculous badgers on farmland, has proven difficult to demonstrate suggesting that other factors such as bought-in cattle and spread from

contiguous herds and residual infection, may play a role as a source of infection in cattle herds (Olea-Popelka *et al.*, 2006).

Most recently, the ISG in its Final Report have suggested that culling of badgers in the way it was carried out in the RBCT would be only beneficial if carried out over large geographical areas and over several years, which would be economically unsustainable. The report also suggests that there are not many other methods of culling which could provide better results than those found in the RBCT. Based on this, and given that the overall effect has been detrimental, the ISG recommends that it would be more beneficial to focus on measures other than badger control, such as cattle movement and more strict testing regime (DEFRA, 2007).

1.5 Aetiology and pathogenesis of bovine tuberculosis in cattle

1.5.1 Aetiology

Bovine tuberculosis is caused by *Mycobacterium bovis* (*M. bovis*). It belongs to a group of mycobacteria known as the *M. tuberculosis* complex of bacteria ("MTBC"), Family Mycobacteriaceae, which comprises four main species: *M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microti* as well as *M. canetti* and *M. caprae* (Neill *et al.*, 2005).

Mycobacterium bovis has a broad range of hosts which include most farmed animals, some wildlife species and humans. Susceptible species include cattle, goats, cats, dogs, pigs, buffalo, badgers, possums, deer and bison, non-human primates and humans (O'Reilly and Daborn, 1995). Some species are spillover hosts only, whilst others maintain and transmit the infection. *M. bovis* is a Gram-positive acid fast rod bacterium with a very thick wall structure which probably enables the bacteria to survive in hostile environments and within the host (Pritchard, 1988). Although cattle-to-cattle transmission is reported to be of low frequency in the field, *M. bovis* can be highly infectious (Menzies and Neill, 2000).

1.5.2 Clinical signs and lesions

Infection with *M.bovis* is usually chronic and can remain sub-clinical for a long period of time. Bovine tuberculosis is mainly a disease of the respiratory tract. Generalised disease can occur but this is rare in countries where there is an eradication control programme in place. Clinical signs are characterised by a soft and chronic cough. If the condition continues, there may be respiratory difficulty and progressive loss of condition.

The first and more predominant immune response is cell-mediated (by T lymphocytes) (Ritacco *et al.*, 1991; Monaghan *et al.*, 1994), which is the cause of the chronic characteristic caseous granulomas. The lesions start by infectious droplet nuclei which form the "primary complex" primarily found in the lungs and thoracic lymph nodes (Neill *et al.*, 2001). Lesions heal or persist with or without progress by interfering with the function of the lungs. The disease then progresses over a number of years. It is in the more advanced stages of the disease that a humoral (antibody production) immune response appears. Infected cattle can become infectious long before they show any clinical signs or lesions typical of the disease (de la Rua-Domenech *et al.*, 2006b).

Most lesions found in naturally infected reactor cattle are located within the lower respiratory tract (LRT) (the lungs and pulmonary and cranial lymph nodes) whilst lesions in the upper respiratory tract (URT) seem to be less frequent (Corner, 1994; Dean *et al.*, 2005). While lesions in the lungs were more common when no measures for control were in place (Menzies and Neill, 2000), differences in prevalence for the presence of lesions in the lungs of reactor cattle have been reported, ranging from 1% to 73% (Neill *et al.*, 1988). From numerous abattoir surveys from naturally infected cattle, lesions in the lungs and associated lymph nodes ranged from 19% to 80% of the total lesions; cranial lymph node lesions ranged from 2% to 55% (Palmer *et al.*, 2002). Within the thoracic lymph nodes, the bronchial and or mediastinal lymph nodes are the most commonly affected. The second most affected nodes are of the head, the retropharyngeal and sub-maxilary nodes frequently affected in the absence of lesions in the lungs (Neill *et al.*, 2001). *M. bovis* has been isolated within the upper respiratory tract, the tonsils, nasal pharynx, trachea and nasal mucus, which

confirms the shedding of the bacterium and the potential to transmit the infection (Neill *et al.*, 1994). It is possible that these lesions are more scarcely reported due to a lower post-mortem examination of heads of reactors in abattoirs (Neill *et al.*, 2005).

The following photos were taken on 14th June 2007 from two reactor cattle at post-mortem inspection in an abattoir in Gloucestershire. The photos present the dissection of lymph nodes at post-mortem inspection with typical bTB lesions.

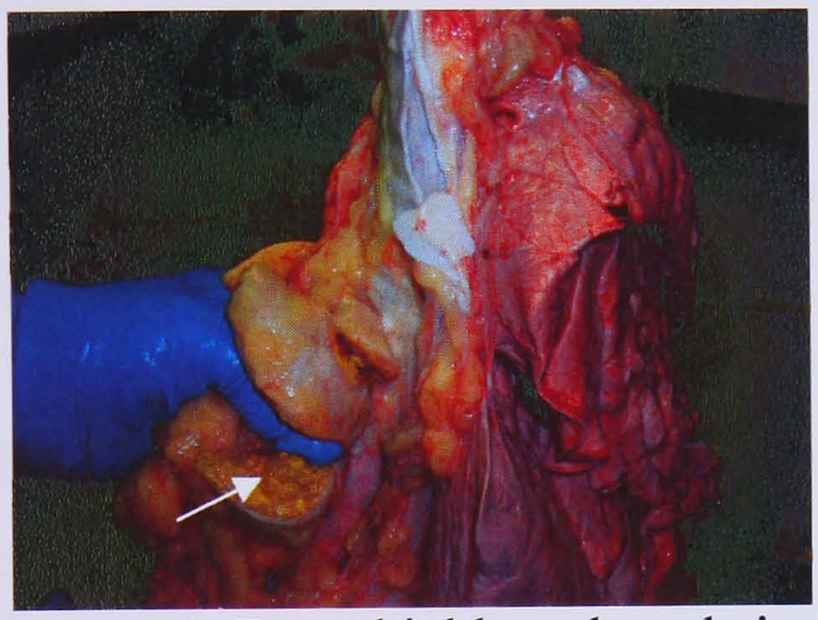


Photo 1.1- Bronchial lymph node in a South Devon female bovine animal 3 years 4 months old

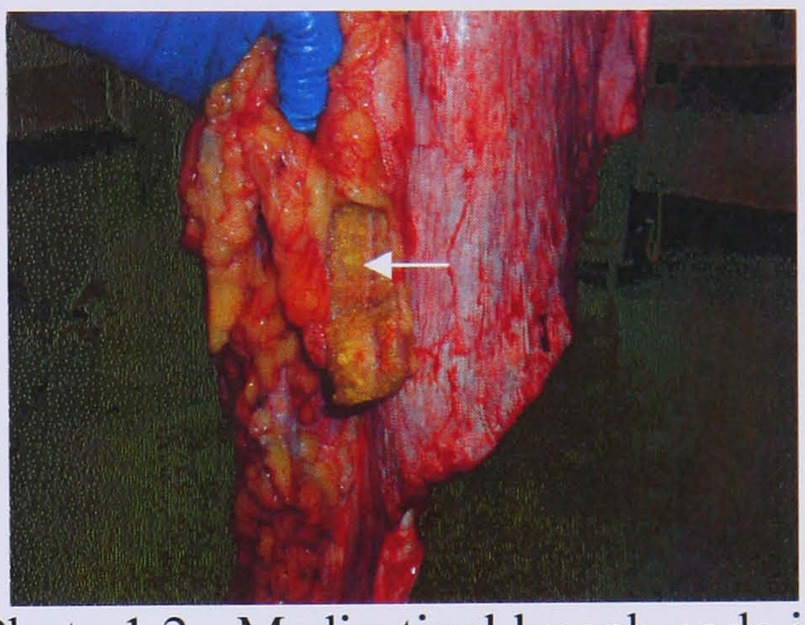


Photo 1.2 - Mediastinal lymph node in a South Devon female bovine animal 3 years 4 months old

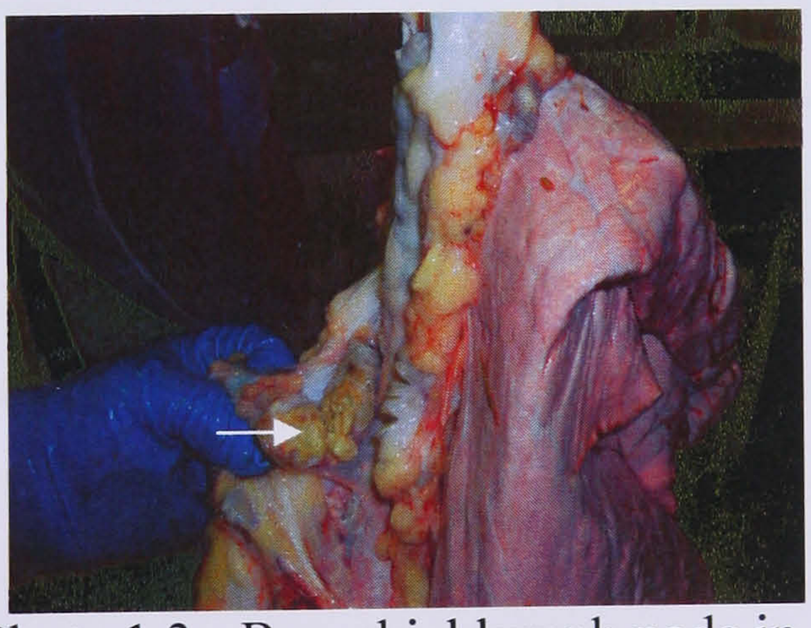


Photo 1.3 - Bronchial lymph node in a Limousin female bovine animal 4 years old

1.5.3 Route of transmission and infective dose

Infection by inhalation is considered as the main route of transmission (Stamp, 1944; Francis, 1947; Collins and Grange, 1983; Pritchard, 1988; Neill *et al.*, 1994). This hypothesis is supported by the observation of the majority of lesions in this tract and the low infectious dose that is needed to cause infection via this route (Dean *et al.*, 2005). Infection by ingestion is more likely to occur in calves suckling tuberculous cows (Francis, 1947; Palmer *et al.*, 2002) or by swallowing infected sputum, and although lesions in the mesenteric lymph nodes are rare, this could be considered the second most common route of infection (Pritchard, 1988). Genital, congenital and vertical transmission are extremely rare (Menzies and Neill, 2000; Neill *et al.*, 2005).

In general, the infective dose needed via respiratory tract is much lower (ranging from one to six bacilli) than that by the oral route (ranging from ten to twenty million bacilli). In aerosol transmission, the size of the inhaled droplet containing the mycobacteria as well as the dose, have been reported to be important in the establishment of the disease (Neill *et al.*, 1991; Menzies and Neill, 2000; Palmer and Waters, 2006). In order to investigate the minimum infectious dose, Dean *et al.* (2005) carried out an experiment using the intra-tracheal route in which they found that one colony forming unit (cfu) was sufficient to cause infection, and lesions were similar to those produced with doses of up to 1,000 cfu, which resembled those observed in natural cases.

Most of the work done on the pathogenesis of *M. bovis* has involved experimental studies. Pollock *et al.* (2006) summarised the findings from nine studies that have reproduced infection and tuberculosis disease in cattle. From these studies, generalised systemic lesions (atypical lesions compared to field cases) were reported when the infection route used was intravenous, sub-cutaneous or oral and with doses between 10^6 and 10^8 cfu. When the intra-tonsillar route was challenged with doses between 10^5 and 10^7 cfu, lesions were mainly found in the URT, with limited lesions found in the LRT (Palmer *et al.*, 1999). Other studies have reported similar lesions to those found in the field after in-contact exposure to intra-nasally infected cattle (Neill *et al.*, 1989; Costello *et al.*, 1998; Cassidy *et al.*, 1999). When the intra-

tracheal route was challenged with doses between 10^3 and 10^5 cfu, generalised lesions were also found using 10^5 cfu while doses of 10^3 cfu produced lesions in the lungs and lymph nodes (Buddle *et al.*, 1994).

Palmer *et al.* (2002) reported the results of the first experimental study in cattle using aerosol transmission by nebulization. In this study, two different strains, one from a white-tailed deer and another from a dairy cow, at doses of 10^3 and 10^5 cfu, were administered by nebulization aerosol to 20 four-month-old calves. Lesions in the URT and LRT (mostly in the lungs and in the tracheobronchial and mediastinal lymph nodes, similar to those found in field cases) were observed in 19 calves after 155 days, regardless of the dosage or strain used. More generalised lesions in the lungs were found when calves were exposed to higher doses, especially when the strain isolated from cattle was used, and the calves with lesions were also positive to the skin test 63-121 days after infection. One calf out of 20 was both negative to the skin test and found with no lesions.

From studies on the transmission of *M. tuberculosis* in humans, the mechanism by which infectious particles containing the bacteria reach the pulmonary alveoli by coughing or sneezing, is well understood (Corner *et al.*, 2004). In the case of bTB, and despite the fact that the respiratory tract has long been considered the main route of transmission, the process of how the bacterium survives once aerosolised after nasal shedding, and the potential for transmission, are poorly understood. Airborne survival could be influenced by field conditions such as temperature, relative humidity and ultraviolet light. In a recent experiment carried out by Gannon *et al.* (2007), the survival of *M. bovis* (a wild-type strain) was assessed using particles with a minimum diameter of 0.3 μm from an aerosolised suspension. The authors showed that 94% of the bacteria were viable after the first ten minutes, in which the bacteria became airborne, and some were still viable after twelve hours.

1.5.4 Excretion of *M. bovis* and survival in the environment

Neill *et al.* (1988) found that after the isolation of *M. bovis* from the nasal tracts of 16% of 25 tuberculous cattle, infection established rapidly, and the period between infection and excretion was very short. All these cattle had been skin tested twice

with a mean interval of six months prior to post-mortem inspection. Subsequently, a mathematical model provided evidence that infection could occur after introducing a single bacillus to the alveoli, with excretion occurring around 87 days after infection (Neill *et al.*, 1991). The detection of *M. bovis* in the nasal mucus and faeces of calves in contact with cattle infected intra-nasally thirty five days after exposure, suggested that excretion by recently infected animals can occur (Cassidy *et al.*, 1999).

In the initial stages of infection, there may be regular excretion of the bacterium in the nasal mucus before this becomes intermittent, although the quantities excreted may vary between animals (Menzies and Neill, 2000). It seems that only certain tuberculous cattle act as effective disseminators and these do so intermittently and only under certain circumstances, but this process is not well understood.

In cases where generalised bTB occurs, *M. bovis* may be excreted in mucus, milk, urine and faeces (Scanlon and Quinn, 2000), however, these routes are considered relatively insignificant for the spread of the disease in developed countries where regular cattle testing is in place (Neill *et al.*, 2005).

The survival of *M. bovis* in liquid cattle slurry protected from the direct sunlight has previously been reported (Scanlon and Quinn, 2000). The latter authors showed that *M. bovis* can survive up to six months in cattle slurry under adequate conditions. For example, the mycobacterium could remain viable in stored slurry because it has a pH close to neutral, which is optimal for *M. bovis* survival. However, on the other hand, it has been suggested that faecal excretion of *M. bovis* may play little role in the transmission of the disease due to the lower evidence of digestive compared to respiratory lesions (Menzies and Neill, 2000).

Some other studies have been carried out to investigate the survival of *M. bovis* in the environment. Wrag in 1975 (reviewed by Menzies and Neill, 2000) reported that *M. bovis* can survive for many months in soil and slurry (from 18 to 332 days) at temperatures between 12 -24°C and up to 700 days when buried in soil protected from the sunlight and mixed with faeces, blood and urine. Young *et al.* (2005) reported that *M. bovis*, which was isolated by using polymerase chain reaction (PCR)

technique from a farm with past history of bTB, may remain viable up to 21 months in soil.

On the other hand, Jackson *et al.* (1995) suggested that it survives poorly on pasture and it was difficult to detect bacilli after four days. More recently, Courtenay *et al.* (2006) detected DNA of *M. bovis* using a species-specific PCR, in soil at badger setts and in faeces at badger latrines, on cattle farms in bTB endemic areas of the UK. The authors reported that the probability of detecting *M. bovis* at the main sett of badger social groups was related to the prevalence of excretion by resident infected badgers.

1.5.5 Understanding how cattle to cattle transmission occurs in the field

The fact that in most cases where a whole herd test results in a HBD involves the disclosure of only one or a few skin positive animals makes it hard to understand how cattle to cattle transmission may occur in field conditions. Innate immunity, host defence, latency and reactivation, could play an important role in understanding why some animals do not become infected and why so few in a herd react positively to the skin test.

Morrison *et al.* (2000) suggested that only a small number of animals may be exposed, and that the transmission rate between cattle is low. An alternative view is that even if a large proportion of animals were exposed to infection, there may exist an innate response sufficient to eliminate the infection before antigen levels produce a reaction due to sensitisation by tuberculin, causing few animals to react to the skin test (Lefford, 1971).

M. bovis has been isolated from skin-test negative animals without gross lesions and also from some animals which had shown a positive response to the gamma-interferon test (Neill *et al.*, 1994). In human tuberculosis, the reactivation of latent infection, as in cases of immuno-suppression, has been reported to play a very important role in the dissemination of the disease. In the case of bTB, as suggested by Francis (1947), a similar explanation could be given by the possibility of lesion latency and the reactivation of these.

Based on the evidence available, animals without visible lesions are not necessarily non-infected (Barlow *et al.*, 1998; Palmer and Waters, 2006) and it has been suggested that all tuberculous cattle should be considered potential excretors (Neill *et al.*, 1991; Morris *et al.*, 1994) and potential sources of infection to other cattle (Menzies and Neill, 2000; Cassidy, 2006).

1.6 Diagnosis and control of bovine tuberculosis

1.6.1 General overview

In order to control the spread of bovine tuberculosis between countries and to allow free international movement, countries should aim to obtain disease free status. In Europe, cattle herds are tested using the single intradermal comparative cervical tuberculin (SICCT) test and animals that react positively to the test are slaughtered, based on the standard interpretation established by the European Legislation (Directive 64/432/EEC). Under the same Directive, a herd obtains the tuberculosis free status if all animals over six weeks of age do not show any clinical signs of the disease; if all have reacted negatively to two intradermal tuberculin tests carried out at six month intervals and if animals that have been introduced into the herd are from officially tuberculosis-free herds.

Despite the current control measures, and whilst the disease has been eradicated in countries where consistently applied, eradication has proven to be difficult in others. The presence of a wildlife reservoir and the low sensitivity of the test may play an important role in the eradication process (Clifton-Hadley *et al.*, 1995). These are the main reasons why further research has been focused on the improvement of current diagnostic techniques and the development of different methods for diagnosis and control (i.e. the gamma-interferon test and vaccination research in both cattle and wildlife).

1.6.2 The tuberculin skin test

The internationally accepted standard method for detection of bTB is the tuberculin skin test, widely used in Europe and other countries in the world. It is not surprising that the current diagnostic tests are based on the measurement of T-cells,

as the cellular mediated immune response is the primary immune response to infection with *M. bovis* (Wood and Rothel, 1994; Neill *et al.*, 2005). The skin test measures a delayed hypersensitivity reaction mediated by sensitised T-cells after the intradermal injection with tuberculin (Monaghan *et al.*, 1994). Hypersensitivity to tuberculin usually occurs in cattle between three and six weeks after infection with tubercle bacilli (Francis, 1947). Therefore it is likely that recently infected animals do not react to the tuberculin test. However, the test detects sub-clinical infection (de la Rua-Domenech *et al.*, 2006b).

There are two versions of the skin test: the single intradermal test (SIT) usually performed at the caudal fold of the base of the tail, but also on the side of the neck, and the single comparative cervical intradermal test (SICCT) on the side of the neck. The first uses tuberculin from *M. bovis* and the second uses tuberculin from *M. bovis* and from *M. avium*. Different sensitivity and specificity results have been reported by different authors, but a sensitivity of the SICCT test of approximately 74% to 95% and a specificity of at least 99% (Monaghan *et al.*, 1994; Costello *et al.*, 1997) and a sensitivity of 83.9% with a specificity of between 75.5% and 99% for the SIT, could be considered as references after the evaluation of various studies (de la Rua-Domenech *et al.*, 2006b). The skin of the neck (used in the cervical test) has been reported to be more sensitive than the skin of the base of the tail (used in the caudal fold site) (Francis *et al.*, 1978; Monaghan *et al.*, 1994).

Whether to use the single or comparative test depends on the prevalence of the disease and the evidence of interaction with other environmental mycobacteria (Monaghan *et al.*, 1994). Since no comparison with the *M. avium* tuberculin is used in the SIT, this can result in the disclosure of more false positives compared to the SICCT. In countries such as USA, New Zealand and Spain the SIT has been used, whilst the SICCT test has been used in the Republic of Ireland, Northern Ireland and the UK.

1.6.3 Current measures in the UK: the SICCT and abattoir surveillance

The SICCT is carried out with two purposes: surveillance testing (routine tests) and disease control, which includes tests on neighbouring herds (contiguous tests) or tracing tests (Mitchell *et al.*, 2006).

The frequency of testing depends on the annual incidence of confirmed herd breakdowns in the area over the previous six years. All herds should be tested every twelve months unless the annual incidence is less than or equal to 1%, every two years if less than 1%, every three years if less than or 0.2 % or every four years if less than 0.1% (DEFRA, 2005a). Increasing the frequency with which herds are tested will help to remove infected cattle, reducing the risk of transmission within the herd as well as the transmission through purchase (Medley, 2003).

Both the SIT and SICCT are designed to detect infection at the herd level (Norby *et al.*, 2004). In the UK, the 60 days after the disclosure of reactors in a herd and the triggered number of tests after the disclosure of any reactors, is believed to increase the sensitivity of the test and detection rate is increased by surveillance at post-mortem inspection in the abattoir (de la Rua-Domenech *et al.*, 2006b).

The procedure and interpretation of the test - The SICCT is carried out in the middle third of the neck with each injection 13 cm apart. The hair of the injection site is clipped and tuberculin is injected intradermally: 0.1ml of avian tuberculin in the upper site and 0.1ml of bovine tuberculin in the lower site. The skin reactions are read 72 hours after the injection by measuring with callipers a fold of skin at both sites. Measurements are recorded. If the bovine reaction is 4 mm greater than the avian reaction, the animal is considered to be infected with bTB and it is known as a positive or reactor. If the bovine reaction is between 1 and 4 mm greater, the animal is termed an inconclusive reactor (IR) and will be re-tested in 60 days, and it is negative or clear if the increase in the skin thickness on the bovine site is less than or equal to the increase in the skin reaction at the avian site of injection. This is what is called "standard interpretation". Inconclusive reactors will be also subjected, if in one or two year testing parish, to the gamma interferon blood test straight after the animal has been found as inconclusive reactor.

If reactors are found with lesions at post-mortem inspection or *M. bovis* is isolated by culture, then the cut-off of the values classed as reactors are lowered. This is called "severe interpretation". Severe interpretation is also used in the follow up tests carried out at 60 days intervals prior to the lifting of movement restrictions (DEFRA, 2005b).

Actions adopted based on the test results - If there is at least one reactor in the herd, movement restrictions on the farm are applied. If there is confirmation by visual lesions at post-mortem inspection or if *M. bovis* is isolated by culture, this triggers other tests on contiguous herds and contact tracings, and a test 60 days later (short interval test) is carried out under severe interpretation (using a lower cut off for the skin reading to increase the sensitivity of the test). A second test 60 days later is repeated, and if negative, restrictions are lifted. If it is positive, or with inconclusive reactors, the sequence of tests follows until the herd is clear. If there is no confirmation by lesions or culture of the first reactor found, a test at 60 days later is carried out and movement restrictions lifted if this is clear or repeated until clear. The same tests at 60 days intervals are carried out if at least one inconclusive reactor is found after the first test (DEFRA, 2005a). The 60 day test interval is used because the injection with tuberculin may give a suppressive response for a period of between 4 and 60 days after injection (Lepper *et al.*, 1977). Reactor cattle are sent for slaughter and in the meantime, farmers are advised that they should be separated from the rest of the herd. Post-mortem examination for all cattle takes place at the abattoir.

Test codes (test types) and VetNet database - Thirty three different test codes described by the State Veterinary Service (DEFRA, 2005b)(Table 1.2), are defined based on the time since the last test, on whether it is a routine or strategic test and whether it is carried out on the herd or on individual animals. The date of the test, the test type and the herd identification (county holding parish number or CPH) are recorded in the recorded on DEFRA's disease national database (VetNet). The individual animal ear tag number is recorded only if the animal is reactor (R), inconclusive reactor (IR), identified with lesions at slaughter (SL) or slaughtered as dangerous contact (DC) or as contact tracing (CT). Individual records for cattle that

test negative are not recorded. Table 1.2 presents the test types and codes described by the State Veterinary Service (2005), fifteen of which were used in this thesis.

The abattoir post-mortem surveillance inspection - The disease is usually detected using the skin test but it can also be detected by routine post-mortem inspection at the abattoir. The aim of the post-mortem examination and bacteriological culture is to assess the severity of the disease, to determine the number of follow-up tests and to support epidemiological evidence by genotyping (de la Rua-Domenech *et al.*, 2006b). In 2004, out of 1,702 confirmed new HBD, 11.4% were first disclosed by post-mortem inspection (Mitchell *et al.*, 2006).

Table 1.2 - Tuberculin test types and VetNet codes: What to use and when

Type of test	VetNet code	When and why	What to test
Herds under bTB restrictions			
Gamma interferon	VE-IFN	A whole blood-based test for bTB used to supplement the SICCT	All bovines over 6 m.o.
90 Day test	VE-90D	Every 90 days only on special premises for fattening/finishing purposes under bTB restrictions	All bovines
Short interval	VE-SI	Sixty days after removal (or effective isolation) of the last reactor or following confirmation of bTB whilst herd under restrictions	All bovines
Herds not under bTB restrictions			
Check test	VE-CT	- Outside the normal testing frequency for the herd, to determine its disease status - When there is suspicion of infection. Following for instance: - voluntary slaughter of an IR identified in an IR-only herd - identification of a clinical case of bTB - disclosure of lesions suggestive of bTB at a slaugterhouse, knacker yard, hunt kennel, VLA regional lab, etc - Back tracing of confirmed reactors found in another herd - Identification of reactors at a routine herd test that included adults only - Identification of culture-positive wild deer within a 3Km radius, in non-annually tested parishes	All bovines except calves under 6 weeks of age, but include calves under 6 weeks where: - there is suspicion that calves could be involved (e.g. tuberculous mastitis) - the VE-CT is due to the identification of reactors - at a VE-RHT that did not include all bovines in the premises
1st Potential hospot check test	VE-CT-HS1	If a holding is within a 3Km radius of an incident which triggers a potential hotspot area	All bovines except calves under 6 weeks of age
Check test following a Badger RTA*	VE-CT-RTA	Outside the normal test frequency for the herd, to determine its disease status following a positive Road Traffic Accident Badger	All bovines except calves under 6 weeks of age
New herd check test	VE-CT-NH1	When a new herd has been identified in a non-annually tested parish, at least 2 months after the introduction of the last animal, but no later than 6 months after the introduction of the first animal	All bovines except calves under 6 weeks of age
Re-formed herd Check test	VE-CT-RH1	When a reformed herd is identified in a non-annually tested parish at least 2 months after the introduction of the last animal, But no later than 6 months after the introduction of the first animal	All bovines except calves under 6 weeks of age

Table 1.2 (cont)

Six-month test	VE-6M	Six months after restrictions have been lifted following a clear short interval test	All bovines except calves under 6 weeks of age
Twelve months test	VE-12M	Twelve months after VE-6M if that test was clear	All bovines except calves under 6 weeks of age
2nd Potential hotspot check test	VE-CT-HS2	twelve months after the VE-CT-HS1 test	All bovines except calves under 6 weeks of age
Contiguous herd test	VE-CON	On herds contiguous to a confirmed bTB incident outside their regular test frequency	All bovines
Contiguous herd test (6M)	VE-CON6	At the discretion of the Divisional Vet Manager (DVM) 6 months after a VE-CON	All bovines except calves under 6 weeks of age
Contiguous herd test (12M)	VE-CON12	Twelve months after a VE-CON or VE-CON6 (if the latter has been carried out)	All bovines except calves under 6 weeks of age
2nd New herd check test	VE-CT-NH2	Twelve months after a VE-CT-NH1	All bovines except calves under 6 weeks of age
2nd Reform herd check test	VE-CT-RH2	Twelve months after a VE-CT-RH1 test	All bovines except calves under 6 weeks of age
3rd New herd check test	VE-CT-NH3	Twenty four months after a VE-CT-NH1 test	All bovines except calves under 6 weeks of age
3rd Reformed herd check test	VE-CT-RH3	Twenty four months after a VE-CT-RH1 test	All bovines except calves under 6 weeks of age
Whole herd test	VE-WHT	Routinely every 12 months in annual testing parishes and in individual herds requiring annual testing, e.g. producer-retailer, dairy herds, bull hirers, heifer rearers, city/open farms, AI centres, etc	All bovines except calves under 6 weeks of age
Second whole herd test	VE-WHT2	Routinely every 24 months in biennial testing parishes at the DVM discretion for epidemiological reasons	All bovines except calves under 6 weeks of age
Routine herd test	VE-RHT	In parishes with a 24, 36 or 48 month testing interval	All bovines except calves under 6 weeks of age Breeding bulls(i.e. entire males over 12 m.o., unless exempted by the DVM) Females which have calved Young bovines which could be used for breeding and purchased since the last herd test (except calves under 6 w.o.)

Table 1.2 (cont)

Tests on individual animals			
Inconclusive reactor retest	VE-IR	On inconclusive reactors identified at an earlier test. To be conducted at least 60 days later. Currently, up to two consecutive retests of the same IR animal are permitted	Individual animals
Traced bovine test	VE-TR	On bovines that have moved from a confirmed incident herd prior to service of restrictions	Individual animals
Slaughterhouse case	VE-SLH	Pseudo-test type recorded once infection in a suspect slaughterhouse case has been confirmed by bacteriological culture	N/A
Query slaughterhouse case	VE-QSLH	Pseudo-code that cannot be used as a Mark Forward code, as it is simply to record a suspicion of disease on the VetNet System	N/A
Export test	VE-EX	On cattle exported from Great Britain	Individual animals
Post-import (Irish) test	VE-PII	On cattle imported from Northern Ireland and the Republic of Ireland 60 days after arrival	Individual animals
Post-import (other) test	VE-PIO	As prescribed by the conditions of the import of license	Individual animals
Test for artificial insemination purposes	VE-AI	On bulls, teasers and embryo donors prior to admission to an artificial insemination centre	Individual animals
Private test	VE-PRI	A test commissioned and paid by the owner and carried out with the DVM agreement	Individual animals
Pre-movement test	VE-PRMT	For a pre-movement test to be carried out in England, Scotland and Wales, 60 days or less prior to movement of an animal(s) form a one or two yearly tested herd	Individual animals
Post-movement test	VE-POSTMT	For a post-movement test to be carried out in England and Wales in circumstances where cattle have been moved into a holding without a required pre-movement test	Individual animals
Pre-movement test	VE-PRMTS	In Scotland where a pre-movement test has not been completed. This test should be undertaken as soon as possible after a movement has occurred from a one or two yearly parish	Individual animals

Source: State Veterinary Service, 2005. Veterinary Instructions, Procedures and Emergency Routines ("VIPER"), Chapter 23, Section G, Defra correspondence.

1.6.4 Discussion of the SICCT test and development of other diagnostic methods

Due to the low sensitivity of the test and the difficulty to control the disease in some countries, other diagnostic techniques to be used in conjunction with the skin test are being developed and are used in various countries. The most recent technique, an *in vitro* blood test, known as the gamma-interferon test, was first used in Australia in the 1980s in conjunction with the SIT using the caudal fold site (Wood *et al.*, 1991). The basis of this test is the same as that for the skin test: the detection of the cellular immune response after stimulation with *M. bovis* and *M. avium,* but the gamma-interferon test uses blood samples which are incubated at 37°C within 28 hours of collection in the presence of the *M. bovis* and *M. avium* antigens for a period of 16-24 hours. The production of gamma-interferon (a cytokine produced by the T-cells) is measured by using a sandwich enzyme-linked immunosorbent assay (ELISA).

The advantage of the gamma-interferon test is that whilst it is only as sensitive as the skin test, it detects infection at earlier stage (one to four weeks post-infection). The skin test remains the international standard test, but used in conjunction with the gamma-interferon test may increase the sensitivity of the skin test. After a trial in the North of Spain, Gonzalez-Llamazares *et al.*(1999) reported that the highest sensitivity (92.9%) was obtained after using the gamma-interferon test and SIT together, compared to that obtained with the SIT only (80.2%) or the gamma-interferon only (84.9%). In Britain, the gamma-interferon test may be used in some circumstances to test inconclusive reactors 60 days after the first retest, and if the herd test is in a one or two year testing area. Other studies carried out in Australia, Brazil, Ireland, Northern Ireland and Italy have reported higher sensitivity of the gamma-interferon compared to the skin test, but the specificity in generally lower than that obtained by the skin test (Neill and Pollock, 2000). Most recently, the ISG has suggested in its Final Report (DEFRA, 2007) that the gamma-interferon when used in parallel with the SICCT test in heavily infected areas, 27% more animals than were identified by the skin test, were confirmed as infected.

Given the economic implications of the removal of too many animals (some not infected given the low specificity), the use of the gamma-interferon only, cannot substitute the present skin test. To increase the specificity of the skin test, work is being carried out towards the use of more specific antigens to produce tuberculin. These more specific antigens would substitute those used in the SICCT test, produced from *M. bovis* (AN5 or Vallee strains) and avian produced from *M. avium* (D4ER or TB56 strains) (Monaghan *et al.*, 1994). For example, genes for the T-cells antigens ESAT-6 and CFP-10 are not present in many environmental, non-tuberculous bacteria, nor in the vaccine strain *M.bovis*-BCG, however, these are present in some other non-tuberculous mycobacteria such as *M. avium* subsp. *paratubeculosis* (causing Johne's disease) (Palmer and Waters, 2006).

Anergic response (or failure to react) from some cattle to the tuberculin injection is also of concern (Pritchard, 1988; Monaghan *et al.*, 1994). Although the reason for this has not fully been elucidated, it could be related to an advanced stage of the disease, generalised disease or pregnancy (Lepper *et al.*, 1977). Other reasons could be desensitisation due to a previous test, or poor application of the test (i.e. poor facilities and/or standards of the personnel carrying out the test) (Moda, 2006).

Other methods, such as ELISA techniques, could be used to screen the disease at a herd level or to detect the disease at a more advanced stage (De Kantor and Ritacco, 2006). The aim of diagnostic technique development is to obtain a sensitive and specific test that is able to detect cattle that have been exposed to *M. bovis*, but have not developed disease; identify cattle that pose a risk of spreading disease and distinguish vaccinated cattle from those infected with *M. bovis* (Neill *et al.*, 2005).

1.7 Risk factors of cattle to cattle transmission

1.7.1 General overview

The dynamics of the transmission of *M. bovis* and the conditions under which cattle become infectious to other cattle are not very well understood, although environmental contamination seems to be a less effective method (Menzies and Neill, 2000). From experimental and epidemiological studies carried out in the past, there is evidence that cattle to cattle transmission does occur (Pollock *et al.* 2006).

On the other hand, it has been claimed that cattle to cattle transmission would not be enough to maintain the disease in the absence of a wildlife reservoir, providing there is a system in place to remove infected animals. Therefore, regular testing of cattle herds and removal of infected and potentially infectious animals are critical in reducing the spread of the disease from cattle to cattle.

The challenge of an epidemiological investigation such as the one presented in this thesis is to elucidate the risk that can be attributed to cattle in a geographical location where wildlife has long been recognised as a reservoir of *M. bovis*, such as the South West of England.

The risk of transmission of *M. bovis* between cattle can be investigated at a herd, individual animal and animal group level. The three levels were investigated in this thesis and as a background, risk factors identified in previous studies associated with the introduction and persistence of *M. bovis* in the herds are presented next.

1.7.2 Farm management and practices risk factors: Aim of this thesis

Most field investigations and case-control studies carried out in the past that investigated risk factors associated with bTB, have been carried out at a herd level in the UK, Ireland, Northern Ireland, New Zealand and the USA.

Purchase of infected cattle and the presence of infected cattle on neighbouring farms have been identified as risk factors (Table 1.3). McIlroy *et al.*(1986) stated that cattle were the major source of bTB infection in Northern Ireland, with infection being introduced from purchasing infected cattle (approx. 30%) and from spread from an infected contiguous herd (40%).

Table 1.3 below shows the results of some of the field investigation studies in which cattle have been identified as the source of bTB.

Table 1.3 - Results from five field investigation studies on bovine tuberculosis from 1983 to 1992

REFERENCE	DESCRIPTION OF INVESTIGATION	PRINCIPAL RESULTS
Wilesmith, 1983	HBD in Great Britain	○ Source of infection was ascribed to 59.5% of breakdowns: 42% purchased cattle (mainly Irish cattle), 51% infected badgers and 6% contiguous spread.
Wilesmith *et al.*, 1986	HBD in South West of England	○ Most cases were attributed to badgers, 10% to purchased cattle and 0.6% to contiguous spread
Griffin *et al.*, 1992	Approximately 4,000 HBD from ten regions in the Republic of Ireland	○ 25% lateral spread, 14% residual infection, 14% wildlife, 11% purchased of cattle and 35% undetermined origin
Schoenbaum *et al.*, 1992	Epidemic in Oklahoma, USA. No cases of bTB had been confirmed. ○ 59 cows and 37 calves were dispersed from a herd at end of 1988. In Aug 1989 one of those animals showed generalised bTB lesions. 52.5% were located and slaughtered and 39% showed bTB lesions. The 13 herds that became in contact with these 12 cows were depopulated. A further 1,969 cattle were traced to 74 additional herds and all were slaughtered.	○ 5 of the in-contact animals were positive on skin test and 7 were found with tuberculous lesions. Giving a total of 12 animals (0.3%) amongst the exposed animals. The low prevalence was attributed to the extensive rearing system used and the relatively short contact time for many of them

Six case-control studies are summarised in Table 1.4 and individual animal and herd risk factors are discussed below.

Individual animal factors - There is no conclusive evidence that sex, age and reproductive stage of the host have a direct influence in the transmission of bTB (Morris *et al.*, 1994). The fact that most reactor animals are found within an older age group, indicates that older cattle have been on the farm for a longer period of time and therefore potentially more exposed to infection, as already suggested by Francis (1947). However, other factors, such as the immune status of individual animals compromised by the presence of other infections, and the nutritional status may affect the susceptibility to infection with *M. bovis*.

Interaction with other infections - Previous or current contacts with other infectious organisms may affect the resistance of cattle to *M. bovis* (Pollock and Neill, 2002). Exposure to environmental mycobacteria could provide a degree of "acquired" protection from tuberculosis through the induction of immune responses to common antigens. On the other hand, infections with viruses are known to have immunosuppressive effects, for example, it was reported that viral infection was associated with rapid progression of tuberculosis in calves (Pollock and Neill, 2002).

Nutritional status - Although mineral deficiency (Griffin *et al.*, 1993) and feeding maize silage (Lanszki *et al.,* 1999: reviewed by Goodchild and Clifton-Hadley, 2001) have been identified as risk factors in previous studies, there is not much information available on the effects of nutritional factors on the susceptibility of cattle to bTB. Metabolic effects were investigated in an experimental study with Albino mice; it was observed that resistance to tuberculosis could be consistently and markedly decreased by adding sodium citrate, known to favour the multiplication of tubercle bacilli *in vitro,* to a variety of diets (Dubos, 1955). Specific nutritional factors, such as zinc and vitamin D have been shown to have important roles in the resistance to tuberculosis in guinea pigs. This effect is considered to be mediated through macrophages, but it is not clear how this can be extrapolated to bTB (Pollock and Neill, 2002). Susceptibility to infection was not observed to be altered in cattle supplied with a restricted diet compared to those fed *ad libitum* during an experiment where cattle were housed in close confinement (Costello *et al.*, 1998).

Herd size and purpose of herd (herd type) - Herd size has been reported previously to be a risk factor for HBD and it has been suggested to be a proxy for an intensive farming system (Pfeiffer and Morris, 1991; Griffin *et al.*, 1996; Goodchild and Clifton-Hadley, 2001) whilst Marangon *et al.*(1998) did not find herd size to be a risk factor**.** Dairy herds are more susceptible to bTB than beef herds, and this could be related to higher levels of stress in dairy herds (Morris *et al.*, 1994 ; Goodchild and Clifton-Hadley, 2001). Dairy cattle remain on farms for longer periods of time compared to beef cattle therefore the exposure to infection is potentially greater. The presence of both beef and dairy cattle in the herd has been associated with an increased risk (Marangon *et al.*, 1998) but found to be a protective by Reilly and Courtenay (2007).

Farm premises - The transmission of the disease between cattle is more likely to occur in conditions of close confinement such as indoors, however, this is difficult to observe in natural farm conditions (Costello *et al.*, 1998). Cattle are more likely to become infected from other cattle when housed in buildings with poor ventilation (Smith, 1905; cited by Phillips *et al.*, 2003) and when the cattle density is high (Neill *et al.*, 1989).

Cattle grazing - Environmental contamination with *M. bovis* has been reported to play an important role in the transmission from badger to cattle. Despite studies reporting contamination of fields where cattle graze, the mechanism of transmission is not very well understood. Cattle could get infected from aerosols created through exhalation if they investigate latrines. It also seems intuitive that ingestion would be the most common route of infection from this source, however lesions in the digestive tract are much less common compared to those in the respiratory tract (Menzies and Neill, 2000).

The production of slurry compared to the production of manure has been associated with a risk of HBD (Griffin *et al.*, 1993). Even if the presence of *M. bovis* excreted in faeces is a less important source of spread, this potentially poses a risk for the maintenance of infection on the farm, especially if spread on grazing fields after being stored in favourable conditions. Recently, Reilly and Courtenay (2007) reported an increased risk for transient HBD on farm that stored manure for more than six months.

In this thesis, the results from the investigation of risk factors associated with the risk of a first HBD in the study farms are presented in Chapter 4.

Table 1.4 - Summary of characteristics of case-control studies from 1991 to 2005 in New Zealand, Ireland, Italy, Northern Ireland and South West of England

REFERENCE	Pfeiffer *et al.*,1991	Griffin *et al.*,1993	Griffin *et al.*,1996	Marangon *et al.*, 1998	Denny *et al.*, 1999	Johnston *et al.*, 2005
Type	Case – control	Case - control	Case - control	Case – control	Case – control	Case - control
Study sample	285 farms 95 cases, 95 random, 95 matched controls	160 farms, 80 cases, 80 controls dairy, herd size and geographical proximity	392 farms, 196 cases, 196 controls	101 farms, 27 cases, 74 controls	427 farms, 215 cases, 212 controls, dairy, >30 cattle/herd	268 farms 151 cases, 117 controls
Definition case	Herd previously free of bTB and under movement restrictions in period 1986-1989	Herd had two breakdowns between 1986- 1990 or continuous breakdowns during last 12 months (in 1990)	196 cases were reactors at TB test in 1998 in a herd that was previously clear	27 breeding herds (1992-95): 3 (1992), 10(1993) 9(1994), 5(1995)	Dairy herd TB positive in 1991-1992, with one or more reactors in homebred, not in purchased animals	HBD in herds prior to FMD 2001in three triplets of the RBCT
Definition control	Random- any enterprise chosen at random in same county Matched- same type enterprise as case and in the vicinity	Herds free of TB since 1982 and dairy was main enterprise	From 2 sources: 27 reactor herds and 171 from clear herds	Herds free of TB for last 3 years- selected at 2 stage sampling	Dairy herd with no reactors between 1980- 1992	Herds with no HBD 12 months prior to HBD in the case farm and within same RBCT triplets as case
Area Location	Waikato area in central North Island, New Zealand	Counties Cork and Kilkenny, Republic of Ireland	Badger removal area since 1989, East Offaly Area, Republic of Ireland	Veneto Region, Italy (good description of geography and farming practices)	Northern Ireland	South West of England within RBCT (proactive treatment)
Aims	Identify risk factors which are associated with the establishment of infection in herds	Provide information on role of farm management practices, environmental factors and farm characteristics	Investigation association between occurrence of TB in herds in East Offaly area and poss. risk factors. Plus expand work done by Dolan et al., 1995 in area prior to badger removal programme	Investigate of role of some husbandry practices and farm characteristics	Identify risk factors related to farm boundaries, neighbouring herds and wildlife	Identify risk factors associated with risk of HBD within the RBCT

Table 1.4 (Cont)

STUDY	Pfeiffer *et al.*,1991	Griffin *et al.*,1993	Griffin *et al.*,1996	Marangon *et al.*, 1998	Denny *et al.*, 1999	Johnston *et al.*, 2005
Data Collection	Questionnaire Period between 1/12/88 and 30/5/90	Questionnaire August - October 1990	Data available form herd files from District Veterinary Office	24 cases-by Local Health Unit 3 cases and controls-by Reg. Epid. Unit. Not time specified.	December 1993 and January 1995 Variables referred to 1990-1992 unless stated	Questionnaire data (TB99) carried out since 1999
Analysis	Univariate analysis- logistic regression screen variables assoc. with outcome $p<0.10$.	Unadjusted OR to test for stat. sign. By a Chi-square test for independence. Using McNemar test for paired data. Means and SD for non categorical variables were tested for stat. sign. by a paired t-test.	Unadjusted OR for categorical factors and evaluated with Chi-square test for independence.	Chi-squared for categorical variables t-test for continuous variables.	Wilcoxon two-sample test was used or analysis of variance as appropriate. OR and Mantel-Haenszel weighted OR were calculated.	Univariable analysis by logistic regression. Screen variables with $p\leq0.15$.
Analysis	Multivariable stepwise logistic regression. Conditional logistic regression used for matched controls. Path analysis to describe causal web of relationships between factors and the outcome variable.	Multivariable modelling – factors associated with outcome, $p<0.15$. Conditional logistic regression used for matched controls.	Multivariable Analysis- Factors associated with the outcome $p<0.10$ were chosen using unconditional logistic regression.	Multivariable screen variables with $p<0.15$, using Unconditional multiple logistic regression. Two models: one with simultaneous entry of all variables, one with backward stepwise with LRT with $p<0.05$.	Multivariable Analysis was done by unconditional logistic regression. Standard stepwise procedures were done using all variables. This did not change the estimated OR.	Multivariable backward elimination considering significance of variables in model and stability if variables removed.

Table 1.4 (Cont)

STUDY	Pfeiffer *et al.*,1991	Griffin *et al.*,1993	Griffin *et al.*,1996	Marangon *et al.*, 1998	Denny *et al.*, 1999	Johnston *et al.*, 2005
Results reported, Not associated with risk of bTB	-Farming experience -Interviewee's relation to enterprise -Water source for livestock -Amount contact with neighbouring farms	-Contact with neighbouring cattle -Poor boundary fencing -Presence substandard cattle housing -Movement of. equipment. or vehicles onto farms -Exposure to water from rivers or streams	-Presence of residual source of infection in a herd- little evidence -Purchase of animals -Badger proximity	-Herd size -Housing system -Summer mountain pasture -Possible contact with wild animals -Indirect contact with other animals	-	-
Results reported associated with risk of bTB	- Cases *vs* controls: Farm size , perseverance, % young stock in total purchased animals, purchased from >3 diff. herds *vs* no purchases - Cases *vs* random controls:% young stock in herd, Controls and random: purchase from >3 herds, % young stock in total purchase,% young stock in herd	Sta. sign. Assoc. ($p<0.05$): -Use of mineral licks -Purchase of a bull -Presence of cubicle housing -Rough grazing areas -Badgers on the farm -Lesser use of hay -Production slurry *vs* manure	At herd level: $P<0.05$, -Total animals in herd -Restriction of one or more contiguous herds within a period of 6 months prior or following the herd under study Animal level: -Cows, heifers and bullocks were more likely to fail	Presence of both dairy and beef animals (OR=4.92, p=0.001) -Introduction of at least one bovid since the last SICTT (OR=5.79,p=0.003)	-Badger setts or carcases on home-farm or out-farm - Home-farm or out –farm contiguous to cattle herds where TB had been confirmed in test reactors.	- Movement of cattle on to farm from markets - Use of either covered yard or other "type" of housing - Spreading artificial fertilizers or farm yard manure.

1.7.3 The role of cattle movement in the spreading of *M.bovis* and within-herd transmission: Aims of this thesis

In many countries, including Great Britain, thousands of cattle movements occur between farms, markets and abattoirs each year. Cattle movements contribute to the spread of infectious diseases. The results of several studies from different countries have highlighted the importance of purchasing cattle infected with *M. bovis* to the introduction of bTB into a herd (Table 1.3 and Table 1.4). The movement of cattle, especially from locations where bTB is present and particularly to locations outside endemic core areas, is a critical factor for the spread of the disease (Gilbert *et al.*, 2005; Green and Cornell, 2005; Carrique-Mas *et al.*, 2007).

A perturbation in the movement of cattle occurred in the UK after the FMD outbreak in 2001 (Gibbens *et al.*, 2001) when depopulated farms were repopulated using varied purchasing practices. In a recent study on the risk of HBD post-FMD in GB, depopulated cattle farms that repopulated with cattle from farms in areas with a high bTB testing frequency, were more likely to break down with bTB than those where cattle were sourced from farms with a low testing frequency, indicating that infected cattle were being purchased (Gopal *et al.*, 2006; Carrique-Mas, 2007).

Cattle movement plays an important role in determining the frequency of testing for individual animals as movements can result in cattle missing tests or being tested more frequently. In a recent study by Mitchell *et al.* (2006) the authors reported that approximately 80% of cattle are never tested. They suggested that if untested, and purchased from infected herds, they could be a reservoir of infection that allows *M. bovis* to persist in the GB cattle population.

Some examples of the importance that animal movement plays in the control of livestock diseases are the strict import/export regulations, the extensive movement restrictions imposed during the 2001 FMD outbreak in GB and, in the case of bTB, the tracing procedures after a new case has been confirmed (Gilbert *et al.*, 2005). In England, pre-movement testing was introduced in March 2006, which requires that all cattle over 15 months old moving from a one or two yearly tested herd must have had a negative bTB test within 60 days prior to movement unless the herd or

movement is exempt. The pre-movement testing was extended to cattle over 42 days of age in March 2007. In Scotland, pre-movement and post-movement (between 60 and 120 days after arrival to the herd) testing was introduced in September 2005. A routine surveillance herd test can count as a pre- or post- movement test as long as it is carried out within the sixty days prior to movement. Farmers can also ask for the whole herd to be tested if the test is to be used as a pre- or post-movement test (DEFRA).

The British Cattle Movement Service (BCMS) is part of the Rural Payments Agency (RPA), an executive agency of Defra. The BCMS was established to manage the Cattle Tracing System (CTS) and they were both created with the aim of ensuring the identification and traceability of individual cattle after the BSE crisis. All cattle born in or imported into Great Britain since 1st July 1996 had to have a cattle passport. The CTS has been in operation since 1998 but it has been a legal obligation since January 2001. It is a legal European requirement that cattle owners keep records of the birth, movements on and off the farms, ear-tag, date of birth, sex, breed and dam identity and the CPH of the source and destination for each animal (DEFRA).

The availability of a database system which holds records of the animals' movements and characteristics of their farm location at different times in their lives is extremely beneficial for epidemiological investigations. In this thesis, the risk of a bovine animal becoming or not a reactor at a herd test using the SICCT was investigated and records from the BCMS and results are presented in Chapter 5.

The role of cattle in the transmission of bTB within cattle herds is not very well understood. A mathematical model showed that within-herd transmission was unlikely to maintain a long-term infection when the caudal fold skin test was used, and external infection and anergic cattle were absent (Barlow *et al.*, 1998). From a Canadian study, the incidence within a dairy herd was 2 to 9.8 cases per 100 cow years for dairy and 2.9 to 20.4 per 100 cow years for beef (Munroe *et al.*, 1999). Perez *et al.* (2002) reported that in the absence of control measures, one infectious animal infects 2.2 cattle per year on an average Argentinean dairy farm, and De Kantor and Ritacco (2006) considered that this low transmission rate was consistent

with the dynamics of a chronic disease with a mean incubation period of 24 months in semi-extensive farming conditions.

The results from the investigation of animal group within-herds are presented in Chapter 6.

1.8 Statistical methods used in this thesis

1.8.1 Survival analysis

Survival analysis is a type of statistical method in which the study unit is followed for a period of time and the outcome (time until the event occurs) is measured based on that time. Using this method, not only the outcome can be assessed based on whether it happens or not, but also when it happens. It is appropriate in longitudinal studies as study units are observed for long periods of time and it has been used in different fields of research.

