An evaluation of Irish cattle herds with inconclusive serological evidence of bovine brucellosis

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ABSTRACT

Since 1998, there has been a steady decline in herd restrictions and de-populations in Ireland due to bovine brucellosis. There is concern that the interpretation of laboratory results may become increasingly problematic, as brucellosis prevalence falls in Ireland. Therefore, the purpose of the current study was to evaluate the infection status of Irish herds and animals with inconclusive serological evidence of bovine brucellosis. During 12 months from September 1, 2004, laboratory and observational epidemiological data were collected from all Irish herds where animal testing identified at least one animal with a complement fixation test (CFT) reading greater than zero and/or a positive result to the indirect enzyme-linked immunosorbent assay (iELISA). Due to the observational nature of the study, we have robust estimates of the relative, but not the absolute, performance of the CFT, iELISA and brucellin skin test (BST). Herds were divided into three categories (Group A, B or C) on the basis of test results at initial assessment. A total of 639 herds were enrolled into the study, and observed for at least two years following enrolment. A rising CFT titre, with a CFT reading of 111 International CFT Units (IU) or greater at the subsequent blood test, was generally associated with herds where other evidence of infection was also available. Knowledge of the CFT reading at the initial and a subsequent blood test proved useful in distinguishing false-positive and true-positive brucellosis results. There was poor correlation between the CFT and iELISA results, and between the CFT and BST results. As a result of this study, national policy has been modified to include re-sampling of all animals with CFT readings of 20 IU or greater. This project has also led to a reduction in the number of herds restricted, as well as restriction duration. It has also contributed to a reduction in the number of herds listed for contiguous tests, and therefore the potential for contiguity testing of false positive results.

KEYWORDS: bovine brucellosis, brucellin skin test, CFT, epidemiology, eradication programme, iElisa, Ireland, MSAT, *Yersinia enterocolitica*

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Irish Veterinary Journal
Volume 62 Number 3 182-190 2009

INTRODUCTION

Progress towards eradication of bovine brucellosis in Ireland

A national programme to eradicate bovine brucellosis in the Republic of Ireland commenced in 1965 with the introduction of milk ring testing for dairy herds. At the outset of the programme, 12% of the 105,000 dairy herds tested positive; a further 3% of tests were inconclusive (Griffin and Collins 1999). At this time, the incidence of disease was higher in the south of Ireland than in the west and north-west. Good progress towards the goal of eradication was achieved over the following 20 years, resulting in a recorded herd prevalence of 0.19% during 1985 and 1986 (Griffin and Collins 1999). Residual disease was limited to north Cork, Limerick and

Tipperary. However, further progress was not achieved, with disease prevalence increasing during the 1990s. A total of 1,081 herds were restricted during 1998 (Griffin and Collins 1999), with disease spreading within, not just Limerick and Tipperary, but also to counties that had been clear for a number of years. A range of policy changes were introduced from February 1998 onwards, including the re-introduction of the pre-movement test, the rapid depopulation of infected herds and the treatment of slurry with lime prior to land spreading (**Figure 1**). In situations where lime treatment was not possible, the herdowner was required to store the slurry or farm yard manure for a prolonged period prior to spreading. Since