When the period of the study is long, there may be some problems such that the study unit does not experience the event before the study ends, it is lost to follow-up or it withdraws from the study. In these cases the study unit is censored, in most cases, right-censored (Kleinbaum, 1996). That is, a censor value (of zero) is given to the study unit when the outcome has not been observed in the study period and once it is already in the study compared to those they are left censored (at the beginning of the study) or in the middle of it (truncated).

One of the requirements in survival analysis is that the study unit is free of disease at the beginning of the study (Kleinbaum, 1996). The assumptions of the Cox proportional hazards regression model requires an identifiable start point and an end point; a common end point; that lost to follow up cases are unrelated to the outcome, and that hazards are constant over time (they must be time independent) (Tibshirani, 1982).

In this thesis, non-parametric Kaplan-Meier estimator of the survivor function and semi-parametric hazard function using Cox proportional hazards regression were used.

The **survival function** ($S(t) = p(T \geq t)$) describes the probability that an individual (or group of individuals), given a lifetime of T, will survive beyond time t. It starts at one and drops to zero. The long-rank test (a large sample chi-square test) is the most commonly used test to assess the statistical significance of differences in the survival curves for two or more groups. It makes use of observed *vs* expected counts over the categories of outcomes, and these categories are defined by each of the ordered failure times (Kleinbaum, 1996). The Kaplan-Meier survival curve provides some initial insight into the possible effect of different variables.

The **hazard function** is the probability of an event occurring at time t given that it had not occurred up to time t. The Cox proportional hazards model is the most commonly used form of multivariable analysis for survival data, also known as the Cox regression model, introduced by D.R.Cox in 1972 (Cox, 1972). It is based on the assumption that the hazard for an individual (or group of individuals) is a product of a baseline hazard (h_0) and an exponential function of a series of explanatory variables: $h(t) = h_0(t)e^{\beta X}$ or equivalently $HR = h(t) / h_0(t) = e^{\beta X}$ where HR is the hazard ratio. Hazard ratios have similar interpretation to odds ratios and risk ratios. They represent the effect of a unit change in the predictor on the frequency of the outcome (which in this case is measured as a hazard). The model assumes that the hazard does not change with time over the period of the study. The likelihood ratio test is used in the Cox proportional hazard model. Deviance residuals used to observe outliers in the data, Schoenfeld residuals test to assess the proportional assumption (the hazards are constant over time) and Cox-Snell residuals to check the model fit, are methods used in survival analysis and were used in this thesis. Cox-Snell residuals are the estimated cumulative hazard for each individual (or observation unit) at its failure (or censoring) time. If the model fits well, these residuals follow a unit exponential distribution, as they are a censored sample from a unit exponential distribution with a mean zero and variance of one (Dohoo *et al.*, 2003).

1.8.2 Multi-level modeling: Generalised Linear Mixed Models (GLMMs)

Epidemiologic data can be clustered in space (such as cows within herds) or in time when repeated measures are made on the same observation unit (such as tests within cows). This is a hierarchical structure. Multilevel modelling (also called

mixed or random effects models or variance components models) takes this structure into account (Green *et al.*, 1998; Goldstein, 2003). Ignoring the hierarchical structure of observations within a population, that is, treating observations as independent when they are not, may lead to the overestimation of statistically significant results (Schukken *et al.*, 2003). Therefore the advantages of using these models are to obtain efficient estimates of regression coefficients; correct standard errors, confidence intervals and significance tests (Goldstein, 2003). Whether the effects of the independent variables on the outcome are "fixed" or they vary across the different levels of clustering, can be evaluated by using these models. They are also used to estimate how much the clustered levels contribute to the total variance of the different variables (Dohoo *et al.*, 2001).

Generalised Linear Mixed Models (GLMMs) are a model class used to deal with multilevel structured data, both continuous and discrete data. Binomial logistic regression with random effects is a typical model within this class, used when the outcome is binary. The equation follows that of a Generalised Linear Model (GLM) but with random effects added on to it. It is formed by two types of parameters: fixed effects (or mean effects) and random effects (which represent the variability around the mean effect). The logit function is used as a linear function of the predictor variables. The probability of the observed data if the estimated values of the predictor variables in the model were the true values is provided by the likelihood ratio test (Leyland and Goldstein, 2001).

An example of a hierarchical Bernoulli model is the one presented in Chapter 5. In that, the binary outcome variable in the model created represents whether a bovine animal was (Y_{ijk} =1) or not reactor (Y_{ijk} =0):

"bovine animal was reactor" = $Y_{ijk} \mid p_{ijk} \sim$ Bernoulli(p_{ijk}) where p_{ijk} = Pr (Y_{ijk} =1) is the probability that the *i*th observation will "select" the binary option =1 where i is the number of observations (animal tests) (level 1), j represents the individual animals within the herds (level 2) and k represents the herds (level 3).

$$Y_{ijk} = \text{logit}(p_{ijk}) = \alpha + \beta_0 + \beta_1 X_{1jk} + \ldots + \nu_{ik} + \mu_{ijk} \quad \text{where}$$

α is the intercept; $\beta_1 \ldots \beta_n$ represent the number of regression coefficients for a given number n of independent variables; $X_1 \ldots . X_n$ are the independent variables; ν_{ik} and μ_{ijk} are the variances between herds and animals within-herds random effects respectively, for a number i of observations. The random effects (or residuals) express the unexplained variation in the probability of the outcome (Leyland and Goldstein, 2001). The random effects for level 3 (herds) and for level 2 (animals within-herds) are assumed normally distributed with mean value of zero and a constant variance in the model: $\nu_k \sim$ Normal $(0, \sigma_\nu^2)$, $\mu_{jk} \sim$ Normal $(0, \sigma_\mu^2)$. An increase in the number of level 2 observation units in the level 3 unit, or an increase in the number of level 1 observation units in a level 2 unit, means there is a lack of information in the unit at level 3 or at level 2 respectively. Then, the best estimate places the predicted residual close to the overall population value as given by the fixed part (Goldstein, 2003). A variance with value of zero means no variation between herds (and therefore no clustering) and a large positive value means a high degree of clustering (Dohoo *et al.*, 2003).

A high or very high value of random effects at level 3 compared to that at level 2 would mean that the majority of the unexplained variation in the probability of a bovine animal becoming reactor is at farm level (level 3). Also, if all cattle were exposed equally to the disease, a high value at level 3 would mean that there is a clustering effect at farm level given by similar characteristics (i.e. attitudes/management) at this level.

Two statistical methods for the estimation of the parameters for the explanatory variables were used in this thesis. These are the deterministic Iterative Generalised Least Squares regression (IGLS), and the stochastic Bayesian method Markov Chain Monte Carlo (MCMC) estimation. Maximum Likelihood Estimation, MLE, is the method used to calculate the logit coefficients in linear mixed models. In GLMM the likelihood function involves an integral over each random effect, making the MLE method computationally very demanding. For this reason, the IGLS is used instead.

The IGLS is an algorithm that works as a block-relation algorithm. There are two blocks of parameters: the first fixes the variance components at some initial value

and maximises the likelihood over the fixed coefficients. The second block fixes the coefficients at their current values and maximises the likelihood over the variance components. The two optimisations are alternated until these converge (Leyland and Goldstein, 2001). Therefore, the process involves iterating between two deterministic steps until two consecutive estimates for each parameter are sufficiently close together, and it is then when convergence is achieved.

An alternative approach to fit models with a discrete outcome is the MCMC estimation, a stochastic procedure. That is, estimation of the different parameters or explanatory variables of interest are calculated using a random sample (a collection of values of the parameter of interest) from a population (or distribution of the parameter).

This method incorporates prior distribution assumptions. One of the procedures used by this method is the Gibbs sampling. This approach involves simulating new random values for each parameter from the conditional distribution of that parameter given all other parameters at their current estimates. The sequence of these random variables is called Markov Chain. After the simulation of each parameter, the new values replace the old estimates and the process is repeated until the estimates converge (Browne, 2004; Sturdivant, 2004). A well-behaved MCMC process will converge towards its "stationary distribution" and this represents the joint posterior distribution of interest (Green *et al.*, 2004). Convergence means that after an initial number of iterations (burn-in period), all the values appear to be sampled from the same distribution, with a constant mean and variance.

The values of the given posterior distribution are used to make inferences about the parameters or explanatory variables of interest. Estimates of the median and mean of the posterior distribution can be computed, as well as confidence intervals, and are interpreted as in the traditional statistical way (Dohoo *et al.*, 2001). Convergence of the MCMC model can be assessed visually by inspecting trace plots and model fit can be assessed by calculating the Pearson's Chi-square test for fitted *vs* observed values suggested by (Hosmer and Lemeshow, 2000).

Chapter 2 - General materials and methods of the study

2.1 Aims

The aim of this chapter is to describe and discuss the study design, recruitment process and data used in the thesis.

2.2 Introduction

The study was designed just after the foot-and-mouth (FMD) disease outbreak in 2001. This offered a unique natural experiment to investigate risk factors associated with the spread of bTB by comparing herds that were depopulated due to FMD and restocked afterwards, with herds that were continuously stocked (or unaffected) by FMD. A Randomised Badger Culling Trial (RBCT) took place in the South West region of England from 1998 to 2006. This offered the possibility to control for the effect of intervention on badgers. The study was designed based on these two selection criteria.

A project team was set up to carry out the farm visits. Good communication between members of the team and with farmers and the skills showed on farms, are of paramount importance for the quality and completeness of the data.

Since the work done for this thesis was carried out using the same study farms, the objective of this chapter is to provide a description of the cohort study design and a description of all the data collected. References in each chapter are made where particular materials and methods are used.

2.3 Study design and sample size

2.3.1 Cohort study design

The study population was farms located in areas where the Randomised Badger Culling Trial (RBCT) was undertaken (Bourne *et al.*, 1999) and where the foot-and-mouth disease (FMD) epidemic of 2001 had led to depopulation of cattle farms

(Gibbens *et al.*, 2001). The areas were in the South West (Cornwall, Devon, Somerset and Gloucestershire) and the West Midlands (Herefordshire and Worcestershire) (Figure 2.1).

Figure 2.1 - Geographical location of study farms

There were thirty trial areas, of approximately 100Km² each, within the RBCT. The areas were identified by ten triplets (from A to J) or geographical areas, and three treatments (reactive culling of badgers, proactive culling of badgers or surveillance of badgers only) in total. In the study, six out of the ten triplets and eleven out of the thirty trial areas were represented. Each trial area and farm was identified by codes and the nature of badger control was not disclosed until the study was completed.

The selected permanent exposure was restocking due to FMD in 2001. Within the study population, farms that were restocked after being depopulated due to FMD in 2001 were selected as exposed, and those that were continuously stocked were selected as non-exposed. Only with the purpose of the selection of farms, one restocked farm was matched with three continuously stocked farms within a trial area. There was a distance of at least 1Km between the restocked and each one of the continuously stocked farms to ensure that the cattle-cattle transmission was not

confounded by nose-nose contact over farm boundaries. No exclusion criteria were applied for herd size and purpose of the herd.

2.3.2 Sample size

The sample size estimated initially was 270 farms, 135 restocked and 135 continuously stocked. This sample size would have provided 80% power and 95% confidence to detect a risk ratio of 3 or more for the impact of repopulation on herd breakdown rate if monitored for two years based on an annual herd breakdown (HBD) incidence of 6% for the regions in the study, at the time when the study was designed.

2.4 Source of farms and access to contact details

The Veterinary Laboratory Agency (VLA) provided a list of 471 farms from the South West and West Midlands regions of England. A total population of 87 restocked farms within the RBCT and a matched random sample of 384 continuously stocked farms made the total of 471 farms provided. Contact details including address, telephone number and County Parish Holding (CPH) number for each farm were given.

2.5 Farmer contact and recruitment period

Farms were recruited between 25th November 2002 and 30th October 2003. The first contact was made by post in December 2002 and January 2003. This included an introductory letter to the study, an invitation to one of the seven meetings organised locally (Appendix 2.1) and a participation agreement form (Appendix 2.2).

Local farmers' veterinary surgeons and local members of the National Farmers Union (NFU) were also invited to the meetings. Farmers were contacted by telephone as many times as necessary to explain the study and encourage participation either when they had not attended a meeting or after the first contact by post. On 10th April 2003, a reminder letter was sent to those farmers not enrolled. On 25th September 2003, the VLA provided twenty five extra farms by request. A number was assigned to each farm in order to keep confidentiality.

2.6 The project team

A project team was set up. This was made up of five field technicians and a database manager. Three other people, one senior and two laboratory technicians worked on the testing of the serum samples for the BBSRC project. The coordination of field and laboratory work as well as communication with farmers, was the author's responsibility. All members signed a confidentiality form in which they agreed to keep farmers' information confidential.

2.7 Source of Data

2.7.1 VetNet database

Outcome variables and history of bTB were obtained from the standard official single intradermal comparative cervical tuberculin (SICCT) test results recorded on Defra's disease national database (VetNet). The database has the records for reactors (R), found with lesions at slaughter (SL), inconclusive reactors (IR), direct contacts (DC) and contact tracings (CT) only. It does not hold records for cattle which tested negative or were not tested. Two updates were requested to the VLA.

2.7.2 British Cattle Movement Service (BCMS) database

Records from the British Cattle Movement Service (BCMS) were provided by the Cattle Tracing System (CTS) for the study farms for the period January 1996 to August 2004. Farms were identified by CPH and cattle by ear-tag numbers. The information used from the database included: date of birth, sex, breed, date of movement (if purchased on to the study farm), date of movement off the farm and number of movements, if any, previous to that on the study farm. Data from this database were used in Chapter 5.

2.7.3 Standardised farmer interview questionnaire

A standardised questionnaire (Appendix 2.3) was designed by the author and Prof. Green, and approved by the Census Survey Group, York, in June 2003. It was pilot tested by the author and four members of the field team, on five farmers between May and June 2003. The pilot study was carried out on selected farms where we

knew the farmers. An extensive questionnaire was first designed and it was refined as we carried out the first pilot visits. Also, in this process, members of the field technicians team and the author met regularly to clarify and re-structure the questions where needed. Where needed, key tables with the answers were printed out and were laminated. To validate the answers, members of the team were sent in different groups, so the interview process was standardised. Coding for subsequent analysis was not required.

Information was collected retrospectively for the period October 2001 to June 2003, and both closed and opened questions were included, that is there were standard answers for the farmers to choose from or farmers could answer freely to the questions. The questionnaire was divided into two main sections.

In the first section (Appendix 2.3 Part 1) questions about how farmers grouped their cattle and the location of these groups within the farm's fields and buildings between October 2001 and June 2003, by month intervals were asked. These questions were non-structured and farmers provided their natural animal groups as managed on the farms. Maps of the farms were printed using the Ordinance Survey 1:10,000 Raster black & white reproduced from the Ordinance Survey mapping with the permission of the Controller of Her Majesty's Stationary Office. Maps were sent out to farmers within two weeks prior to the visit for them to allow time to think about the questions we asked. Instructions were sent together with the maps (Appendix 2.4). See example of a map from fields from a study farm in Appendix 2.5.

Here the alternative hypothesis being tested using these data (Chapter 6) was that there were factors that would affect the risk of an animal group having at least one reactor animal in the group. The information from the questionnaire was made into questions such as: was the animal group in a field with observed presence of badgers during the study period; in buildings and in fields with a number of other animals in the group; in fields where slurry had been spread during the study period?, and all these questions were asked for monthly periods prior to the test date.

The second section (Appendix 2.3 Part 2) covered the main aspects of farm management and practices. It was divided into ten practices/management areas. The alternative hypothesis being tested using these data (Chapter 4) was that there were factors that would affect the risk of a herd breaking down with bTB. The main questions are specified in Table 4.1 and these refer to management practices such as: did the use of different types of manure/slurry and the type of storage, made any difference in the risk of HBD; and the use of certain types of feeding stuff; and the purchase from different sources?, etc.

2.7.4 Building survey

A building survey was designed to complement the building information from the farmer interview questionnaire. It was carried out after all questionnaires had been completed by the members of the field team. The building information sheet from the main questionnaire was photocopied and taken on the farm visit when performing this survey. This ensured that all buildings previously recorded were also surveyed. Seven surveys could not be completed due to the withdrawal of seven farms. Information from this survey could be used as part of further work in Chapter 6.

2.7.5 Reactor animal location

For each farm, a list of ear-tag numbers of animals disclosed as reactors (as recorded in VetNet) between January 2000 and November 2004 was produced together with a list of animal groups as defined by farmers in the questionnaire. Farmers were asked to identify each of the reactor animals with an animal group. A list of the location of these animal groups within the farms' fields and buildings were also added into the same document to help farmers with the identification of the reactors. An example is given in Appendix 2.6. Data from this section were used in Chapter 6.

2.8 Data collection and storage

2.8.1 Farm visits

Visits were arranged two weeks in advance. Flexibility of visit times for farmers was always a priority, but where possible, matched farms were visited within the same one-month period. Each visit was made by two members of the team.

2.8.2 Data input

Data were entered into a Microsoft Access Database as soon as possible after the visit was completed and by the same field technician who did the farmer interview. A sheet was completed by the technician, stating the name, date of entry and any notes for any further revision.

2.8.3 Data quality

During the pilot of the study and at the beginning of the farm visits, the team met regularly to standardise the questionnaire by discussing the type of questions and answers given by farmers. I helped in doing so, by attending at the beginning to a large number of visits. Data recorded in the database, as described above, were double-checked by one and the same technician who had been involved in the visits and right from the beginning of the study. If there was missing information, technicians telephoned farmers or revisited them to complete the data.

2.9 Results

2.9.1 Farm recruitment

The recruitment period lasted eleven months, from November 2002 to October 2003, with most farms recruited within the first five months (November 2002 - April 2003). Farmers meetings were organised between 25th November 2002 and 4th February 2003: two in Gloucestershire, four in Devon and one in Somerset. The number of farmers who agreed to participate at the meetings was approximately 20% of the total enrolled. The rest of the farmers were contacted by telephone. In most cases, follow-up telephone calls were made to allow farmers time to consult with

their own veterinary surgeon and/or other farmers on whether to participate or not. Sometimes, extended conversations were needed to respond to the demands of farmers regarding the details and outcomes of the study and more generally, to listen to their concerns about bovine tuberculosis. From the final reminder letter sent to farmers on 10th April 2003, four farmers agreed to participate.

From a total of 471 farms provided by the VLA, 468 were successfully contacted and 148 farms were recruited. Table 2.1 shows the outcome from farmers' contacts. The response rate, as the proportion enrolled from the total 369 eligible farms, was 40%.

Table 2.1 - Results from study farms recruitment

Farm status from list	**Number of farms**	**% from 468**
Enrolled	148	31
Not enrolled	323	69
From not enrolled		*% from 323*
No longer farming	89	28
Refused to participate	221	68
Duplicate farm from VLA	10	3
Letter returned-addressee unavailable	2	1
Out of study region	1	0

Out of 87 farms categorised as depopulated in 2001, 20 (23%) did not restock. Farms that were matched (one restocked and three continuously stocked and all within the same trial area from the RBCT) were first contacted within the same week of the recruitment period. Table 2.2 presents the results of this matching, by quads (one restocked and three continuously stocked), triplets (one restocked and two continuously stocked) and pairs (one restocked and one continuously stocked). Some farms were unmatched.

Table 2.2 - Results from recruitment by RBCT and restocking matching criteria

RBCT identification *				Matched farms			Unmatched farms	
Triplet	County	Intervention Treatment	Trial area	Quads	Triplets	Pairs	Restocked	
							Yes	No
A	Gloucs. & Hereford	Reactive	A1	4	2	0	0	1
A	Gloucs. & Hereford	Proactive	A3	0	1	0	0	0
I	Gloucs.	Survey	I3	1	0	1	0	0
I	Gloucs.	Reactive	I1	2	0	0	0	9
B	Devon & Cornwall	Proactive	B2	4	3	1	2	0
B	Devon & Cornwall	Reactive	B1	1	1	0	0	4
B	Devon & Cornwall	Survey	B3	0	0	0	1	2
J	Devon	Survey	J3	6	0	0	1	0
J	Devon East	Proactive	J1	1	0	0	0	5
C	Cornwall	Reactive	C1	1	0	0	0	0
H	Devon & Somerset	Survey	H3	3	0	0	0	6
Total	**5**	**3**	**11**	**23**	**7**	**2**	**4**	**27**

* Source: (Bourne *et al.*, 2004)

2.9.2 Maintenance of farms and withdrawals from the study

One hundred and fourteen farmers (all except thirty three who had only growing cattle for beef production and one that left the study after completing the questionnaire), also participated on the investigation of five other endemic diseases study, as mentioned in the introduction of this chapter, regardless of their bTB status. Feedback of serology test results was sent to farmers in December 2003 and regular communication was kept with farmers to assist with the collection of blood samples. The team's skills shown on farms were perceived as an advantage to completing the visits and on the quality of the data collected. In October 2004, a letter was sent to farmers thanking them for their collaboration in the study and for the data provided until then.

Eleven farmers withdrew from the study, but the questionnaires had already been completed by the time these farmers left. Eight of these sold the herd or stopped farming; one was rejected from the study due to the farmer's rudeness towards members of the team; one farmer passed away and one farmer decided to stop participation because in his opinion, the study had not given a solution to the bovine tuberculosis problem!

2.9.3 Farm visits and completion of data collection

Farmer interviews were completed between 17th June 2003 and 18th February 2004. Visits were spread out during the eight months of data collection. Despite farmers being very busy during the summer months due to agricultural work, seventy questionnaires were completed between 17th June and 15th September 2003 and the other seventy eight were completed by 18th February 2004. Building surveys were completed between 12th October 2003 and 30th September 2005. Reactor animal information within animal group location was collected between 6th December 2004 and 23rd August 2005, with information completed for most farms between January and April 2005. On some occasions, building surveys and reactor information coincided with blood sampling visits if not many cattle were to be sampled, as this way was more convenient for some farmers.

2.10 Discussion

2.10.1 Study design and selection criteria

The study investigation focused on the identification of risk factors with bTB in cattle and the role that these play in the spread and maintenance of *M. bovis* in the herds. However, consequently controlling for badger interactions from the RBCT was important to control for any effect of badgers on bTB. Restocked and continuously stocked farms were matched within triplets from the RBCT with the aim of recruiting similar ratios of farms in all triplets. The RBCT was started in 1998, almost three years before commencement of this study, and during the FMD outbreak in 2001, the RBCT suffered some disruption. Consequently some farms requiring a land survey were not visited (Le Fevre *et al.*, 2005); personal communication with farmers).

The disruption of the RBCT could have affected the validity of the results of this study. However, there were two factors that could have helped to improve this problem: the long duration of the trial and the inclusion of questions in the standard questionnaire regarding badger activity. These two factors could have helped to decrease the detrimental effect from the disruption of the RBCT elucidating some association with bTB in the study herds if there was any.

The first results from the RBCT were reported in December 2003 (Donnelly *et al.*, 2003). The design of the study could have been different if these results had been available when the study was being designed. For example, farms could have been recruited from targeted specific farm locations where the results of the trial were reported to have an effect on the risk, rather than in the whole geographical region. On the other hand, the naïve knowledge of the effect from the RBCT was regarded as a positive effect on the avoidance of biases towards the RBCT.

The recruitment of restocked farms was limited by the number available within the RBCT and the number that, once depopulated, were restocked again. Because of this reason, the proportion of recruited restocked farms was lower (24%) compared to continuously stocked (76%). This had not been anticipated when the study was designed. However, given that the incidence rate of HBD in the study region, was approximately three times higher during the study period (over 20%, DEFRA, (2004b) compared to 6% initially estimated), the study sample obtained was thought to be still large enough to detect statistically significant factors associated with the risk of HBD, reducing type II errors, that is, reducing the probability of obtaining not statistically significant factors when they really are. Based on a sample size calculation that was done *a posteriori* where the incidence rate used was 20%, the required number of farms was 51 for each exposed and non-exposed (Thrusfield, 2005). Although the total recruited number of farms was above this, the number of exposed farms was still lower than required by this calculation.

2.10.2 Farm recruitment

Recruitment was a difficult process. Many farmers, some of them having had to manage their herd with bTB for years, were very reluctant to collaborate. Many

farmers argued that in their opinion, policy-makers were not handling the eradication process of bTB in an appropriate way and consequently they could not see the usefulness of the study if, after all, little action was going to be taken towards eradication. The main reason why some farmers took longer to be recruited was because they were to participate in the study only if blood samples were taken from their herds for the investigation of the other five endemic diseases, and prior to the completion of the questionnaire. The expectation of the results from the blood tests became essential as an encouragement to continue participation. Despite the time it took to complete the recruitment process, there was no interference with the collection of data and once farmers had decided to collaborate, they showed patience through the completion of the questionnaire and throughout the three years of the study.

The attendance by a few farmers' veterinary surgeons at the local meetings encouraged some farmers to enrol in the study. A few farmers would only participate if their veterinarians considered the objectives of the study to be useful. This was observed in one of the meetings and in conversations with farmers. Some NFU representatives attended the meetings and gave their points of view and welcomed the study. This could have had a positive effect on the perception of the study from the farmers' point of view as the NFU representatives were there to defend their interests.

2.10.3 Maintenance of farms in the study and data quality

Good communication between team members and with farmers, as well as the time flexibility given to them, was much appreciated and was seen as successful in the maintenance of the study. The team spent all the time necessary in listening to farmers' concerns in order to complete the required data for the questionnaire; they were helpful in handling cattle around the farm if required at the time when they were taking blood samples, which in turn gained respect for the project members and willingness in providing detailed questionnaire data.

Quality of the data collected was achieved by the interview and data input being carried out by the same technician and by continuous communication kept between

members of the team. Compared to other studies, where more staff may be involved in the data collection, the way data were collected and handled for this study was seen as an advantage.

Due to the withdrawal of the eleven farmers (7% of the total in the study), seven building surveys could not be complete but all farmer questionnaires had been completed before these farmers left.

2.10.4 Questionnaire design

Information in the interview questionnaire was requested retrospectively from October 2001 to June 2003. Last cases of FMD in the South West region occurred in June 2001(DEFRA). Therefore, October 2001 was selected as the start of the study because by this time farms that were depopulated due to FMD had started restocking. The questionnaire included specific questions referring to specific dates or time periods. Questions directed to the farmer (rather than based on the interviewer observations) and questions referring to specific time periods, were combined with questions which allowed farmers to describe their herds in the way they managed them (i.e. animal groups within-herd as farmer grouped them instead of asking for pre-structured animal groups). This implied more manipulation of the data once collected, but it reflected more truly the practices on the farm.

Data collected in the way it was done in the farmers' questionnaire Part 1, had not been previously collected in any other previous studies and thought to be worth investigate. The author was in particularly interested in the identification of reactors within the animal groups as a new contribution to the epidemiology of the disease in cattle. The data of animal groups in buildings and fields through monthly periods within the study period was done to identify risk factors that may help to elucidate the risk of bTB given that, in general, herds are managed in groups and those are potentially exposed to different risk factors when they are in buildings and in fields.

Data that were asked in the farmers' questionnaire Part 2 were based on results from an extensive general literature review, including descriptive and case-control studies that investigated risk factors at herd level. There was a compromise between the amount of data collected and the way it was analysed. In that, detailed

information was gathered to avoid too general and vague inferences from results, but the analysis was carried out carefully using stepwise analysis and in such a way that potential biological risk factors were identified and analysed by management groups.

A positive experience for myself together with Prof. L. Green, was to be able to revise critically in August 2004, the design of a revised version of the TB99 (the official questionnaire used by the State Veterinary Service (DEFRA) to investigate bTB at a national level). Comments referring to the avoidance of recall biases by not requesting farmers to give numbers that were difficult to remember (such as neighbour's CPH) as well as the avoidance of using pre-determined structured animal groups for farmers to fit their animals, were suggested to be useful for improvement of the national questionnaire.

Chapter 3 - Description of study farms

3.1 Aims

The aim of this chapter was to characterise the study farms by using the study design criteria and the general information from the standard farmer's questionnaire. Results from the SICCT test carried out during the study period and a brief description of farmers' opinions about bTB are also presented.

3.2 Introduction

Data used in this thesis are original field data collected using a questionnaire specifically designed for this study. Here, a description of the study farms is presented to set the scene and provide information on the farm selection criteria (areas affected by FMD in 2001 and participation in the RBCT), the purpose of herd (herd-type) and herd size.

In addition, since the interpretation and types of SICCT test used, are outcome variables and covariates throughout the thesis the results of herd and animal tests are presented for the period from October 2001 to November 2004.

3.3 Description of study farms

3.3.1 Geographical location

Farms were located within four counties of the South West (Cornwall, Devon, Somerset and Gloucestershire) and one (Herefordshire and Worcestershire) of the West Midlands regions of England. The last region is just on the border with that of Gloucestershire. Table 3.1 presents the number of total cattle holdings registered in the Census June 2002 for the counties in the study, the number of farms provided by the Veterinary Laboratory Agency (VLA) and finally recruited in the study.

Table 3.1 - Number of total cattle holdings registered in Census June 2002 by county and number and percentage recruited from herds initially provided

County	Number of total cattle holdings*	Herds in the study within FMD and RBCT areas		
		recruited	provided	% recr/prov
Cornwall	14,555	11	34	32.35
Devon	22,899	84	284	29.58
Somerset	11,433	4	17	23.53
Gloucestershire	5,344	44	121	36.36
Hereford & Worcester	9,596	5	14	35.71

*Source: Agricultural Census June 2002, Defra.

The percentage of total farms represented in the study from the total from all counties based on the Census June 2002, was 0.23%. It varied for each county from 0.03% in Somerset to 0.82% in Gloucestershire. Of those provided from each county, the percentage recruited varied between 23.5% in Somerset to 36% in Gloucestershire. From the 148 farms recruited, over 57% were in Devon, 30% in Gloucestershire and 13% in the other three counties (Figure 3.1).

Figure 3.1 - Distribution of 471 farms provided and 148 study farms recruited by county.

3.3.2 Restocked *vs* continuously stocked farms

In the FMD epidemic in 2001, over four million animals, 14% of which were cattle, were slaughtered as one of the measures to control FMD. Approximately 18% of cattle slaughtered were from the study areas. There was a total of 2,030 infected

herds (Infected Premises or IPs) in Great Britain during the outbreak, 326 (16%) of which were within the counties included in this study and of these, 234 (11.5% of the total IPs) were cattle farms (www.defra.gov.uk). Infected herds as well as contiguous, dangerous contact and other herds within the control areas, were depopulated.

The number of cattle farms provided by the VLA that were depopulated was 87/471 but only 77% restocked with cattle. In total, 36/87 (41%) restocked farms and 112/384 (29%) continuously stocked farms were recruited. In Figure 3.2 the percentage of study farms by restocking status per geographical region is shown.

Figure 3.2 - Distribution of 148 study farms among restocked and continuously stocked on the study counties.

As shown above, the representation of farms in the study varied between counties within the continuously stocked farms (from 72.6% in Devon to 91% in Cornwall) and within the restocked farms (from 9% in Cornwall to 27% in Devon).

3.3.3 Distribution of farms within the Randomised Badger Control Trial areas

From the 471 farms initially provided, 36.5% were in the reactive, 24.4% in within the proactive and 39% within the survey only treatments of the RBCT. There were study farms in eleven out of the thirty trial areas and in six out of the ten triplets (Table 3.2). The percentage of farms represented from the total number of farms in

trial areas of the RBCT varied considerably, between 21.79% (trial area I1, reactive in Gloucestershire) and 2.33% (trial area B3, survey in Cornwall).

Table 3.2 - Number and percentage of total herds in the RBCT, provided by the VLA and recruited in the study by trial area of the RBCT

Trial area	Total in RBCT*	Recruited	Provided	% (recruited/ provided)	% (recruited/ total in RBCT trial)
A1-reactive	135	23	60	38.33	17.04
A3-proactive	74	3	5	60.00	4.05
B1-reactive	90	11	47	23.40	12.22
B2-proactive	153	29	85	34.12	18.95
B3-survey	129	3	14	21.43	2.33
C1-reactive	151	4	15	26.67	2.65
H3-survey	136	18	75	24.00	13.24
I1-reactive	78	17	50	34.00	21.79
I3-survey	103	6	20	30.00	5.83
J1-proactive	116	9	25	36.00	7.76
J3-survey	129	25	75	33.33	19.38

*Source: Defra, 2004

Of the 148 study farms, 37% were in reactive trial areas, 28% in proactive and 35% in survey only. Figure 3.3 below shows the distribution by county and intervention treatment of the RBCT.

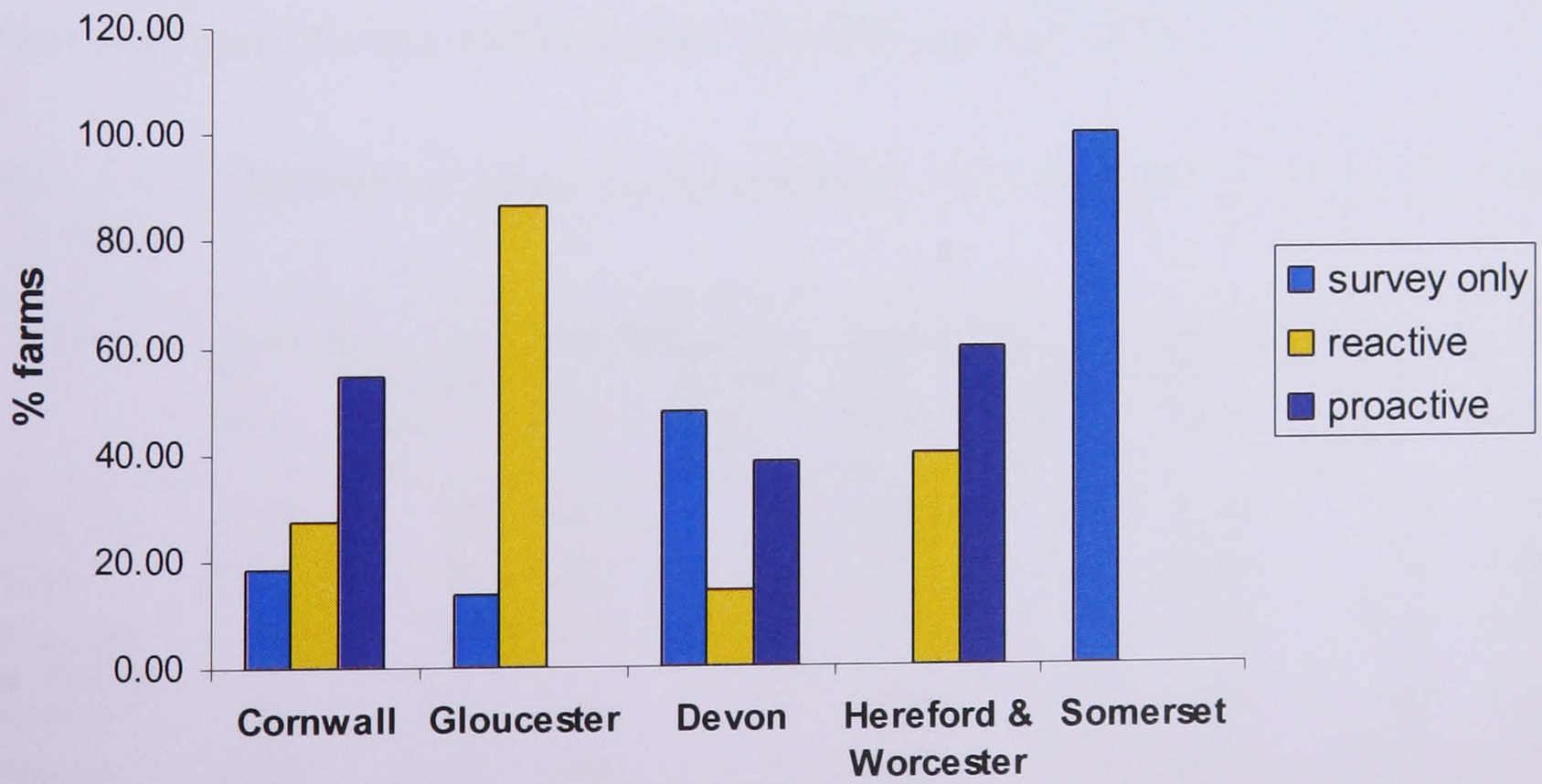


Figure 3.3 - Percentage of study farms by county and RBCT intervention treatment

In Table 3.3 the number of farms per county by RBCT treatment and restocking status is presented.

Table 3.3 - Number of study farms by geographical location, intervention treatment of the 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

Information about which RBCT treatment the farm was on was not requested from farmers during the study. Rather it was the objective for this not to disclose the RBCT treatment at the time of the farmer's questionnaire completion. However, farmers inevitably mentioned participation: 75% said they were aware of their farm being enrolled, 17% thought they were not (when probably they meant they were in the survey only areas) and 8% were unsure.

3.3.4 Herd size and purpose of herds

The numbers of holdings and cattle per county are provided by the National Census (www.defra.gov.uk). In Table 3.4, an estimated average of herd size is presented based on the total number of holdings and cattle.

Table 3.4 - Number of total cattle holdings, and average of herd size from Census June 2002

County	Dairy only		Dairy and grower		Suckler only		Suckler & grower		Grower stock	
	No. farms	Average herd size	No. farms	Average herd size	No. farms	Average herd size	No. farms	Average herd size	No. farms	Average herd size
Cornwall	1,395	82	3,676	75	2,215	26	3,717	59	3,552	45
Devon	2,273	88	5,801	79	3,343	27	5,848	59	5,634	46
Gloucester	493	97	1,374	77	771	24	1,379	55.5	1,327	43.5
Hereford & Worcester	591	83.5	2,485	61	1,584	25	2,532	56	2,404	42
Somerset	1,381	104.5	2,895	91	1,521	25	2,879	54.5	2,757	43
Total	6,133	91	16,231	77	9,434	26	16,355	57.5	15,674	44

Herd size of all ages of cattle ranged from 3 to 847, with a median of 145.5. In the 148 study farms, 35% had up to one hundred cattle, 33% between one hundred and one and two hundred, and 31% over two hundred cattle. Figure 3.4 shows the distribution of farms by herd size and restocking status.

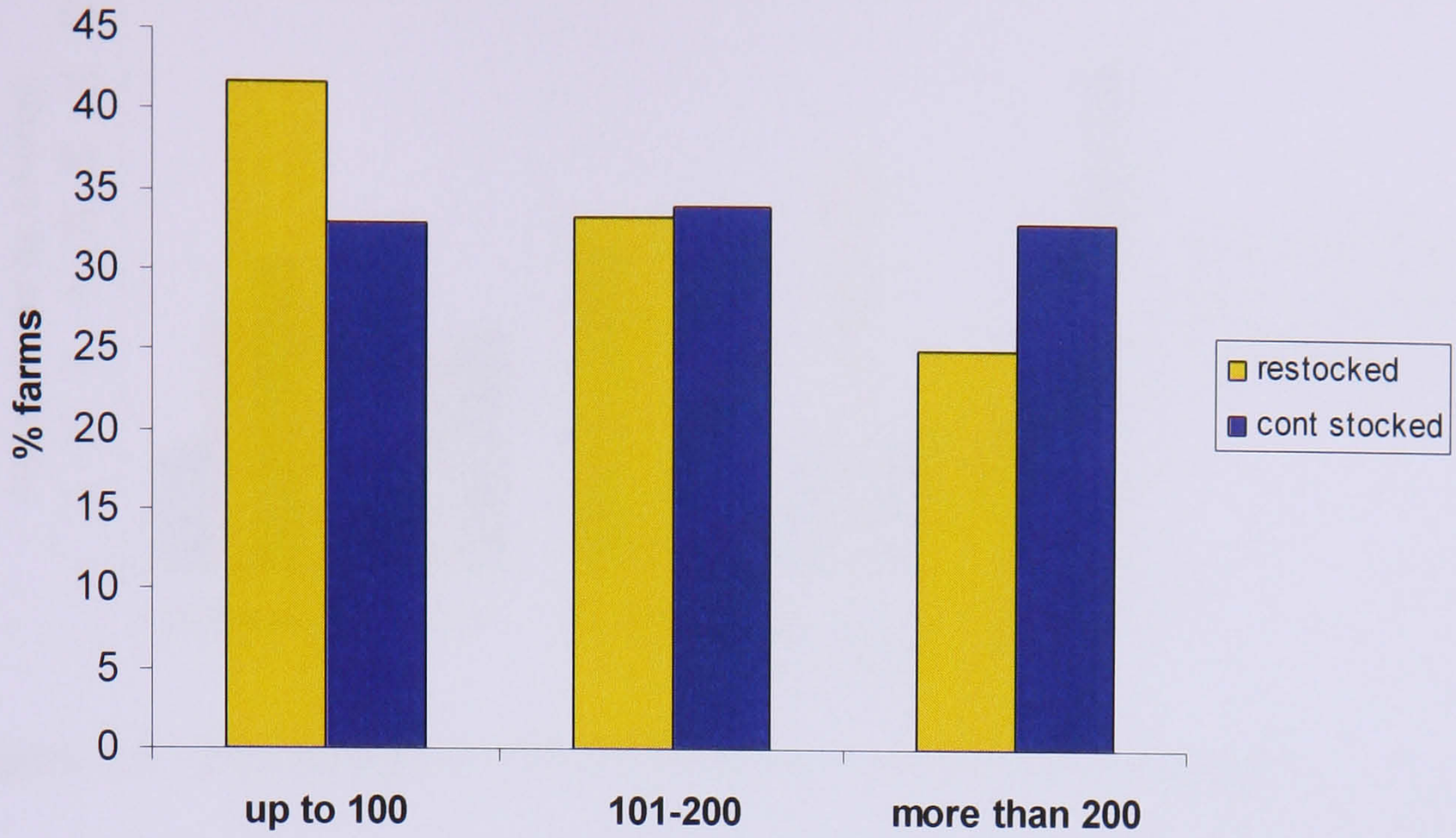


Figure 3.4 - Percentage of 148 study farms by herd size and restocking status.

Herd size was approximately evenly distributed within continuously stocked farms with 33% within each, up to one hundred cattle, between one hundred and one and two hundred, and more than two hundred cattle per herd. Within restocked farms, 42% had up to one hundred cattle, 33% of the farms had between one hundred and one and two hundred, and 25% had more than two hundred.

There was more variation within the counties in the study. Herds with up to one hundred cattle varied between 18% in Cornwall and 48% in Gloucestershire. There were no herds with between one hundred and one, and two hundred cattle in Somerset, 60% of those were in Hereford and Worcester and approximately 35% in each of the other counties. Larger herds, those with more than two hundred cattle varied between counties, with 75% of these in Somerset to 16% in Gloucestershire (Figure 3.5).

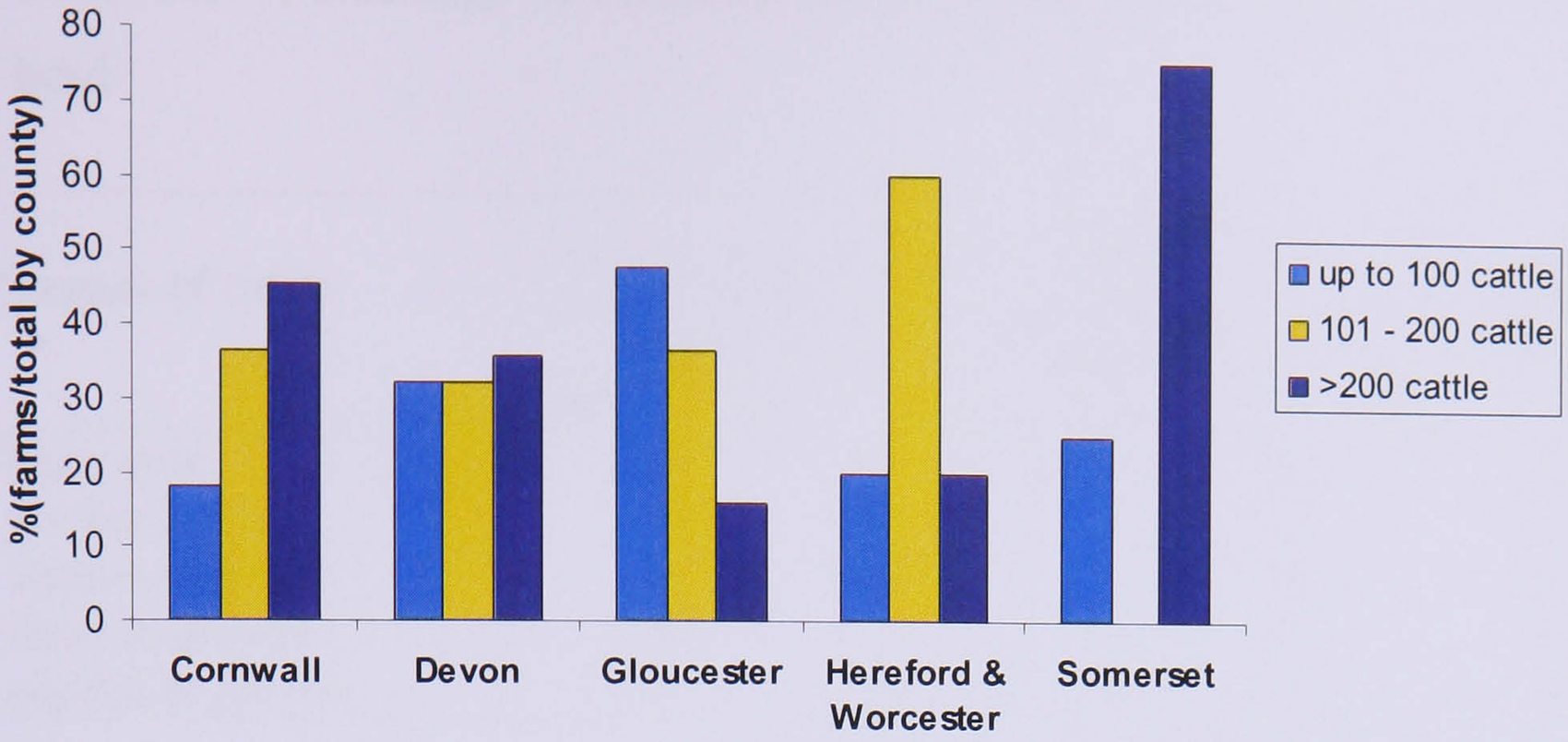


Figure 3.5 - Percentage of 148 study farms by herd size and county

The purpose of the herds or herd types were classified into dairy, suckler (or breeding) herds and grower cattle for beef production or replacement dairy heifers. Of the 148 herds, 40% had adult and grower cattle (62% suckler and grower and 38% dairy and grower) and 60% had adult cattle only, of which 38.5% had dairy only 24% suckler only and 37.5% had grower cattle only. Figure 3.6, shows the percentage of herds by herd purpose and restocking status and in Table 3.5 a summary of all farms by restocking status, herd size and purpose of herds is presented.

Figure 3.6 - Percentage of 148 study farms by restocking status and purpose of herd

Table 3.5 - Percentage of study herds by restocking status, herd size and purpose of herd

Purpose of cattle	**herd size**					
	up to 100		101-200		more than 200	
	restocked		restocked		restocked	
	yes	no	yes	no	yes	no
dairy only	7	11	33	24	22	38
grower only	33	41	25	18	22	3
suckler only	33	24	0	16	0	3
dairy & grower	0	3	8	24	11	30
suckler & grower	27	21	34	18	45	26

3.3.5 Withdrawal of farms from the study

During the period of the study, but after the farmer questionnaire had been completed, eleven farms stopped participation. Five were in Gloucestershire, four in Devon, one in Cornwall and one in Somerset. Six had been restocked after FMD. Three were in the survey only, five in the reactive and three in the proactive intervention treatments of the RBCT. Two farms had grower cattle only, four dairy, one suckler and four were mixed (two were dairy and grower and two were suckler and grower). Six out of the eleven herds had at least one HBD after 2001 but by the time they stopped the study. The reasons for these non-random withdrawals were given in Chapter 2.

3.3.6 Other farm characteristics

Most farms had only cattle, but 19.5% had other type of stock. Of these, 76% had sheep, 21% had some kind of poultry and 10% had horses.

Water for cattle was supplied from different sources: 74% of farms used mains (48% had this as the only source). In Table 3.6 the different sources of water used for cattle in the study farms are presented.

Although there is not previous evidence nor a reason to investigate different source of drinking water and the influence on the risk of bTB in cattle herds, this was thought to provide some information on how cattle come close to each (i.e. by drinking from water troughs they could become closer to each other).

Table 3.6 - Source of water supply in 148 study farms

Source of water	Number of farms	% from total 148
Mains only	71	47.97
Mains and other	39	26.35
bore hole	7	4.73
stream	20	13.51
spring	9	6.08
well	3	2.03
Other than mains	38	25.68
bore hole	15	10.14
spring	19	12.84
stream	8	5.41
well	2	1.35

3.4 Description of bTB in study farms based on the SICCT

3.4.1 Types of test

England is divided into counties, parishes, and holdings. The inter-test interval for bTB tests depends on the annual incidence of confirmed herd breakdowns in the parish over the previous six years. All herds should be tested every twelve months unless the annual incidence is less than or equal to 1%, every two years if less than 1%, every three years if less than 0.2% or every four years if less than 0.1% (State Veterinary Service, 2005). The cattle tested and test interpretation vary by farmer purchasing behaviour, age of cattle and purpose (e.g. cattle for beef consumption, killed young, may not be tested, whereas a replacement heifer of the same age may be tested because she will be kept alive for a longer time), inter test interval, herd bTB history and whether the test is routine or triggered by a herd breakdown (HBD) for the herd in question or a neighbouring herd (Pritchard, 1988; Green and Cornell, 2005). The date, test type and number of cattle tested are stored in the national database (VetNet) together with the identification of positive reactor cattle. Although the procedure for all tests is standard as stated by the European Legislation (Directive 64/432/EEC), different codes are used in the database to highlight the frequency of testing (as described above) and whether tests target herds or individual cattle.

3.4.2 Tests used in the study period

Type of test - Fifteen different tests out of 33 possible types (Chapter 1, Table 1.2) were carried out in the study period; twelve were herd tests and three were individual tests (Table 3.7 below). 35% of the tests used a short interval test (VE-SI), 16.5% were inconclusive tests (VE-IR) and 11% and 9.5% were whole herd test (VE-WHT) and a six month interval test (VE-6M) respectively. Between 6.5% and 2.5% of the tests were VE-CT, VE-CON12, VE-TR, VE-CON6, VE-CON and VE-12M. Less than 1% of the tests were VE-SLH, VE-PRI, VE-PII and VE-PR.

Table 3.7 - Percentage of tests out of a total of 921 tests by test type and year of the study

Test type	Year 1		Year 2		Year 3	
	Restocked		Restocked		Restocked	
	No	Yes	No	Yes	No	Yes
VE- 12M	4.21	0.00	2.40	0.00	3.03	0.00
VE- 6M	7.89	7.69	6.51	4.35	14.39	15.94
VE- CON	2.11	7.69	3.77	4.35	2.65	1.45
VE- CON12	7.37	0.00	2.40	2.90	7.20	11.59
VE- CON6	4.21	0.00	5.48	4.35	1.89	2.90
VE- CT	3.16	42.31	5.14	28.99	1.14	7.25
VE- IR	20.00	3.85	15.75	14.49	17.80	14.49
VE- PII	0.00	0.00	0.00	0.00	0.38	1.45
VE- PR	0.00	0.00	0.00	0.00	0.38	0.00
VE- PRI	0.00	0.00	0.00	4.35	0.38	0.00
VE- RHT	2.11	0.00	1.03	0.00	0.38	0.00
VE- SI	30.53	26.92	42.81	37.68	32.95	31.88
VE- SLH	1.05	0.00	0.68	1.45	0.38	0.00
VE- TR	2.11	7.69	2.05	4.35	7.20	2.90
VE- WHT	15.26	3.85	11.99	8.70	9.85	10.14

In the first year of the study, the highest percentage of tests used a short interval test (VE-SI) in continuously stocked herds (after being triggered by other tests) and check test (VE-CT) in restocked herds. In the second and third years, short interval tests were the most frequently used.

Number of reactors per test - Out of the 921 tests, 75% (687/921) were negative and 25% (234/921) were positive with at least one reactor disclosed at the test. Of these, 49.5% were confirmed by lesions at slaughter and or culture in the laboratory. Table 3.8 presents the percentage of negative and positive tests by test type. Within

the negative tests, six were VE-SLH (cattle were clear at the SICCT test but found with lesions at post-mortem inspection).

Table 3.8 - Percentage of negative and positive tests out of 921 in the study period by number of reactors at the test and type of test.

Test Type	No reactors		One reactor		Two reactors		More than two reactors	
	Number of tests	%	Number of tests	%	Number of tests	%	Number of tests	%
VE-12M	17	2.47	3	3.41	0	0.00	3	3.30
VE-6M	60	8.73	13	14.77	4	7.27	11	12.09
VE-CON	22	3.20	2	2.27	3	5.45	1	1.10
VE- CON12	44	6.40	3	3.41	0	0.00	3	3.30
VE-CON6	27	3.93	1	1.14	2	3.64	4	4.40
VE-CT	48	6.99	5	5.68	5	9.09	2	2.20
VE-IR	131	19.07	14	15.91	5	9.09	2	2.20
VE-PII	2	0.29	0	0.00	0	0.00	0	0.00
VE-PR	1	0.15	0	0.00	0	0.00	0	0.00
VE-PRI	4	0.58	0	0.00	0	0.00	0	0.00
VE-RHT	6	0.87	2	2.27	0	0.00	0	0.00
VE-SI	199	28.97	37	42.05	32	58.18	57	62.64
VE-SLH	6	0.87	0	0.00	4	7.27	8	8.79
VE-TR	35	5.09	1	1.14	0	0.00	0	0.00
VE-WHT	85	12.37	7	7.95	0	0.00	0	0.00
Total	687	100.00	88	100.00	55	100.00	91	100.00

Restocked *vs* continuously stocked - Out of the 921 tests, 81% were done on continuously stocked and 19% on restocked herds. During the first year, the percentage of number of tests in continuously stocked herds was higher than in restocked and vice-versa in the second and third years of the study (Figure 3.7).

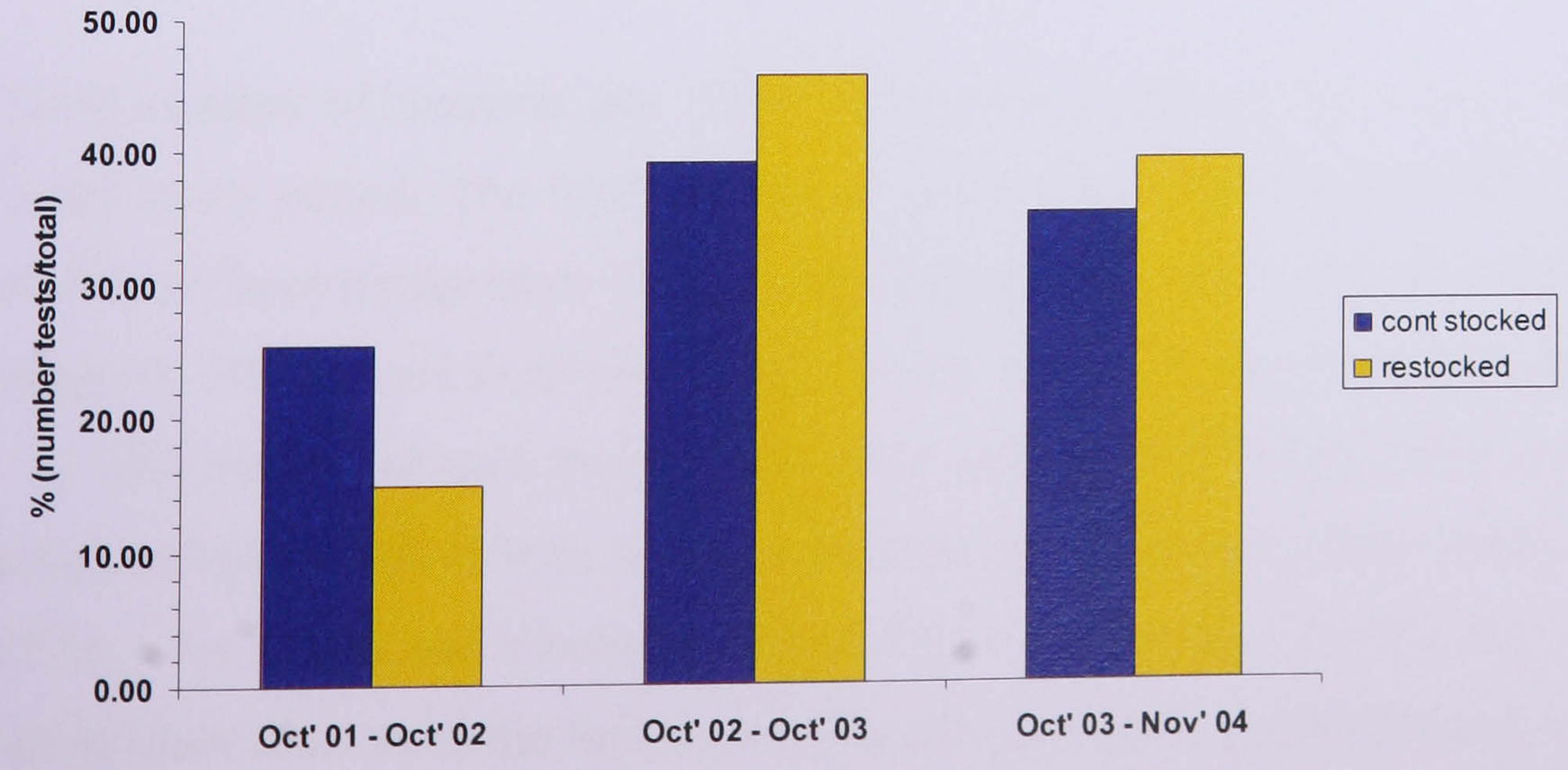


Figure 3.7 - Percentage of tests per study year from a total 921 tests by restocking status.

In the first three months of the study (between 1st October 2001 and 18th December 2001) only 16 herds were tested. Out of the 921 tests, 19 (2%) were carried out during that period; 128 (14%) between January and June 2002; 130 (14%) between July and December 2002 and 644 (70%) between January 2003 and October 2004).

3.4.3 Tests on unrestricted herds

Type of test - Out of the 112 HBD disclosed during the study period, 25% were disclosed by a VE-6M test, 17% by a VE-WHT, 16% by VE-IR (most of these triggered by VE-WHT, VE-CT and VE-6M herd tests) and 8% by VE-CT. Approximately 5% of HBD were disclosed each of VE-12M, VE-CON, VE-CON12 and VE-CON6. Approximately 4% were disclosed by VE-SI and 4% by VE-SLH. None of the HBD was disclosed by VE-PII, VE-PR or VE-PRI. The test type was not specified for one HBD.

Number of reactors per test disclosing a HBD - There was a total of 356 reactors at the disclosing tests. There were 41% HBD disclosed with only one reactor at the test; 21% of the HBD were disclosed by two reactors, 11% by three and 27% by more than 3 reactors; 3.5% (4/112) of HBD were disclosed at post-mortem inspection at the slaughterhouse (VE-SLH) (Table 3.9).

Total number of reactors per HBD - There was a total of 753 reactors in all HBD in the study period. The total number of reactors per HBD in the total 112 had a median of three (range from 0 to 90). Confirmed HBD had a median of four reactors (range 0 - 90) and not confirmed HBD (33 out of 112), had a median of one (range 0 - 7). The number of zero reactors was given at four HBD which were confirmed at post-mortem (VE-SLH test) and at three other no confirmed HBD tested with VE-CON, VE-CON12 and VE-6M tests (see Table 3.9 above). The VE-SLH tests does not disclose reactors at the test but rather positive animals at post-mortem inspection. The reason why there were not reactors in the last three tests is unknown, and it could have been due to a data entry error in VetNet.

Table 3.9 - Percentage of HBD out of a total 112 in the study period by test type and number of reactors per disclosing test

Test Type	No reactors		One reactor		Two reactors		More than two reactors	
	Number of tests	%	Number of tests	%	Number of tests	%	Number of tests	%
VE-12M	0	0.00	3	6.52	0	0.00	3	8.82
VE-6M	1*	14.29	13	28.26	4	16.67	10	29.41
VE-CON	1*	14.29	2	4.35	3	12.50	1	2.94
VE-CON12	1*	14.29	3	6.52	0	0.00	3	8.82
VE-CON6	0	0.00	0	0.00	2	8.33	4	11.76
VE-CT	0	0.00	3	6.52	5	20.83	1	2.94
VE-IR	0	0.00	11	23.91	5	20.83	2	5.88
VE-PII	0	0.00	0	0.00	0	0.00	0	0.00
VE-PR	0	0.00	0	0.00	0	0.00	0	0.00
VE-PRI	0	0.00	0	0.00	0	0.00	0	0.00
VE-RHT	0	0.00	2	4.35	0	0.00	0	0.00
VE-SI	0	0.00	1	2.17	1	4.17	2	5.88
VE-SLH	4**	57.14	0	0.00	0	0.00	0	0.00
VE-TR	0	0.00	1	2.17	0	0.00	0	0.00
VE-WHT	0	0.00	7	15.22	4	16.67	8	23.53
Total	7	100.00	46.00	100.00	24.00	100.00	34.00	100.00

(*) As recorded on VetNet. The number of reactors at these tests should have been at least one.
(**) At a VE-SLH test the number of reactors at test is zero. HBD is disclosed by post-mortem inspection at slaugterhouse.

Appendix 3.1 presents the number of HBD per herd and test date when it was disclosed, together with the number of animals tested and disclosed as reactors.

Confirmed HBD - Out of the 112 HBD, 69% were confirmed by lesions at post-mortem inspection and/or culture, 29% were not and 2% was not specified in the VetNet database. Confirmed HBD by restocking status are described below.

Restocked *vs* continuously stocked - During the study period (1st October 2001 to 1st November 2004), 50% (18/36) restocked herds and 54% (61/112) continuously stocked herds had a HBD. Of the 18 restocked herds, 67% had one HBD, 28% had two and 5% had three. And of the 61 continuously stocked herds, 66% had one HBD, 26% had two and 8% had three.

Out of the 112 HBD, 29.5% were disclosed each in the first and third years and 41% in the second year. As with the percentage of tests by year of the study (Figure 3.8), during the first year, the percentage of number of HBD in continuously stocked

herds was higher than in restocked and *vice-versa* in the second and third years of the study (Figure 3.8). However, the difference in the number of HBD between years on restocked and continuously stocked herds, was not statistically significant (Chi-square = 2.82, df = 2, p =0.24).

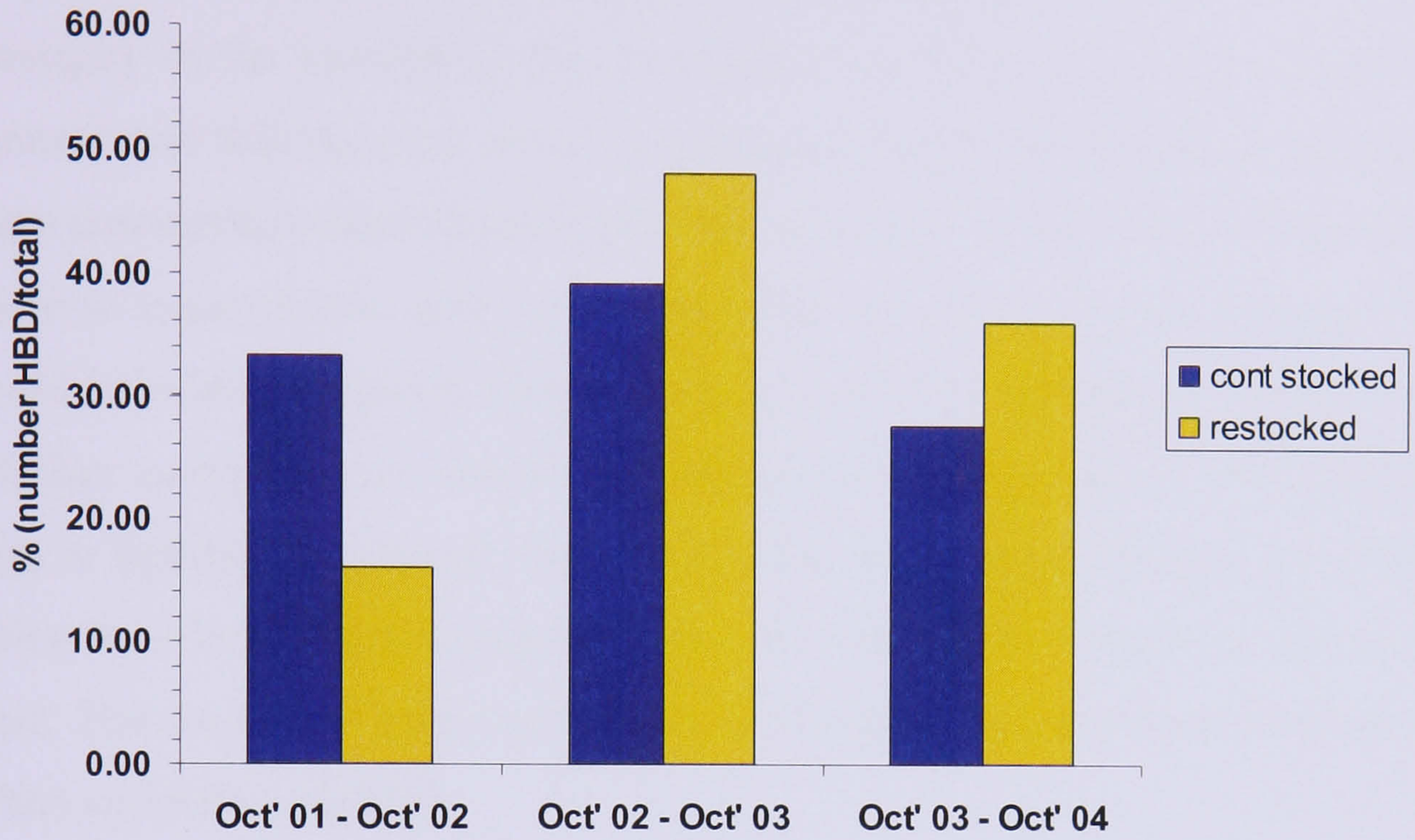


Figure 3.8 - Percentage of HBD per study year from 112 HBD in the total study period by restocking status

During the study, 1,923 cattle were recorded in VetNet with test results such as DC (dangerous contact), CT (contact tracing), IR (inconclusive reactor), R (reactor) or SL (disclosed at slaughter). In Table 3.10, the numbers of IR and R are presented, as well as the number and percentage of reactors which were confirmed by lesions at slaughter and or culture.

Table 3.10 - Number and percentage of Inconclusive Reactors (IR), reactors (R) and confirmation by lesions and or culture from 1,923 animal tests

Test result	**% (number/total)**	**Restocked**	
		Yes	**No**
IR	56% (1078/1923)	11% (120/1078)	89% (958/1078)
R	72% (774/1078)	9% (73/774)	91% (701/774)
Confirmation of reactors			
VL*	29% (223/774)	40% (29/73)	28% (194/701)
VL* and culture of *M.bovis*	80% (179/223)	86% (25/29)	79% (154/194)
NVL and culture of *M. bovis***	13% (28/208)	4% (1/26)	15% (27/182)

*VL=visible lesions at post-mortem inspection; **NVL=no visible lesions

3.4.4 The farmers' perception of bTB and the SICCT

At the end of the standard questionnaire, farmers were asked whether they wanted to give any comments on why they thought their herds had or did not have bTB. A summary of the answers given is presented in Table 3.11. Approximately 60% of farmers said that they had never experienced bTB in their herds in the past. Most of them commented about the test, the role of badgers on the farm and the attraction of these to maize fields, and the benefits that vaccination of both badgers and cattle could have on eradication. Others were critical about the lack of measures taken and whether more input on farmer education and investigation of other diseases would help in fighting the disease, whilst just a few thought that farmers and officials were doing their best. Some wondered about the possibility of transmission by birds and deer. The impact of cattle movement after FMD was also mentioned as having an effect on HBD with bTB.

Table 3.11 - Reasons why farmers thought their herds had or not had bTB in the past

Reasons why had/not bTB	Herd had bTB in the past	Herd never had bTB
wildlife (mostly badgers)	25.00%	16.60%
purchasing practices	12.50%	18.30%
don't know	38.50%	35.00%
other reasons	15.00%	21.60%

Among other reasons, farmers were quite concerned about the current test practices. Some claimed that the test should not be allowed to be postponed by the farmer at his/her convenient time; that there was a need for a more accurate test and grower cattle for beef production should not miss the test.

They were also asked about what they thought was the best and the worst thing about the test. Approximately 30% responded to the first question and 40% to the second. Some farmers saw the test as a measure to reduce the public health risk; as a good time to treat cattle for other diseases and for sorting out ear-tag numbers whilst cattle were restrained. Others thought that standards of the test were high when veterinary surgeons always performed the test in the same way and others saw the completion of the test in a short period of time as an advantage. The test was seen by many farmers as a stressful time for the cattle and as a disruption for the herd.

3.5 Discussion

3.5.1 Description of farms

The number of farms initially provided, within the selection criteria for the study, varied considerably between counties. However, approximately 30% (36%-23.5%) of farms provided per county were recruited. All counties were therefore approximately equally represented in the study sample. Due to the lower number of restocked farms initially provided, the total number of restocked was only 24% compared with 76% continuously stocked, with a variation from 9% to 27% of the restocked farms represented in the counties of Cornwall and Devon respectively, and a variation between 72.6% and 91% of continuously stocked farms represented in Devon and Cornwall respectively.

The different three treatments from the RBCT were approximately equally distributed with 32% of the farms in the reactive, 35.5% in proactive and 28% in survey trial areas. The first two areas were mostly in Devonshire and the survey was mostly in Gloucestershire, however, what was most important was the evenly distributed number of farms by intervention treatment, which would have been implemented in the same way in all the counties.

The herd-types were represented in both restocked and continuously stocked herds. Within the 60% that had only one type, dairy, suckler and young stock herds were represented from 38.5%, 24% and 37.5% respectively. A 40% of the herds were mixed. Herd size was similar in restocked and continuously stocked herds, but there were differences within counties, with the highest percentage of large herd sizes (over 200 cattle) and none of between one hundred and one and two hundred cattle in Somerset.

3.5.2 Description of the tests

The percentage of HBD increased between the first and the second year of study, especially on restocked herds, and decreased towards the third year (Figure 3.7) accompanied by an increased number of tests in the last two years (Figure 3.8).

The FMD outbreak in 2001 caused a delay in the bTB routine testing and movement restrictions from 31st January 2002 were imposed for herds that were overdue a test (Le Fevre *et al.,* 2005). Because of these reasons, it is possible that cattle infected remained undetected posing a risk to other cattle (also suggested by Phillips *et al.,* 2003). In fact, between January and February 2001, 31 study herds were tested; only 10 were tested between March and September 2001 and 16 were tested for the first time during the first three months of the study. Therefore it is not surprising to see a higher percentage of HBD in continuously stocked herds in the first year of the study, and a decrease thereafter as herds were starting to be tested as the frequency of testing increased until returning to normal testing routine.

Restocked herds were newly formed herds compared to continuously stocked, where cattle could have been bought from different sources and from geographical areas with a different testing regime. This is a likely reason why, compared with continuously stocked herds, in which there was a possibility of infected cattle not being removed due to the reasons mentioned above, restocked herds broke down less in the first year. Herds that were overdue a test due to FMD, were given priority to be tested earlier, and this would have been the case in continuously stocked herds. The increase in the last two years in the restocked herds could be explained by both an increase in testing and a residual infection (environmental) that was left from before FMD in 2001 could have been sufficiently high to serve as infection to newly purchased cattle.

Despite 89% of the animal results being within the continuously stocked herds, the percentage of inconclusive reactors and reactors in restocked and continuously stocked herds were very similar (58% and 56% were inconclusive reactors and 35% and 40% were reactors respectively), which could be a sign of consistency in the interpretation of the test throughout restocked and continuously stocked herds.

To summarise, all tests in the study period from recruited restocked farms from a population sample and continuously stocked farms from a matched and randomly selected sample, were used to give an overall description of the study sample. The process of recruitment (as described in the previous chapter), the description of tests

and detailed characteristics of the study farms would have hopefully provided a good description of the study subjects used in the following chapters of this thesis.

As the objective of this study was to make inferences on the risk of HBD in herds from a population of farms affected by FMD *vs* randomly selected farms unaffected and both controlled for the effect from the RBCT, the results that follow in the next chapters should be taken with caution when extrapolated to all farms in the region or in the country, as FMD only occurred in some areas of the country and the RBCT was only in the South West region. However, they can be used as very valuable set up for the investigation of the behaviour of *M. bovis* in cattle herds having controlled for the effect of the main recognised wildlife reservoir.

Chapter 4 - Risk factors investigation for first herd breakdown on unrestricted study herds

4.1 Aims

The association of farm risk factors with first herd breakdown (HBD) with bTB on unrestricted herds over the three-year study period was explored in this chapter. The information used was original field data collected using a standard questionnaire. Kaplan-Meier plots for observation on probability of survival until first HBD if occurred or until the end of the study and Cox proportional hazard models for multivariable model building were used.

4.2 Introduction

Risk factors associated with HBD or presence of bTB in cattle herds have been investigated in the past using case-control and descriptive studies, the majority being carried out in Ireland, Northern Ireland, New Zealand and the UK (some studies are presented in Chapter 1, Table 1.4). The use of cohort studies and the analysis using proportional hazards regression models have previously been suggested (Morris *et al.*, 1994).

This chapter presents the investigation of risk factors associated with farm management practices and time to first HBD, in a geographical area of the country where some farms went through an unusual restocking process due to depopulation during the FMD outbreak in 2001 and where the badger intervention trial (RCBT) was taking place since 1998.

Studies of animal health and production often involve the investigation of many explanatory variables. So, it is important to carry out a careful screening of variables or for example, grouping by farm management factors (Dohoo *et al.*, 1996).

Data used are original field data collected using a questionnaire specifically designed for this study. Questions about herd and farm management were asked for

the period October 2001 to June 2003. Survival analysis was used to examine factors associated with HBD.

4.3 Materials and Methods

4.3.1 Source of data

The second section of the farmer questionnaire used in this chapter was described in Chapter 2. Table 4.1 presents a summary of the farm management/practices.

Table 4.1 - List of main aspects of farm management and practices from the farmer questionnaire

Risk factor group	Description
General	Purpose of use of cattle, herd size, ownership
Manure/slurry	animal origin, where produced, type, storage, use of spreader and whether shared, spreading time and time of storage
Bedding	type, where produced, where stored, wildlife presence in stores
Feeding	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
Diseases	persistence of disease, presence of BVDV, IBR, Johne's disease, Neosporosis or Leptospirosis, and other clinical signs since January 2000
Vaccinations	reasons, type of vaccines given since January 2000
Purchase of cattle	number and type of cattle bought since January 2000, source, country or area of origin, TB status of source herd
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

4.3.2 Statistical analysis

4.3.2.1 Type of analysis

Survival analysis was carried out using S-Plus version 6.2 (Selvin, 1998). Assumptions made by the Cox regression model are that there must be an identifiable start point and a common end point, that withdrawals or losses to follow up are not related to the outcome and that the hazard is proportional during the study period, (so it is time independent). The first three assumptions were controlled for in the study design.

Staggered entry time into the study was used. All herds entered the study on 1st October 2001 except thirteen herds that were under restriction with a HBD on that

date and entered the study between 10th October 2001 and 21st August 2003. The end of the study was the 1st November 2004.

Eleven farmers withdrew from the study but the farmer questionnaires had already been completed by the time the farmers dropped out. Eight of these sold their herd or stopped farming and the other three stopped due to other different reasons. Herds that had not experienced a HBD by 1st November 2004 were censored.

4.3.2.2 The outcome and explanatory variables

The outcome variable - The study unit was the herd. The outcome variable, time to first HBD (failure) on unrestricted herds, was observed from 1st October 2001 to 1st November 2004. A HBD was confirmed if there was at least one reactor in the disclosing test which had been confirmed either by *post mortem* examination at the slaughterhouse and or by culture in the laboratory. All first HBD on unrestricted herds, whether confirmed or not, were considered in the analysis. Restocked farms in the study had started repopulating by the time the study started.

The explanatory variables - The interview questionnaire asked data retrospectively for the period October 2001 to June 2003. There were 190 identified explanatory variables. These were managed within areas of farm practices (Table 4.1). Continuous variables were checked for linearity. Some variables (i.e. the treatment within the RBCT) were categorical. Most variables were not exclusive (i.e. most farms would store manure/slurry in more than one form) and in the analysis were entered as binary variables rather than categorical to avoid ending up with very low number of observations within each class. Some variables were re-grouped. For example: the type of storage of manure/slurry was grouped into two categories based on the environmental conditions of storage of the manure/slurry and protection from exposure to sunlight which compared to outdoor conditions, could favour the survival of *M. bovis*. These variables were: "stored indoors/close containment", if the manure/slurry was stored in buildings or in a pit, silo, spreader or tank or "outdoors" if stored in fields, yards, heap or not stored.

Questionnaire data were entered into Tables in an Access database. Tables were then imported into S-Plus. Binary variables were coded as 1/0 for yes/no

answers. Categorical variables were coded as numbers (0,1,2,etc) based on an alphabetical order of the names of the categories.

4.3.2.3 The analysis process

Variables were screened at univariable level using Kaplan-Meier plots to observe patterns over time. For all plots in the study, time was represented by the number of days the farm was in the study, from date of entry to first HBD or to the censoring date. Cox proportional hazards regression models were built to examine variables at univariable and multivariable levels. The risk of HBD was investigated for the whole study period (1st October 2001 to 1st November 2004).

The analysis was carried out in five steps, using a stepwise regression (Dohoo *et al.*, 2003). First, univariable analysis was done on all initially identified variables. Then forward selection on small models by farm practices/management group. In that, variables were selected even if they were not statistically significant at $p \leq 0.20$ at univariable level. The baseline within the group was selected if the number of observations was nine or more and statistically significant at $p \leq 0.20$. Six preliminary models were built. Thirdly, a pre-final model was created using a stepwise forward selection with variables from all the preliminary models selected using the same criteria as before for the number of observations and p value. Fourthly, the final multivariable model was created using a backward elimination.

4.4 Results

4.4.1 Description of first HBD on unrestricted herds during the study period

By 1st November 2004, 75 (50%) of unrestricted study herds had broken down at least once with bTB (Figure 4.1). Thirteen herds were under restriction with a HBD on 1st October 2001 and entered the study between 10th October 2001 and 21st August 2003. By the end of November 2004, seven out of the thirteen had broken down once more.

Out of the 75 HBD, approximately 79% (59/75) occurred in continuously stocked herds and the other 21% in restocked herds. That is, 53% of continuously stocked

herds and 44% of the restocked herds experienced a HBD when the herds were unrestricted, with no statistical difference between the two groups (chi-square = 0.9 p=0.34).

In the first year, from October 2001 to October 2002, 41% broke down (13% of those were restocked). In the second and third year, the proportion of restocked herds that broke down increased, with 28.5% out of the 47% that broke down in the second and 22% out of the 12% that broke down in the last year being restocked..

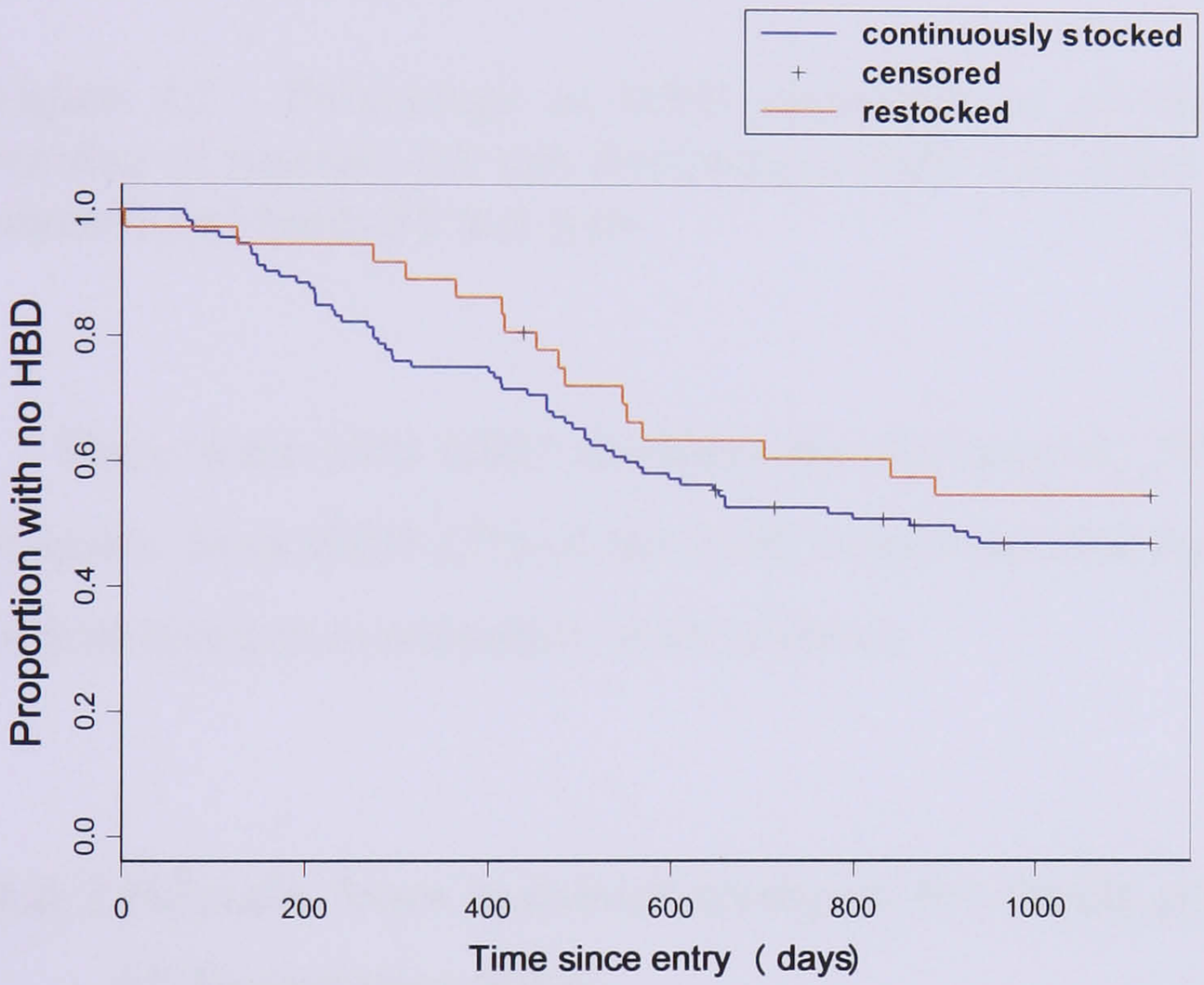


Figure 4.1 - Kaplan-Meier survival curve for 148 study herds on the time to survival until the first HBD from 1st October 2001 to 1st November 2004 for all herds, restocked and continuously stocked herds

During the study period, some herds were more frequently tested than others prior to their first HBD. Out of the 75 herds which had a first HBD, approximately 55% (41/75) were tested since 1st October 2001 at least once, before they broke down: 19 herds were tested once, 13 twice, 5 three times, 2 four times and 2 five.

To show the type of test that was used to disclose the 75 HBD and how many reactors were disclosed by each type, Figure 4.2 is presented. A total of 283 reactors were disclosed at these 75 HBD.

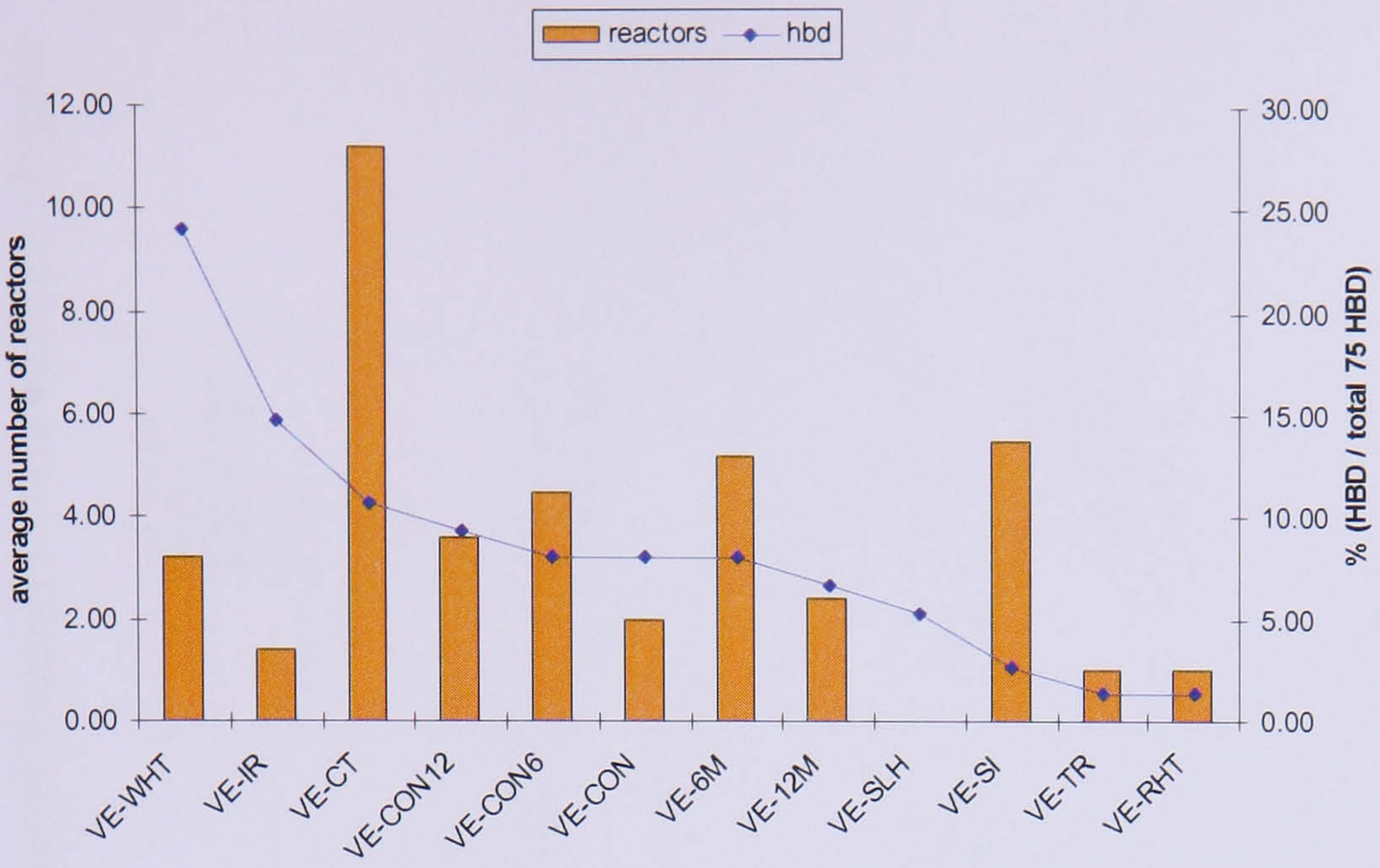


Figure 4.2 - Percentage of HBD disclosed out of the 75 first HBD and average number of reactors per test disclosing a HBD out of the 75 first HBD in the study on unrestricted herds by test type.

There were 35% HBD disclosed by one reactor, 25% by two and 11% by three reactors. Four HBD (5% of the total) were disclosed by cattle with lesions observed at post mortem examination at the abattoir.

4.4.2 Results from survival analysis for study period 1st October 2001 to 1st November 2004

4.4.2.1 Univariable results

Variables were screened at univariable level. The results are presented in Appendix 4.1 and include the number of observations per variable. Kaplan Meier curves were plotted to explore variables at univariable level and some variables that were statistically significant at this level are presented in Figure 4.3.

Figure 4.3 - Kaplan-Meier survival plots for purpose of herd (Figure 4.3.1), herd size (Figure 4.3.2), purchase of livestock from markets (Figure 4.3.3) and storage of manure/slurry indoors/closed containment (Figure 4.3.4).

Fig.4.3.1

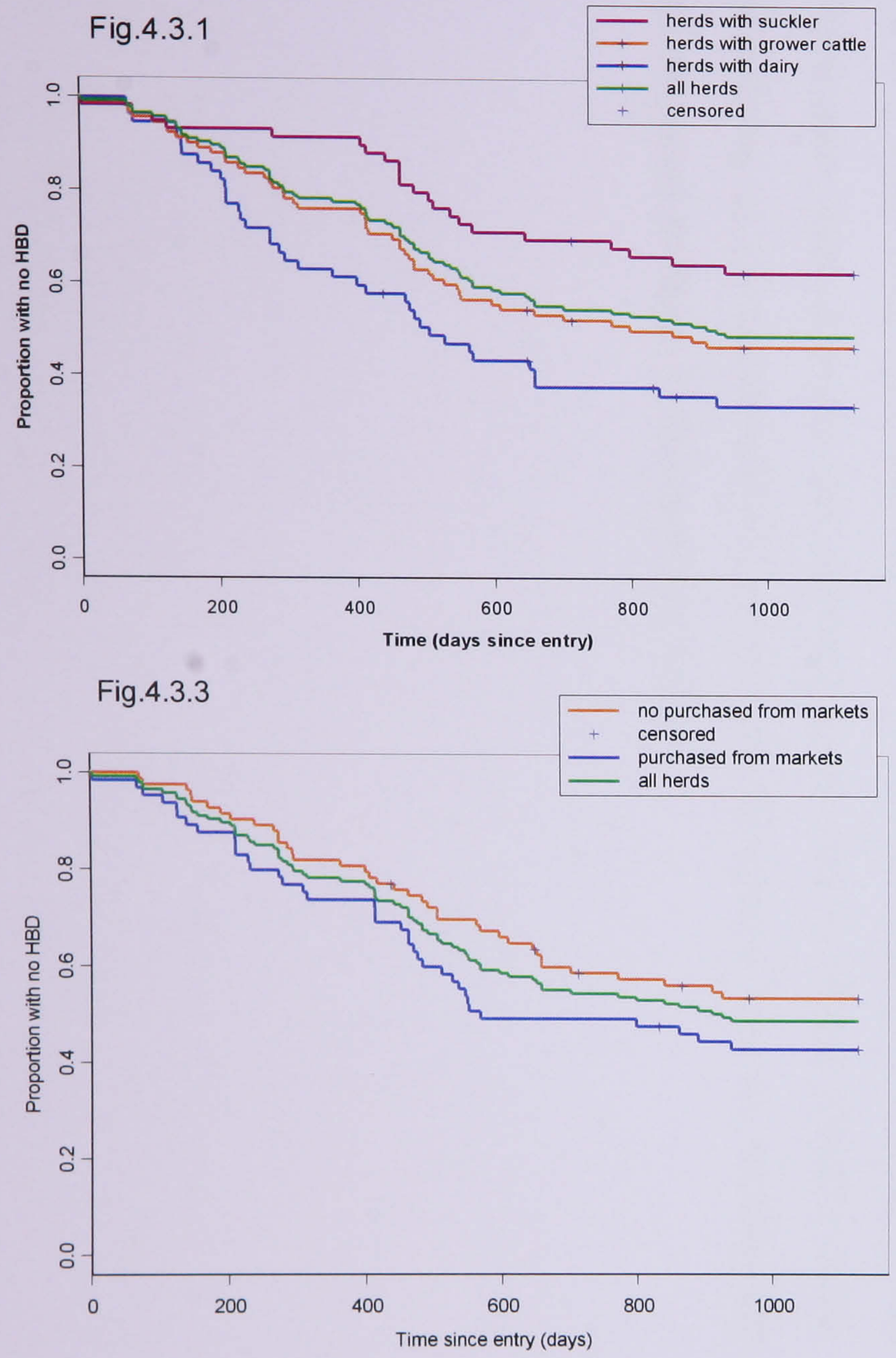


Fig.4.3.2

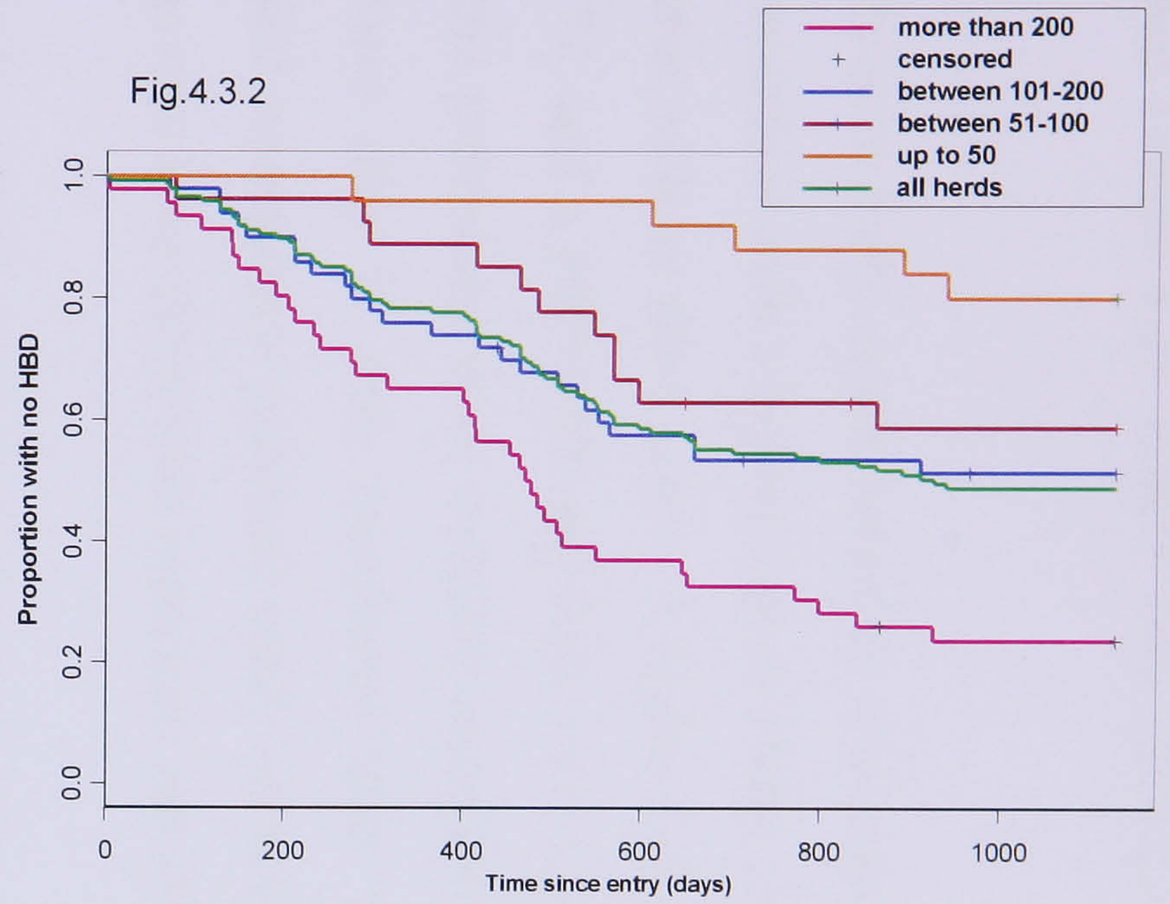
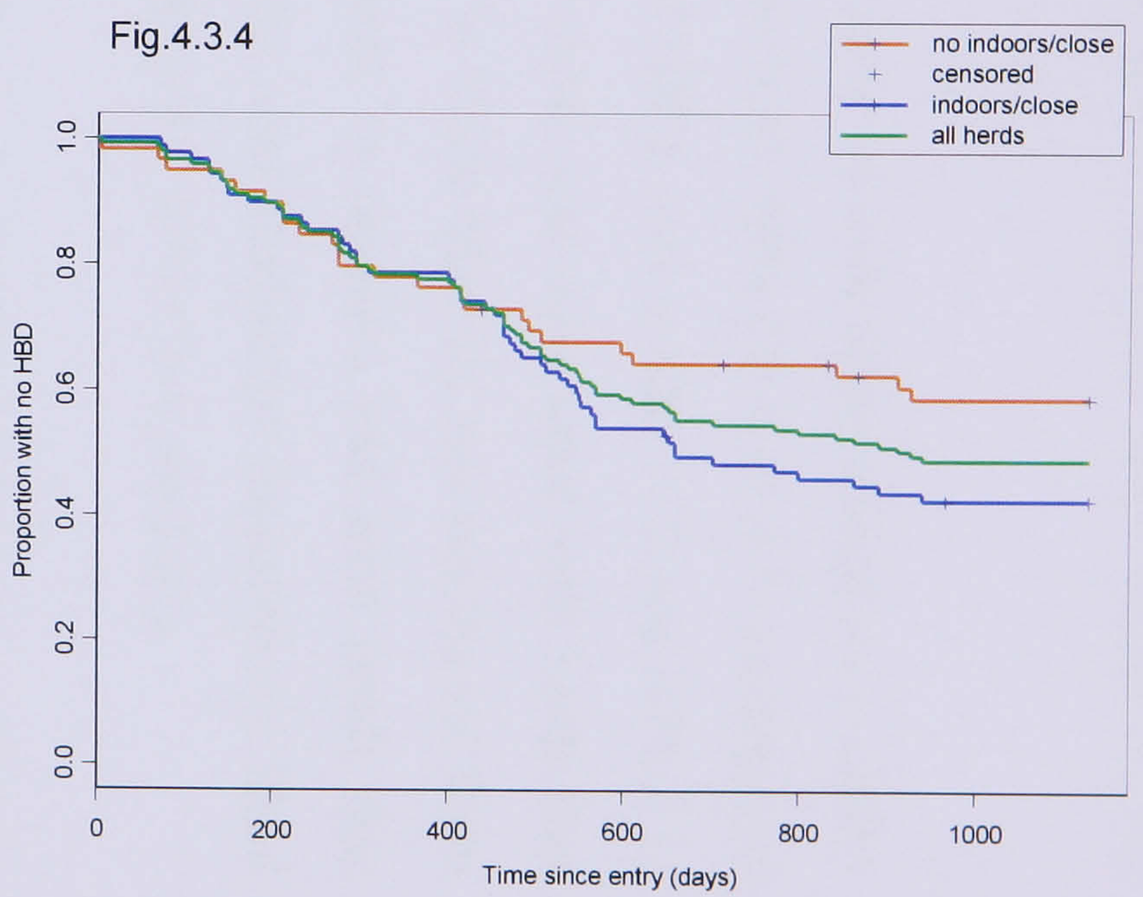


Fig.4.3.3

Fig.4.3.4



4.4.2.2 Multivariable results

A multivariable model using Cox's proportional hazards regression was developed (Table 4.2). RBCT and restocking status variables were forced in the model.

Herds with dairy cattle (whether with dairy only or with dairy and grower cattle) were associated with a higher risk of HBD, (HR=2.52, 95% CI=1.41-4.51) compared with herds without dairy cattle. A higher risk of HBD, with a HR=1.72 and 95% CI= 1.06-2.77, and a HR=1.93 and 95% CI= 1.05–3.56, was associated with herds that had cattle purchased from markets compared to those that did not purchased from that source (that is, they purchased from farms or dealers) and with herds that purchased young male castrated cattle (steers) for beef production respectively. Herd size was also associated with increased risk (HR=2.01, 95% CI=1.44-2.81).

Farmers stored manure and slurry outdoors or in buildings/close containment (such as in a pit, silo, spreader or tank). The latter was associated with an increased risk of HBD with a HR=2.18 and 95% CI=1.24-3.82. Herds that provided cattle with vitamins and minerals (these in the form of licks) *vs* those that did not, were associated with a decreased risk of HBD with a HR=0.49, 95% CI=0.29-0.80. Both the RBCT treatment and restocking status were not significantly associated with risk at a p value of ≤ 0.05. Within the areas defined by triplets in the RBCT, herds in the county of Somerset were at lower risk of break down (HR=0.74 and 95%CI= 0.59-0.92), whilst being in Gloucestershire/Hereford and Worcester was not statistically significant compared to those in Devon/Cornwall..

Table 4.2 - Results of a multivariable Cox's proportional hazards model for time to first HBD on 148 study herds from 1st October 2001 to 1st November 2004

Variable	Covariate	n	coef	S.E.	*P*	HR	95% CI
Purpose of cattle	Dairy cattle	57	0.92	0.29	<0.01	2.52	1.41 - 4.51
	No dairy cattle	91	ref				
Herd size	Ln (number of cattle)	148	0.70	0.17	<0.001	2.01	1.44 - 2.81
Type of storage	Indoors/close containment	99	0.77	0.14	<0.01	2.18	1.24 - 3.82
of manure/slurry	Not indoors/close containment	49	ref				
Use of feedingstuffs	Minerals/vits/licks	72	-0.71	0.25	<0.01	0.49	0.29 - 0.80
	No minerals/vits/licks	76	ref				
Purchase practices	From market	65	0.54	0.24	0.02	1.72	1.06 - 2.77
	Not from market	83	ref				
	Castrated male cattle(steers)	40	0.66	0.31	0.03	1.93	1.05 - 3.56
	No castrated male cattle	108	ref				
RBCT treatment	Reactive	55	0.14	0.21	0.52	1.15	0.75 - 1.76
	Proactive	41	0.09	0.1	0.38	1.10	0.88 - 1.36
	Survey only	52	ref				
RBCT area	Gloucester/Hereford&Worcester	49	0.44	0.29	0.13	1.55	0.88 - 2.74
	Somerset	18	-0.30	0.11	<0.01	0.73	0.59 - 0.91
	Devon and Cornwall	81	ref				
Restocking status	Restocked	36	-0.38	0.31	0.22	0.68	0.37 - 1.26
	Continuously stocked	112	ref				

R^2=0.34

4.5 Discussion

4.5.1 Results from the multivariable Cox proportional hazards model

4.5.1.1 Purpose of the herd

Dairy herds have previously been reported to be associated with an increased risk of infection (Morris *et al.*, 1994). The presence of dairy cattle in the herd was associated with an increased risk of HBD in the multivariable model. Dairy as well as suckler or breeding cattle in general, remain on the farm for longer periods of time compared to growing beef cattle. Based on this, dairy cattle could be potentially more exposed to infection than beef cattle. Based on the length of time cattle spend on the farm, suckler cattle would be expected to be at higher risk compared to beef cattle, but suckler cattle were not associated with a risk in the analysis. In the study, suckler herds were less frequently tested compared to dairy. Also, the exposure of the latter to a greater amount of stress compared to other type of cattle has previously been reported although, there is little evidence to support this.

The purpose of the herd (herd type) was categorised based on the presence of dairy, suckler or grower cattle for beef in the herd, regardless whether they were mixed or not. The Kaplan-Meier survival curve in Figure 4.3 shows that dairy herds (including those mixed with growing cattle for beef) had a higher risk of HBD compared with herds that did not have dairy cattle. This would be on line with the results reported by Marangon *et al.*(1998) where the presence of both beef and dairy cattle in the herd was reported to be associated with an increased risk of bTB and they based their findings in the probability of introduction of infection into the dairy herd through the purchase of beef cattle.

4.5.1.2 Herd size

Herd size has been a reported risk for a bTB breakdown in previous studies (Pfeiffer and Morris, 1991; Griffin *et al.*, 1996; Olea-Popelka *et al.*, 2004) whereas Marangon *et al.*(1998) did not find it statistically significant. As reported in these studies, herd size could be a proxy for an intensive farming system. However, in our models we adjusted for those variables that are associated with intensive systems (i.e. dairy herd as a type of herd, use of concentrates, purchase of cattle, length of manure/slurry storage, etc). Another plausible explanation is that it could be related to the performance of the test. Larger herds could be more susceptible to a lower standard of test performance due to reasons such as the difficulty in the handling of wilder animals and the time involved in completing the test (as observed in the field when a test has been carried out). Therefore, reactor animals from larger herds could remain undetected and this could result in a positive test the following time the herd is tested.

4.5.1.3 Purchasing practices

The purchase of steers (young castrated male cattle used for beef) was associated with an increased risk of HBD. The purchase of cattle (Griffin *et al.*, 1992; Marangon *et al.*, 1998; Johnston *et al.*, 2005) and younger cattle (Pfeiffer and Morris, 1991; Griffin *et al.*, 1993) has been reported previously as a risk factor for bTB. If steers were purchased from beef herds only, instead of mixed herds, they would have been less frequently tested. It is also possible that they moved between herds without

being tested: Mitchell *et al.* (2006) reported that at national level, approximately 80% of cattle were not tested in their lifetime.

Farms that purchased cattle from markets compared to farms that did not, were at higher risk of HBD (reported also by Johnston *et al.,* 2005). From the total 148 farmers in the study, 68% did not know the bTB status of the source of the cattle that they bought from and this was more likely to be the case when purchasing from markets.

4.5.1.4 Storage of manure

In the multivariable model, the storage indoors/close containment was associated with an increased risk of HBD compared to farms that did not store manure/slurry in this way. We believe this is an important finding, since the survival of *M. bovis* would be favoured if protected from the sunlight, by high water content, the amount of organic matter present and slightly alkaline pH of the cattle slurry. The prolonged survival of any microbial pathogens, including *M. bovis*, is possible in stored slurry (Menzies and Neill, 2000; Scanlon and Quinn, 2000).

The length of time for manure/slurry storage was not associated with a risk. Reilly and Courtenay (2007) reported an increased risk for transient HBD on farms that stored manure for more than six months. Based on previous suggestions that longer periods of storage may decrease the survival of *M. bovis* and based on the results presented here, the result by the last authors could be interpreted as a proxy for the type of storage (i.e. indoors/close containment) rather than the length of the storage period. To support this argument, Figure 4.3.4 shows that herds that stored manure/slurry in buildings/close containment were at higher risk than those which did not store in this way. These broke down after some time of being in the study, which could be interpreted as the long term effect of the storage of manure/slurry.

In previous studies, the production of slurry *vs.* manure was associated with an increased risk (Griffin *et al.*, 1993) and the spread of farm yard manure was associated with a decrease risk (Johnston *et al.*, 2005). In the preliminary analysis, the production of whole slurry and separated liquid was associated with an increased

risk and the use of farm yard manure with a decreased risk of HBD, but these were not statistically significant at the multivariable level.

4.5.1.5 Other variables

Since the study was designed in areas of the RBCT and the permanent selected exposure was restocking *vs* continuously stocked herds, it was thought to be most appropriate to force these variables into the model, despite none of them being associated with a risk in the whole period of the study. When these variables were removed from the model, the coefficients for the main variables in the model did not change significantly. Based on these two arguments, these two variables used in the design were left in the model.

The use of vitamins and minerals as licks was associated with a decreased risk of HBD, contrary to the findings by Griffin *et al.* (1993). In the preliminary univariable analysis, feeding of maize silage was associated with an increased risk for HBD, previously reported by Goodchild and Clifton-Hadley (2001). However, this was not statistically significant in the final model.

Preliminary analysis also showed that within the type of contacts with cattle from other herds (returns from markets, breaks in and out from and to contiguous farms, cattle walking through the farmland, and hiring bull out), hiring a bull in, was associated with a decreased risk of HBD compared to herds that did not have this contact. The hiring of a bull for reproduction purposes could reflect good management practices if farmers always made sure they hired from a free bTB status herd. On the other hand, it could be argued that "closed" herds, understood as those that do not hire bulls and use their own, could be reducing the risk on introduction of diseases into the herd, including bTB. However, in areas like the SW of England where bTB is endemic, hiring a bull may not necessarily pose a risk if the source farm is not in the high risk area.

Other factors such as the presence of wildlife in bedding and feeding stores; the use of different types of bedding and of hired equipment, were investigated but none were found to be associated with a risk in the final multivariable model.

Collinearity was checked for by using correlation coefficients between variables in the model and variables that in preliminary sub-models showed to be statistically significant with $p \leq 0.1$ (Table 4.3).

Table 4.3 - Correlation coefficients for variables in the multivariable model and other variables from preliminary sub-models with $p \leq 0.1$

Correlation Coefficients	Restocking status	RBCT treatment	RBCT location	Dairy cattle	Herd size	Slurry/ manure stored indoors/ close cont.	Use of minerals vitamins licks	Purchase from markets	Purchase of steers
Restocking status	-	0.03	0.06	-0.16	0.00	0.01	-0.11	0.04	0.19
RBCT treatment	0.03	-	0.14	0.20	0.07	-0.04	0.04	-0.02	-0.12
RBCT location	0.06	0.14	-	0.16	0.23	-0.28	0.08	-0.06	-0.08
Dairy cattle	-0.16	0.20	0.16	-	0.40	-0.15	-0.05	-0.08	-0.29
Herd size	0.00	0.07	0.23	0.40	-	-0.12	-0.05	0.01	0.09
Slurry/manure stored indoors/close containment	0.01	-0.04	-0.28	-0.15	-0.12	-	0.10	0.05	0.15
Minerals/vitamins /licks	-0.11	0.04	0.08	-0.05	-0.05	0.10	-	0.06	-0.14
Purchased from market	0.04	-0.02	-0.06	-0.08	0.01	0.05	0.06	-	0.23
Purchased steers	0.19	-0.12	-0.08	-0.29	0.09	0.15	-0.14	0.23	-
Other purpose of cattle									
Suckler cattle	0.09	-0.08	-0.07	-0.64	-0.16	0.27	0.23	0.03	0.09
Slurry/manure management									
Stored all year	-0.04	0.21	0.01	0.56	0.34	-0.17	-0.02	0.07	-0.10
Spread all year	-0.04	0.17	-0.05	0.25	0.18	0.09	0.06	0.12	-0.07
Use of separated liquid	-0.16	-0.01	-0.04	0.20	0.16	-0.03	-0.17	-0.04	-0.06
Use of whole slurry	0.01	0.10	0.23	0.61	0.42	-0.22	0.08	-0.05	-0.19
Use of feedingstuffs									
Maize silage	0.02	0.09	-0.06	0.28	0.27	-0.07	-0.13	0.02	-0.05
Grass silage (clamp)	-0.04	0.03	0.06	0.47	0.56	-0.06	-0.09	-0.09	0.02
Grass silage	-0.07	-0.02	0.14	0.21	0.39	0.05	0.03	-0.05	0.08
Wheat straw big bales	-0.10	0.18	-0.29	0.25	0.12	0.03	-0.03	0.05	0.00
Hay big round bales	0.07	0.14	0.22	-0.06	-0.17	0.14	0.17	0.12	-0.11
By-products	-0.02	0.11	-0.18	0.13	0.10	-0.03	-0.03	-0.08	-0.09
Use of hay rack	0.08	0.03	0.01	0.06	0.26	0.02	0.13	0.03	0.13
Contact with other cattle									
Bulls hired in	-0.06	0.12	0.11	0.14	0.00	0.06	0.02	0.01	-0.16
Bulls hired out	-0.01	-0.03	-0.02	-0.20	-0.09	-0.04	0.22	0.06	0.13
Returns from markets	0.03	0.12	0.08	-0.04	-0.15	-0.05	0.06	-0.02	-0.10
Other purchased cattle									
Purchase of heifers	0.23	0.12	-0.09	0.00	0.12	0.14	-0.07	0.29	0.32
Equipment hired and staff working with herd									
Maize harvesting	-0.06	0.15	-0.05	0.31	0.27	-0.12	-0.14	-0.09	-0.05
Staff working with herd	-0.05	-0.11	0.10	0.14	0.38	-0.08	-0.07	-0.09	0.12
Herd had bTB prior Oct'01	-0.30	0.10	0.07	0.11	0.22	-0.06	-0.03	0.01	-0.04

4.5.2 Study design

Since risk data extended from October 2001 to June 2003, and the outcome between October 2001 and November 2004, it could be argued that farm management and practices could have changed between June 2003 and November 2004. However, given the detail of the data collected, and the temporal dynamics of HBD due to the natural history of bTB, it is highly likely that the data reflect the farm practices carried out by farmers during the relevant risk period.

No exclusion criteria were applied for the type of test used to disclose a HBD (as both herd and individual tests could disclose a HBD) and whether the HBD had reactors confirmed by post-mortem examination and or culture. This is appropriate since firstly, 75% of the HBD were confirmed, and secondly since movement restrictions are applied to herds whether or not the HBD has been confirmed.

4.5.3 The statistical analysis, model assumptions and model fit

One of the requirements in survival analysis is that the study unit is free of disease at the beginning of the study (Kleinbaum, 1996). The bTB disease status of a herd is based on the last test carried out. Most herds, over 80%, had been tested within the previous year and a half prior to October 2001, with 50% of these tested within the previous year. Movement restrictions apply when there is a HBD on the farm, and here, all herds were unrestricted when they entered the study. Given that the disease can develop as a chronic disease, and given the sensitivity of the skin test is only approximately 74%, the real disease status of the herds is difficult to assess. However, the study was carried out using the only available test data and on a geographical area where herds are tested at least annually.

The proportionality assumption for the Cox proportional hazards regression model was checked by using the test and plots of Schoenfeld residuals. The plot of the deviance residuals was used to detect outliers and model fit was assessed by the Cox-Snell residuals plot.

The Schoenfeld residuals global test showed that the assumption was violated ($p=0.01$) when restocking status and RBCT variables were in the model (Table 4.4).

However, for most variables the assumption was met and this was made more obvious when restocking and RBCT variables were excluded (p=0.20) (Table 4.5). As mentioned before, these variables were only included in the models because they were used in the study selection criteria.

Table 4.4 - Results from the Schoenfeld residuals test for all variables in the Cox proportional hazards model

Variables	**rho**	**chisq**	***p***
Restocking status	0.15	2.18	0.14
Dairy cattle	-0.11	1.06	0.30
Ln (number of cattle)	-0.19	3.17	0.07
Minerals/vits/licks	0.12	1.19	0.27
Manure/slurry stored indoors/close containment	0.15	2.04	0.15
RBCT treatment			
Reactive	-0.01	0.02	0.89
Proactive	0.25	6.00	0.01
Survey only	ref		
RBCT area			
Glouces/Her&Worc	-0.11	1.40	0.23
Somerset	0.08	0.57	0.45
Devon/Cornwall	ref		
purchase of steers	-0.04	0.13	0.72
purchase from market	-0.07	0.32	0.57
GLOBAL	NA	23.35	0.01

Table 4.5 - Results from the Schoenfeld residuals test for variables in the Cox proportional hazards model excluding restocking status and RBCT variables

Variables	**rho**	**chisq**	***p***
Dairy cattle	-0.06	0.33	0.56
Ln (number of cattle)	-0.10	0.74	0.39
Minerals/vits/licks	0.14	1.44	0.23
Manure/slurry stored indoors/close containment	0.20	3.22	0.07
purchase of steers	-0.02	0.04	0.84
purchase from market	-0.04	0.14	0.70
GLOBAL	NA	8.55	0.20

Smooth curves were flat when the scaled Schoenfeld residuals were plotted for each one of the predictor variables (Appendix 4.2.). Overall they seemed to be distributed around zero, indicating no trend in the residuals over time (Dohoo *et al.*, 2003).

Deviance residuals were plotted to identify outliers (Figure 4.4). Most residuals seemed to be distributed around zero. That is, most residuals would be expected to

follow a standard normal distribution, with a mean of zero. Residuals with values outside 2 and -2 could be considered outside the expected range. Farm 114 was identified as an outlier, having the highest residual out of the 148 herds. When the model was run excluding farm 114, no statistical significance difference for the explanatory variables in the Cox proportional hazard model was observed. Therefore, all farms were left in the model.

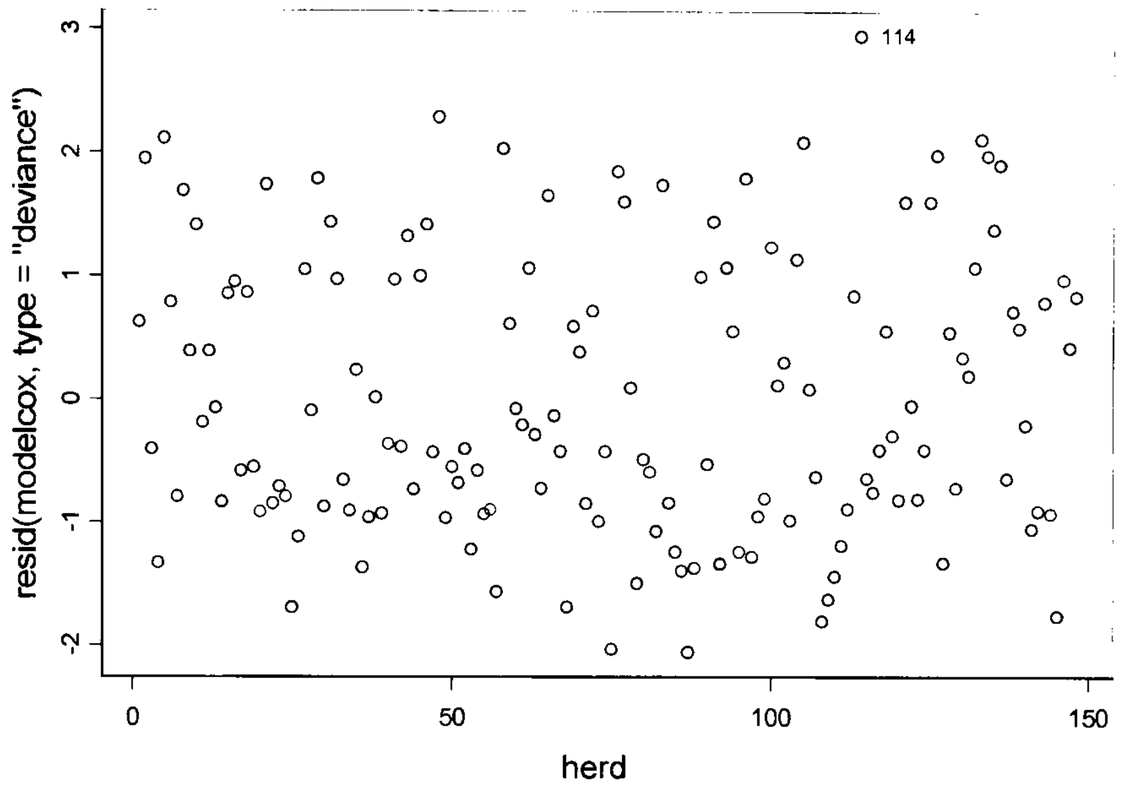


Figure 4.4 - Deviance residuals plot for the 148 study herds

To check the model fit, Cox-Snell residuals were plotted (Figure 4.5). If the model fit is good, the residuals have a mean of zero and variance of one, which indicates a unit exponential distribution. When this is the case, then the cumulative hazard should be a straight line with an intercept of zero and a slope of one (Dohoo *et al.*, 2003). Residuals from the Cox proportional hazards model seemed to be approximated to a straight line. When these were calculated and plotted excluding farm 114, no differences were observed in the distribution of the residuals in the plot.

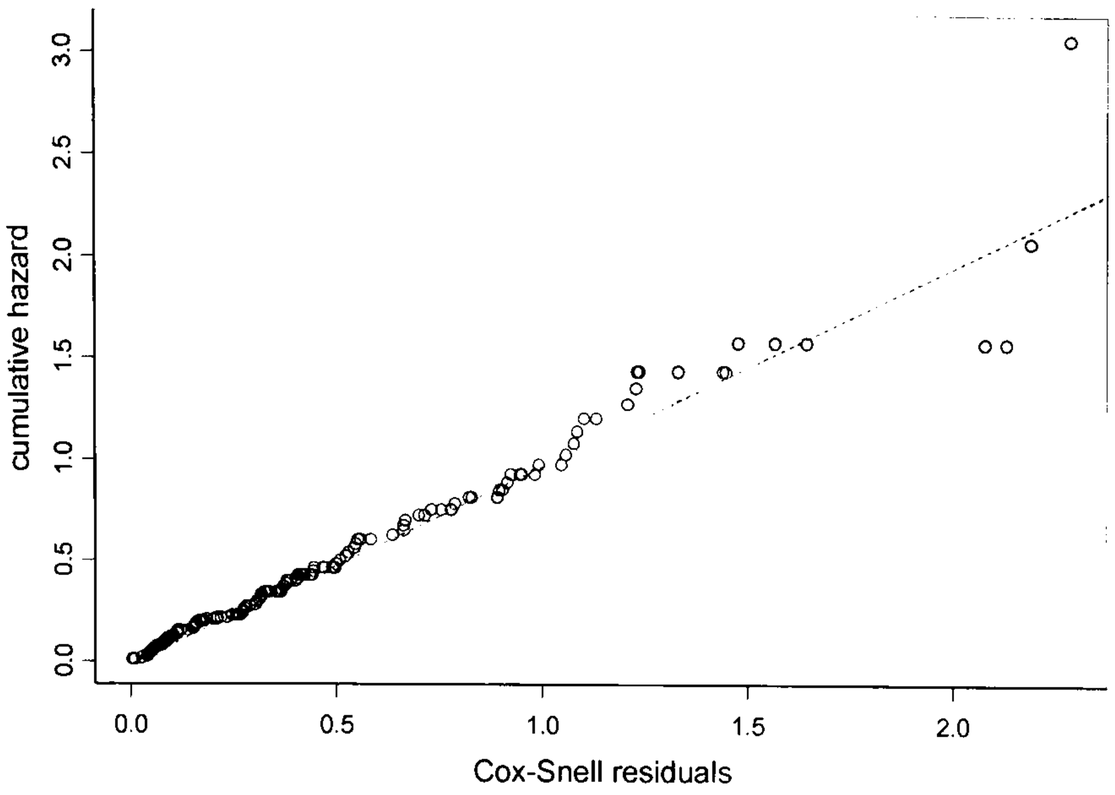


Figure 4.5 - Cox-Snell residuals plot for the Cox proportional hazards model fit
Note: Line (----) has been plotted as a straight line as reference indicating perfect fit

In this chapter some conclusions have been drawn on the association of some farm practices and the introduction and persistence of *M. bovis* in cattle herds. The main findings have highlighted the importance of some factors that were suggested in previous studies but in areas of an unusual restocking of herds and of an intervention on the main wildlife reservoir. The effects of restocking and from the RBCT were not associated with a risk of HBD. The investigation presented in the following chapters would help to elucidate further this effect at individual bovine animal and at animal group within-herd levels.

Chapter 5 - Risk factors for bovine tuberculosis skin test reactivity in 48,055 cattle on 148 herds

5.1 Aim

The aim of this chapter was to develop a multilevel model to investigate risk factors at herd, individual bovine animal and test levels associated with the risk of a bovine animal becoming reactor at a herd SCCIT using records from the British Cattle Movement System (BCMS).

5.2 Introduction

The use of cattle movement records has proven to be very useful in recent studies on the investigation of bTB (Green and Cornell, 2005; Carrique-Mas *et al.*, 2007; Gopal *et al.*, 2006). The main objective of this chapter was to investigate movement factors associated with the risk of an individual bovine animal becoming a reactor at a herd bTB test. In particular, we were interested in the risk of becoming a reactor as associated with exposure to reactors, i.e. the potential for cattle-cattle transmission. During the time a bovine animal was on a source farm (for purchased cattle) until it left that farm and the time a bovine animal was on the study farm up to the test date (time between birth or purchase on the farm and date of the test), other cattle within the herd could be disclosed as reactors, potentially posing a risk to non-infected cattle. In some cases, there were no tests carried out during this time, and therefore the potential exposure to reactors was unknown. In principle, the longer a bovine animal is on the farm, the more chances it would have to become exposed to infection. By the time cattle were tested they would have been potentially exposed through their lives to a number of other reactors, from both source farms and from study farms.

Herd-level sensitivity (HSe) is the probability that a positive herd gives a positive herd result, and herd-level (HSp) is the probability that a negative herd gives a negative herd result (Martin *et al.*, 1992). The skin test is a useful test to detect infection at herd level. However, whereas the specificity of the test is approximately 99%, a number of animals infected would be likely to remain undetected as the

sensitivity of the test is of approximately 74% to 95% (Monaghan *et al.*, 1994; Costello *et al.*, 1997). Apart from the sensitivity and specificity of the test, other factors are likely to affect the HSe and HSp such as the number of animals tested and the herd cut off value (Christensen and Gardner, 2000).

A mixed model with three hierarchical levels was fitted to the test outcome data. Bayesian Monte Carlo Markov Chain (MCMC) estimation procedure using Gibbs' sampling was used to analyse the data.

5.3 Materials and Methods

The general materials and methods of the study have been presented in Chapter 2.

5.3.1 The study sample

The study sample was formed by all cattle that moved onto or were born on the study farms between 1st July 1996 and 4th August 2004, and were tested on these farms between 1st June 2001 and 19th August 2004 with a herd test, based on the assumption that cattle that were on the farm on the day there was a herd test were tested according to the exclusion criteria as explained below. In the study sample, the first test was carried out on 25th June 2001 and the last on 3rd August 2004. No exclusion criteria were applied for herd size or type.

The dataset comprised 48,055 bovine animals tested with 697 herd tests on 144 farms. The hierarchical structure was arranged in three levels: 156,562 animal tests (level 1), 48,055 bovine animals (level 2) and 144 herds (level 3). The data-set was created using Access Database, Microsoft Corp. US.

5.3.2 Source of data

Data were extracted from two sources: the British Cattle Movement Service (BCMS) and the VetNet databases. Records from the BCMS were available from the 1st July 1996 to the 4th August 2004 and from VetNet from the 1st January 1995 to the 19th August 2004.

5.3.2.1 The BCMS database

A total of 135,472 cattle were registered in the BCMS database and passed through the 148 study farms at some point between the 1st July 1996 and the 4th August 2004. Less than 3% of the cattle had been born before 1st January 1998 as recorded in the database. Cattle were either purchased or born on the farm and had either left or were still on the farm at the end of the study period.

For cattle that had left the farm before the end of the study, the length of time spent on the farm was defined by the date the animal was born or moved onto the farm to the date that it died or was moved off to other premises.

Cattle were assumed to be on the farm at the end of the study when there were no records for movements off the farm. Cattle were assumed to have been tested if they were present on the farm when a herd test took place, except those excluded for some types of tests due to young age. Before any tests were excluded, a total of 161,782 cattle tests were identified in the database for the study animals. Where errors in the data were obvious, these were corrected.

5.3.2.2 The VetNet database

The herd and animal test databases were used. The latter records results for reactor cattle (R) only. The VetNet database does not have records for cattle that tested negative to the SICCT test. There are two types of test: those that target whole herds (herd tests) and those that target individual animals (animal tests). Using the test type definition and test criteria, described by the State Veterinary Service (2005) (Table 1.2, Chapter 1), individual animal tests such as VE-IR, VE-PII, VE-PRI, VE-SLH or VE-TR, and herd tests such as VE-6M, VE-12M, VE-WHT or VE-WHT2 when bovine animals were calves under six weeks old, were excluded (Figure 5.1).

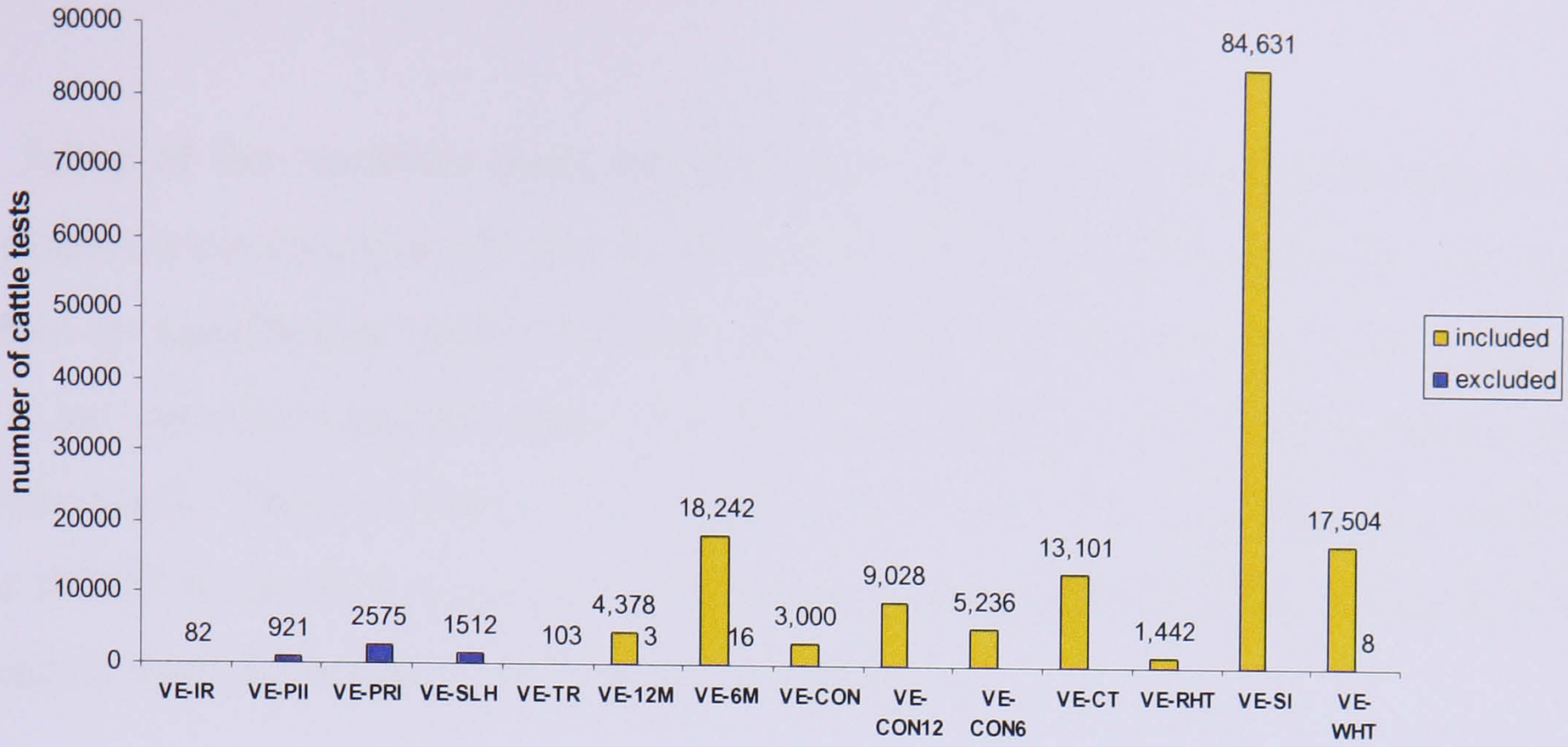


Figure 5.1 - Number of cattle tests by test type and exclusion criteria out of 161,782 initially identified tests.

5.3.2.3 The reactor animals

A total of 776 reactors were disclosed during the study period, 723 of which were reactors at herd tests. To identify cattle that were reactors in the study sample, the ear-tag numbers from the BCMS were searched within the animal ear-tag numbers within the animal test database from VetNet. Two of the 776 reactors were not identified in the BCMS database, therefore they were excluded. Approximately 60% of the reactors' ear-tag numbers, although it was obvious to be the same number, had been recorded slightly different in the BCMS and VetNet (e.g.: different spaces between herd and individual animal numbers) and all were rectified in the database.

5.3.3 Statistical analysis

5.3.3.1 The outcome variable

The outcome variable was binary: a bovine animal was a reactor or not at a test. A bovine animal could be tested several times, but it could be reactor only once.

5.3.3.2 The explanatory variables

Twenty seven explanatory variables were investigated initially. The use of the data held in the national databases were initially explored and then new variables were created to test for the risk of cattle-to-cattle transmission based on the presence of infected cattle whilst the study animal was on the farm.

Some of the variables were initially explored using different categories. Binary variables were coded as 1/0 for yes/no answers and categorical variables were coded based on alphabetical order. Variables chosen for the analysis were animal variables (i.e. sex, breed) except for those that were herd variables (i.e. RBCT, restock status of the herd). Dates of birth or movement on to farms (study or source farms) held in the BCMS database were especially useful to create variables such the length of time animals were in the study until the date of the test.

After initial analysis of the eleven trial areas from the RBCT represented in the study, these were grouped into the three intervention treatments (reactive, proactive, survey). The type of test was divided into three groups: yearly, if it was a routine test; short-interval if carried out sixty days after a previous test and other strategic tests if tests were carried out with a control purpose, for example due to a contiguous herd HBD. Yearly tests included tests coded as VE-WHT, VE-RHT, VE-12M and VE-CON12 and strategic tests included tests coded as VE-CT, VE-6M, VE-CON and VE-CON6.

From the VetNet database the number of reactors disclosed during the period of time an animal was on the farm was used. The potential exposure to other reactors on source and study farms was investigated using a categorical variable based on the total number of reactors disclosed whilst the individual bovine animal was present on during a test. For purchased cattle, this includes tests on both the most immediate and previous source farms and on the study farm prior to the date of the test. Animals born on study farms were only exposed in their natal herd. The final categories used were: not exposed to reactors or unknown number of reactors (if the animal had not been on a farm when a test occurred), exposed to one to five, six to twenty, and more than twenty reactors.

Tables and queries in a relational Access database were used to produce a final query which was then exported into MLwiN.

5.3.3.3 Multilevel analysis

The final query produced in the Access was exported as a text file and imported into MLwiN. Herd, animal and test dates were sorted.

Logistic regression with random effects was carried out using the statistical package for Multilevel Modelling, MLwiN, Version 2.0 (Rasbash *et al.,* 2004). The analysis was implemented by using a Generalised Linear Mixed Model (GLMM) with a 3-level hierarchical structure. Monte Carlo Markov Chain (MCMC) with Gibb's sampling was used to adjust biases in a final multivariable model. In that, an IGLS estimation procedure was used as the prior distribution for the MCMC model.

The model was initially run for the default number of 5,000 iterations and 500 as the burn-in period. It was improved with 70,000 iterations and a burn-in period of 5,000.

5.4 Results

5.4.1 Descriptive analysis

5.4.1.1 Cattle tests by restocking status and RBCT

Approximately 80% of the cattle tests were carried out on continuously stocked herds. Figure 5.2 shows the percentage of cattle tests by restocking status and treatment within the RBCT.

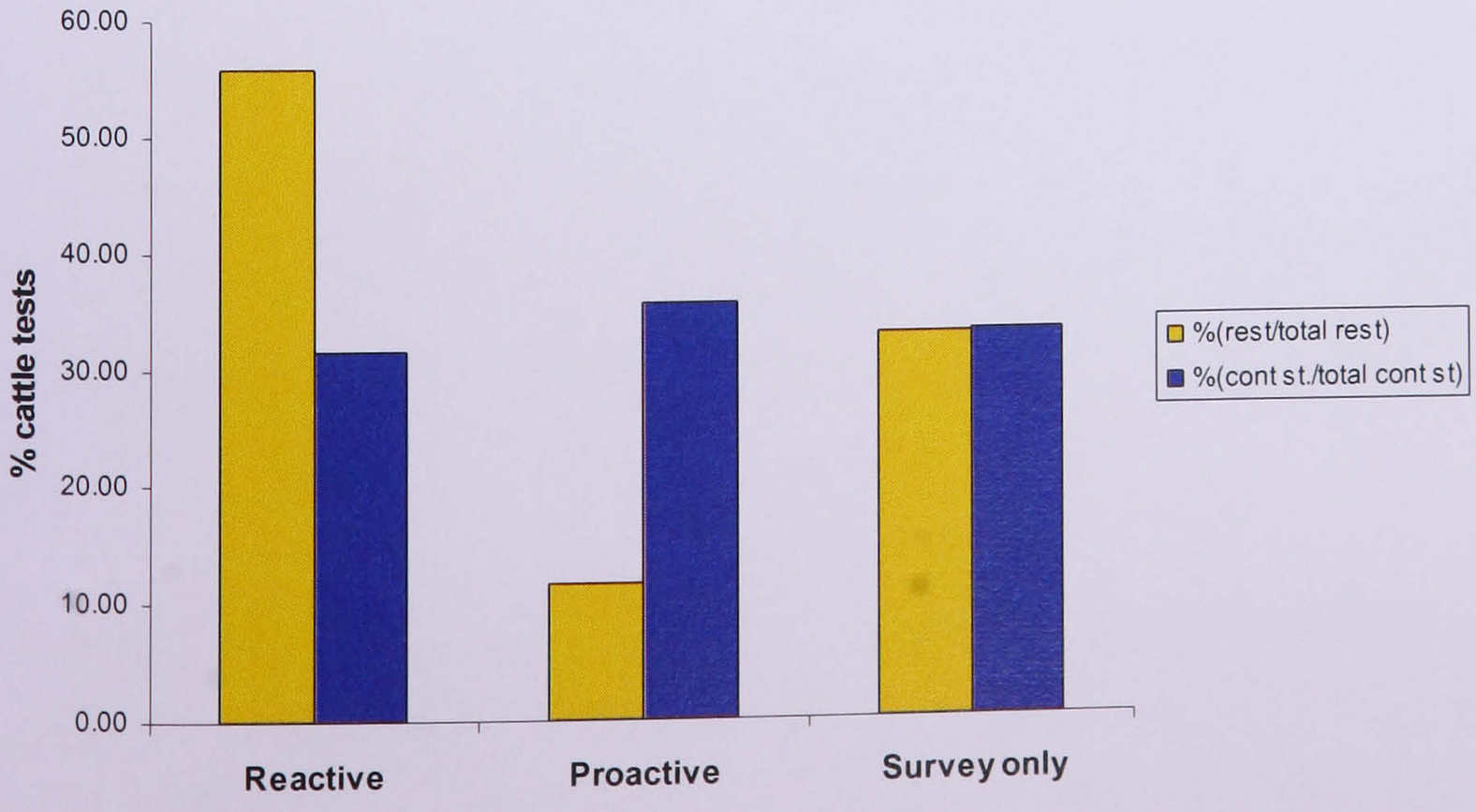


Figure 5.2 - Percentage of cattle tests out of a total of 156,562 by restocking status and RBCT intervention treatment.

Reactive treatment was carried out in four triplets, proactive in three and survey only in four triplets. Figure 5.3 shows the percentage of cattle tests carried out on restocked and continuously stocked herds and in eleven trial areas out of the total thirty areas of the RBCT.

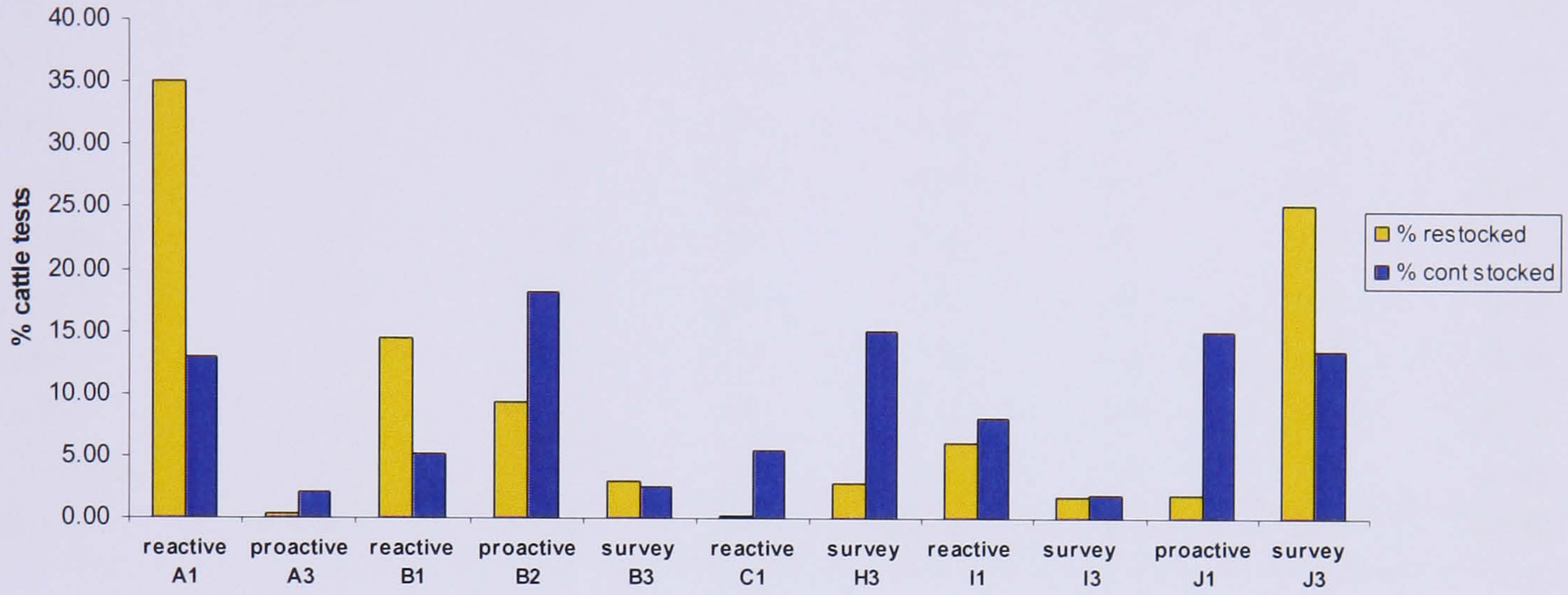


Figure 5.3 - Percentage of cattle tests by restocking status and RBCT trial area.
Key: A1, A3: Gloucestershire and Herefordshire; B1, B2, B3: Devon and Cornwall; C1: East Cornwall; H3: Somerset and Devon; I1, I3: Gloucestershire; J1, J3: Devon

5.4.1.2 Herd tests

There were 697 SICCT herd tests carried out on the study farms between 1st June 2001 and 19th August 2004. All were included in the study. Out of the 148 herds, four were not tested with a herd test during this period, two of these had individual animal tests (VE-SLH and VE-TR) and both were negative. The other two herds were last tested before June 2001.

The number of tests per year varied. Out of the 697 herd tests, 4% were carried out in 2001, 29% in 2002, 45% in 2003 and 22% in 2004. The decrease in the number of tests in 2001 was due to the disruption caused by the FMD outbreak that year. Consequently, many herds that were due a routine test during that year were not tested.

As presented below in Table 5.1, over 50% of the cattle tests were carried out with a VE-SI test and this represented approximately 45% of the total 697 herd tests. Over 50% (392/723) of reactors were disclosed with a VE-SI test however, the

percentage of reactors out of the cattle tests was highest when tested with a VE-CT (check test).

Table 5.1 - Distribution of 156,562 cattle tests in 697 herd tests and 723 reactors by test type

Test type	Number of cattle tests	% (cattle tests/ total cattle tests)	Number of herd tests	% (herd tests/ total herd tests)	Number of reactors	Reactors/ cattle test x100	Reactors/ herd test
VE-SI	84,631	54.06	310	44.48	392	0.46	126.00
VE-CT	13,101	8.37	61	8.75	109	0.83	179.00
VE-6M	18,242	11.65	85	12.20	84	0.46	99.00
VE-CON	3,000	1.92	28	4.02	13	0.43	46.00
VE-CON6	5,236	3.34	32	4.59	28	0.53	87.50
VE-WHT	17,504	11.18	102	14.63	56	0.32	54.90
VE-RHT	1,442	0.92	8	1.15	2	0.14	25.00
VE-12M	4,378	2.80	23	3.30	15	0.34	65.00
VE- CON12	9,028	5.77	48	6.89	24	0.27	50.00
TOTAL	156,562	100.00	697	100.00	723	3.79	732.40

There were 133 herd tests on 34 restocked herds and 564 herd tests on 110 continuously stocked herds. The median number of tests during the study period was three and four respectively for restocked and continuously stocked herds.

The percentage of herd tests that were carried out in the study in restocked and continuously stocked herds by test type is shown in Figure 5.4. In the multilevel analysis, tests were classified into three main groups according to the frequency and purpose of the test. These groups are: SI (short interval), strategic tests (excluding VE-SI) and including VE-CT, VE-6M, VE-CON and VE-CON6 tests and yearly or routine tests (VE-WHT, VE-RHT, VE-12M and VE-CON12).

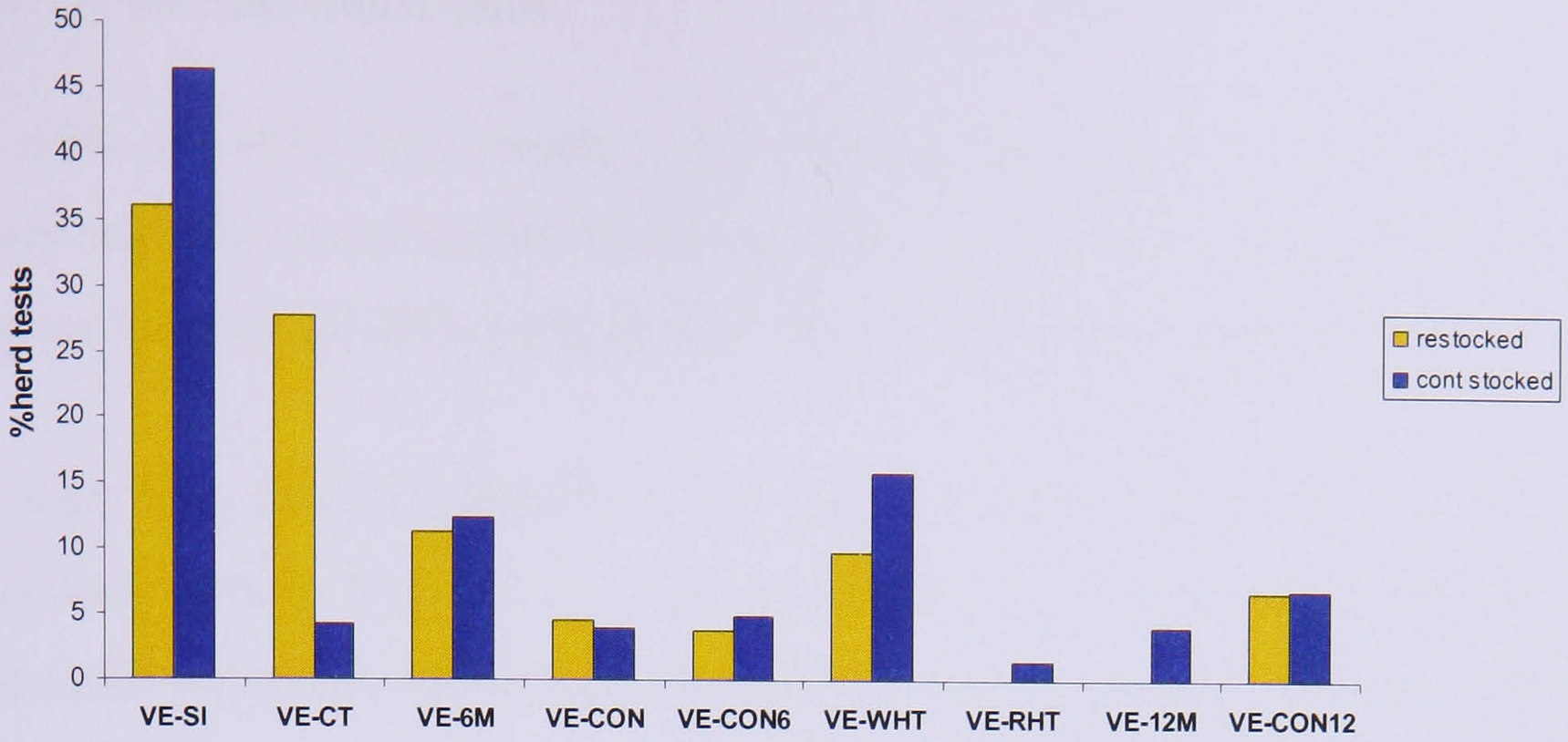


Figure 5.4 - Percentage of herd tests from 697 herd tests by test type and restocking status.

Eight out of the 697 were VE-RHT, carried out on seven study herds, three of which were had grower cattle for beef production only, three had suckler cattle only and two were mixed herds.

5.4.1.3 Purpose of the herd

The number of cattle tests carried out by herd type and number of reactors within each type is presented in Table 5.2. Approximately 1% of the cattle tested were reactors. The highest percentage of tests was carried out on dairy only or mixed herds, and consequently, the majority of reactors were disclosed on those herds.

Table 5.2 - Number and percentage of cattle tests and reactors from a total of 156,562 cattle tests and percentage of reactors out of the total 723 by herd purpose

Purpose of herd	**Number of cattle tests**	**% of total cattle tests**	**Number of reactors**	**% of reactor/ cattle tests**	**% of total reactors**
Suckler only	6,810	4.35	12	0.18	1.66
Dairy only	51,046	32.60	272	0.53	37.62
Young stock <30 m.o. only	19,598	12.52	47	0.24	6.50
Young stock and suckler	35,458	22.65	136	0.38	18.81
Young stock and dairy	43,650	27.88	256	0.59	35.41
TOTAL	156,562	100.00	723	0.46	100.00

5.4.1.4 The individual cattle

61% of cattle were female, 54% were grower cattle for beef production, 44% dairy and 2% mixed breeds. From the total 156,562 cattle tests, 73.5% were carried out on females and 44% were on beef, 54% dairy, and 2% mixed breeds.

Less than 1% (376/48,055) of the cattle moved between the study farms during the study period. There were 1.4% for which date of birth was not recorded; 47% of the cattle were born on the study farms 53% were purchased. Of the purchased cattle, 39% and 59% moved on to the study farm when they were less and more than one year old respectively. The age of the other 2% was not recorded. Out of the total tests, 46% were carried out on cattle born on the farm and 54% on purchased cattle.

The number of times a bovine animal was tested (Figure 5.5) varied considerably during the study period with a median of twice (range 1 - 21). Approximately one third of the cattle (16,507/48,055) were tested only once during the study period. Of the 156,562 cattle tests, 20% (31,313 /156,562) were carried out on restocked and 80% (125,249/156,562) on continuously stocked herds.

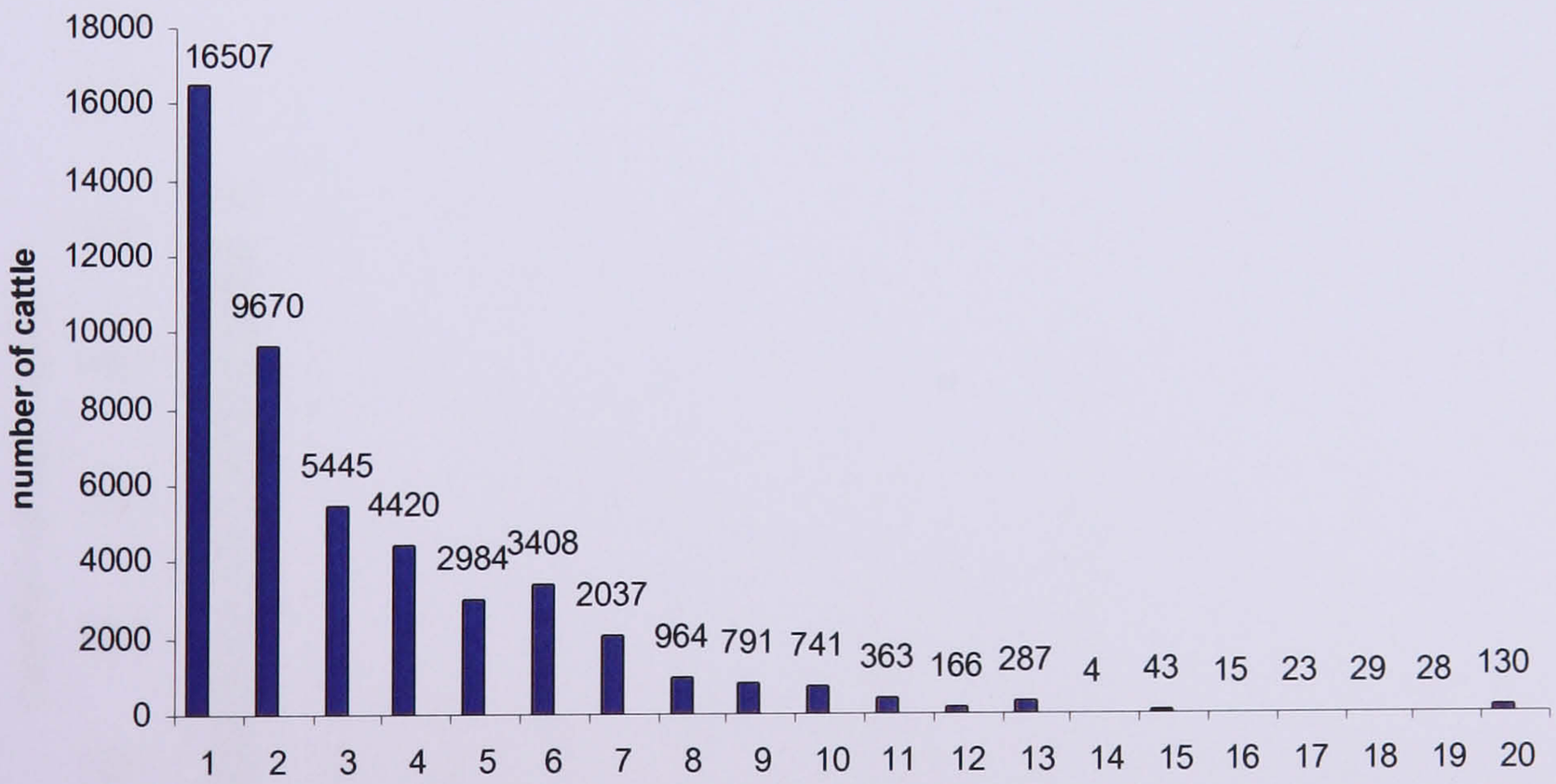


Figure 5.5 - Number of times 48,055 cattle were tested with herd tests during the study period.

Approximately 29% of the tests were carried out on cattle under one year old, 25% between one and two years old and 46% from two years old and older (Table 5.3).

Table 5.3 - Number and percentage from 156,562 cattle tests and 723 reactors by age of the individual bovine at test

Age (years) at test	Number of cattle tests	% of cattle tests	Number of reactors	% of reactors from total reactors	% of reactors/ cattle tests
up to 1 year	44,959	28.72	49	6.78	0.11
> 1 - 2	38,695	24.72	68	9.41	0.18
> 2 - 3	19,222	12.28	102	14.11	0.53
> 3 - 4	13,381	8.55	118	16.32	0.88
> 4 - 5	11,067	7.07	116	16.04	1.05
> 5 - 6	8,622	5.51	81	11.20	0.94
more than 6	20,616	13.17	189	26.14	0.92
TOTAL	156,562	100.00	723	100.00	4.60

5.4.1.5 The reactor cattle

There were 1.5% reactors (723 out of the total 48,055 cattle) in the study and they were all disclosed on 29% (204/697) of the herd tests. The number of reactors per positive test ranged from 1 to 78 (note the discontinuous scale). Out of the positive herd tests, 33% had only one reactor. Figure 5.6 shows the number of reactors disclosed per herd test.

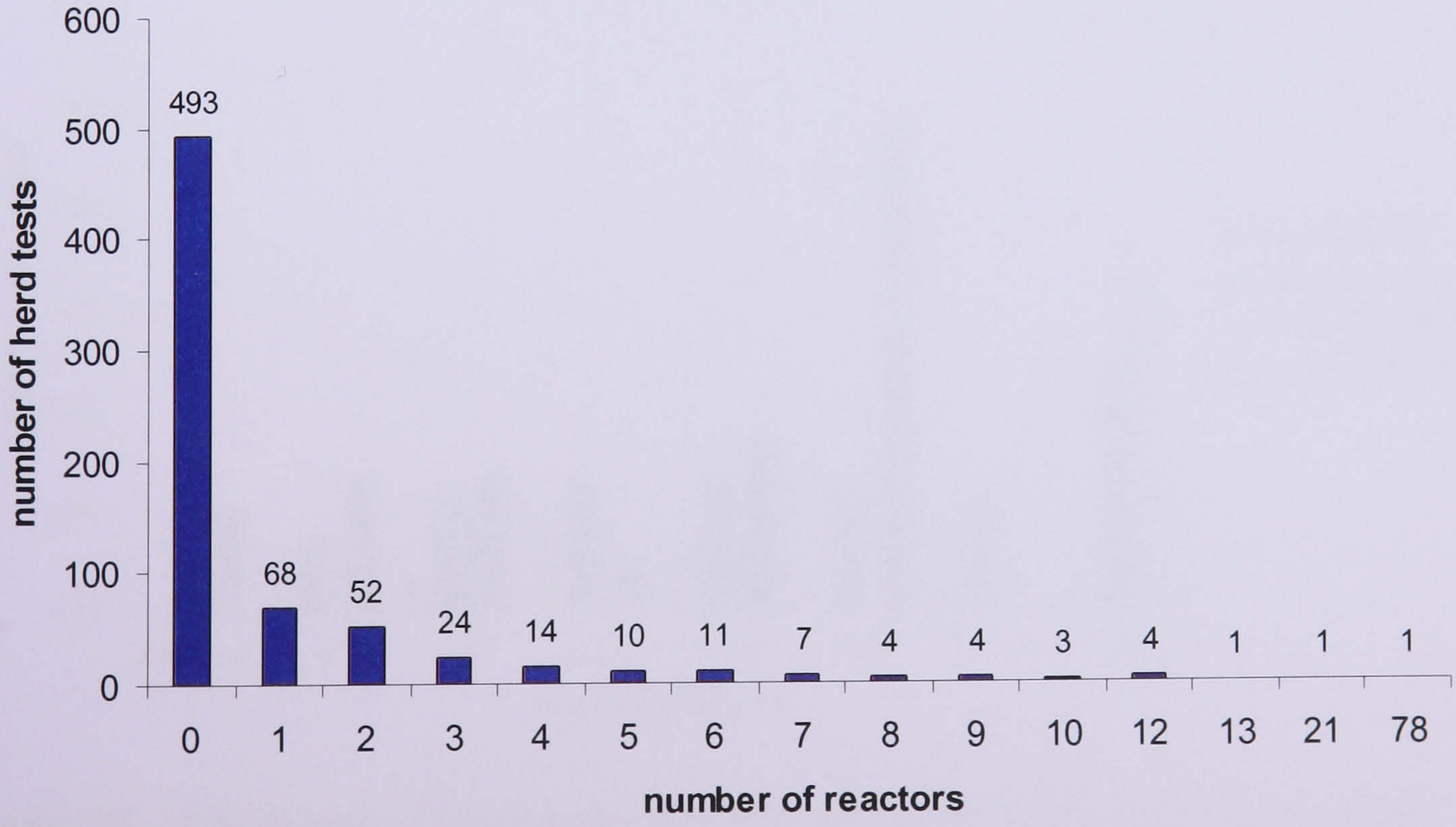


Figure 5.6 - Number of reactors per test from 697 herd tests.

5.4.1.6 On farm exposure to other reactors

Figures 5.7 to 5.10 below show the percentage of reactors disclosed out of the total number of animal tests, by the potential exposure to other reactors on source farms for purchased cattle and on the study farms for both cattle purchased and born on the farm.

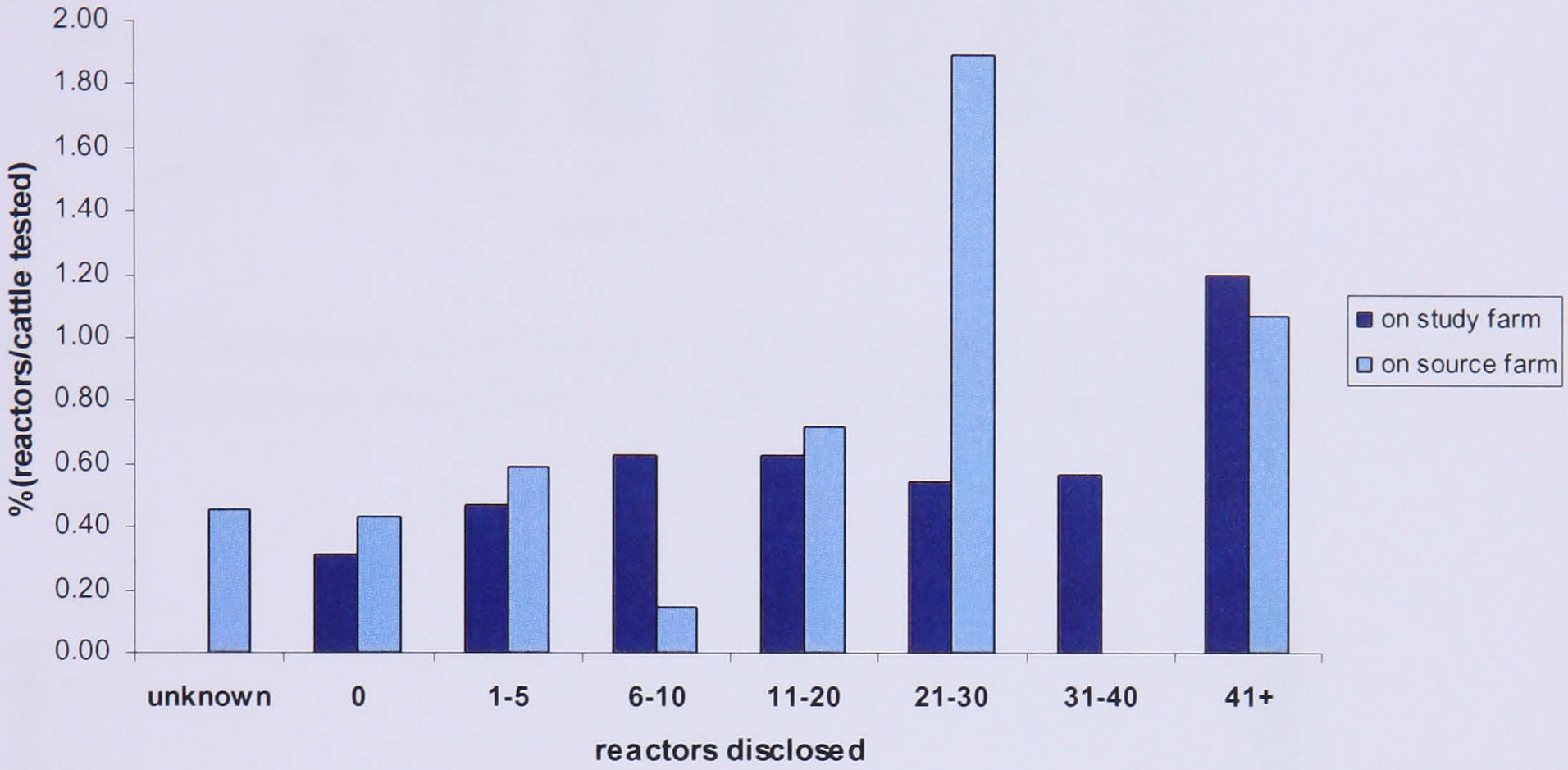


Figure 5.7 - Percentage of reactors by previous exposure to unknown and to other disclosed reactors on study and source farms.

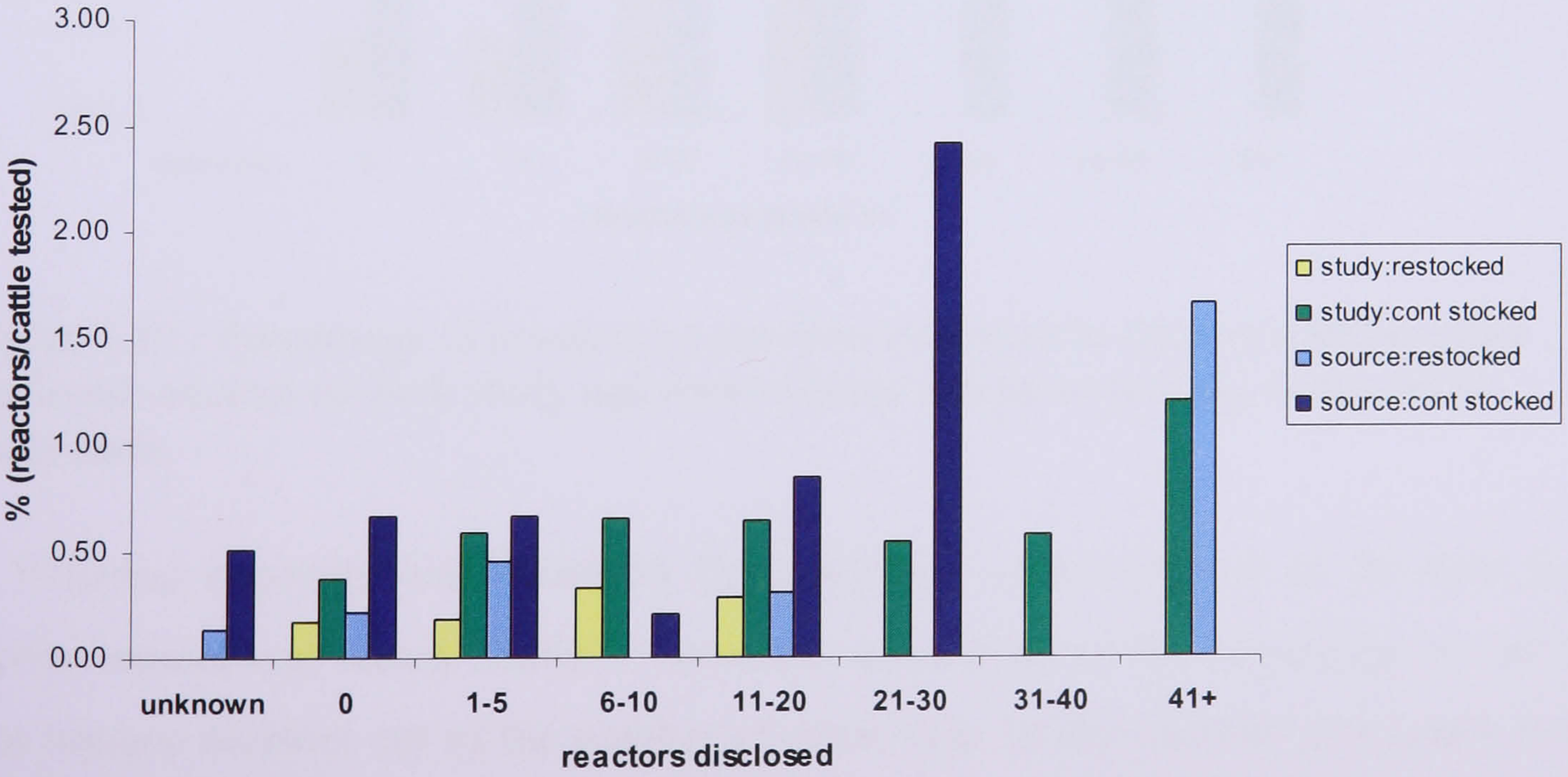


Figure 5.8 - Percentage of reactors by previous exposure to unknown and to other disclosed reactors on study and source farms and by restocking status of the study farm.

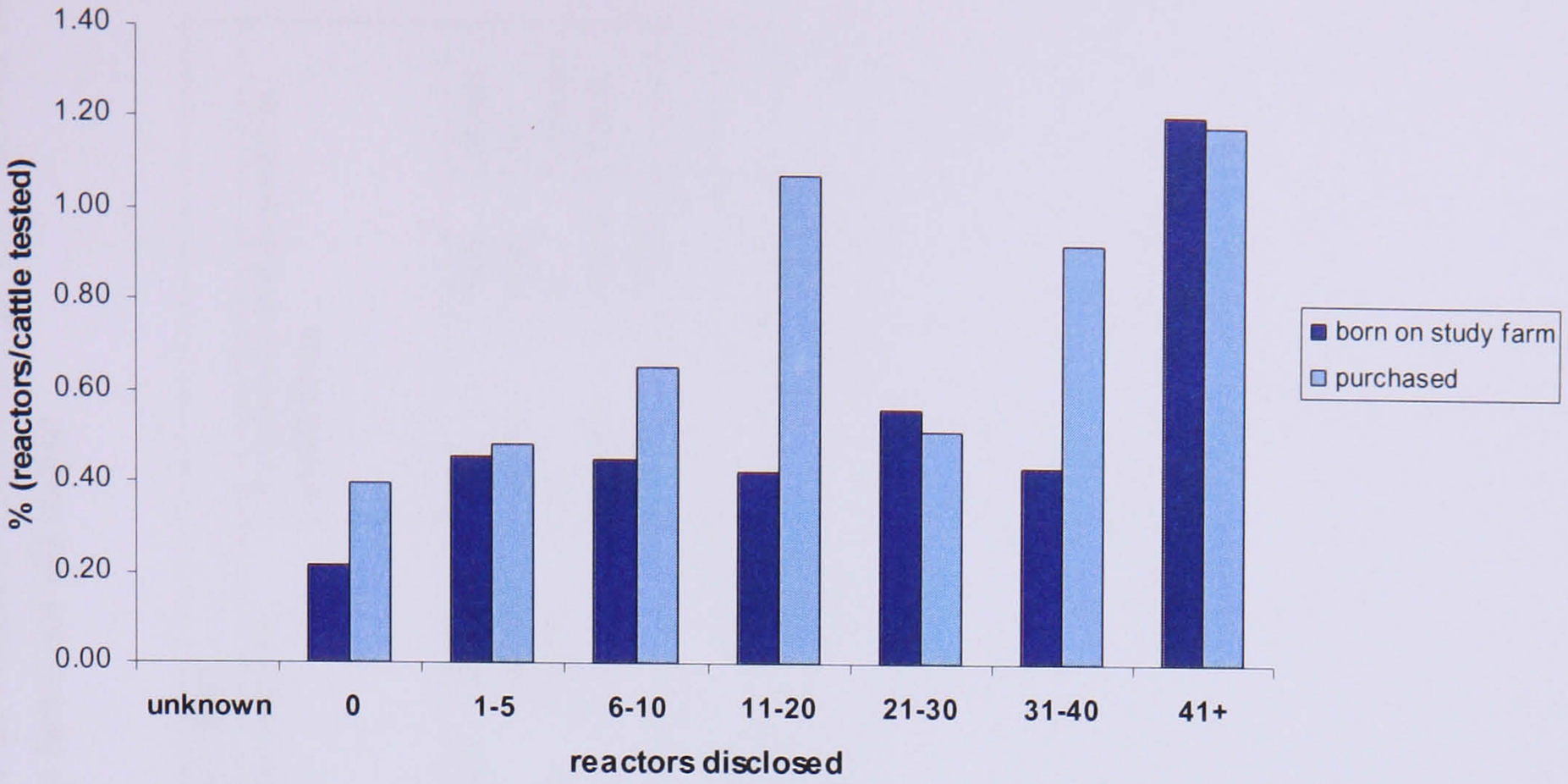


Figure 5.9 - Percentage of reactors by previous exposure to unknown and to other disclosed reactors on study farm by purchased or born on the study farm.

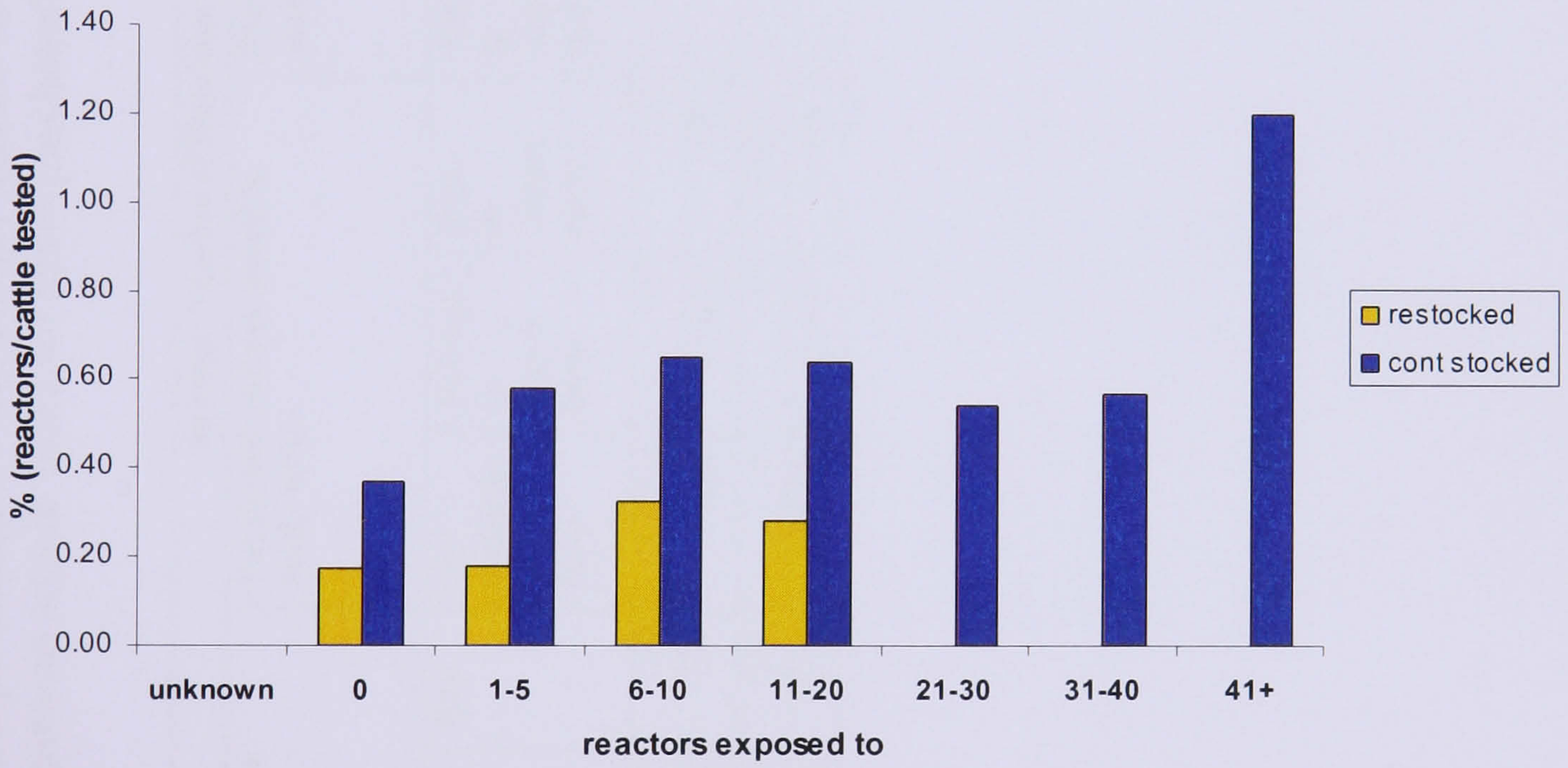


Figure 5.10 - Percentage of reactors by previous exposure to unknown and to other disclosed reactors on both study and source farms and by restocking status of the study farm.

Potential exposure was based on the disclosure of reactors up to the date the bovine animal was tested. Table 5.4 presents a summary of the percentage of cattle that became reactors out of the number of cattle tests by the number of reactors that were disclosed at the current test and by exposure to previously disclosed reactors on the farms (both source and study farms) up to the date of the test. The risk of a bovine animal becoming reactor increases if at the current test there is more than one single reactor.

Table 5.4 - Number of herds, herd tests, reactors and percentage of reactors per animal tests by number of reactors disclosed at the current test and by the potential previous exposure to other reactors on source farms for cattle purchased on the study farm for all cattle

Number of reactors	Number of herds with maximum number of reactors	Number of herd tests	Average Number of Reactors/ Number of tests (x100)											
			Born in study farm			Purchased and not tested in study farm			Purchased and not exposed in study farm			Purchased and exposed in study farm		
			Not tested	Not exp.	Exp.	Not tested in source farm	Not exp. in source farm	Exp. in source farm	Not tested in source farm	Not exp. in source farm	Exp. in source farm	Not tested in source farm	Not exp. in source farm	Exp. in source farm
0	74	484	0	0	0	0	0	0	0	0	0	0	0	0
1	13	72	0*	0.02	0.07	0.00	no tests	0.00	0.05*	0.05*	0.03*	0.10	0.08	0.37
2	7	55	0	no tests	0.51	0	0	no tests	no tests	no tests	no tests	0.94	1.24	1.25
3+	50	86	0	no tests	1.75	no tests	no tests	no tests	no tests	no tests	no tests	2.99	2.78	2.96

(*) the reactors in these categories were singletons

Table 5.5 presents the average number of reactors per cattle test, number of reactors and number of cattle tests by the age at which cattle were tested, and by the total number of reactors potentially exposed to.

Out of the 17 reactors (Table5.5) that had not been potentially exposed to other reactors on the study farms, 70.5% of them were confirmed by lesions and or by culture.

Table 5.6 and Table 5.7 present the number of reactors, tests and average number of reactors out of the number of tests, for animals born in study and purchased, that were disclosed during the first six months and during the first year of being in the study farms. The average of reactors per animal tested disclose in the first six months and first year is greater for animals purchased than in those born on the study farms. The same was observed when comparing the number of reactors disclosed in the six and twelve months out of total disclosed in the study: within animals born on the study farms, only 2% (7/339) during the first six months and 6% (20/339) during the first year were disclosed as reactors out of the total disclosed during the study, however, for purchased animals, 9.1% (35/384) during the first six months and 27% (104/384) during the first year were disclosed.

Table 5.5 - Average number of reactors from individual animal tests by potential previous exposure in both study and source farms and by age at the test

Potential exposure to reactors		**Age at test**							**Totals**
Not Exposed (reactors not disclosed whilst study animal present on farm)		**<1y.o**	**12y.o.**	**2-3 y.o.**	**3-4 y.o.**	**4-5 y.o.**	**5-6 y.o.**	**>6 y.o.**	**All ages**
Born in study and not exposed	(reac/animal tests)x100	0.01	0.03	0	0	0	0.52	0	0.02
	reactors	1	1	0	0	0	2	0	**4**
	animal tests	12,208	3,885	1,399	860	541	384	179	**19,456**
Purchased and not exposed in either farm	(reac/animal tests)x100	0	0	0.14	0.12	0	0	0.15	0.05
	reactors	0	0	3	1	0	0	1	**5**
	animal tests	1,224	4,248	2,097	803	526	372	679	**9,949**
Born in study and not tested	(reac/animal tests)x100	0	0	no tests	no tests	no tests	no tests	no tests	0
	reactors	0	0	0	0	0	0	0	**0**
	animal tests	98	0	0	0	0	0	0	**98**
Purchased and not tested in neither farms	(reac/animal tests)x100	0	0	0	0	0	no tests	0	0
	reactors	0	0	0	0	0	0	0	**0**
	animal tests	7	11	32	1	1	0	3	**55**
Purchased and not exposed in study farms and not exposed in source farm	(reac/animal tests)x100	0	0.05	0	0	0	0	0.18	0.05
	reactors	0	2	0	0	0	0	5	**7**
	animal tests	4,786	4,319	1,230	545	327	398	2,729	**14,334**
Purchased and not tested in study farm and not exposed in source farm	(reac/animal tests)x100	0	0	0	0	0	0	no tests	0
	reactors	0	0	0	0	0	0	0	**0**

	animal tests	8	44	4	4	1	1	0	**62**

Table 5.5 (cont.)

Potential exposure to reactors		**Age at test**							**Totals**
Exposed to number of reactors from study and or source farms		**<1y.o**	**1-2y.o.**	**2-3 y.o.**	**3-4 y.o.**	**4-5 y.o.**	**5-6 y.o.**	**>6 y.o.**	**All ages**
Total	% reac/tests	0.18	0.25	0.68	1.05	1.2	1.06	1.07	0.63
	reactors	48	65	99	117	116	79	183	**707**
	tests	26,628	26,188	14,460	11,168	9,671	7,467	17,026	**112,608**
One to five reactors									
	% reac/tests	0.07	0.18	0.47	0.39	0.77	0.48	0.59	0.29
	reactors	11	24	28	13	23	11	35	**145**
	tests	16,149	13,506	5,900	3,327	2,971	2,274	5,901	**50,028**
Six to twenty reactors									
	% reac/tests	0.24	0.3	0.92	1.06	1.11	0.98	1.31	0.73
	reactors	20	26	48	45	39	26	81	**285**
	tests	8,502	8,743	5,200	4,264	3,516	2,658	6,190	**39,073**
More than twenty reactors									
	% reac/tests	0.86	0.38	0.68	1.65	1.7	1.66	1.36	1.18
	reactors	17	15	23	59	54	42	67	**277**
	tests	1,977	3,939	3,360	3,577	3,184	2,535	4,935	**23,507**

Table 5.6 - Number of reactors, animal tests and average of reactors that were disclosed during the first six and twelve months of born animals being on the study farm by exposure to number of reactors

Number of reactors exposed to	**Total number of reactors during the study**	**Born on study farm**					
		Up to six months on the study farm			**Up to one year on the study farm**		
		Number of reactors	**Number of total animal tests**	**Number reactors/total tests(x100)**	**Number of reactors**	**Number of total animal tests**	**Number reactors/total tests(x100)**
None or None source & not tested study or None in study & not tested source	4	0	7,739	0.00	1	12,306	0.008
One to five	41	0	6,728	0.00	1	12,156	0.008
Six to twenty	118	0	2,874	0.00	6	7,235	0.08
More than twenty	176	7	355	2.00	12	1,509	0.79
Totals	339	7	17,696	0.04	20	33,206	0.06

Table 5.7 - Number of reactors, animal tests and average of reactors that were disclosed during the first six and twelve months of purchased animals being on the study farm by exposure to number of reactors

Number of reactors exposed to	**Total number of reactors during the study**	**Purchased onto study farm**					
		Up to six months on the study farm			**Up to one year on the study farm**		
		Number of reactors	**Number of total animal tests**	**Number reactors/total tests(x100)**	**Number of reactors**	**Number of total animal tests**	**Number reactors/total tests(x100)**
None or None source & not tested study or None in study & not tested source	12	4	11,232	0.04	6	15,843	0.04
One to five	104	13	6,207	0.21	37	12,059	0.31
Six to twenty	167	12	2,596	0.46	49	4,921	1.00
More than twenty	101	6	697	0.86	12	2,012	0.60
Totals	384	35	20,732	0.17	104	34,835	0.30

5.4.2 Results from multilevel analysis: a binomial logistic regression with random affects

Variables were first investigated at univariable level (Appendix 5.1). The number of cattle tests, reactors and percentage of reactors from cattle tests for each category are presented in the table.

The results from the multivariable model are presented in Table 5.8. The effect from restocking was protective (OR=0.49, CI =0.19-0.97) compared to continuously stocked farms. The treatment from the RBCT was not statistically significant, however it was forced into the model as it was one of the variables that was used for the selection criteria in the study design. The use of a short interval test was associated with a decreased risk of a bovine animal becoming reactor (OR= 0.40, CI=0.38-0.67), whilst other strategic tests slightly increased the risk (OR=1.04, CI=1.02-1.80) compared to those tested with a yearly test.

Bovine animals that had been potentially exposed to reactors whilst on the farm in the past and up to the date of the test were associated with an increased risk of becoming reactors compared to those that were not exposed. Within cattle not exposed on the study farms, there was a decreased risk of becoming reactor if born on the study farms (OR=0.35, CI=0.10-1.16) when compared to those purchased.

The Chi-square test to assess the significance of the variance between animals within-herds (χ^2 = 22.48, df=2, p<0.05) suggested that the difference in the probability of a bovine animal becoming reactor varies between animals within-herds and the same interpretation comes from the same test for variance between herds (χ^2= 17.32, df=2, p<0.05).

Table 5.8- Results from the multivariable multilevel logistic regression with random effects analysis from 156,562 cattle tests using 697 herd tests in 144 herds and 48,055 individual cattle

Variable	obs	reactors	No.reactors/ obs (x100)	OR	Coef	M.C. SE	95% credibility interval lower	upper
Restocked status								
Cont. stocked	125,249	666	0.5	ref				
Restocked	31,313	57	0.2	0.49	-0.71	0.31	0.19	0.97
RBCT- Treatment								
Survey	51,560	193	0.4	ref				
Reactive	56,956	270	0.5	1.24	0.22	0.28	0.78	3.57
Proactive	48,046	260	0.5	1.23	0.21	0.3	0.76	3.77
Test type								
Yearly test	32,352	97	0.3	ref				
Short interval	84,631	392	0.5	0.4	-0.92	0.13	0.38	0.67
Other strategic tests	39,579	234	0.6	1.04	0.04	0.14	1.02	1.80
Potential previous exposure to reactors								
Purchased onto study farm and not exposed in study farm	28,283	13	0	ref				
Born on study farm And not exposed	19,456	4	0	0.35	-1.06	0.62	0.10	1.16
Born on study farm and exposed	65,545	335	0.5	9.21	2.22	0.33	4.80	17.65
Purchased onto study farm and exposed in study farm and not tested in source	26,331	229	0.9	17.5	2.86	0.33	9.11	33.61
Purchased onto study farm and exposed in study farm and not exposed in source farm	11,876	90	0.8	18.12	2.90	0.34	9.23	35.56
Purchased and exposed in study and exposed in source farm	4,839	52	1.1	19.45	2.97	0.36	9.62	39.32
Born or purchased and not tested and not tested on study farm (missing)	232	0	0					
Variance								
Between herds					0.93	0.22		
Between animals within herds					0.05	0.02		

In a second model, the exposure to total number of reactors from both study and source farms was replaced by two different variables. One was born in study, purchased or tested and purchased and not tested, and the other was the total number of reactors that cattle were exposed to. The results from this model were very similar to those presented in Table 5.8, showing that purchased cattle, were at higher risk of becoming reactors compared to born on the study farm and exposure to a greater number of reactors increases the risk of a bovine animal becoming a reactor

compared to bovine animals not exposed or not exposed in study and not tested in source (results presented in Table 5.5). In this second model, when the type of test used was other strategic test this decreased the risk when compared to yearly test, whilst in the model in Table 5.8, it was only borderline statistically significant.

5.5 Discussion

5.5.1 Results from the multivariable mixed logistic regression model

5.5.1.1 Potential previous exposure to infected cattle (reactors)

The importance of exposure to infected cattle as a risk to naïve cattle has been suggested in the past (Francis, 1947; Morris *et al.*, 1994). The potential exposure of individual cattle to reactors disclosed on both source and study farms prior to the test date (or current test) was a significant finding in the present study as presented in the results of the multilevel model (Table 5.8). It was not possible to measure with certainty the quantity of exposure as it occurred in the field, so therefore, the term "potentially exposed" was used. Figures 5.7 to 5.10 show that there is a pattern with the increase in the percentage of bovine animals that became reactors and the number of reactors exposed to up to the date of the test.

Evidence of current infection - The disclosure of other reactors on the same day the bovine animal was tested (current test) indicates presence of infection. In Table 5.4 the number of reactors disclosed out of the number of tests by previous exposure and number of reactors disclosed at the current test shows that the percentage of bovine animals that became reactors was higher if there were two or more reactors at the current test.

The data as presented in this table must be interpreted with caution as categorising by the number of reactors disclosed at a test may lead to misinterpretation of the positive tests out of the total tested in that group. However, it can be used to provide further evidence that it is more likely that a bovine animal becomes a reactor if there are some other infected cattle in the herd, these being also disclosed on the day of the test.

A low percentage of positive tests were singletons (one only reactor disclosed at the test) compared to the percentage that had more than one reactor, which emphasises the importance of the transmission that may occur between cattle.

Age of cattle at test - The majority of the cattle tests were carried out on cattle two years old and under. However, the percentage of reactors from cattle tests was higher if cattle were over two years old (Table 5.3). When the age at test was compared to the potential exposure to reactors on the farm, there was an increase with age on the percentage of reactors disclosed out of the number of tests if there had been previous exposure. The risk increased with the number of reactors the bovine animal had been exposed to (Table 5.5).

Despite the large percentage of cattle tested up to the age of one year old (42%), the majority of reactors were three to five years old when they were tested. This could be explained by the higher risk of being potentially exposed to a higher number of other infected cattle on the farm, the greater chances of being tested, and the higher susceptibility to infection at an older age due to lower immunity resistance could also be a reason. The slight decreased risk from five years old onwards could be explained as either a cattle effect, i.e. anergic reaction to the skin test injection (Pritchard, 1988; Neill *et al.*, 2005), or as a management effect, i.e. that reactors are removed at younger ages. The effect of an increased risk with animal age and no effect of type of herd were also reported by (Munroe *et al.*, 1999).

Cattle purchased *vs* born on the study farm - Purchased cattle were shown to be at higher risk of becoming reactors compared to those born on the study farm given that they had been exposed to reactors on their previous farms (Table 5.7). In a second model where born *vs* purchased was investigated, purchased cattle were associated with an increased risk compared those born on the farm. Figures 5.7 – 5.9 show the exposure that cattle had to other reactors on source farms as evidence of potential exposure on these farms. Also, when the number of reactors disclosed during the first six and twelve months of the study was investigated (Table 5.6 and Table 5.7), the percentage of reactors in those time periods was higher for purchased animals than for born on the study farm. Assuming that once on the study farm, the exposure for all cattle was the same, the results suggest that the exposure on source

farms could have contributed to the risk and this could be the reason why purchased cattle were disclosed earlier as reactors compared to those born on the farm.

5.5.1.2 The SICCT tests

In this study, the highest percentage of reactors was disclosed using short interval tests (VE-SI). In the multivariable model this test was associated with a decreased risk of a bovine animal becoming reactor if compared to cattle tested with a yearly test. The use of other strategic tests slightly increased the risk compared to yearly, however the effect was borderline statistically significant. The higher frequency of testing has been previously suggested to increasing the chances of finding infection (Medley, 2003) and this could explain the reason why short interval tests are associated with a decreased risk. Barlow *et al.* (1998) suggested that reducing the testing interval from 36 to 24 months gave a 45% reduction in the percentage of herds with movement controls after ten years. Based on these arguments, it is not surprising to find that the majority of reactors were disclosed on short interval or other strategic tests. This could be interpreted as a herd risk factor, meaning that if herds were tested with a short interval test, this would remove infected cattle for the farm, reducing the risk to other cattle and therefore decreasing the chances of an individual bovine animal (as in this study) becoming reactor. Since the increase in the frequency of the SICCT tests was associated with a decrease risk compared to a yearly testing frequency, the results of the model suggests that the removal of recent infection reduces the risk of an individual bovine animal becoming a reactor and residual infection may be undisclosed given the low sensitivity of the test.

When a bovine animal was tested with a strategic test, other than short interval test, this was associated with a slightly increased risk of becoming reactor if compared to cattle tested with a yearly test. The same argument given above on the frequency of testing could apply here as strategic tests are carried out as a control measure or when the disease is suspected. However, the regularity with which the tests are carried out is lower for other strategic tests than it is for short interval or yearly tests. This could be a speculative reason for the increased risk associated with other strategic test compared to yearly tests.

Using the test type definition described in the State Veterinary Service (DEFRA, 2005b) individual animal tests such as VE-IR, VE-PII, VE-PRI, VE-SLH or VE-TR, were excluded from the dataset (98 tests in total which would have been applied to 5,193 cattle in the study) (Figure 5.1). Some animals tested under these targeted tests were recorded in the animal database (i.e. those that become reactors or inconclusive reactors), and therefore it was obvious that the animal had been tested. However, cattle tested under targeted tests having a negative result were not recorded in the VetNet database. The inclusion of these tests would have biased towards reactor cattle, therefore were excluded leaving only herd tests in the study sample. Moreover, calves under six weeks old are not tested with a herd test such as VE-6M, VE-12M, VE-WHT or VE-WHT2. Under this criterion, 20 tests were excluded which would have been applied to 27 bovine animals.

The VE-CON12 was included within the yearly tests since this test, although carried out every 12 months due to a contiguous herd HBD (therefore it could be considered as a strategic test), would have been similar to having a routine yearly test, given the frequency of testing in the area.

Based on the type of farming, some cattle would remain longer than others on the farm. From the total 135,472 cattle that passed through the study farms between July 1996 and August 2004, only 48,055 (35%) were tested under the assumption used in this study, and between June 2001 and August 2004, 59,114 which passed through these farms, 59% were tested. As this study involved only tests carried out between June 2001 and August 2004, it is obviously possible that those which were not tested here could have been tested on other premises or sent to slaughter before June 2001. Also, those that were not included in the analysis could have been tested using individual animal tests, which were excluded here to avoid biases towards the disclosure of reactors. Even so, this could also imply that a large proportion of cattle could move between farms without being tested ever in their lives (Mitchell *et al.*, 2006). The follow up of cattle after they left the study farms was not an objective of the present study.

All herds in this study were expected to be tested at least once a year. However, tests in eight out of the 697 herd tests, which were carried out on 695 animals, were

VE-RHT tests, that is, a routine test carried out every 2, 3 or 4 years. This test type was not expected to be found in these farms, four of which had beef only, two had suckler only and two other were mixed. Although the number of VE-RHT tests is almost negligible, this reinforces the view that beef herds could be less regularly tested than dairy herds.

5.5.1.3 Purpose of herd

Cattle were tested on 144 out of the 148 study farms. Three out of the four not included in this study had beef only and one had beef and suckler cattle. Only 1% of the cattle tested were reactors. The highest percentage of tests was carried out on dairy only or mixed herds, and consequently, the majority of reactors were also disclosed on these herds. Dairy herds were at higher risk of break down with bTB compared to herds without dairy cattle in the investigation presented in Chapter 4. Various authors have reported the same result and suggestions have been made about the possibility of dairy herds being under higher stress due to management pressure compared to beef or breeding herds. In this study, from the total 156,562 cattle tests, 73.5% were carried out on female cattle and 54% were on dairy, 44% were on beef and 2% mixed breeds.

5.5.1.4 Restocking status

Both the restocking status after being affected by FMD in 2001 and participation of the farms in the RBCT were used as the farm selection criteria. The FMD outbreak in 2001 was a "natural experiment" which offered a unique opportunity to investigate diseases such as bTB. Given the importance of animal movement as the potential to spread infectious diseases in general, and in particular in the case of bTB, the potential of spread from locations where bTB is present and particularly to locations outside endemic core areas (Gilbert *et al.*, 2005; Green and Cornell, 2005), the risk of restocking to individual cattle as well as to herds (Chapter 4) and animal groups (Chapter 6) was investigated as the main hypothesis. A bovine animal being tested on a restocked farm was associated with a decreased risk of becoming reactor if compared with bovine animals tested on a continuously stocked farm. This could be explained by the fact that cattle in restocked farms were within a newly formed herd and even if in an endemic area, these could have not been long enough on the

farm to be as at high risk as those in continuously stocked farms. Figure 5.10 shows the difference on the percentage of reactors disclosed on restocked herds *vs* continuously stocked. Moreover, the fact that restocking status was not statistically significant at herd level, (Chapter 4) indicates that the risk from restocking is associated to an animal rather than to a herd level effect, and that the animal effect are the other infected cattle as exposure to infection. The results from Carrique-Mas (2007) suggest that there was an increased risk of HBD after the first test post FMD on farms that had purchased animals from "high risk" areas. The same authors reported an increased risk of HBD if there was a history of bTB in the herd in the area of the South West, but not in the restocked herds in the North of England. The latter suggests the endemnicity of the disease in the South West and the difficulty to elucidate some risk factors in this area.

5.5.1.5 The intervention treatments within the RBCT

The decision for grouping into the three main treatments was based on the univariable results from the eleven trial areas. Only trial area B1 (reactive treatment in Devon and Cornwall), was associated with a decreased risk of a bovine animal becoming reactor at a test. All the other ten trial areas were not associated with a decreased risk. Therefore, this variable was re-categorised into the three main intervention treatments.

To avoid the spread of bTB as infection could have not been detected due to disruption of bTB regular testing, movement restrictions from 31st January 2002 were imposed on herds that were overdue a skin test (Le Fevre *et al.*, 2005). The disruption in the RBCT trial would have had higher impact in areas where badger removal was carried out as in reactive and proactive compared to survey areas as no action was taken on badgers in the latter. Despite the fact that results from the trial showed an increased incidence in cattle herds in reactive trial areas compared to survey only (Donnelly *et al.*, 2003) and further results showed a decreased incidence in proactive areas compared to survey (Donnelly *et al.*, 2006), in the present study, the RBCT was not associated with a change risk of an individual bovine animal becoming reactor at a test. The same results were observed when the risk of HBD was investigated at herd level (Chapter 4).

5.5.1.6 Variance between herds and between animals within herds

The variances between herds (level 3) and between animals within-herds (level 2) indicated that there are some unexplained risks of a bovine animal becoming reactor associated with these two levels which were not explained by the model. They also suggest some level of clustering at the two different levels. The unexplained risk at herd level could be associated with management factors such as those presented in Chapter 4 and the unexplained risk at animal level could be related to the immune status of the individual animal.

5.5.2 Statistical methods and model fit

Multilevel analysis in the present study was used to investigate risk factors for an individual bovine animal becoming reactor, for two main reasons. The first was because the risk of cattle testing positive to the bTB skin test (SICCT) could vary between herds and between cattle within the same herds. The second was because the use of repeated measures (tests) on the same bovine animal could be incorporated into the model, forming the lower level of the hierarchical structure of the data. Using this method, independence between clustered individuals in a study sample was not assumed, avoiding the underestimation of standard errors and confidence intervals (McDermott and Schukken, 1994).

A deterministic multivariable model, using the Iterated Generalised Least Squares (IGLS) as the estimation method, was carried out. First order MQL as the linearization approximation procedure was used. This procedure offers the crudest approximation and could have led to estimates biased downwards, especially when there are a few observations within a unit at a given level (Goldstein and Rasbash, 1996; Dohoo *et al.*, 2001). As this was not a major concern in the study, the procedure was accepted. First and second order PQL procedures were attempted but these methods were not stable.

An alternative approach to fit models with a discrete outcome is the MCMC estimation, which uses a stochastic (random or simulation) procedure. Here, Gibbs sampling was used to develop the MCMC model. That is, the starting values for the different parameters were given from the IGLS model developed prior to the

MCMC, and from there, there was a sampling from the conditional posterior distributions.

The model was run for 70,000 iterations with a burn-in period of 5,000. Convergence to the joint posterior distribution, where the mean of the explanatory variables were obtained from, was assessed using diagnostic and traceability plots (Appendix 5.2). The precision of the estimates was assessed by using kernel density plots. In some of these, the value zero was included in the kernel plot, indicating that the parameter could increase as well as decrease the risk of an animal becoming a reactor. This was the case for the parameters that were not shown to be associated with a risk in the multivariable model, but were forced into the model (such as the intervention treatment from the RBCT). A strong auto-correlation in the sequential values in a chain generally indicates poor mixing (Green *et al.*, 2004). Autocorrelation coefficients were checked on the diagnostics and they all indicated good mixing of the chains. The model was run with a data point with a large residual value (a herd with 78 reactors disclosed on a test), absorbed in the model as a dummy variable and checked that the model estimates did not change. The final four parameters in Appendix 5.2 show co-linearity, i.e. their estimates are highly correlated. This is to be expected because they all define the magnitude of the effect of past exposure on risk of being a reactor. An alternative approach would be to re-parameterise the model so that the effect of exposure was estimated independently, and introduce contrast parameters to estimate the relative effect of different types of exposure. However, there is no indication in the model diagnostics that this co-linearity had any adverse affect on chain mixing.

The observed and expected values were divided into deciles (Appendix 5.3) Model fit was assessed by calculating the Pearson's Chi-square test suggested by Hosmer and Lemeshow (2000) at both level 3 (herd) and level 2 (animals within herds). A result of a $\chi^2 = 9.12$ and $p = 0.42$ for residuals at level 3 and a $\chi^2 = 14.84$ and $p = 0.09$ for level 2 indicated that there was no statistical difference between the observed and predicted values from the model, suggesting that the model fitted the date well.

5.5.3 The effect of history of bTB on the study farm and farm management risk factors

Farms with and without a history of bTB prior to June 2001 - The same model as presented in Table 5.7 was run for cattle tests carried out on farms that did and did not have bTB prior to June 2001 and since 1995 (when SICCT test results were first recorded in VetNet). The results obtained for the model using cattle tested on the farms that had bTB prior to June 2001 (a total of 109, 527 animal tests from which 529 reactors were disclosed), were not significantly different to those presented for all farms (Table 5.7).

However, when the model was run for farms without a history of bTB prior to June 2001 (using a total of 47,035 animal tests from which 184 were reactors) the effect from restocking after FMD was not statistically significant. This may be explained by the fact that whereas there is infection on the farm, being restocked will not decrease the risk, as there is already infection, although a history of bTB on the farm was not associated with a risk in the multivariable model.

Risk factors from the herd level investigation into the main multilevel model- The farm risk factors from the herd level investigation were not statistically significant and did not change the significance of variables in the multivariable model. Since the multilevel model suggested some degree of clustering and therefore a difference between herds in the risk of an animal becoming a reactor, some of the risk factors from the herd level analysis would have been expected to be associated with a risk in this model. The variables chosen were the ones that were significant in the herd level analysis final model and no attempt was made to include others. There are two plausible explanations for this: that other risk factors associated with the risk at herd level, different from the ones presented in the results in Chapter 4 could have influenced the risk or, that the herd level risk factors are not significant when there are factors associated at lower level and this is the same level as the outcome (animal level).

5.6 Conclusions

By exploring risk factors at animal level, we have been able to elucidate some factors that contribute to the risk of a bovine animal becoming reactor that otherwise it would have been difficult to find. The risk of potential exposure to infected (reactor) cattle was the major finding in the present analysis. Although the study farms are in an annual testing area and as shown by the model, the risk of a bovine animal becoming reactor decreases with a higher frequency testing (short interval tests) when compared to yearly tests, suggesting a reduction of the infection, due to the test characteristics, undetected infected animals could pose a risk and could increase the persistence of *M. bovis* in the herd.

Chapter 6 - Risk factors for reactor cattle identified in 738 animal groups on 148 herds

6.1 Aims

To identify factors associated with the risk of an animal group having at least one reactor animal identified within the group, using the location of the animal groups within the farms' buildings and fields, by the month of the study period.

6.2 Introduction

Little is known about the transmission of bTB in animal groups within herds and most of the work done at this level has involved mathematical modelling (Barlow *et al.*, 1998; Munroe *et al.*, 1999).

The investigation at animal group level could provide further information on how bTB behaves in cattle herds, after investigating this at herd and individual bovine animal levels.

Multilevel or cluster analysis was used, where the three levels were: the herds (level 3), the animal groups within herds (level 2) and the animal group tests (level 1). The study sample used in the analysis presented in this chapter has three main components: the animal groups described by farmers and the identification of reactors within each group, the location of these groups within fields and buildings and the months when the animal groups were located in the fields and buildings.

6.3 Materials and Methods:

6.3.1 The study sample

The study sample was formed by 2,372 animal group tests, comprising 140 herds, 738 animal groups and 404 herd tests that were carried out between 9th October 2001 and 30th June 2003. The study unit was the animal group. The study period was from 1st October 2001 to 30th June 2003.

6.3.2 Source of data

6.3.2.1 Standardised farmer questionnaire

Data collected in the first section of the farmer's questionnaire (Appendix 2.3 part one) and a map of the farm's fields (see example in Appendix 2.5) were used as described in Chapter 2.

Firstly, farmers were asked to identify and number the fields using the maps provided and to draw sketches of the buildings that were used by cattle during the study period. Highlighter colour pens were used to distinguish the different types of field boundaries (Appendix 3.4). Information about slurry spread, growth of maize and presence of wildlife in the fields was asked at this time also using the maps.

Secondly, a description of the herd's natural animal groups was requested. Farmers were asked to identify from October 2001 to June 2003 by monthly interval, the location of each of the animal groups (and an approximate number of animals in the group) in the fields and buildings previously described by the farmer. Interestingly, the maps proved invaluable in eliciting this information: farmers were able to recall the use to which fields were put at different times.

There were a total of 794 animal groups described initially by farmers, 33 were sheep and two others were undefined in the questionnaire. There were 21 groups excluded due to the testing exclusion criteria as described below. In total, 738 bovine groups were considered for the analysis.

6.3.2.2 Identification of reactors in animal group and within fields and buildings

Once the data collected in the standard questionnaire were recorded in the database, farmers were interviewed to find out the identification of reactors in animal groups. To help to recall the information, a full list of all individual animal test results (including results from animals slaughtered as reactors, dangerous contacts, as well as inconclusive reactors and disclosed at slaughter) between January 2000 and August 2004 together with their ear tag numbers was provided. Together with this, a list of animal groups and locations as described by farmers in the questionnaire was included (see example in Appendix 2.6).

6.3.2.3 VetNet Database

Animal groups were assumed to be tested if there was a herd test on the farm during the study period. There were a total of 507 tests carried out in study farms between October 2001 and June 2003, 103 of which were excluded due to the test type criteria. The tests that were excluded were animal tests: VE-IR, VE-PR, VE-PRI, VE-SV, VE-TR and VE-SLH. There were six reactors disclosed at VE–SLH (lesions observed at post-mortem inspection).

Eight farms did not have any herd tests during the study period. They were all grower cattle herds except one that was suckler. They all had tests in 2000 and before, and in 2003 and 2004, but not within the study period.

Reactor animals - There were a total of 549 reactors on the study farms between 1st October 2001 and 30th June 2003 disclosed by herd and animal tests. Out of these, 73 were excluded: 17 were disclosed at animal tests and 56 could not be identified with an animal group by the farmer. In total, 476 reactors were used.

6.3.3 Statistical analysis

The statistical approach taken was the same as in Chapter 5.

6.3.3.1 The outcome variable

The outcome variable was binary: there was or was not at least one reactor animal disclosed in the animal group on the test date that the animal group was assumed to be tested.

An animal group could be tested more than once, and the group could have at least one reactor animal in the group more than once, but a bovine animal could be reactor only once.

6.3.3.2 The explanatory variables

Explanatory variables were divided into three main groups: herd variables (such as restocking status and treatment from the RBCT), location of animal groups in fields and in buildings, and some characteristics of both fields and buildings. An

animal group was tested when it was at a particular location (a particular field or building) when the month for the location in the questionnaire coincided with the month the animal group was assumed to be tested. From the date of the test, calculations for the location of the animal groups were done up to eighteen months prior to the date of the test. For example: the group had or had not been in fields within 6 months prior to the test; the group had been in fields for a number of months within the 6 months to the test. Most variables were binary (coded as 1/0 for yes/no answers).

Variables chosen for this analysis were particularly related with the characteristics of the location (i.e. spread of slurry in fields) rather than characteristics related to the animals (i.e. breed).

A relational Access database was created. Queries were created based on the information on location of the animal groups in space (buildings or fields) but backwards on time as from the date of the test. A final query was then exported as a text file and imported into MLwiN. Herd, animal group and test dates were sorted. A three-level multilevel dataset was created.

6.4 Results

6.4.1 Descriptive results

6.4.1.1 Description of animal groups

The number of animal groups per farm ranged from 1 to 15 (median=5) (Figure 6.1).

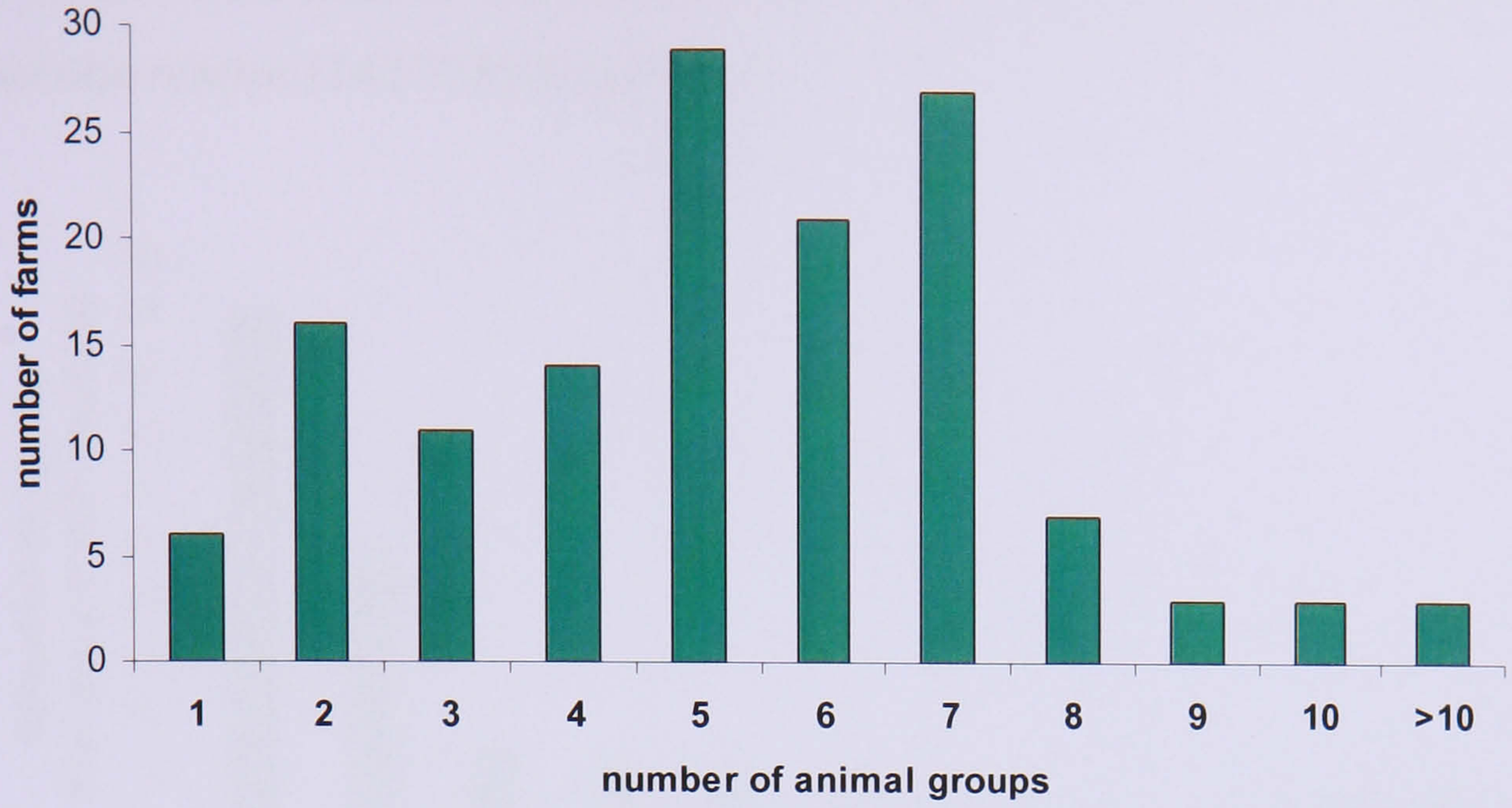


Figure 6.1 - Number of animal groups per farm from 140 farms and 738 animal groups

Type of cattle- The percentage of animal groups with reactors out the total number of animal groups by the type of cattle is presented in Figure 6.2. The groups are presented in the figure as they were defined by farmers, so it can be seen the variety of names that are given when in fact the purpose of the cattle may be the same. For example: steers, yearlings, bulls, youngstock, beef, stores, stock, finishers and fattening could all have the purpose of a grower/beef herd.

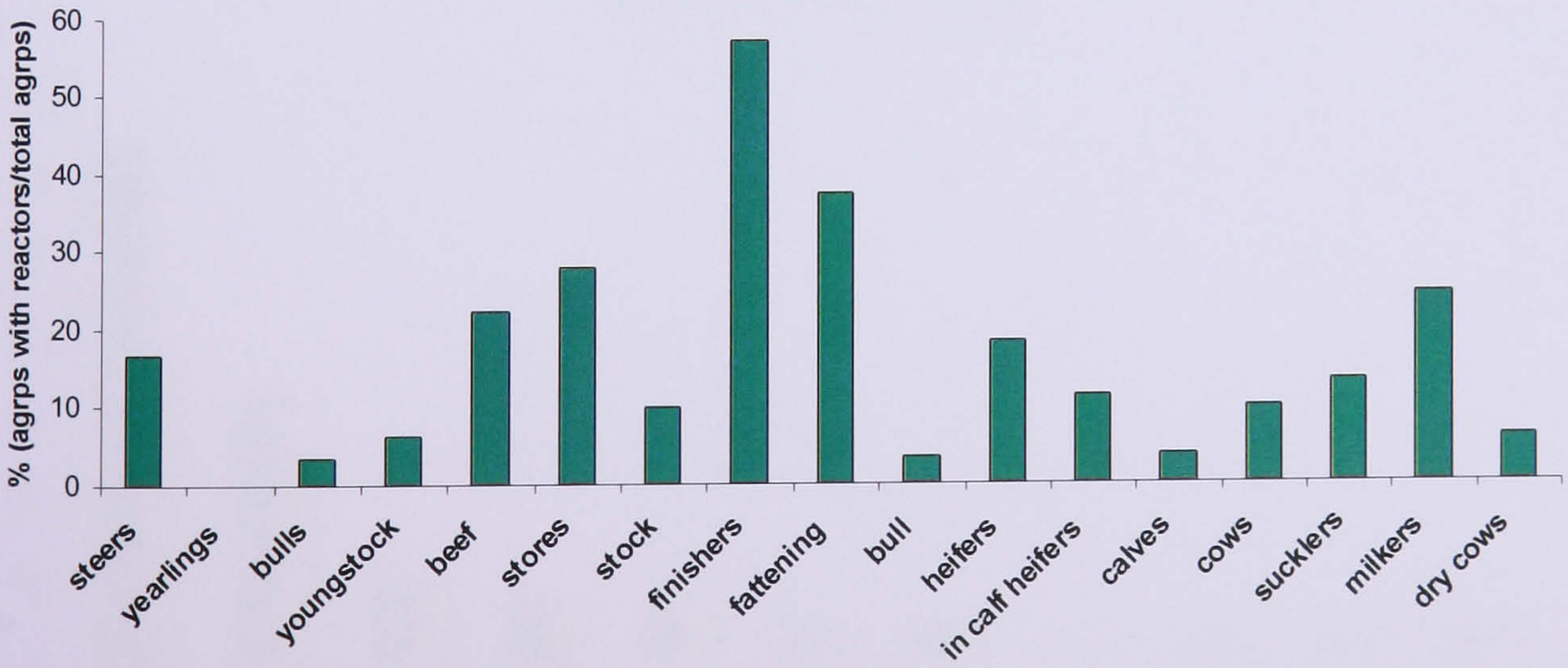


Figure 6.2 - Percentage of animal groups with at least one reactor out of the total numbers of animal groups by type of cattle

Reactors within animal groups - A total of 476 reactors were disclosed and identified during the study period.

There were a total of 738 animal groups in the study sample, of which 19% had at least one reactor (142/738) (Figure 6.3).

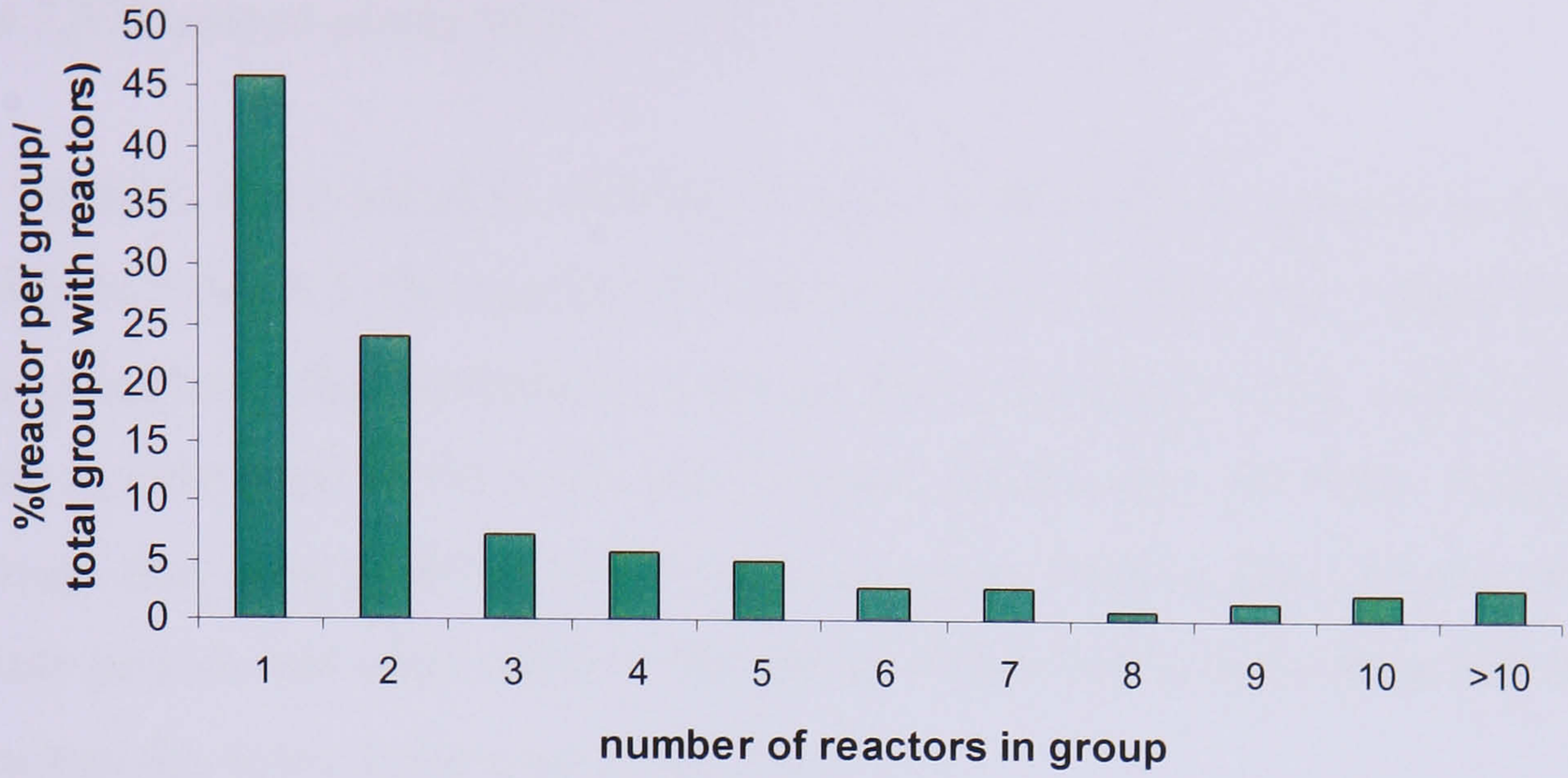


Figure 6.3 - Percentage of reactors per animal group from the 142 animal groups with at least one reactor in the group at the current test during the study period.

As the animal group could be tested several times, at each test, the number of reactors in the group could vary. Therefore, an animal group could be in more than one category based on the number of reactors in the group. The number of positive animal groups and farms by the number of reactors per animal group is presented in Figure 6.4.

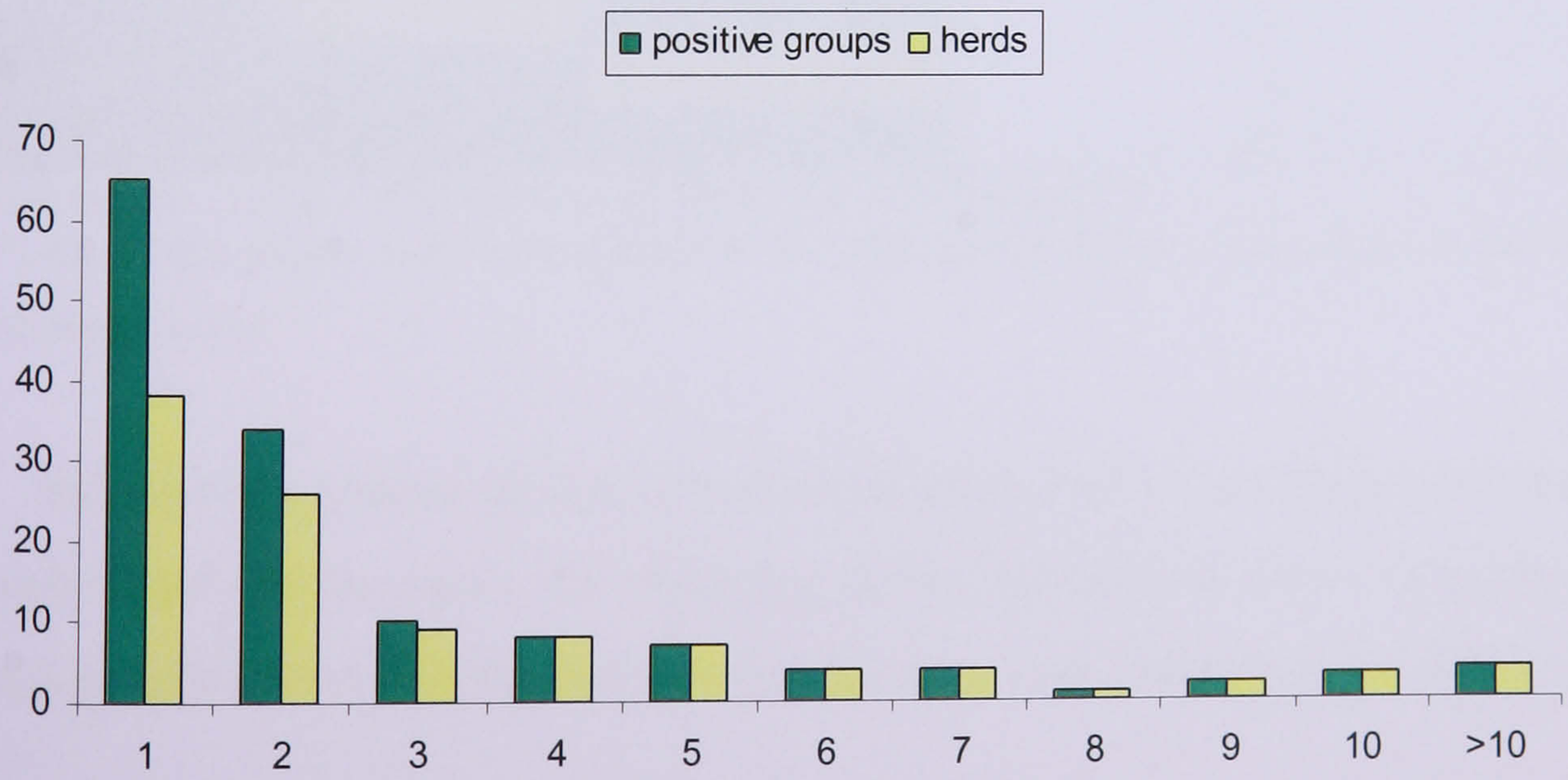


Figure 6.4 - Number of animal groups and farms by the number of reactors per group during the study period

Out of the 2,372 animal group tests, 93.5% did not have any reactors at the animal group current test, 3% had one, 1.5% had two and 2% had from 3 to 63 reactors (median =10). In total, 21% (155/738) had at least one reactor in the group at one of the 2,372 animal group tests.

Farmers were asked to identify reactors within animal groups as from January 2000. In Table 6.1, the number of positive groups (i.e. they had at least one reactor in the group) and the percentage of groups that were positive out of the total positive, nine months prior to the study and fourteen months after the study, is presented. All groups that were positive on all three occasions, that is, prior, during and after the study period, had adult cattle in the group. Three had suckler, three had milkers and one had dry cows at the time of the test.

Table 6.1- Number and percentage of animal groups that had at least one reactor in the group nine months prior to study, during the study and fourteen months after the study period

Nine months prior study* (01/01/01-01/10/01)	Study period (01/01/01-30/06/03)	Fourteen months after study (30/06/03-30/08/04)	Number of animal groups	Percentage out of 738 animal groups
			7	0.95
			11	1.49
			2	0.27
			19	2.57
			27	3.66
			50	6.78
			33	4.47
			589	79.81

Key: yellow colour = bTB positive; green colour = bTB negative
(*) the animal groups in restocked farms in this period were different from those in the study and after the study period

Because the animal groups in restocked farms before and during the period of the study were not the same, the objective of the table above was to present data on the history of bTB on the farms given that reactors were identified as from January 2000.

The number of times an animal group was tested ranged from one to eleven times, (mean=5.22 times, median =4.5).

The number of animal group tests also varied over the months. Figure 6.5 below shows the number of reactors disclosed per month and the percentage of animal group tests.

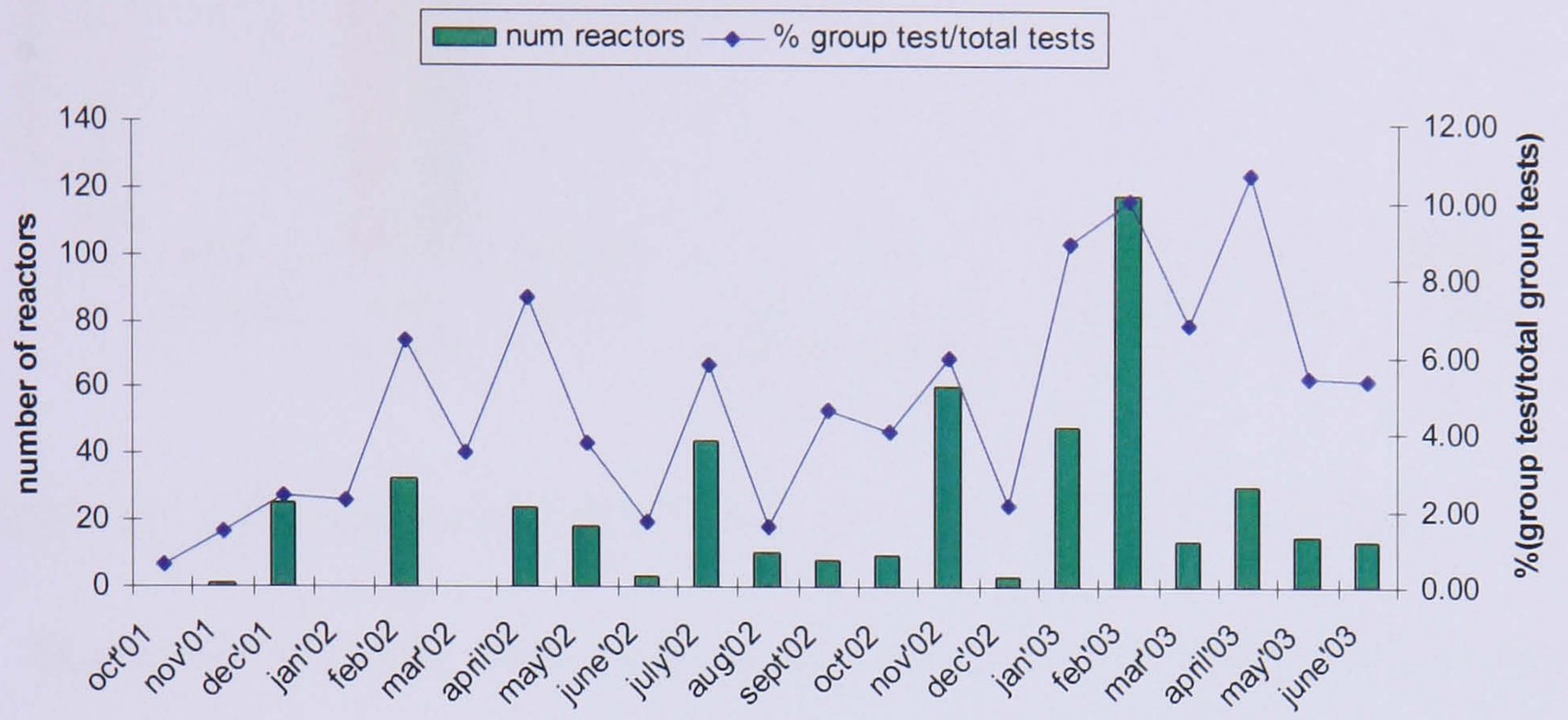


Figure 6.5 - Number of reactors and percentage of animal group tests out of the total animal group tests per month in the study period

6.4.1.2 Description of fields

There were a total of 3,172 fields on the study farms. The number of fields per farm varied from 2 to 53 (with a median of 19).

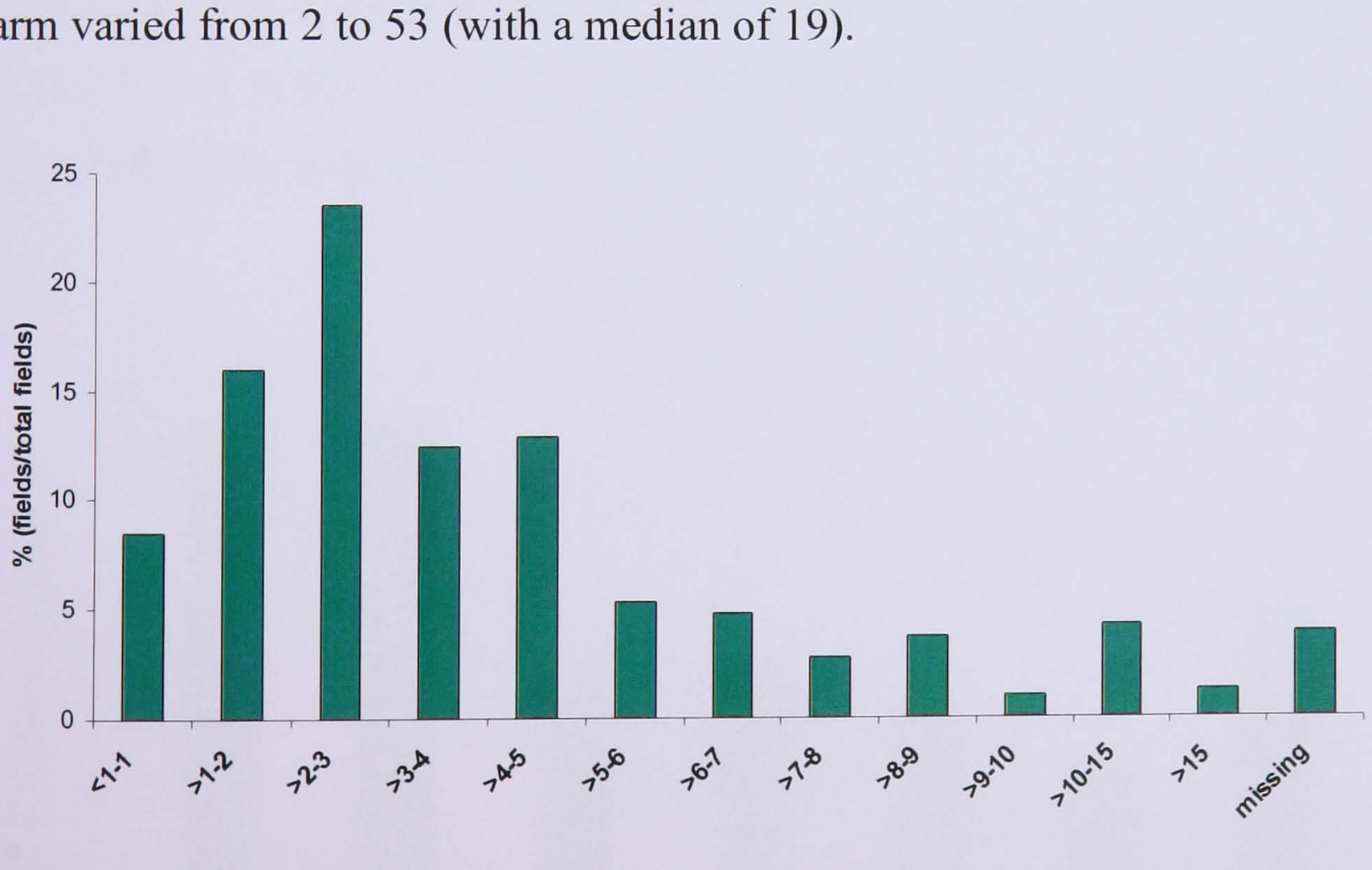


Figure 6.6 - Percentage out of 3,172 fields in the study farms by size in hectares

In 2000, 80% of the fields were used for grazing, 83% in 2001 and 96% in 2002 (Figure 6.7).

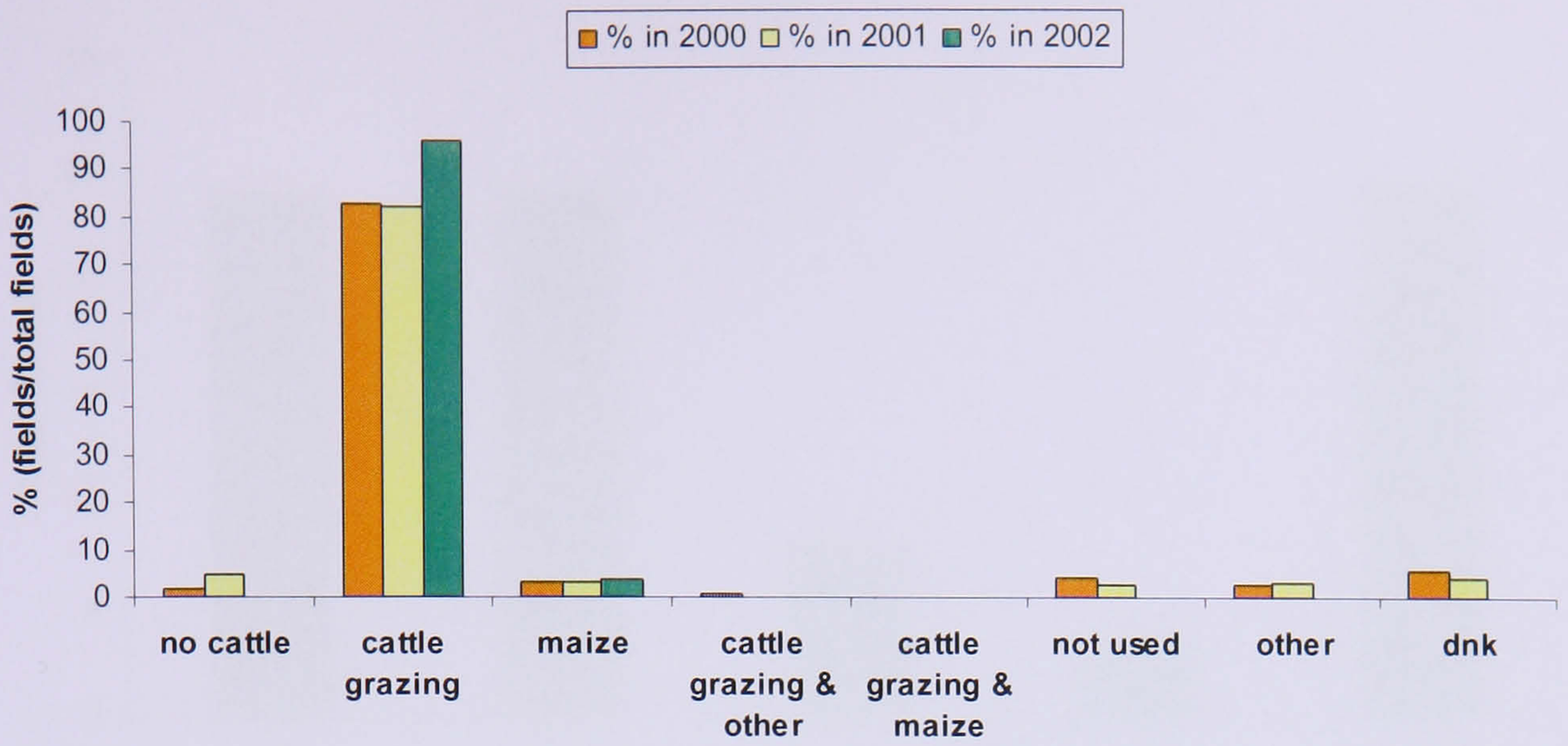


Figure 6.7 - Percentage of fields by use type in 2000, 2001 and 2002

Out of 3,172 fields, 41% were spread with slurry in 2002. 71% of the fields had troughs as the main source of water for cattle, 23% had a stream and the other 6% were supplied by river, spring, ditch, pond or reservoir. According to farmers, 0.8% had an unknown source of water and 0.5% had none.

Most fields had hedges as boundaries. Some boundaries had spaces (i.e.: hedges_sp) through which animals from adjacent fields could pass. Figure 6.8 shows the type of boundaries in the fields and Figure 6.9 the type of wildlife that was present in the fields during the year 2002.

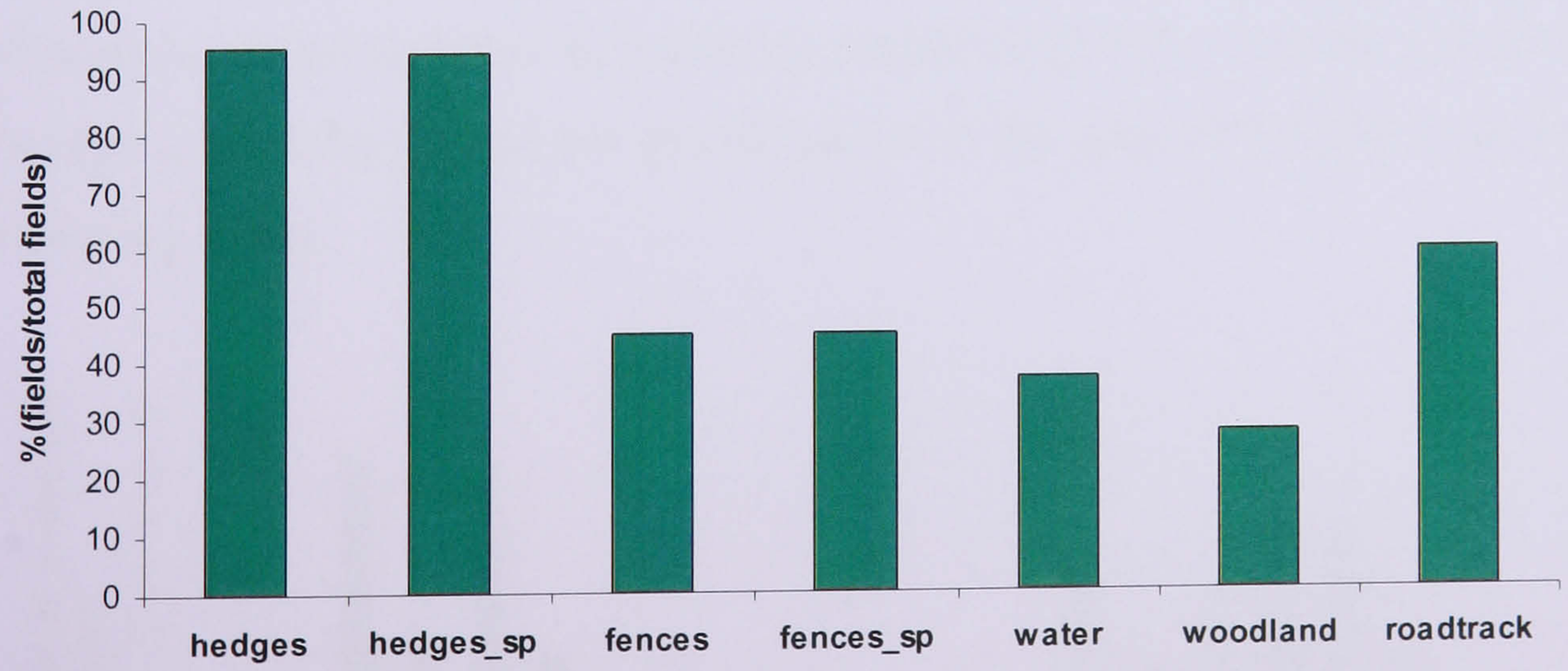


Figure 6.8 - Percentage of fields from 3,172 in the study farms by type of boundary

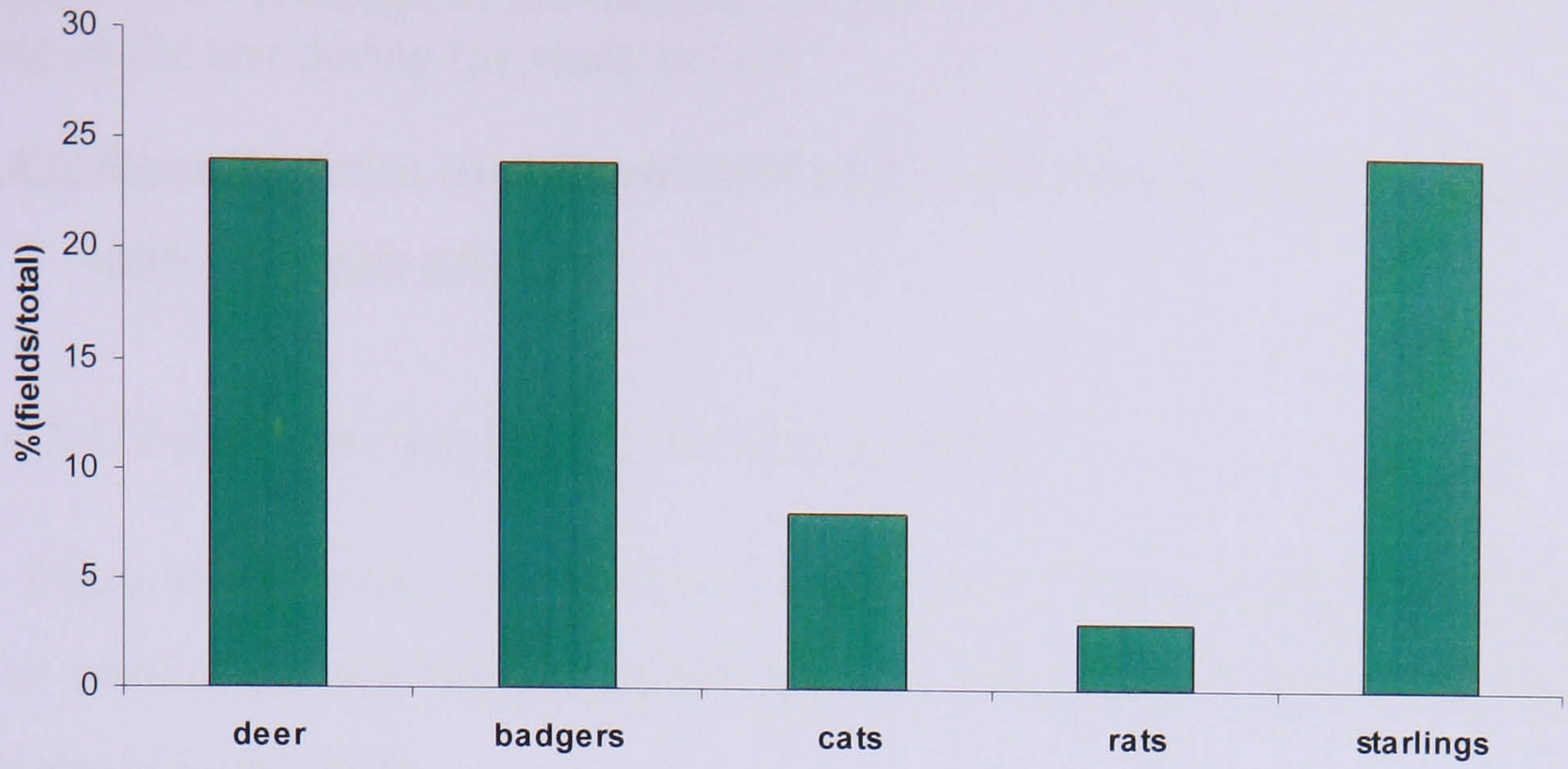


Figure 6.9 - Percentage of fields from 3,172 in the study farms by type of wildlife present on the fields in 2002

There were 71% fields which were owned by the farmer; the remainder were rented.

Out of 2,478 neighbouring fields in 2002, 82% had cattle present, 41.5% had slurry spread and 3.7% had maize grown.

6.4.1.3 Description of buildings

There were a total of 747 buildings. The average per farm was 4. During the same month period, animal groups could have been in different buildings with a different number of animals per group (i.e. animals came in and out the group). The average of animals per group when in buildings ranged from 550 - 1 (with a mean of 30). The average size of the groups per month period at the time of the test is presented in the following figure.

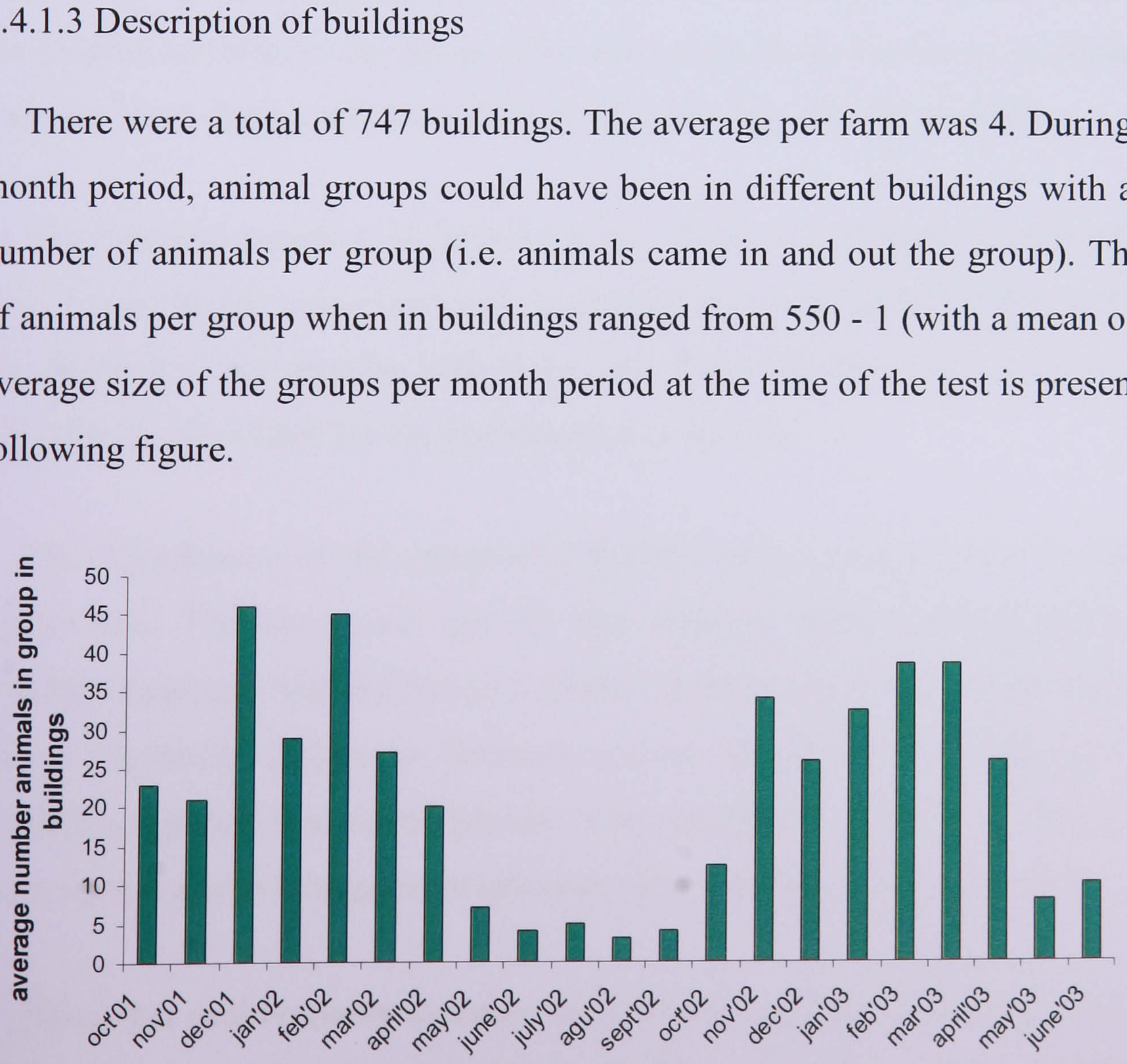


Figure 6.10 - Average of the number of cattle per animal group in buildings at the time of the test during the study period

6.4.2 Results from multilevel analysis: a binomial logistic regression with random effects

6.4.2.1 Univariable results from multilevel analysis

There were a total of 63 variables investigated at univariable level (Appendix 6.1). The number of total animal groups and the percentage of which were positive are presented in the table.

6.4.2.2 Multivariable results from multilevel analysis

The results from the multivariable model are presented in Table 6.2. In this model, variables related to the location of animal groups in buildings and in fields are included. Restocking status and the treatment from the RBCT were not statistically significant. However, as discussed in the previous chapters these two variables were forced into the model as they were used for the selection criteria in the study design. The risk of an animal group having at least one reactor in the group increased with the number of cattle in the group when the groups were located in the buildings. The risk was much higher when the size of the group was over 90 animals.

The reported presence of badgers in the fields was associated with an increased risk of a group having at least one reactor in the group, when the group had been in the fields for two months (OR=3.39, CI=1.18-9.73) and five months (OR=6.59, CI=2.06-21.10) within the six months prior to the test.

The significance of the variance at the two levels was assessed by using a chi-square test. The chi-square test for the variance between herds (χ^2=2.54, df=2, p=0.28) suggested that difference between herds was not significant, whereas, there was a statistical difference between animal groups within-herds (χ^2=7.51,df=2, p=0.02) suggested that the difference in the probability of a reactor being disclosed in an animal group is significant between animal groups within the herds.

Two other multivariable models were created. One model included field variables and the other model included building variables only (Appendixes 6.2 and 6.3). The

main findings from these two models highlighted the importance of the number of animals in the groups, both in buildings and in fields as suggested from the model presented in Table 6.2. The presence of badgers in the fields was not associated with a risk in the field multivariable model.

Table 6.2 - Results from multivariable multilevel logistic regression with random effects from 2,372 animal group tests using 404 herd tests in 140 herds and 738 animal groups

Variable	Number of observations	Number of animal groups with at least one reactor	coef	Odds Ratio	M.C.S.E.	95% credibility interval	
						lower	upper
Herd							
Restocking status							
cont.stocked	1,952	420	ref				
restocked	140	15	-0.98	0.37	0.70	0.10	1.46
RBCT treatment							
survey	860	51	ref				
reactive	886	57	-0.10	0.91	0.60	0.28	2.94
proactive	626	47	0.07	1.07	0.60	0.33	3.50
Number of cattle in group in buildings							
1-30	30	10	ref				
31-60	25	11	1.54	4.66	0.53	1.64	13.18
61-90	16	5	1.56	4.75	0.75	1.09	20.62
91-120	18	8	4.04	56.88	0.81	11.65	277.73
121-550	24	11	3.61	36.86	0.71	9.11	149.08
Presence of badgers in fields							
Number of months within 6 months prior to test							
0	1,574	65	ref				
1	211	10	-0.67	0.51	0.73	0.12	2.15
2	189	21	1.22	3.39	0.54	1.18	9.73
3	143	19	0.96	2.61	0.60	0.80	8.49
4	85	13	0.67	1.95	0.87	0.36	10.69
5	89	15	1.89	6.59	0.59	2.06	21.10
6	81	12	0.27	1.31	2.24	0.02	106.51
Variance							
Between herds			1.70		1.07		
Between animal groups within herds			3.22		1.41		

Risk factors from the herd level investigation into the main multilevel model - Another model using variables in Table 6.2 and those found associated with a risk of HBD as presented in Chapter 4, was built (results are presented in Appendix 6.4). The use of vitamins and minerals (as licks) decreased the risk of an animal group having at least one reactor in the group and purchasing cattle from markets increased the risk. The effect of both factors coincided when the risk of HBD was investigated.

However, none of the other factors associated with the risk of HBD, (presence of dairy cattle, storage of manure/slurry in buildings/close containment and the purchase of steers), were associated with a risk at animal group level.

6.5 Discussion

6.5.1 Results from the multivariable mixed logistic regression model

The increase in the number of cattle in the group in a categorical scale increased the risk of a group having at least one reactor in the group. The effect of the size of the animal group coincides with the results from the investigation of the risks associated with HBD (Chapter 4) and from other previous studies (Pfeiffer and Morris, 1991; Griffin *et al.*, 1996) where herd size was associated with an increased risk. The number of cattle per group when this was located in fields, was also investigated. However, the model would not converge if both the number of cattle per group in fields and buildings were introduced into the model. Because of the higher confinement provided by the environment of a building, the latter was chosen for the final model. However, the effect of the animal group size was highlighted in the two separate, fields and buildings models.

The presence of badgers on a farm has previously been reported as a risk for HBD (Griffin *et al.*, 1993; Denny and Wilesmith, 1999). This was associated with an increased risk when the animal groups had been on fields for two or five months within the prior six months to the test where badgers were present. However, the effects from the treatment from the RBCT was not associated with risk, which could suggest that the risk can be only detectable if investigated at closer level (i.e. presence of badgers on fields known to be grazed by cattle during a period of time). Moreover, the presence of badgers in fields was not statistically significant when only field variables were included in the model. It is worth mentioning at this point that badger activity on the study farms was used as reported by farmers (we did not confirm this). The presence of badgers on the farm would be very likely to be reported by farmers if they had been having bTB for years or if they had been purchasing from infected areas.

Interestingly, animal groups grazing in fields where slurry had been spread during the 18 months prior to the test was not associated with a risk. When the storage of manure/slurry variable from the risk factor analysis at herd level (Chapter 4) was included in the model, this was not statistically significant.

Restocking status and the treatment from the RBCT were both not associated with risk, results that coincide with those from the investigation of the risk of HBD (Chapter 4). As discussed previously, these two variables were forced into the model.

The variances between herds and between animal groups within-herds, were both very high, suggesting a high degree of clustering at these two levels and a lack of explanation in the model for the risk associated with the an animal group having at least one reactor.

6.5.2 Modelling the dynamics of bTB infection within animal groups

The advantage of the data collected at animal group level was mainly that this was done based on the location within the main different parts of the farms and on a monthly time period. In the farmer's standard questionnaire, as well as the number of cattle in the groups, the number of cattle that came into the group and left, at different particular times, was also recorded. This information could be used to further develop the dynamics of infection at group level. In that, the study population would be open (there would be new animals joining and leaving the group), so the susceptible animals in the group could conform a dynamic cohort with a population-at-risk changing over time (Rothman and Greenland, 1998).

6.6 Conclusions

The results from the investigation of the risk of an animal group having at least one reactor in the group suggest that the number of cattle in the group in housed conditions and the presence of badgers as a potential source of infection on the fields where cattle were grazing during some time within the previous six months to the test, are associated with an increased risk. Other variables associated with a risk of a herd HBD such as the use of minerals and licks, and the purchase form markets, also decreased and increased the risk respectively of an animal group having at least one

reactor in the group, when they were included in the model. Other variables such as the storage of manure/slurry in buildings/close containment were not associated with risk.

Chapter 7 - General Discussion and Conclusions

7.1 Introduction

This thesis uses three approaches with the objective of further understanding the epidemiology of bovine tuberculosis in cattle. Field data were collected for the purpose of this study and combined with national data. Although farm risk factors associated with the risk of herds becoming infected with *M. bovis* have been reported in the past, there is no epidemiological evidence to date of studies carried out at individual animal and/or animal group level using the same study farms. The study presented here is based on a cattle population area with a historical association with wildlife as a potential reservoir for infection with bTB. Also, in the same area, some farms were forced to carry out an unusual purchase of cattle due to a recent epidemic of foot-and-mouth disease. This study provides a deep investigation of the disease in cattle using these two criteria.

7.2 Overview

Over the years, efforts have been put into the control and eradication of bovine tuberculosis worldwide. However, in the UK and other parts of the world, it remains an important animal disease with a high economic cost. Due to the introduction of milk pasteurisation as a method of control in humans, the risk to public health is less important in developed countries, but it is not so in developing countries where control measures are scarce.

Cattle are the main species affected by *M. bovis* and the respiratory tract has been consistently recognised as the main route of transmission. It is on this basis that restrictions of cattle movement on affected herds are applied. Despite the old implementation and acceptance of this measure by farmers and some professionals involved in control programmes, the role that cattle play in the transmission of *M. bovis* and how the disease occurs in the field has not been completely understood. Moreover, in areas where a wildlife reservoir is identified, cattle could have been underestimated as an important source of infection.

There are several explanations that could be given to highlight the difficulties in understanding and controlling the disease. These are: the sub-clinical presentation; the interaction with a wildlife population and the provision of a vaccine for cattle, badgers or both that enables differentiation between diseased and vaccinated animals; and the lack of an improved diagnostic testing method.

With this frame in mind, epidemiological studies as presented in this thesis can help to elucidate farm risk factors associated with the disease and to understand how it occurs in the field.

7.3 The cohort study and statistical analysis approach

Ideally, as in a cohort longitudinal study, the questionnaire data should have included repeated measures of the same explanatory variables over the period of the study. However, given the detailed information recorded in the first farm visit in the farmer's questionnaire, consideration was taken into whether it was feasible to visit the farms again to collect the same information or to collect further different data. Farms were re-visited to collect information on the reactor animal location within the animal groups previously identified by the farmer and to carry out a building survey to complement the farmer's questionnaire. The use of the SICCT over the three years period as used in the analysis carried out in Chapters 5 and 6, justifies the cohort longitudinal study.

The majority of studies that have been done in the past to investigate risk factors for bTB in cattle herds, have had the herd as the unit of study, using case-control studies and logistic regression as the statistical method. In this thesis, when the risk of bTB was investigated at herd level, survival analysis was carried out as an alternative approach to multivariable analysis using proportional hazards regression models already suggested by Morris *et al.* (1994).

Data were set up in a hierarchical structure to investigate risk factors having the individual animal and animal groups as the study units. The statistical method used was logistic regression with random effects. Since the disclosure of disease, using the international standard skin test, occurs as rare events, other methods such as Poisson regression could have also been justified as an alternative method. However, the

logistic regression approach was attempted first and proved to be adequate to identify robust estimates of variables for risk factors where the outcomes were binary. At any rate, and despite the wide use and ease of use of odds ratios in interpreting epidemiological data, these should be used with caution. In that, they should be taken as incidence-ratio estimates and not as summaries of effect in themselves (Greenland, 1987).

The main hypothesis being tested in this thesis was that some factors affected herds, animal groups and individual animals with the risk of bTB. It could be argued that given the low response rate (40%) some risk factors could have not been detected due to a low power of the study (as discussed in Chapter 2, Section 2.10.1). This could be the reason why variables such as the intervention from the RBCT were not seen as a risk of HBD. The low sensitivity of the test was also a drawback which could have been reflected in a lower number of positive outcomes. However, this is the only standard test which is available.

7.4 Identification of risk factors

The results from the proportional hazards model that was built to investigate factors at herd level have suggested an association of some farm management factors with the risk of bTB, some of which coincided with results from previous studies. These are: the increased risks associated with herds having dairy cattle, with the increase in the herd size, and with the purchasing of steers and from markets. The use of vitamins and minerals (licks) reported by Griffin *et al.* (1993) to increase the risk of HBD was associated here with a decreased risk.

Although some experimental studies suggest that the survival of *M. bovis* in cattle slurry could be favoured under adequate conditions (Scanlon and Quinn, 2000), the storage of manure/slurry in close containment associated with an increased risk in the study presented here, had not been reported before. This is an important finding as a risk factor contributing to the persistence of the bacteria in the farm environment. But interestingly, when this variable was introduced in the analysis of animal groups, it was not statistically significant and neither was the grazing of cattle in fields that had been spread with slurry. The only association with the management of manure, was the increased effect that the variable spreading manure/slurry all year round in

the fields had on the risk of an animal group having a reactor. However, it was not associated with a risk of HBD in the Cox model. In contrast, factors that were related to the introduction of *M. bovis*, such as purchasing new cattle from markets and to the provision of vitamins and minerals (licks) in the feeding ration, were significantly associated with the risk of an animal group having at least one reactor in the same was as they were for the risk of a herd breaking down with bTB.

The transmission of infection between cattle has been demonstrated from experimental studies, but not from field studies such as the one presented here. The results from the multilevel analysis at animal level strongly suggest an association between the potential exposure to infected (reactor) cattle and the increased risk of a bovine animal becoming reactor. The risk of a bovine animal reacting to the SICCT test was divided into two groups: those that did have potential exposure to other reactors in previous tests (98% of all reactors) and those that were not previously in a herd where reactors had been disclosed during the time the bovine animal was on the farm. The risk increased with age as would be expected given the greater chance of coming into contact with other reactors, and also if there were other reactors disclosed at the same test. The latter could be used as an indication of cattle being a source of infection themselves or they could reveal a farm-specific risk (environment).

Individual animal characteristics, such as age, sex and breed, are factors which can be explained by the potential exposure to infection. In that, some cattle would be potentially more exposed to infection than others: cattle of younger age would have been less time on the farm and therefore potentially less exposed than older cattle by the time they are tested; female cattle used for dairy or breeding purposes would be longer on the farm than grower cattle used for beef purposes. The lack of evidence that sex, age or reproductive state have direct influence on the risk of an animal becoming infected has previously been reported (Morris *et al.*, 1994).

Given that all the tests (regardless of the test code applied) are carried out in the same manner, the inclusion of the test type in the multilevel model at animal level indicated that the cattle tested with a short-interval test, were associated with a decreased risk compared to those tested with a yearly test. Although the

interpretation of the result may be difficult (as the test carried out in the animal is the same in all cases), a plausible explanation is that the higher frequency testing does contribute to removing infected cattle, and therefore removing a source of infection for others in the herd. The reduced testing during FMD and subsequent increase in bTB diagnosis supports this.

The investigation at animal group level highlighted the importance of the number of cattle in the group as a risk factor for having at least one reactor animal in the group. The information collected at this level has not previously been recorded elsewhere and provides a unique chance of following the animal groups within the farms by location in fields and buildings by monthly periods during the study period. The risk from the reported presence of badgers in the fields as a wildlife reservoir was associated with an increased risk if the group had been in the field for two or five months within the previous six months to the test. The fact that the effect of a wildlife source was associated with a risk here could be because it was known to be located in a particular field/building with presence of badgers. However, the treatment from the RBCT was not statistically significant in any of the three investigations. As described previously in this chapter, the study was set up within the areas of the RBCT but none of the stages of the trial was confirmed by us. Deer and other wildlife animals were not associated with a risk at herd and animal group levels.

On the other hand, it is not clear why keeping animals in a larger group should increase the risk of reactor presence. For most infectious disease, risk of transmission increases with increased population density, and this is certainly a possible explanation in terms of the risks associated with housing cattle in (poorly ventilated buildings, although this was not a risk in the animal group investigation) buildings. However, if the environment (e.g. badgers) is a source of infection, then it is not clear why a larger group is at greater risk. Most likely, there are several processes operating: initial infection from the environment (as associated with reported badger activity) and transmission between cattle (as associated with larger group size).

Restocking, although it was not associated with the risk of a herd breaking down or an animal group to have at least one reactor in the group, it decreased the risk of a

bovine animal becoming a reactor. The most plausible explanation for the latter is that cattle in the newly formed herds would have not have sufficient time on the study farms to be in-contact with other infected cattle, despite any risk present from the environment. Although these could have been infected in the source herds, the risk was lower when compared to animals in the continuously stocked herds.

When the risk factors identified with the risk of HBD (Chapter 4) were included in the multilevel model of a bovine animal becoming a reactor as the outcome variable (Chapter 5), none of the risk factors were statistically significant. This emphasises the advantage of investigating the disease at this deep level and importance of the findings at animal level.

The benefit from this study will be to use the findings as evidence for implementation of future measures for control. However, can we extrapolate the results from this study to other regions, and especially to regions where there is not a recognised wildlife reservoir? Given that the study was set up in an area where a randomised badger trial was taking place for approximately eight years, the effect of this wildlife as a source of infection was controlled for and furthermore, no effect was observed. The last study reported from that trial suggest that if culling of badgers is established as a method of control of bTB in cattle, this would be only beneficial if carried out systematically over large areas and long period of time (Donnelly *et al.*, 2007). Based on this, and on the findings presented in this thesis, it would be very beneficial from the disease control point of view, not to overlook the importance that cattle play in the transmission of the disease. The implications of bTB in cattle if bTB in wildlife disappeared, was not a question addressed in this thesis. However, from the results presented here there is enough evidence to suggest that the cattle to cattle transmission could enhance the persistence of infection and therefore measures directed to reduce the infection in cattle as a very important source of infection to other cattle. This is in agreement with the recommendations that the ISG has just recently published in its Final Report (DEFRA, 2007), in which the increased use of the gamma-interferon test in parallel with the SICCT and even depopulation in chronic heavily infected herds, and the tighter movement controls are recommended.

7.5 Further work

Two main areas that have been identified could be explored further. These are: the study of the transmission dynamics within animal groups as discussed in Chapter 6 and the investigation of the immune status of individual cattle for other diseases such as Bovine Viral Diarrhoea Virus and Johne's disease, but also others such as Neosporosis, Leptospirosis and Infectious Bovine Rhinotracheitis. Serological test results from serum samples were taken from the study farms (BBSRC project) and could be used at herd level but also could be used to elucidate further individual animal factors that could be associated with an immunosuppressive effect. This investigation would be a unique contribution to the knowledge of bTB in cattle given the detailed identification and data available at individual animal level.

7.6 Conclusions

Bovine tuberculosis remains an important disease of cattle with a high economic impact in the UK. Epidemiological studies can help to understand further how *M. bovis* behaves in cattle herds, by investigating farm risk factors associated with the presence of the disease in the herd. Whilst more work towards understanding the immunity of bTB and the development of other control measures will contribute to the control of the disease, the work presented in this thesis provides new, robust and sound evidence of the significant role that cattle play as a source of infection to other cattle. These results can be used to inform future control measures in the UK and possibly in other parts of the world where the bTB is present.

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APPENDICES

Appendix 2.1 – Introductory letter to the study

Dear Sir or Madam

THE UNIVERSITY OF WARWICK

HOW IMPORTANT ARE COWS IN TRANSMISSION OF TB?

Bovine tuberculosis (TB) is currently increasing in many areas of the country.
We, independent scientists at the University of Warwick, are initiating research into the way TB is transmitted between cattle.

Your participation in this project is essential to its success.
As you will be aware, we need to understand more about TB to reduce its occurrence. Our aim is to obtain the most valuable information to establish recommendations for future prevention and control of this expanding disease.

So, we would kindly ask to interview you and collect data from your farm and your farm records. With your permission we would like to use data from electronic sources including the British Cattle Movement Scheme, the State Veterinary Service, the Veterinary Laboratory Agency, your vet and any production records. The information from these sources will not be reported back to any organisation.

As part of the project, where possible, we would like to collect blood samples from adult cattle. This will enable us to investigate other diseases that may interact with TB, such as BVD and IBR. All the information collected will be strictly confidential. However, results will be given confidentially to individual farms on request, as well as interim and final study results.
We are a large team of researchers and will try to work around your time to minimise the extra work we place upon you. For example, to save you some time, we could arrange our visit on the same day as the TB or brucellosis test and assist you with this whilst we collect blood from your cattle.
We would be most grateful if you would participate in this project.

Please find enclosed a copy of the agreement form that we will be asking all participating farmers to sign. In the coming weeks we are organising evening meetings at local venues where we will be describing the study. Please come; it will give you a chance to discuss any queries that you may have. At this stage, we would simply like to know whether you would, in principle, agree to participate and whether you will be attending one of the meetings. We have set up a free telephone number to enable you to contact us (0800 389 1578).

The date and venue for the meeting in your area is:
On Tuesday 4th February at the "EXETER INN", BAMPTON

We look forward to meeting you in the near future,
Yours sincerely
Ana Ramirez MRCVS
Laura Green MRCVS PhD
Graham Medley PhD

Appendix 2.2 – Participation Agreement Form

THE UNIVERSITY OF
WARWICK

UNIVERSITY OF WARWICK TB STUDY PARTICIPATION AGREEMENT

I (Mr/ Mrs)
from _____
and CPH number ___ ___/ ___ ___ ___/ ___ ___ ___ ___,
agree to participate in the University of Warwick TB Study. I give my consent for the Study Team to:
access information on TB testing records held at the regional Animal Health Office from the State Veterinary Service which relates to my farm;
access information from the British Cattle Movement Scheme;
blood sample cows on my farm and use the blood for further research on diseases that may interact with TB;
contact my local veterinary practice and Veterinary Laboratory Agency to gather information about other diseases in my herd;
ask me about my cattle and their management and my farm.

Signature: Date:

My vet is: _____
Address: _____

We researchers at University of Warwick undertake to
treat all the above information obtained about your farm, from whatever source, in the strictest confidence;
act and conduct research according to the highest scientific standards with the aim of improving of British Agriculture.
Signature: Date:

Print Name: _____
(for and on behalf of the University of Warwick TB Study Team)

The TB Study Team, Ana Ramirez, Dr Laura Green & Dr Graham Medley,
Dept. Biological Sciences, Warwick University, Coventry CV4 7AL;
Free Tel: 0800-3891578; Fax: 0247652 4619; E-mail: tbproject@bio.warwick.ac.uk

Appendix 2.3 - Farmer questionnaire Part 1

Please find enclosed a map of your fields. Let us know if this map is not all appropriate and we will send you the right one.
Please use the marker pens to highlight the boundaries of each individual field that has been used by cattle from October 2001 to Turn Out 2003.
Please use the marker pens to highlight all the boundaries:
Blue pen to mark in water
Green pen to mark in hedges (please mark with hyphens if accessible by animals)
Pink pen to mark in fences (please mark with hyphens if accessible by animals)
Yellow pen to mark in woodland
Orange pen to mark in road or track
Please number each field by writing 1, 2, in the middle of the field.
Please indicate in which fields maize was grown in 2002 by writing M in the field
Please indicate in which fields slurry and/or manure was spread in 2002 by writing S in the field
Please indicate in which fields you saw wild deer in 2002 by writing D in the field.
Please indicate fields where you saw badgers or signs of them in 2002 by writing B in the field
Please indicate fields where you saw cats or signs of them in 2002 by writing C in the field
Please indicate fields where you saw rats or signs of them in 2002 by writing R in the field
Please indicate fields where starlings were active in 2002 by writing ST in the field

What signs of badgers did you see?
What signs of cats did you see?
What signs of rats did you see?
What signs of starlings did you see?

Please record the following for each field that was used by cattle in 2002, as well as those where maize was grown in 2002, including the off ground and rented land. Use the field numbers as identified in the map. Tick all that apply unless otherwise stated.

Field Number	F	F	F	F	F	F	F	F	F
What is the size? (acres / hectares)									
Was it owned(O) or rented(R)									
If there were cattle did they drink from: Stream									
Trough									
Other (state)									
Was this used in same way in 2001? yes/No (specify use)									
Was this used in same way in 2000? yes/No (specify use)									
Field Number	F	F	F	F	F	F	F	F	F
What is the size? (acres / ha)									
Was it owned (O) or rented(R)									
If there were cattle did they drank from: Stream									
Trough									
Other (state)									
Was this used in same way in 2001? yes/No(specify use)									
Was this used in same way in 2000? yes/No (specify use)									

We would like to know where you kept your cattle and sheep from Winter 2001, during 2002 and up to Turn Out in 2003.
Please complete the summary of cattle location form. Write, by drawing an arrow down, which type of cattle by group (and sheep) were in which field and building (or pen), number, and when .You may need your diary for this.
Please at the same time as the summary form, complete this following table:

Give the list of group of cattle as you have entered in the summary form. Please split the groups as neccesary	Did individuals join the group? How many?	If individuals joined group, where did they come from?	Did individuals leave the group? How many?	If individuals left group, where did they go?
i.e.:: suckler cows, while in Fields 2 and 4, from March-Sept'02	Yes, 3	From market	No	N/A

FARM BUILDINGS- Period Winter 2001 to Turn Out in 2003

Please make a diagram of your farm buildings and pens within the buildings, and yards. Please label each building with a name, and if there are any pens in the building, label those with an individual number. State also the type of separation between the pens in the building;, any passageways, feeding areas, etc.
Also record
What else was kept in the pen (other stock, forage, feed, equipment etc.) in 2002
Please indicate in which pens or buildings you saw signs of wild deer in 2002 by writing D in the sketch
Please indicate in which pens or buildings badgers were active in 2002 by writing B in the sketch
Please indicate in which pens or buildings cats were active in 2002 by writing C in the sketch
Please indicate in which pens or buildings rats were active in 2002 by writing R in the sketch
Please indicate in which pens or buildings starlings were active in 2002 by writing ST in the sketch

FARM BUILDINGS- Period Winter 2001 to Turn Out in 2003

For each building, please record its length, breadth and maximum height on the Table below, and indicate whether you consider the ventilation good, adecuate or poor.

Name of building (specify if split in pens)	What was the use of the building in the winter of 2001 to turn out in 2002?	What was the use of the building from turn out in 2002 to winter 2002?	What was the use of the building from winter 2002 to turn out in 2003?	What is the age of the building in years?	Length	Breadth (give min-max)	Maximum height (give min-max)	Ventilation Good = G Adequate = A Poor = P
e.g. cubicle house	Milking cows			15	50 ft	30 ft	15 ft	G

Please identify each one of your neighbours' fields that touch your land on the map

Use the first letter of the farmer's surname following by a number (eg. if the field belongs to Mr Roberts please write in the fields R1, R2, R3, etc).
For each of these fields please do the following:

If maize was grown in the field in 2002 write an M
If manure and/or slurry were spread in the field in 2002 write an S
Please indicate in which of your neighbours fields you saw wild deer in 2002 by writing D in the field
Similarly, Please indicate fields where badgers were active in 2002 by writing B in the field
Please indicate fields where cats were active in 2002 by writing C in the field
Please indicate fields where rats were active in 2002 by writing R in the field
Please indicate fields where starlings were active in 2002 by writing ST in the field

Please use the previous information about your neighbours' fields to complete the following table. Please write only about fields that had cattle or spread slurry and/or manure in 2002.

NEIGHBOURING FIELD IDENTIFICATION	(eg..R1)					
Were there cattle in this field in 2002?						
If yes, in which months?						
Was manure or slurry spread in 2002?						
If yes, in which months?						

NEIGHBOURING FIELD IDENTIFICATION						
Were there cattle in this field in 2002?						
If yes, in which months?						
Was manure or slurry spread in 2002?						
If yes, in which months?						

Summary of Cattle Location by Group From Winter 2001 to Turn Out 2003
Please think about where the animals were located during this period of time and we will complete this table when we visit you

Groups ID: i.e. milking cows, 8-14m.o. male calves, heifers, etc.

Group ID ⇒			
Month of year/day/ period	N animals	Location	If group sharing field/building at same time please give details
Oct'01			
Nov'01			
Dec'01			
Jan'02			
Feb'02			
March'02			
Apr'02			
May'02			
June'02			
July'02			
Aug'02			
Sep'02			
Oct'02			
Nov'02			
Dec'02			
Jan'03			
Feb'03			
March'03			
Apr'03			
May'03			
June'03			

Appendix 7.1 – Farmer's questionnaire Part 2

FARMER INTERVIEW ON THE MOVEMENT, MIXING AND CONTACT BETWEEN CATTLE AND OTHER POSSIBLE SOURCES OF M. BOVIS

Date of interview		Dd/mm/yy
Name of interviewee		Name of farmer
Name of Warwick interviewer		Name
Questionnaire ID		Place stamp

Thank you for agreeing to participate in this study. We want to build up a picture of how you manage your farm using maps of the farm and your knowledge. We will ask questions from October 2001 to May 2003. Next year when we come back we will just ask about 2003 -4. We do ask for detail on some aspects, please do tell us when you are unsure of your answer. We hope you enjoy this!

General questions

We would like to start by asking a few general questions

Question	State answer and unit		Unit for answer
What is your role on the farm?			Job description
How long have you been on this farm?			Number of years
Do you own this farm?			Yes / no
Do you own any extra land that you regularly run this herd on? If yes, what is the CPH number of this land that you own?			Yes / no County/parish/holding number
Do you rent in any extra land that you regularly run this herd on?			Yes/ no
Do you own other farms?			Yes / no
If yes, How many?			Number
If yes, please give the cph numbers			County/parish/holding number
What is the acreage of this farm?			Number of acres / hectares
How much of this land is used for cattle?			Number of acres / hectares
What type of cattle do you have?			Dairy Beef Both
Do you have any other stock than cattle? Yes No If, yes What do you have? How many of these? (prompt for each species)	Sheep		Number
	Poultry		
	Pigs		
	Farmed deer		
	Horses		
From where do you get drinking water for your cattle?			Source of water well, spring mains

Table 1 - List of manures used on farm

We'd like to record which types of manure you have used and stored on your farm since October 2001

Manure ID	Which animals did the manure come from? Type pf cattle Sheep etc	Was the manure produced on this farm? Yes / No	What type of manure was it? manure whole slurry separated solid separated liquid	Where was it stored? Pit, Silo Tank, field ID Building, yard other	When was the manure made? state period or months/all year		When was it spread? months	In which fields was it spread? Field ID	Did you use your own spreader? Yes/No	If no, How many other cattle farms use this equipment? Number or n k
					month of first addition	month of last addition				
S										
S										
S										
S										
S										
S										

So, these are all the types of slurry and manure you used from October 2001 – May 2003? If yes, go to Table 2, if no, complete

Table 2 – Feed

Tick	Which raw ingredients did you use?	Which years?	Was it home produced?	Was this machine mixed?	Where was this stored?	To which cattle was it fed?	What did cattle eat this from?		Did you see evidence of the following in feed whilst stored? Yes/ No			
If used		2001, 2002, 2003	Yes / no	Yes / no	Silo, pit, barn, etc.	Record groups from buildings and fields	Floor, Troughs, bucket,etc	Specify by group	Badger	Cats	Rats	I
	Maize silage											
	Grass silage - clamp											
	Grass silage - Big Round Bale											
	Grass Silage – Big Square Bale											
	Hay Big Round Bale											
	Hay Big Square Bale											
	Hay Small Bale											
	Wheat straw Big bale											

Which of the following raw ingredients did you feed to your cattle from October 2001?

Tick	Which raw ingredients did you use?	Which years?	Was it home produced?	Was this machine mixed?	Where was this stored?	To which cattle was it fed?	What did cattle eat this from?		Did you see evidence of the following in this feed whilst stored? Yes/ No			
If used		2001, 2002, 2003	Yes / no	Yes / no	Silo, pit barn, etc	Record groups from buildings and fields	Floor, troughs, bucket,etc	Specify by group	Badger	Cats	Rats	Deer
	Wheat straw small bale											
	Barley straw Big bale											
	Barley straw Small bale											
	Concentrates											
	Straights											
	By-products											
	Milk Powder											
	Whole milk											
	Minerals & Vitamins incorporated											
	Minerals & Vitamins – lick											

Table 3 Bedding

What type of bedding material did you use for your cattle from October 2001?

Tick If used	Bedding material	Where was it stored? In building, in field, record ID	Was it home produced? yes/no	When did you use this? 2001, 2002, 2003	For which cattle was this bedding used?	Did you see evidence of cats, rats, badgers, or deer in the bedding store ? Yes/ No specify
	Hay Big Round Bale					
	Hay Big Square Bale					
	Hay Small Bale					
	Wheat straw Big bale					
	Wheat straw Small bale					
	Barley straw Big bale					
	Barley straw Small bale					
	Sand					
	Wheat Straw Round Bale					

Table 4 - Milking Practices from October 2001 to May 2003

	How long did it take from gathering to return ? Hours	Number of milking times per day?	How much concentrate did you feed? kg / cow, to yield or none	Gathering yard before milking			Standing yard after milking			Did you see rats, badgers, cats or deer in any areas? Yes/ No, specify which animals		
				Did you use a gathering yard before milking? Y/N	How often did you clean it? per day	Did you use this yard for other purposes? eg:feeding	Did you use a standing yard after milking? Y/N	How often did you clean it? per day	Did you use this yard for other purposes? eg:feeding	Parlour	Gathering Yard	Stanc Yard
Oct '01												
Nov '01												
Dec '01												
Jan '02												
Feb												
Mar												
Apr												
May												
Jun												
Jul												
Aug												

	How long did it take from gathering to return ? Hours	Number of milking times per day?	How much concentrate did you feed? kg / cow, to yield or none	Gathering yard before milking			Standing yard after milking			Did you see rats, badgers, cats or deer in any areas? Yes/ No, specify which animals		
				Did you use a gathering yard before milking? Y/N	How often did you clean it? per day	Did you use this yard for other purposes? eg:feeding	Did you use a standing yard after milking? Y/N	How often did you clean it? per day	Did you use this yard for other purposes? eg:feeding	Parlour	Gathering Yard	Stanc Yard
Sep												
Oct												
Nov												
Dec												
Jan '03												
Feb												
Mar												
Apr												
May												

Table 5 - People & Equipment Contacting this Herd since October 2001

We'd now like to record how many staff, visitors and hired equipment contacted your herd since October 2001?

Staff working with your herd	Number	Unit of time
How many people worked with your herd? including farmer, family members, relief milkers, full time and part-time staff		
How many other cattle herds did your staff come into contact with in 2002?		
Visitors contacting your herd	Number	
How many people visited your farm per week/month/etc in 2002 including:		
Vets		
Milk recorder or Salespeople (specify)		
Other (specify)		
Equipment	Number	How long was this equipment used for in this herd? week/ year/day
Which of the following equipment did you hire or share?		
Cattle truck		
Crush		
Contract baling (hay or straw) equipment		
Contracted maize silage equipment		
Contracted grass silage equipment		
Other:		

Table 6 – Contact with other cattle outside this herd since October 2001
Have your cattle had any contact with any other cattle outside this herd?
If yes,

Which cattle outside your herd did your cattle contact? Add others as necessary	How many cattle from column 1?	Which group of your cattle was contacted?	Did these cattle break into your / your neighbours maize fields?	How often? Times /week /month /year	On average, how long did the event last? Time in minutes/hours/weeks
Bulls hired in					
Bulls lent out					
Breakouts					
Break-ins					
Cattle walk through this farm					
You walk cattle through another cattle farm					
Return from markets					
Return from shows					
Return from abattoirs					
Other:					

Table 7a – Results of Last TB Test
TB and TB Testing in this herd:

1. Have you ever had a TB breakdown reactor cattle in this herd? If NO go to Q10-	Yes No DNK
2. When was the most recent herd breakdown?	Date:
3. How many reactors did you have? Were the reactor animals homebred or bought in?	
4. Was this breakdown confirmed with visible lesions?	Yes No DNK Reactors still alive Awating results
5. Was this breakdown confirmed by culture?	Yes No DNK Reactors still alive Awating results
6. When was the earliest breakdown in this herd? If same as Q2, go to Q10	Date:
7. Was this breakdown confirmed with visible lesions?	Yes No DNK
8. Was this breakdown confirmed by culture?	Yes No DNK
9. Have you ever had a breakdown with confirmed lesions?	Yes No DNK
10. Have you ever had an inconclusive reactor in this herd? If no, go to Q12	Yes No DNK
11. When was the most recent inconclusive reactor in this herd?	Date :

12. Was this herd tested in 2002?	Yes No DNK
13. Was this herd tested in 2001?	Yes No DNK
14. Was this herd tested in 2000?	Yes No DNK
15. When was this herd last tested?	Date :
16. What was the reason for the last test?	

What is your current TB status?

Why do you think your herd broke down / was clear? ask as appropriate, record all farmer says

Are you participating in the badger culling trial? Yes No

Table 7b – Records of the last TB test

Which groups of cattle, including bulls, were/ were not tested in the last TB test?

Which group of cattle?	How many cattle from this group were tested?		How many cattle from this group were not tested?		Reactor animals			Inconclusive Reactors	
	Exactly	Approx.	Exactly	Approx.	How many were reactors?	Were they separated from the rest of the herd? yes/no	How long did it take to have them removed?	How many were IR?	Were they separated from the rest of the herd?

If any cattle were not tested, which groups (from table 7b) and why they were not tested?

Table 7c- Reasons why cattle were not tested

Group of cattle	Why were they not tested?

Table 7d- TB Test technique

1. Is it always the same vet who does the test? Yes No
2. Do you ask for the test to be done by the same vet? Yes No
3. Do you know how the test has to be done? Yes No
4. If you have had more than one test on this farm, is it always done in the same way? Yes No N/A DK
5. Are you satisfied with the way it is done? Yes No
6. What is the best thing about how the test is done?

7. What is the worst thing about how the test is done?

8. Is there anything that you would change? Please give your opinion

Table 8 –Diseases Other than TB in this Herd

Table 8a- History of BVD in this herd (Bovine Viral Diarrhoea)

Have you ever had BVD confirmed in this herd? Yes No if no go to next page

If yes,

Which group of cattle was affected?	When was it last seen? Year	Was it diagnosed by Self / Vet / Laboratory?	Since January 2000, how common has been problem in this group Persistent / occasional

Table 8b- History of IBR in this herd (Infectious Bovine Rhinotracheitis)
Have you ever had IBR confirmed in this herd? Yes No if no go to next page

If yes,

Which group of cattle was affected?	When was it last seen? Year	Was it diagnosed by Self / Vet / Laboratory	Since January 2000, how common has been problem in this group Persistent / occasional

Table 8c- History of Johnes Disease in this herd

Have you ever had Johnes Disease confirmed in this herd? Yes No if no go to next page

If yes,

Which group of cattle was affected?	When was it last seen? Year	Was it diagnosed by Self / Vet / Laboratory	Since January 2000, how common has been problem in this group Persistent / occasional

Table 8d- History of Leptospirosis in this herd

Have you ever had leptospirosis confirmed in this herd? Yes No if no go to next page

If yes,

Which type of cattle was it affected?	When was it last seen? Year	Was it diagnosed by Self / Vet / Laboratory	Since January 2000, how common has been problem in this group Persistent / occasional

Table 8e- History of Neospora in this herd

Have you ever had neospora confirmed in this herd? Yes No if no go to next page

If yes,

Which type of cattle was it affected?	When was it last seen? Year	Was it diagnosed by Self / Vet / Laboratory	Since January 2000, how common has been problem in this group Persistent / occasional

Table 8f- General Conditions seen since January 2000 up to now, excluding BVD, IBR, Leptospirosis, Johnes', Neospora
Please read from key 1.

What clinical signs from Key 1 have you seen in your herd had since Jan'00?	Which group of cattle were affected?	When was this last seen? Month, year, period	How many animals out of the group were affected?	Was it diagnosed by Self / Vet / Laboratory

Code	Clinical Signs	D17	Skin Problems
D1	Abortion		
D2	Chronic Weight Loss		
D3	Diarrhoea		
D4	Eye Discharge		
D5	Husk		
D6	Hypomagnesaemia Hypomag		
D7	Lameness		
D8	Listeria circling		
D9	Listeria silage eye		
D10	Mastitis		
D11	Milk Drop		
D12	Milk Fever		
D13	Nervous Signs		
D14	Pneumonia		
D15	Respiratory Disease		
D16	Rotavirus		

Table 9 – Vaccinations given to animals in this herd since January 2000
see Key 2

Which vaccines have you used in your herd since January? From key 2	When was it last given? Month, Year	Which groups of cattle were vaccinated?	What was the reason for Vaccination? Routine / Outbreak Response / "please state"

Key 2 - Vaccines

Vaccine	Code	Vaccine	Code
Blackleg Vaccine	V1	Rispoval RS	V22
Blackleg Vaccine BP Vet	V2	Rotavec K99	V23
Bovidec	V3	Spirovac	V24
Bovilis Huskvac	V4	Tetanus Antitoxin Vericore	V25
Bovilis IBR + P13 live	V5	Tetanus Antitoxin Behring	V26
Bovilis IBR	V6	Tetanus Toxoid	V27
Bovisan DPS	V7	Tetanus Toxoid Concentrate	V28
Bovivac	V8	Torvac	V29
Bovivac Plus	V9	Tracherine	V30
Covexin 8	V10	Tribovax T	V31
Ecosan	V11	Vaxall Leptospira Hardjo Vaccine	V32
Grovax	V12		
Imuresp	V13		
Imuresp RP	V14		
Lactovac	V15		
Lambisan	V16		
Leptavoid-H	V17		
Louping III vaccine Coopers	V18		
NOBIVAC Rabies	V19		
Pastobov	V20		
Ringvac Bovis LTF - 130	V21		

Table 10a – Replacement / restock groups for your herd from January 2000

Can we use your written records to record your replacements since January 2000? Yes No
Have you bought any cattle since January 2000? Yes No
If yes:

Replacement Group	What type of cattle were they?	How many did you buy?	What age were they? Months / years range if necessary	What sex M/F	What month and year when they moved on to your farm?	Where were they purchased from? Farm/Neighbouring farm/ Market / Dealer /Separate holding	What was the county of origin? County	Had the herd that these cattle came from ever had TB? Yes, no, don't know	Did you have a pre-pur TB test carried out? yes/no if yes, date the last tes in the purchased stock
R									
R									
R									
R									
R									
R									
R									

Table 10b –Replacement / restock groups for your herd from January 2000

This table follows Table 10a.
As animals arrived to the farm:

Replacement Group	Where were the cattle kept when you brought them onto your farm? Building / field number	How long were they kept there? Weeks / Months	Which group of cattle did they join?	When did they join the group Immediately / after how many weeks / days?	What diseases did you test them for? TB; Lepto; BVD; IBR; JD; Neo	What vaccines did you give them? From Key 2	Did they have any disease outbreaks? From Key 1	Where are they n On farm / abattoi
R								
R								
R								
R								
R								
R								
R								

Farmer's comments_
Please write down any comments that could be relevant in the control of TB, measures he may have taken, etc.

Interviewer's comments_
Please write down any comments that you think may be useful to help you understand how the farm is managed, etc.

Appendix 2.4 - Intructions to farmers for Part 1 of questionnaire

Dear Sir/Madam:

PREPARING FOR THE QUESTIONNAIRE

THE UNIVERSITY OF WARWICK

As arranged, we will visit you within the next month to complete the TB questionnaire. We enclose some parts of the questionnaire that we would like you to complete before we visit you. We have asked you to fill in information on your fields and buildings. The information on how you used your fields and buildings since October 2001 is one of the longest aspects of the questionnaire, but hugely important to improve our understanding of how your cattle mix with each other, wildlife and neighbouring cattle. Please fill this in as accurately as you can.

We hope that you may find this quite interesting. If you fill in these forms before we visit you, the interview will last about 90 minutes. However if you would rather wait until we visit you we will fill in the forms with you, the interview will then take longer.

If you have any enquiries, please do not hesitate to contact us.

Fields: Please use the map you think is more suitable and follow the instructions that we have provided.

Buildings: Please sketch a diagram and put information into the tables provided following the example given.

Summary of Location of Animals: We would like to go through this with you, so you may prefer to think about this part and leave it to complete when we visit you.

OTHER INFORMATION WE NEED

When we visit you we will ask you about personnel and equipment that you used with your herd since October 2001; e.g. silage contractors, hiring of bulls; last TB test results, and, disease information and movement records since 2000. It would be very helpful if we could have a copy of your movement records from January 2000 if possible.

We look forward to seeing you in the very near future.

Yours sincerely

The TB team

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tbproject@bio.warwick.ac.uk

Appendix 2.5 - Map of a farm's fields

Appendix 2.6 - Reactor animal information

CPH 07 023 0014 FarmID **27-2** FarmName: **Allin**

Eartag Number	TestDate	Break Date	TestRes	Action	LesSH	Cult	Group	Location
UK K6594 00525	24/11/2006	21/07/2003	IR	I				
UK NK0449 04297	21/07/2003	21/07/2003	R	S	VL	Ms		

GroupID	Group Name
1	Milkers
2	Dry cows and Bulling heifers
3	Calves (0 - 10 w.o)
4	Calves (10 w.o - 14 m.o)
5	Bull
6	

B1 HOUSING MILKING COWS
B2 DRY COW SHED
B3 YOUNG STOCK 1
B4 YOUNG STOCK 2
B5 YOUNG STOCK 3
B6 YOUNG STOCK 4
B7 YOUNG STOCK 5
F1
F2
F3
F4
F5
F6
F7
F8
F9
F10
F11
F12
FS1
FS2
FS3
FP1
FP2
FP3
FG1
FG2

Appendix 3.1 – Number of HBD per herd that had at least one HBD during the study period, the date of the HBD was disclosed, number of animals tested and number of reactors disclosed at the test.

herd id	break date	number animals tested	number reactors disclosed
1	21/07/2003	140	1
2	17/12/2001	304	1
2	13/01/2004	309	4
3	19/02/2002	254	3
3	16/09/2003	278	8
3	25/10/2004	missing	missing
4	23/04/2002	438	8
5	26/02/2002	312	1
5	27/05/2003	366	1
6	12/03/2003	2	2
7	23/08/2004	2	2
7	29/04/2002	134	6
7	06/10/2003	153	1
8	21/07/2003	1	1
9	04/02/2003	345	4
10	13/04/2004	301	1
11	08/03/2004	1	1
11	28/05/2002	1	1
12	29/03/2004	194	5
13	19/11/2002	272	2
14	02/07/2002	3	2
15	30/04/2002	315	6
16	07/04/2003	256	2
17	09/04/2002	204	1
18	20/05/2003	0	0
19	18/11/2002	164	0
19	25/02/2002	158	1
20	24/02/2003	215	1
21	19/10/2004	168	1
22	14/01/2003	2	1
23	10/09/2002	1	1
24	11/11/2002	223	10
25	08/07/2003	2	1
25	14/06/2004	182	2
26	18/02/2002	269	4
27	07/01/2003	169	1
28	06/03/2002	158	0
29	20/01/2003	354	2

30	04/02/2002	133	2
30	19/05/2003	2	2
30	15/03/2004	163	1
31	03/04/2003	157	1
31	27/04/2004	156	2
32	07/01/2003	302	3
33	08/12/2003	214	2
34	19/01/2004	184	2
35	30/09/2002	74	2
35	10/11/2003	76	1
36	18/11/2002	180	3
37	27/01/2003	389	13
38	31/03/2003	1	1
38	02/02/2004	101	4
39	26/04/2004	8	1
40	02/09/2003	40	1
40	27/04/2004	26	1
41	18/02/2003	540	78
41	20/07/2004	462	4
42	02/07/2002	30	0
42	19/04/2004	39	1
43	01/04/2003	220	7
43	21/06/2004	246	1
43	08/07/2002	100	3
44	25/06/2002	68	1
45	05/02/2002	152	2
46	22/07/2002	71	2
47	22/11/2002	0	0
48	17/05/2002	52	1
49	14/07/2003	207	2
50	12/05/2003	139	1
50	30/04/2002	5	1
51	23/07/2002	12	2
52	22/04/2003	3	3
53	27/01/2003	20	2
54	10/12/2001	378	21
55	25/05/2004	317	1
55	21/05/2002	354	7
55	28/04/2003	373	1
56	17/11/2003	216	2
56	25/11/2002	239	1

57	10/02/2003	277	2
58	25/03/2003	6	1
59	20/04/2004	3	3
59	23/06/2003	13	1
60	06/08/2002	148	5
61	22/04/2003	1	1
62	21/03/2003	161	1
63	18/07/2003	69	3
63	15/07/2002	64	2
64	02/06/2003	0	0
65	02/12/2003	142	1
65	22/04/2003	1	1
65	18/05/2002	25	2
66	09/02/2004	55	5
67	21/06/2004	92	1
67	06/10/2003	138	1
68	02/07/2002	163	2
68	19/04/2004	7	1
69	11/12/2001	167	3
69	07/10/2002	137	1
70	10/11/2003	56	3
70	17/12/2001	78	9
71	18/02/2003	133	1
72	05/12/2003	68	4
72	19/11/2002	72	3
73	17/12/2002	183	1
74	05/11/2002	224	2
75	11/11/2003	260	9
76	08/03/2004	30	1
77	03/05/2004	338	3
77	15/01/2002	0	0
78	12/08/2002	146	5
79	07/01/2003	53	3

Appendix 4.1 -Univariable results for time to first HBD on 148 study herds from 1st October 2001 to 1st November 2004 and values of p≤0.2

Variable	n	HR	coef	SE	p	low 95% CI	high 95% CI
ln (herd size)	148	1.82	0.59	0.14	<0.001	1.38	2.37
herd has dairy cattle	57	2.22	0.8	0.23	0.001	1.41	3.50
herd has suckler cattle	58	0.51	-0.68	0.25	0.007	0.31	0.83
herd ever had a HBD prior to Oct'01	45	1.41	0.35	0.24	0.15	0.88	2.28
RBCT							
Geographical location							
Glouces/Hereford&Worcester	49	1.07	0.07	0.18	0.70	0.74	1.54
Somerset	18	0.89	-0.11	0.08	0.18	0.76	1.05
Devon/Cornwall	81	ref					
Treatment							
Reactive	55	1.12	0.10	0.14	0.43	0.85	1.47
Proactive	41	1.02	0.02	0.08	0.79	0.87	1.20
Survey only	52	ref					
Manure/slurry management							
manure stored all year	67	2.23	0.8	0.23	0.001	1.41	3.53
manure not stored	12	0.39	-0.93	0.59	0.11	0.12	1.25
manure stored from 7-11 months	12	1.66	0.5	0.37	0.18	0.79	3.45
spread all year	37	2.05	0.72	0.24	0.003	1.27	3.31
not spread	9	0.15	-1.89	1.01	0.06	0.02	1.08
own spreader used	95	1.41	0.34	0.25	0.16	0.87	2.29
type farm yard manure	134	2.18	0.78	0.51	0.13	0.80	5.97
type separated liquid	11	2.51	0.92	0.37	0.014	1.21	5.24
type whole slurry	68	1.68	0.52	0.23	0.025	1.07	2.65
origin sheep	42	1.48	0.39	0.25	0.11	0.91	2.40
stored indoors/close containment	89	1.22	0.2	0.12	0.1	0.95	1.56
Bedding use and management							
presence mice	90	1.59	0.46	0.25	0.065	0.97	2.60
stored in barn	126	1.77	0.57	0.37	0.13	0.85	3.68
barley straw	91	1.44	0.37	0.25	0.14	0.88	2.36
no wildlife present	46	0.7	-0.35	0.27	0.19	0.42	1.18
Feeding use and management							
maize silage	35	2.27	0.82	0.24	0.001	1.41	3.67
grass silage clamp	87	2.19	0.78	0.25	0.002	1.33	3.61
minerls & licks & vitamins	72	0.55	-0.59	0.24	0.012	0.35	0.88
grass silage all types	126	3.16	1.15	0.46	0.013	1.27	7.82
wheat straw big bale	22	1.84	0.61	0.29	0.034	1.05	3.25
haybig round bale	65	0.61	-0.49	0.24	0.039	0.38	0.98
byproducts	18	1.85	0.62	0.31	0.044	1.02	3.38
whole milk	45	1.61	0.47	0.24	0.049	1.00	2.58
straights	48	1.54	0.43	0.24	0.067	0.97	2.45
barleysmall bale	11	1.92	0.65	0.38	0.083	0.92	4.01
grass silage big square bale	32	1.57	0.45	0.26	0.084	0.94	2.62

wheat all types	21	1.59	0.46	0.3	0.12	0.89	2.84
grass silage big round bale	109	1.51	0.41	0.29	0.15	0.86	2.66
fed method trough	116	1.57	0.45	0.31	0.15	0.85	2.91
fed method ring feeder	87	0.73	-0.31	0.23	0.18	0.46	1.15
fed method hay rack	52	1.52	0.42	0.23	0.072	0.96	2.41
stored in silo	29	1.88	0.63	0.26	0.016	1.13	3.14
stored in pit	38	1.53	0.42	0.25	0.088	0.94	2.49
Contact with other cattle							
bulls hired in	40	0.64	-0.45	0.28	0.11	0.37	1.11
no contacts	57	1.45	0.37	0.23	0.11	0.92	2.29
returns from markets	10	0.45	-0.8	0.59	0.17	0.14	1.42
Variable	n	HR	coef	SE	P	low 95% CI	high 95% CI
Diseases since jan'00							
milk fever	59	2.27	0.82	0.23	<0.001	1.44	3.57
Persistence of disease	28	2.24	0.81	0.26	0.002	1.35	3.72
none immunosupr.clinical	72	0.49	-0.71	0.24	0.003	0.31	0.78
rotavirus	18	2.42	0.88	0.31	0.004	1.33	4.40
BVDV	27	2.12	0.75	0.27	0.005	1.26	3.58
stillborns	43	1.88	0.63	0.24	0.009	1.17	3.02
		2.3					
Johne's Disease	13	6	0.86	0.34	0.012	1.21	4.61
lameness	93	1.89	0.64	0.26	0.013	1.14	3.14
none external clinical signs	39	0.48	-0.74	0.31	0.016	0.26	0.87
none metabolic signs	58	0.59	-0.53	0.25	0.033	0.36	0.96
mastitis	80	1.62	0.48	0.24	0.042	1.02	2.58
milk drop	10	2.25	0.81	0.4	0.042	1.03	4.92
hypomagnesemia	19	1.59	0.46	0.32	0.14	0.86	2.95
abortion	16	1.54	0.43	0.34	0.2	0.79	3.00
Vaccinations since Jan'00							
vaccination as routine	72	2.62	0.96	0.24	<0.001	1.63	4.21
vac.rsv	11	3.66	1.3	0.36	<0.001	1.81	7.41
vac.worming.none	67	0.43	-0.84	0.25	0.001	0.27	0.70
vaccination not given	70	0.47	-0.75	0.24	0.002	0.29	0.76
vac.bvd	23	2.03	0.71	0.28	0.012	1.17	3.54
vac.lepto	42	1.82	0.6	0.24	0.013	1.13	2.92
vac.lungworm	13	2.34	0.85	0.34	0.013	1.20	4.56
vac.rotavirus	14	1.55	0.44	0.31	0.16	0.84	2.87
Purchase practices							
purchase of steers	40	1.73	0.55	0.24	0.025	1.07	2.78
purchase of heifers	65	1.59	0.46	0.23	0.046	1.01	2.51
purchase from markets	65	1.38	0.32	0.23	0.16	0.88	2.18
number groups animals purchased	127	1	0	0	0.16	1.00	1.01
Staff working with the herd							
1-2	60	1.29	0.26	0.13	0.05	0.99	1.69
3-4	63	1.28	0.25	0.09	0.006	1.07	1.53

more than 5	24	ref					
Hire of equipement							
hire maize equipment	31	2.25	0.81	0.25	0.001	1.38	3.69
hire silage equipment	88	1.57	0.45	0.25	0.068	0.97	2.53
hire bailing equipment	87	0.74	-0.3	0.23	0.2	0.47	1.17

Appendix 4.2 - Figures 4.1 – 4.11 - Schoenfeld residuals plots for each of the variables in the Cox proportional hazards model

Fig. 4.1-restocking status

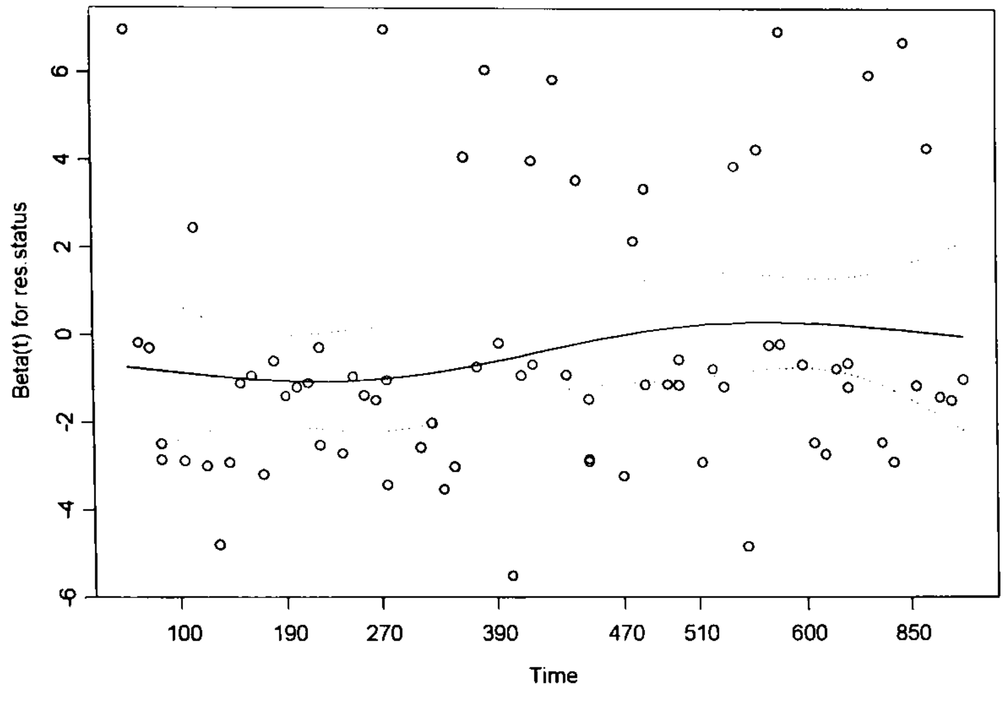


Fig. 4.2-dairy herd

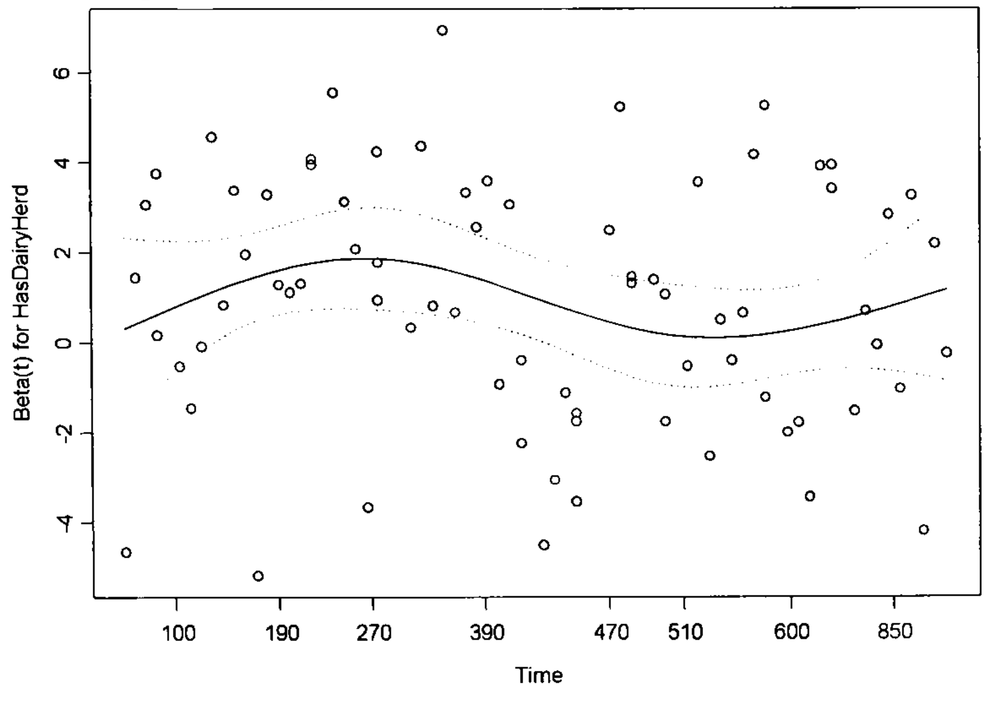


Fig 4.3-herd size

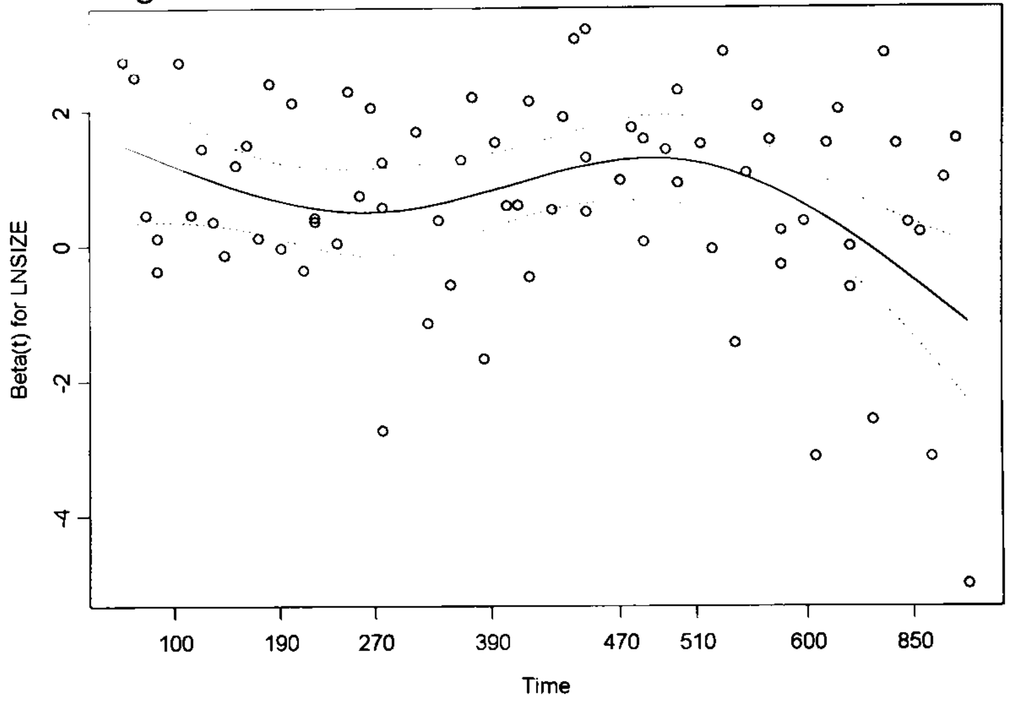


Fig. 4.4-minerals and vitamins as licks

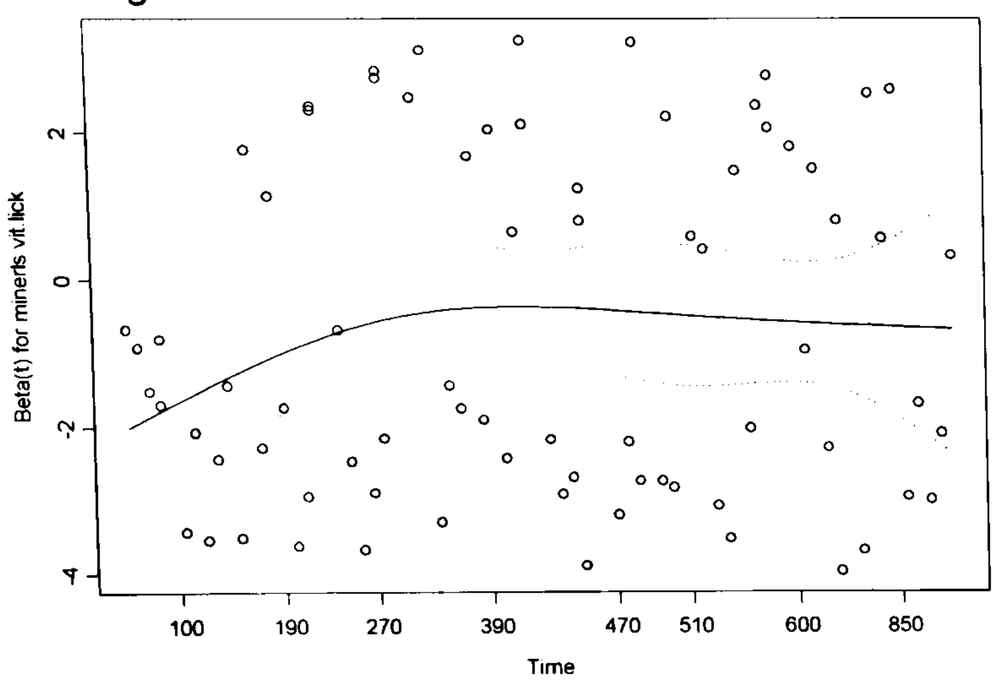


Fig 4.5-manure in buildings/close containment

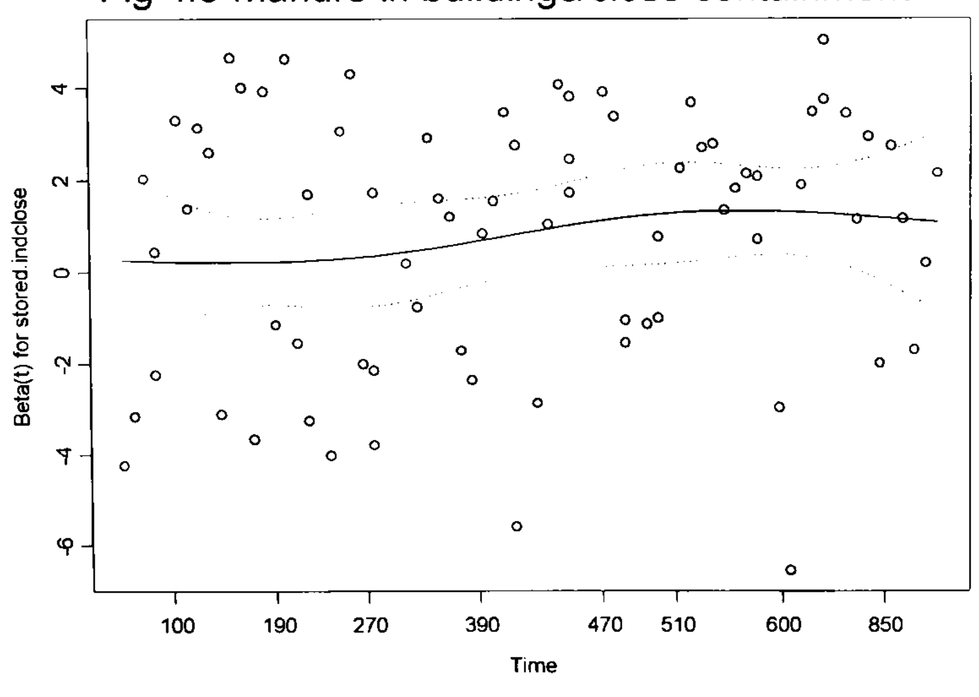
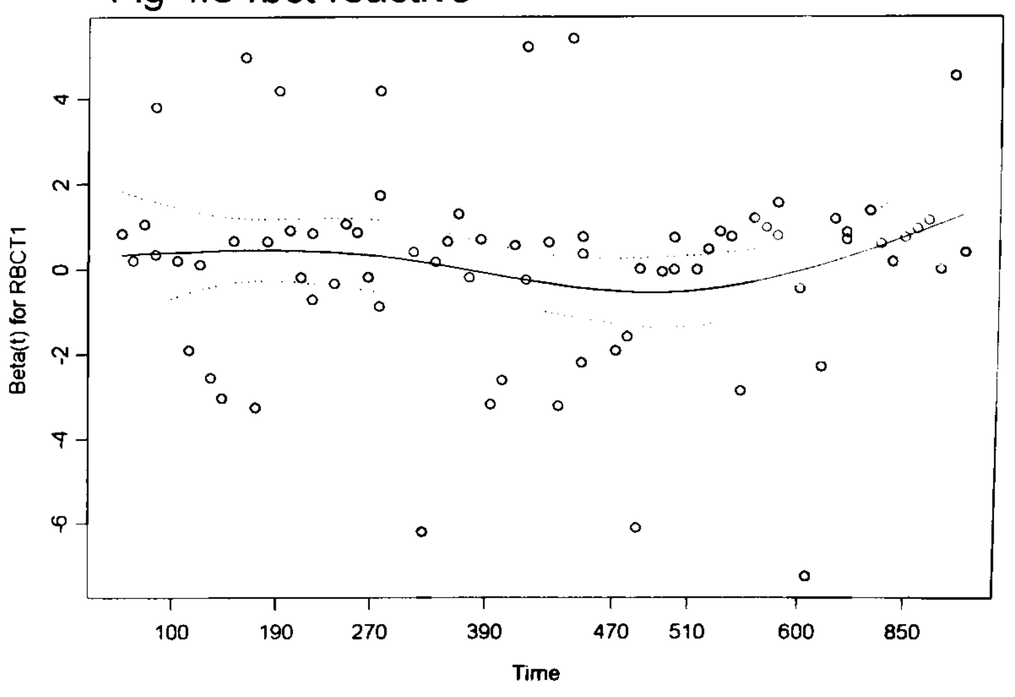


Fig 4.6-rbct-reactive



Appendix 4.2. (cont)

Fig. 4.7-rbct-proactive

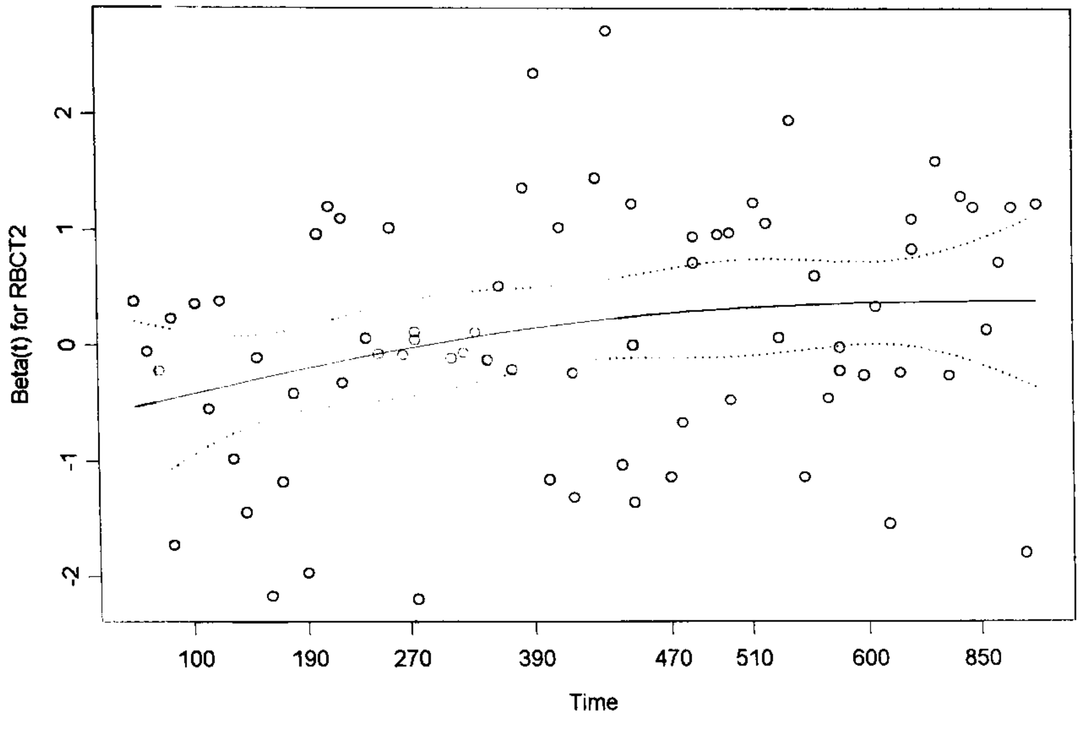


Fig. 4.8-location Gloucs/Hereford/Worcester

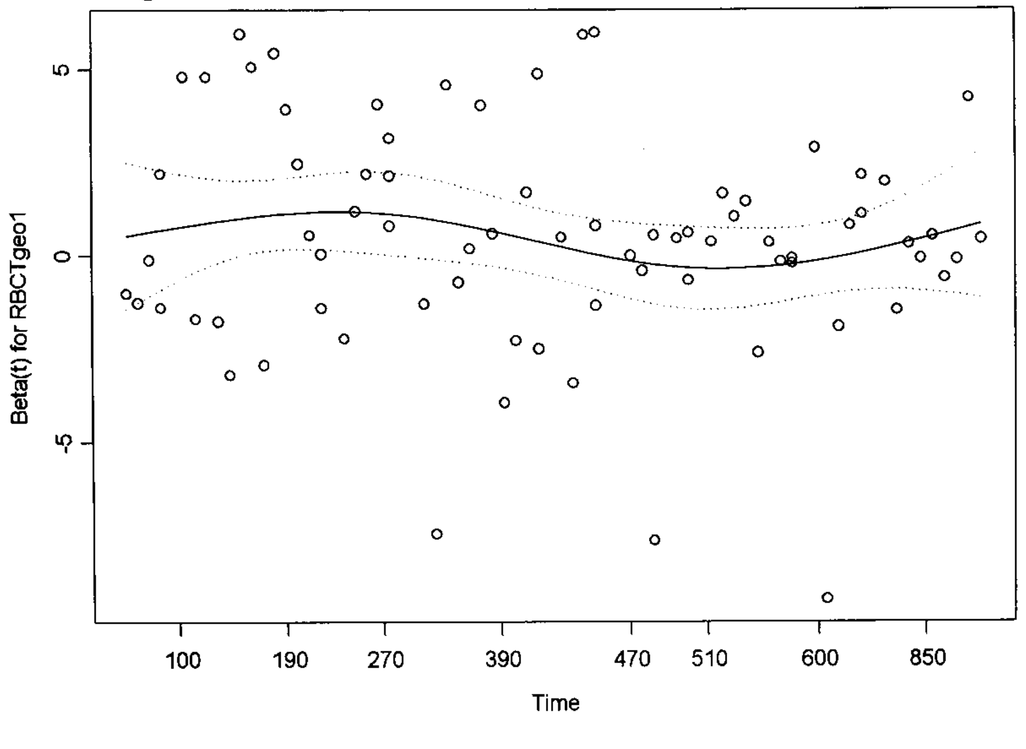


Fig. 4.9-location Somerset

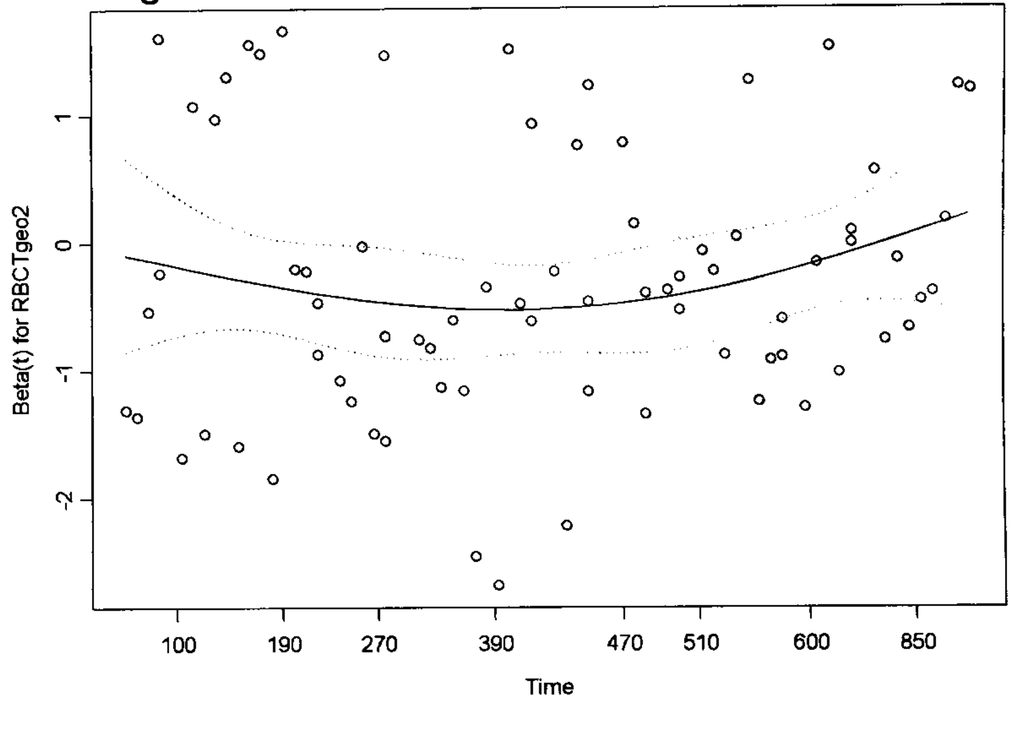


Fig.4.10-replacement of steers

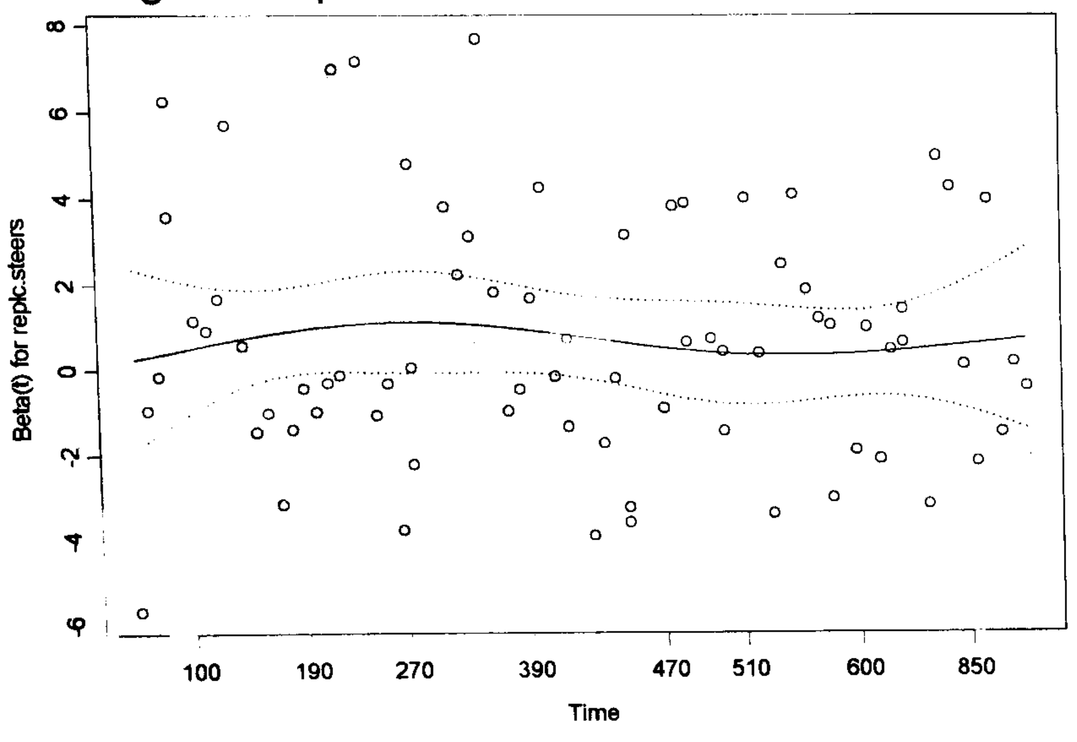
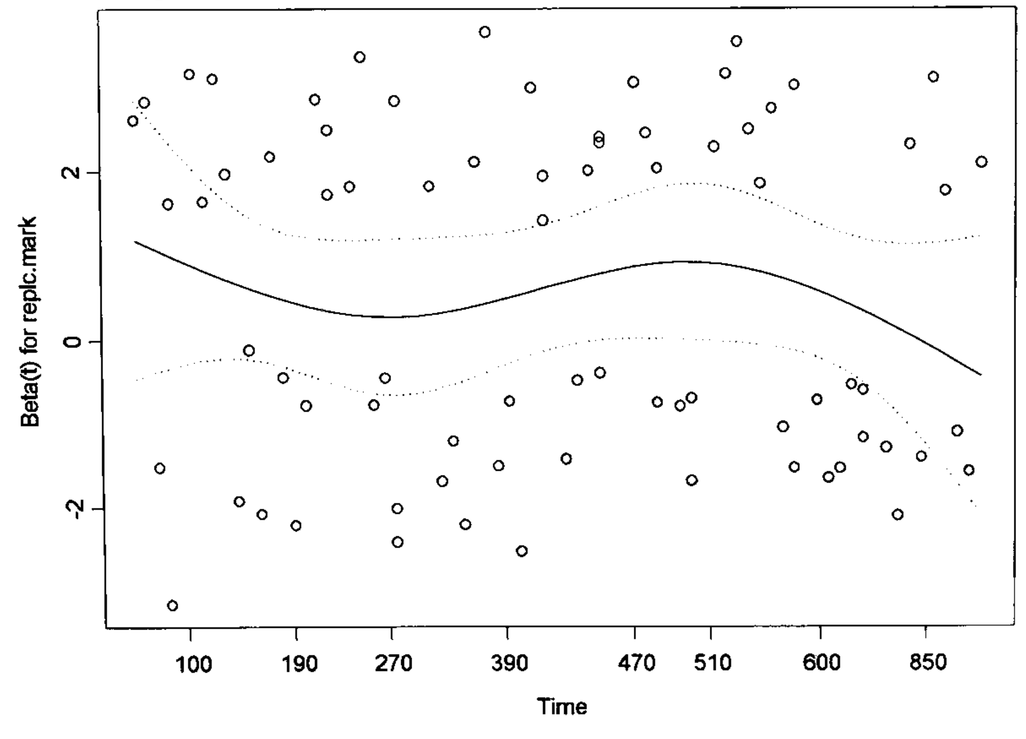


Fig.4.11-purchased from markets



Appendix 5.1 - Results from univariable multilevel logistic regression with random effects analysis from 156,562 cattle tests using 697 herd tests in 144 herds and 48,055 individual cattle

Variable	Obs	Num reactors	Perc. of reac/obs	OR	coef	SE	95% credibility interval	
							lower	upper
Individual cattle								
Sex								
female	115,067	650	0.6	ref				
male	41,495	73	0.2	0.33	-1.11	0.14	0.25	0.44
Type cattle								
beef	69,070	194	0.3	ref				
dairy	84,237	510	0.6	2.70	1.00	0.13	2.10	3.49
mixed	3,255	19	0.6	2.50	0.92	0.27	1.49	4.20
Cattle breed								
hibrid	63,742	177	0.3	ref				
pure	91,822	537	0.6	3.93	1.37	0.43	1.69	9.12
mixed	998	9	0.9	2.42	0.88	0.12	1.90	3.07
Previous movements								
born on study	85,099	339	0.4	ref				
one	28,984	121	0.4	1.48	0.39	0.15	1.10	2.00
twice	14,456	72	0.5	2.46	0.90	0.17	1.77	3.42
three and more	8,230	25	0.3	2.27	0.82	0.21	1.49	3.45
Born on farm								
no	71,463	384	0.5	ref				
yes	85,099	339	0.4	0.49	-0.72	0.11	0.39	0.60
Age moved on to farm								
born on farm	85,099	339	0.4	ref				
up to one year old	22,019	45	0.2	0.66	-0.41	0.20	0.45	0.98
over one year old	46,239	313	0.7	2.30	0.84	0.10	1.88	2.83
Years on farm up to test date								
up to 1year	68,041	124	0.2	ref				
between 1 - 2 years	40,641	169	0.4	2.45	0.90	0.13	1.90	3.17
between 2 - 3 years	20,910	156	0.7	4.28	1.45	0.14	3.24	5.64
between 3 - 4 years	13,096	116	0.9	5.63	1.73	0.15	4.20	7.55
4 and over	13,874	158	1.1	8.62	2.15	0.15	6.49	11.45
Herd								
Restockins status								
cont. stocked	125,249	666	0.5	ref				
restocked	31,313	57	0.2	0.53	-0.64	0.33	0.28	1.01
RBCT intervention treatments								
survey	51,560	193	0.4	ref				
reactive	56,956	270	0.5	1.60	0.47	0.32	0.86	2.97
proactive	48,046	260	0.5	1.42	0.35	0.34	0.73	2.77
RBCT trial areas								
A1	27,195	120	0.4	ref				

A3	2,688	9	0.3	0.68	-0.38	0.91	0.11	4.08
B1	10,989	13	0.1	0.20	-1.60	0.61	0.06	0.67
B2	25,765	96	0.4	0.75	-0.29	0.41	0.33	1.69
B3	4,010	11	0.3	0.41	-0.89	0.87	0.07	2.28
C1	6,818	29	0.4	0.85	-0.16	0.78	0.19	3.88
H3	19,820	96	0.5	0.93	-0.07	0.46	0.38	2.30
I1	11,954	108	0.9	2.17	0.77	0.45	0.89	5.28
I3	2,926	7	0.2	0.84	-0.18	0.74	0.19	3.59
J1	19,593	155	0.8	1.76	0.56	0.52	0.64	4.85
J3	24,804	79	0.3	0.61	-0.50	0.43	0.26	1.42
RBCT geographical location								
Gloucestershire	44,763	244	0.5	ref				
North Devon	21,468	97	0.5	0.63	-0.46	0.44	0.27	1.51
South Devon	90,331	382	0.4	0.54	-0.61	0.30	0.30	0.96
SICCT tests								
Herd size								
<151	28,926	109	0.4	ref				
151-300	56,117	273	0.5	1.15	0.14	0.18	0.81	1.62
>300	71,519	341	0.5	1.00	0.00	0.19	0.69	1.44
Test type								
Yearly tests	32,352	97	0.3	ref				
SI (short interval)	84,631	392	0.5	0.69	-0.37	0.13	0.53	0.90
other strategic tests	39,579	234	0.6	1.61	0.48	0.13	1.25	2.08
Number reactors at test								
one	17,114	65	0.4	ref				
two	13,680	105	0.8	1.88	0.63	0.26	1.13	3.13
three and over	25,181	553	2.2	5.89	1.77	0.22	3.85	9.01
none (missing)	100,587	0	0.0	n/a				
Age at test								
up to 1 year old	44,959	49	0.1	ref				
1-2	38,695	68	0.2	1.81	0.59	0.20	1.22	2.69
2-3	19,222	102	0.5	5.38	1.68	0.19	3.71	7.81
3-4	13,381	118	0.9	8.62	2.15	0.19	5.96	12.46
4-5	11,067	116	1.0	11.12	2.41	0.19	7.71	16.05
5-6	8,622	81	0.9	10.26	2.33	0.20	6.97	15.09
six and over	20,616	189	0.9	10.22	2.32	0.18	7.21	14.48
Birth location and herd tested								
Born on study farm	85,001	339	0.4	ref				
Purchased and herd not tested	40,665	236	0.6	2.08	0.73	0.12	1.65	2.62
Purchased and herd tested	30,664	148	0.5	2.02	0.7	0.14	1.54	2.64
History of bTB on farm								
History prior birth/purchase								
purch & study & source not tested	27,679	174	0.6	ref				
born on farm & hist	58,679	164	0.3	0.25	-1.38	0.16	0.18	0.34
born on farm & no hist	25799	173	0.7	1.21	0.19	0.16	0.90	1.65

born + study not tested	282	2	0.7	2.75	1.01	0.76	0.63	12.12
purch & study not tested & hist source	16351	79	0.5	0.68	-0.38	0.17	0.48	0.95
purch & study not tested & not hist source	27049	131	0.5	0.73	-0.31	0.15	0.55	0.98
Ever bTB on study farm								
prior birth/purchase								
born & not hist	25972	173	0.7	ref				
born & hist	58843	164	0.3	0.21	-1.56	0.18	0.15	0.30
purch & no tested	71463	384	0.5	0.70	-0.36	0.14	0.53	0.92
born & no tested	284	2	0.7	2.28	0.83	0.75	0.52	10.00
Ever bTB on source farm before cattle left								
no	27,180	131	0.5	ref				
not tested	27,853	174	0.6	1.26	0.23	0.14	0.95	1.66
born on farm	85,099	339	0.4	0.53	-0.64	0.14	0.40	0.70
yes	16,430	79	0.5	0.89	-0.11	0.17	0.64	1.26
Ever TB before June'01 & restocking								
not bTB & cont. stocked	36,125	163	0.5	ref				
not bTB & restocked	10,910	21	0.2	0.61	-0.50	0.47	0.24	1.52
bTB & contstckd	89,124	503	0.6	1.00	0.00	0.29	0.56	1.78
bTB & restocked	20,403	36	0.2	0.45	-0.79	0.48	0.18	1.15
Years since previous source herd								
last bTB positive								
tested negative	27049	131	0.5	ref				
not tested	22,315	161	0.7	1.35	0.30	0.15	1.01	1.81
born in study farm	84,760	339	0.4	0.53	-0.63	0.14	0.40	0.70
same year as current test	741	30	4.0	0.58	-0.54	0.24	0.37	0.93
one year since current test	4,655	28	0.6	1.21	0.19	0.25	0.74	1.98
two years since current test	2,311	11	0.5	1.21	0.19	0.32	0.65	2.28
three years since current test	1,653	8	0.5	1.22	0.20	0.37	0.59	2.52
four and over years since current test	4355	15	0.3	0.90	-0.11	0.28	0.52	1.55
Years since study herd last bTB positive								
tested negative	25,972	173	0.7	ref				
not tested	284	2	0.7	2.24	0.8	0.75	0.51	9.78
purchased	71,463	384	0.5	0.70	-0.4	0.14	0.53	0.93
same year as current test	34,432	75	0.2	0.15	-1.9	0.22	0.09	0.22
one year since current test	8,663	36	0.4	0.34	-1.1	0.28	0.20	0.60
two years since current test	6,177	22	0.4	0.33	-1.1	0.32	0.18	0.62
three years since current test	4,428	20	0.5	0.42	-0.9	0.35	0.21	0.83
four and over years since current test	5,143	11	0.2	0.34	-1.1	0.36	0.17	0.68
Years since all farms last bTB positive								
tested negative	53,152	304	0.6	ref				
no tested	22760	163	0.7	1.15	0.1	0.13	0.90	1.47
same year as current test	43,203	105	0.2	0.23	-1.5	0.16	0.17	0.32
one year since current test	13,346	64	0.5	0.59	-0.5	0.19	0.40	0.85
two years since current test	8,499	33	0.4	0.56	-0.6	0.23	0.36	0.88
three years since current test	6,089	28	0.5	0.66	-0.4	0.25	0.40	1.09
four and over years since current test	9,513	26	0.3	0.58	-0.5	0.22	0.38	0.90

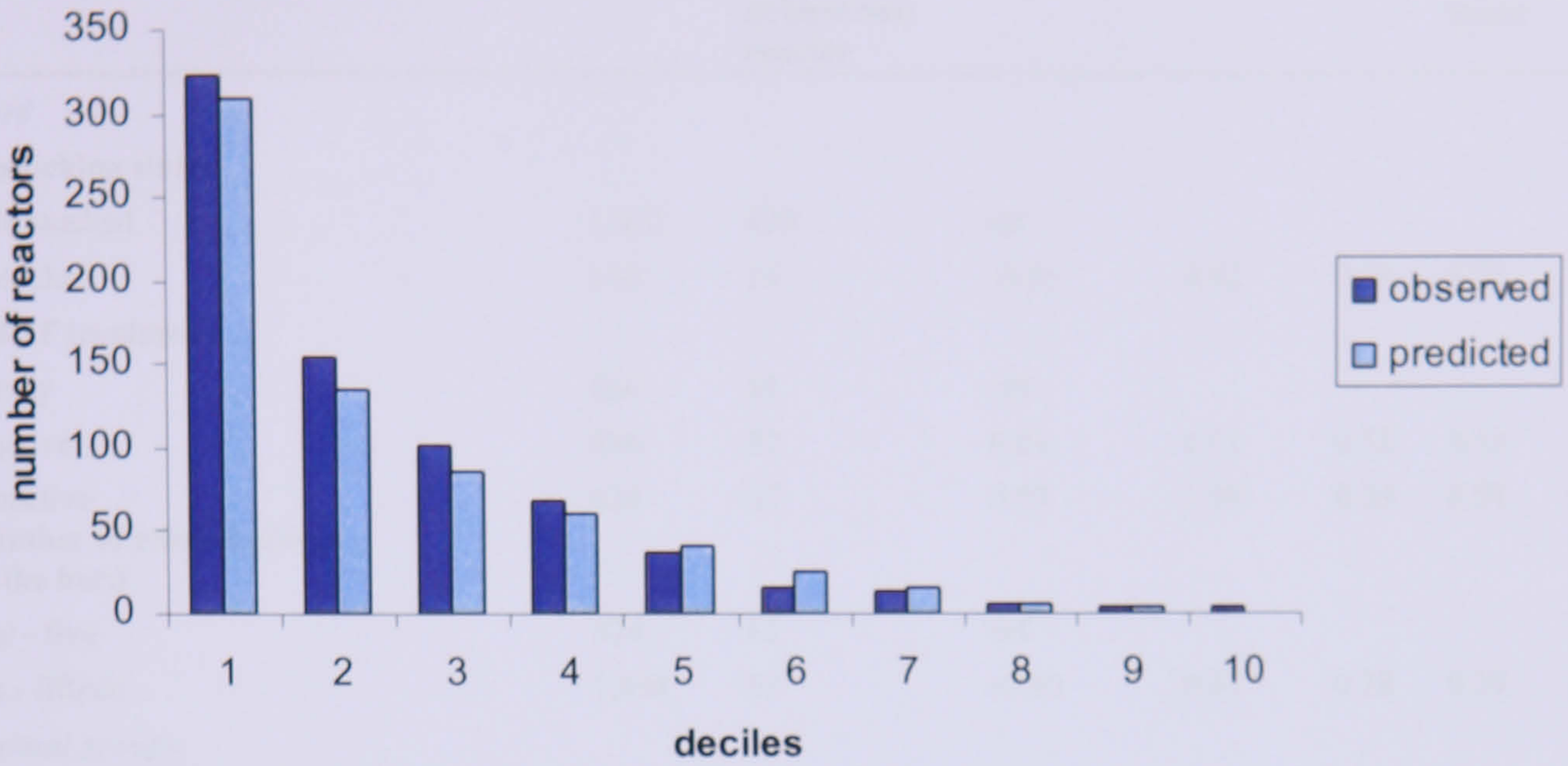
Potential previous exposure to reactors								
Exposure on study farm								
born on farm & expos	65,545	335	0.5	ref				
born on study farm & no expos	19,456	4	0.0	0.60	-0.5	0.17	0.43	0.84
purchased & no expos	28,283	13	0.0	1.89	0.6	0.14	1.43	2.50
purchased & expos	43,046	371	0.9	2.07	0.7	0.57	0.13	6.30
missing: no tested study	232	0	0.0	1.00				
Exposure on previous sources								
Born on study farm	85,099	339	0.4	ref				
purchased& not tested	40,720	236	0.6	2.08	0.7	0.12	1.66	2.62
purchased & no exposed	21,887	95	0.4	2.02	0.7	0.15	1.51	2.71
purchased & exposed	8,856	53	0.6	2.02	0.7	0.19	1.38	2.96
Exposure on all farms								
Purch & no exp on study farm	28,283	13	0.0	ref				
born on study +no exp on study farm	19,456	4	0.0	0.39	-0.9	0.50	0.15	1.04
born & exp on study farm	65,545	335	0.6	6.42	1.9	0.27	3.82	10.79
puch & exp on study & not tested on source	26,331	229	1.1	13.12	2.6	0.27	7.80	22.05
puch & exp on study & not exp on source	11,876	90	0.9	13.46	2.6	0.28	7.82	23.17
puch & exp on study & exp on source	4,839	52	1.3	14.08	2.6	0.31	7.72	25.71
born /purch & not tested on study (missing)	232	0	0.0	n/a				
Total previous exposure to reactors								
None or								
none in one farm and unknown in the other	43,054	16	0.37	ref				
One - five	50,028	145	0.29	7.06	1.9	0.24	4.44	11.24
Six - twenty	39,073	285	0.73	15.13	2.7	0.24	9.47	24.17
More than twenty	23,507	277	1.18	24.85	3.2	0.25	15.28	40.41

Appendix 5.2 - Trajectories and kernel density plots of the parameters estimated in the multilevel multivariable model for the risk of a bovine animal becoming reactor

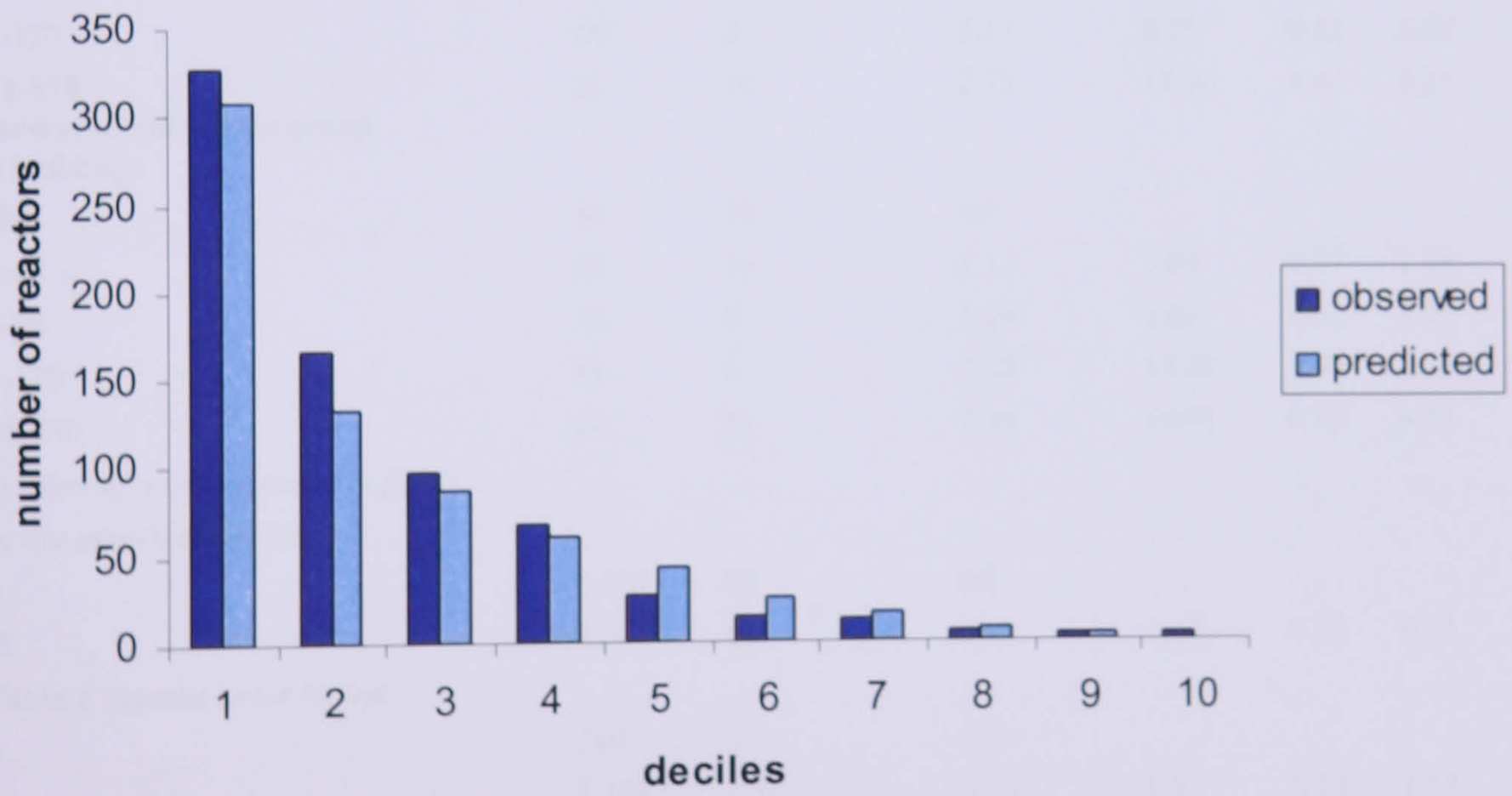
Key for plots (from top to bottom): intercept; restocked; rbct-reactive; rbct-proactive; test-SI; test-other strategic; born study- not exposed; born study-exposed; purchased-exposed study-not tested in source; purchased-exposed study-not exposed source; purchased-exposed study- exposed source.

Appendix 5.3 - Distribution of the number of reactors observed vs predicted by the multilevel MCMC model by deciles and at levels 3(herds) and 2(between animals within herds).

Distribution of the number of reactors observed *vs* predicted by the multilevel MCMC model by deciles and at level 3.



Distribution of the number of reactors observed *vs* predicted by the multilevel MCMC model by deciles and at level 2



Appendix 6.1 – Results from univariable multilevel logistic regression with random effects from 2,372 animal group tests using 404 herd tests in 140 herds and 738 animal groups

Variable	obs.	Number of groups with at least one reactor	coef	OR	SE	95% credibility interval	
						lower	upper
Herd							
Restocking status							
cont.stocked	1,952	420	ref				
restocked	140	15	-0.65	0.52	0.38	0.25	1.11
RBCT treatment							
survey	860	51	ref				
reactive	886	57	0.01	1.01	0.33	0.53	1.94
proactive	626	47	0.07	1.08	0.35	0.54	2.13
Number of animal groups in the herd							
one - five	674	62	ref				
six - fifteen	1,698	93	-0.40	0.67	0.28	0.39	1.17
Animal groups							
Number of animals in group in fields							
1-30	29	9	ref				
31-60	21	7	0.80	2.23	0.45	0.92	5.38
61-90	9	3	2.18	8.85	0.50	3.32	23.57
91-120	10	3	2.17	8.76	0.62	2.60	29.52
121-550	21	10	2.75	15.64	0.47	6.23	39.30
Number of animals in group in buildings							
1-30	30	10	ref				
31-60	25	11	1.11	3.03	0.37	1.48	6.21
61-90	16	5	1.29	3.61	0.52	1.31	9.94
91-120	18	8	2.60	13.42	0.49	5.19	34.73
121-550	24	11	2.39	10.91	0.46	4.47	26.62
Location of animal groups in fields							
On the month of the test							
no	1,661	93	ref				
yes	711	62	0.11	1.12	0.22	0.73	1.70
Within 6 months prior to test							
no	999	35	ref				
yes	1,373	120	0.60	1.82	0.23	1.17	2.85
Within 6-12months prior to test							
no	1,249	57	ref				
yes	1,123	98	0.34	1.41	0.21	0.94	2.11
Within 12-18 months prior to test							
no	1,990	110	ref				
yes	463	45	0.26	1.30	0.24	0.81	2.07
Cat_ Within 18 months prior to test							
none	820	25	ref				
within 6m prior to test	420	31	0.66	1.94	0.31	1.05	3.57
within 12m prior to test	131	7	0.44	1.56	0.48	0.60	4.00
within 6m and 12m prior to test	538	47	0.72	2.04	0.29	1.15	3.62
within 18m prior to test	5	1	1.52	4.59	1.69	0.17	124.66
within 12m and 18m prior to test	43	2	0.36	1.43	0.79	0.31	6.66
within 6m, 12m and 18m prior to test	411	42	0.81	2.25	0.32	1.21	4.18

within 6m and 18m prior to test-missing	4	0					
Number months within the 6 months prior to test							
0	999	35	ref				
1	33	16	0.12	1.13	0.34	0.58	2.22
2	359	29	0.50	1.65	0.31	0.90	3.01
3	236	25	0.91	2.49	0.32	1.34	4.64
4	137	16	0.85	2.35	0.39	1.09	5.04
5	149	17	0.78	2.18	0.38	1.04	4.59
6	157	17	0.67	1.96	0.41	0.88	4.36
Number months within the 6-12 months prior to test							
0	1,249	57	ref				
1	167	16	0.52	1.68	0.36	0.83	3.40
2	119	11	0.26	1.29	0.45	0.54	3.09
3	135	12	0.36	1.43	0.41	0.64	3.21
4	171	12	0.29	1.33	0.38	0.63	2.79
5	280	24	0.22	1.25	0.32	0.67	2.32
6	251	23	0.44	1.55	0.32	0.83	2.88
Location of animal groups in fields with slurry							
On the month of the test							
no	1,928	107	ref				
yes	444	48	0.33	1.39	0.25	0.86	2.26
Within 6 months prior to test							
no	1,515	68	ref				
yes	857	87	0.61	1.83	0.22	1.19	2.84
Within 6-12 months prior to test							
no	1,653	86	ref				
yes	719	69	0.38	1.46	0.22	0.95	2.26
Within 12-18months prior to test							
no	2,123	129	ref				
yes	249	27	0.18	1.20	0.31	0.65	2.21
Cat_ Within 18 months prior to test							
none	1,358	61	ref				
within 6m prior to test	284	25	0.55	1.74	0.31	0.95	3.18
within 12m prior to test	125	5	-0.03	0.97	0.51	0.36	2.63
within 6m and 12m prior to test	356	38	0.70	2.01	0.28	1.16	3.46
within 12m and 18m prior to test	26	2	0.72	2.06	0.80	0.43	9.87
within 6m, 12m and 18m prior to test	212	24	0.59	1.81	0.35	0.91	3.58
within 18m prior to test -missing	6	0					
within 6m and 18m prior to test-missing	5	0					
Cat_ Number of months within the 6 months prior to test							
0	1,515	68	ref				
1	227	12	0.11	1.11	0.37	0.54	2.28
2	229	25	0.71	2.03	0.32	1.09	3.77
3	134	19	1.02	2.78	0.35	1.40	5.51
4	102	12	0.67	1.94	0.43	0.83	4.53
5	89	8	0.35	1.42	0.50	0.54	3.76
6	76	11	0.72	2.06	0.51	0.76	5.55
Cat_Number of months within the 6-12 months prior to test							
0	1,653	86	ref				
1	123	11	0.52	1.68	0.40	0.77	3.68
2	101	10	0.35	1.41	0.46	0.57	3.49
3	85	9	0.38	1.46	0.49	0.56	3.81

4	114	9	0.36	1.44	0.43	0.62	3.33
5	167	16	0.21	1.23	0.38	0.58	2.61
6	129	14	0.56	1.75	0.38	0.82	3.72
In fields not being used for cattle							
On same month of the test							
no	1,454	115	ref				
yes	918	40	-0.40	0.67	0.23	0.42	1.06
Within 6 months prior to test							
no	1,350	105	ref				
yes	1,022	50	-0.37	0.69	0.23	0.44	1.08
Within 6-12 months prior to test							
no	1,359	109	ref				
yes	1,013	46	-0.39	0.68	0.23	0.43	1.07
Within 12-18 months prior to test							
no	1,450	121	ref				
yes	922	34	-0.51	0.60	0.24	0.38	0.95
In fields being used for cattle							
On same month of test							
no	836	68	ref				
yes	1,536	87	-0.30	0.74	0.21	0.50	1.11
Within 6 months prior to test							
no	182	10	ref				
yes	2,190	145	0.09	1.10	0.38	0.52	2.32
Within 6-12 months prior to test							
no	412	32	ref				
yes	1,958	123	-0.11	0.90	0.25	0.55	1.46
Within 12-18 months prior to test							
no	1,074	86	ref				
yes	1,298	69	-0.21	0.81	0.20	0.55	1.19
Cat_ Within 18 months prior to test							
within 6m prior to test	403	31	ref				
within 12m prior to test	135	7	-0.24	0.79	0.49	0.30	2.04
within 6m and 12m prior to test	526	48	0.06	1.06	0.29	0.60	1.88
within 18m prior to test	7	1	0.69	1.99	1.55	0.10	41.39
within 12m and 18m prior to test	40	2	-0.18	0.83	0.80	0.17	3.98
within 6m, 12m and 18m prior to test	1,247	66	-0.21	0.81	0.27	0.48	1.37
within 6m and 18m prior to test-missing	4	0					
In fields with water troughs as source of water							
On same months of test							
no	847	73	ref				
yes	1,452	82	-0.27	0.77	0.21	0.51	1.15
Within 6 months prior to test							
no	338	24	ref				
yes	2,010	131	-0.02	0.98	0.30	0.55	1.75
Within 6-12 months prior to test							
no	581	47	ref				
yes	1,791	108	-0.19	0.83	0.23	0.53	1.29
within 12-18 months prior to test							
no	1,145	94	ref				
yes	1,227	61	-0.30	0.74	0.20	0.50	1.11
Cat_ Within 18 months prior to test							
none	185	17	ref				
within 6m prior to test	378	30	-0.24	0.79	0.46	0.32	1.93
within 12m prior to test	133	5	-0.81	0.44	0.63	0.13	1.52

within 6m and 12m prior to test	449	42	-0.19	0.82	0.45	0.34	1.98
within 6m, 12m and 18m prior to test	1,178	59	-0.52	0.60	0.42	0.26	1.35
within 12m and 18m prior to test missing	31	0					
within 18m prior to test - missing	13	0					
within 6m and 18m prior to test -missing	5	0					
Size of the fields							
On same months of the test							
no	1,894	109	ref				
yes	478	46	0.26	1.30	0.24	0.81	2.08
Number of months within 6 months in field <3hec.							
0	1,419	70	ref				
1	236	14	0.26	1.30	0.35	0.66	2.56
2	255	21	0.32	1.38	0.33	0.72	2.66
3	170	18	0.74	2.09	0.34	1.07	4.11
4	91	9	0.63	1.89	0.46	0.77	4.61
5	114	11	0.36	1.44	0.45	0.60	3.46
6	87	12	0.64	1.90	0.48	0.74	4.90
Number of months within 6 months in field >3hec.							
0	1,212	41	ref				
1	303	17	0.32	1.38	0.34	0.71	2.66
2	308	27	0.68	1.97	0.31	1.07	3.61
3	204	24	1.05	2.86	0.32	1.52	5.37
4	118	15	0.96	2.60	0.40	1.18	5.75
5	120	17	1.00	2.72	0.39	1.26	5.86
6	107	14	0.89	2.43	0.45	1.01	5.86
Number of months within 12 months in field <3hec.							
0	1,595	90	ref				
1	138	12	0.30	1.35	0.40	0.62	2.97
2	125	11	0.14	1.15	0.44	0.49	2.72
3	89	7	0.16	1.17	0.44	0.50	2.76
4	122	8	0.02	1.02	0.45	0.42	2.49
5	165	14	0.01	1.01	0.40	0.46	2.20
6	138	13	0.42	1.52	0.38	0.72	3.23
Number of months within 12 months in field >3hec.							
0	1,400	61	ref				
1	155	15	0.55	1.74	0.37	0.84	3.59
2	115	12	0.50	1.65	0.43	0.71	3.85
3	115	11	0.38	1.47	0.44	0.61	3.51
4	144	13	0.57	1.76	0.38	0.84	3.70
5	245	22	0.26	1.29	0.33	0.67	2.49
6	198	21	0.60	1.81	0.34	0.94	3.51
Presence of badgers in fields							
On the same month as test							
no	1,974	106	ref				
yes	398	49	0.38	1.47	0.26	0.89	2.42
Within 6 months prior to test							
no	1,574	65	ref				
yes	798	90	0.76	2.15	0.23	1.37	3.36
Within 6-12 months prior to test							
no	1,689	86	ref				
yes	683	69	0.34	1.41	0.23	0.90	2.20
Within 12-18 months prior to test							

no	2,084	126	ref				
yes	288	29	0.13	1.14	0.30	0.63	2.07
Cat_ Within 18 months prior to test							
within 6m prior to test	237	26	ref				
within 12m prior to test	91	6	-0.22	0.81	0.53	0.29	2.28
within 6m and 12m prior to test	315	37	0.00	1.00	0.34	0.52	1.95
within 18m prior to test	6	1	0.41	1.51	1.72	0.05	43.83
within 6m and 18m prior to test	5	2	2.07	7.94	1.21	0.75	84.25
within 12m and 18m prior to test	36	1	-1.10	0.33	1.07	0.04	2.72
within 6m, 12m and 18m prior to test	1,682	82	-0.59	0.55	0.30	0.31	1.00
Number of months within 6 months prior to test							
0	1,574	65	ref				
1	211	10	0.07	1.07	0.39	0.50	2.32
2	189	21	0.73	2.08	0.34	1.06	4.06
3	143	19	0.96	2.61	0.35	1.31	5.20
4	85	13	1.01	2.75	0.43	1.18	6.37
5	89	15	1.10	3.01	0.41	1.36	6.66
6	81	12	0.86	2.37	0.47	0.95	5.91
Number of months 6-12 months prior to test							
0	1,689	86	ref				
1	125	15	0.62	1.85	0.38	0.88	3.91
2	89	10	0.34	1.41	0.49	0.54	3.69
3	86	9	0.28	1.33	0.50	0.49	3.55
4	84	10	0.56	1.76	0.46	0.72	4.31
5	169	13	-0.13	0.88	0.43	0.38	2.01
6	130	12	0.52	1.69	0.39	0.78	3.65
Location of animal groups in buildings							
On the same month as test							
no	958	66	ref				
yes	1,414	89	0.05	1.06	0.21	0.70	1.58
Within 6 months prior to test							
no	368	19	ref				
yes	2,004	136	0.26	1.29	0.31	0.70	2.40
Within 12 months prior to test							
no	948	48	ref				
yes	1,424	107	0.22	1.25	0.21	0.83	1.88
Within 18 months prior to test							
no	1,275	71	ref				
yes	1,097	84	0.18	1.20	0.20	0.82	1.75
Cat_Within 18 months prior to test							
within 6m prior to test	524	23	ref				
within 12m prior to test	56	4	0.28	1.32	0.65	0.37	4.69
within 6m and 12m prior to test	467	32	0.24	1.27	0.31	0.69	2.32
within 6m and 18m prior to test	175	13	0.34	1.40	0.40	0.64	3.06
within 12m and 18m prior to test	63	3	-0.18	0.84	0.73	0.20	3.49
within 6m, 12m and 18m prior to test	1,066	80	0.32	1.38	0.26	0.82	2.30
within 18m prior to test -missing	21	0					
Number of months within 6 months prior to test							
0	368	19	ref				
1	203	17	0.34	1.41	0.42	0.61	3.24
2	181	15	0.47	1.60	0.43	0.70	3.68
3	321	33	0.57	1.77	0.37	0.86	3.63
4	377	23	0.07	1.08	0.39	0.50	2.29

5	364	24	0 21	1 23	0.38	0.59	2 60
6	558	24	-0.10	0 91	0 38	0 43	1 93
Number of months within 6-12 months prior to test							
0	948	48	ref				
1	387	27	0 07	1 07	0 29	0 60	1 90
2	244	17	0 22	1 24	0 33	0 65	2 38
3	163	18	0 51	1 66	0 36	0 82	3 35
4	188	21	0 54	1 72	0 34	0 89	3 32
5	125	14	0 41	1 50	0 41	0 65	3 34
6	317	10	-0 35	0 70	0 38	0 33	1 47
Number of months with 12-18 months prior to test							
0	1,275	71	ref				
1	111	11	0 36	1 43	0 42	0 62	3 28
2	150	15	0 38	1 46	0 36	0 71	2 97
3	118	11	0 46	1 58	0 40	0 73	3 44
4	247	18	0 03	1 03	0.34	0 53	1.99
5	228	22	0 34	1 40	0 32	0 75	2 61
6	243	7	-0 61	0 55	0 42	0 24	1 24
Presence of badgers in buildings							
On same month as test							
no	2,334	148	ref				
yes	38	7	0.61	1 84	0.76	0 42	8 12
Within 6 months prior to test							
no	2,313	143	ref				
yes	59	12	0.97	2 63	0 67	0.71	9 71
Within 12 months prior to test							
no	2,330	147	ref				
yes	42	8	0 57	1 77	0 78	0 38	8 23
Within 18 months prior to test							
no	2,339	149	ref				
yes	33	6	0 43	1 53	0 83	0.30	7.74
Number of months within 6 months prior to test							
0	2,313	143	ref				
2	11	2	0.72	2 04	1.15	0 21	19.59
3	10	5	1.62	5.03	1 03	0 68	37 52
4	11	1	1.12	3.06	1 06	0 38	24 50
5	14	3	0.72	2 05	1.06	0.26	16.45
6	6	1	0.99	2.70	1 42	0.17	43 22
1 - missing	7	0					
Poor ventilation of buildings							
On same month as test							
no	2,013	134	ref				
yes	359	21	-0.02	0.98	0 32	0.52	1 84
Within 6 months prior to test							
no	1,965	130	ref				
yes	407	25	0.02	1.02	0 31	0 55	1.88
Within 6-12 months prior to test							
no	2,019	132	ref				
yes	353	23	0.12	1.13	0 31	0 61	2 08
Within 12-18 months prior to test							
no	2,050	135	ref				
yes	322	20	-0.01	1.00	0 32	0 53	1.87
Cat_ Within 18m prior to test							

none	1,946	130	ref				
within 6m prior to test	57	1	-1.11	0.33	1.00	0.05	2.35
within 6m and 12m prior to test	38	4	0.56	1.76	0.68	0.46	6.71
within 6m and 18m prior to test	11	1	-0.18	0.84	1.54	0.04	17.01
within 6m, 12m and 18m prior to test	301	19	0.03	1.03	0.34	0.53	1.99
within 12m prior to test-missing	9	0					
within 18m prior to test-missing	5	0					
within 12m and 18m prior to test-missing	5	0					

Appendix 6.2 - Multivariable multilevel logistic regression with random effects from 2,372 animal group tests using 404 herd tests in 140 herds and 738 animal groups with field variables only

Variable	Obs.	Number of groups with at least one reactor	Coef.	OR	SE	95% credibility interval	
						lower	upper
Herd							
Restocking status							
cont.stocked	1,952	420	ref				
restocked	140	15	-1.50	0.22	0.91	0.04	1.33
RBCT treatment							
survey	860	51	ref				
reactive	886	57	0.21	1.24	0.56	0.41	3.71
proactive	626	47	-0.58	0.56	0.68	0.15	2.09
Number of animals in group in buildings							
1-30	30	10	ref				
31-60	25	11	0.79	2.21	0.44	0.94	5.18
61-90	16	5	2.13	8.43	0.48	3.27	21.77
91-120	18	8	2.10	8.13	0.62	2.42	27.36
121-550	24	11	2.73	15.36	0.47	6.08	38.83
Variance							
Between herds			2.46		0.73	2.77	49.29
Between animal groups within herds			0.00		0.00	1.00	1.00

Appendix 6.3 - Multivariable multilevel logistic regression with random effects from 2,372 animal group tests using 404 herd tests in 140 herds and 738 animal groups with building variables only

Variable	Obs.	Number of groups with at least one reactor	Coef.	OR	SE	95% credibility interval	
						lower	upper
Herd							
Restocking status							
cont.stocked	1,952	420	ref				
restocked	140	15	-0.64	0.53	0.45	0.22	1.28
RBCT treatment							
survey	860	51	ref				
reactive	886	57	-0.05	0.96	0.40	0.44	2.09
proactive	626	47	0.05	1.05	0.41	0.48	2.33
Number of animals in group in buildings							
1-30	30	10	ref				
31-60	25	11	1.13	3.11	0.37	1.51	6.38
61-90	16	5	1.27	0.52	0.48	1.38	9.19
91-120	18	8	2.62	13.68	0.49	5.25	35.67
121-550	24	11	2.43	11.30	0.46	4.60	27.79
Variance							
Between herds			0.50		0.37	0.80	3.41
Between animal groups within herds			2.15		0.62	2.57	28.86

Appendix 6.4 - Multivariable multilevel logistic regression with random effects from 2,372 animal group tests using 404 herd tests in 140 herds and 738 animal groups with risk factors associated with the risk of HBD

Variable	Obs.	Number of groups with at least one reactor	coef.	OR	SE	95% credibility interval	
						lower	upper
Herd							
Restocking status							
cont.stocked	1,952	420	ref				
restocked	140	15	-0.66	0.51	0.39	0.24	1.09
RBCT treatment							
survey	860	51	ref				
reactive	886	57	0.08	1.08	0.33	0.57	2.05
proactive	626	47	0.23	1.25	0.35	0.64	2.48
Number of animals in group in buildings							
1-30	30	10	ref				
31-60	25	11	-0.64	0.53	0.30	0.29	0.95
61-90	16	5	0.42	1.52	0.30	0.84	2.74
91-120	18	8	0.36	1.43	0.48	0.56	3.69
121-550	24	11	1.74	5.69	0.46	2.33	13.88
Presence of badgers in fields							
Number of months within 6 months prior to test							
0	1,574	65	ref				
1	211	10	0.02	1.02	0.41	0.45	2.29
2	189	21	0.78	2.18	0.35	1.11	4.28
3	143	19	1.10	3.02	0.35	1.51	6.01
4	85	13	1.11	3.04	0.43	1.32	7.04
5	89	15	1.20	3.30	0.41	1.47	7.42
6	81	12	1.03	2.80	0.49	1.08	7.27
Risk factors for HBD							
No minerals/vits/licks	76		ref				
Minerals/vits/licks	72		-0.75	0.47	0.28	0.27	0.82
Not from market	83		ref				
From market	65		0.74	2.10	0.28	1.21	3.65
Variance							
Between herds			0.35		0.26	0.85	2.37
Between animal groups within herds			2.37		0.48	4.15	27.57