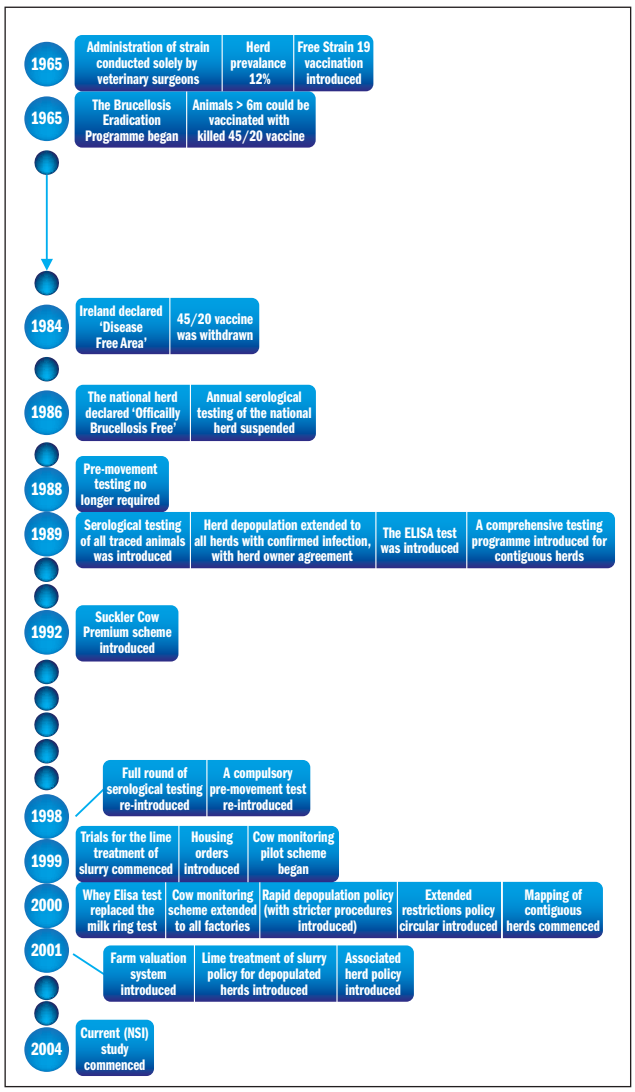

Figure 1: National policy changes, relevant to the eradication of bovine brucellosis, between 1965 and 2004.

1998, there has been a steady decline in herd restrictions and de-populations. In 2004, 2005, 2006 and 2007, the number of herds depopulated as a result of confirmed or suspected brucellosis was 68, 27, 3 and 0, respectively (**Figure 2**). If the current disease situation is maintained, Ireland will be eligible to achieve Officially Brucellosis Free (OBF) status in April 2009.

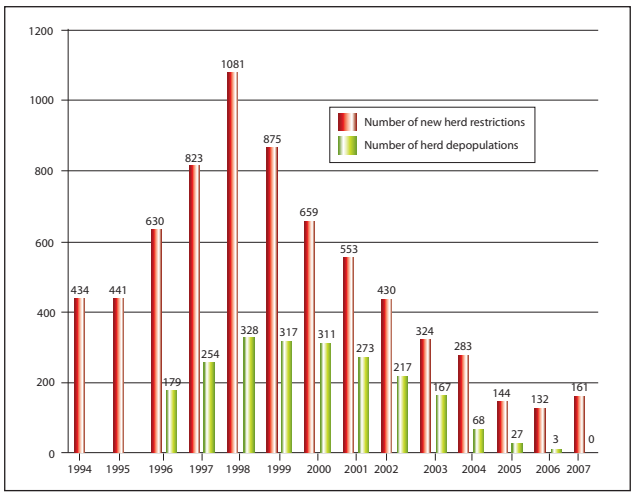

Figure 2: New herd restrictions and depopulations due to bovine brucellosis in Ireland during 1994 to 2007. Herd depopulation data was not available for 1994 and 1995.

Challenges faced as disease prevalence falls

There is a fall in the positive predictive value of sero-diagnostic testing with reducing disease prevalence. In other words, false positive serological reactors become increasingly problematic as disease levels fall. This issue, which has been reported in the European Union and New Zealand (Pouillot *et al.* 1998; Godfroid *et al.* 2002) in association with the latter stages of brucellosis eradication programmes, presents a range of challenges for programme decision-makers. These include the imposition of potentially unnecessary herd restrictions, related trade implications and testing requirements for herds contiguous to those with false positive reactor animals (Godfroid *et al.* 2002). Efforts to distinguish false and true positive reactors have been conducted, based on detailed epidemiological investigations, laboratory testing and measures of cellular immunity (Godfroid *et al.* 2002; Saegerman *et al.* 2004), and improvements in the specificity of a national brucellosis testing programme have recently been reported (McGiven *et al.* 2008).

Study objectives

The correct interpretation of annual test results from all female and male cattle over 12 months of age is a critical component of the national programme in Ireland. However, the interpretation of these results may become increasingly problematic, as brucellosis prevalence falls in Ireland. The purpose of the current study was to evaluate the infection status of Irish herds and animals with inconclusive serological evidence of bovine brucellosis.

MATERIALS AND METHODS

The Irish programme

As part of the national brucellosis eradication programme, blood is collected annually from all female and entire male cattle aged 12 months and over, for serological testing at the Blood Testing Laboratory, Model Farm Road, Cork. The protocol for annual herd testing in Ireland is presented in **Figure 3**. Initially, serum is

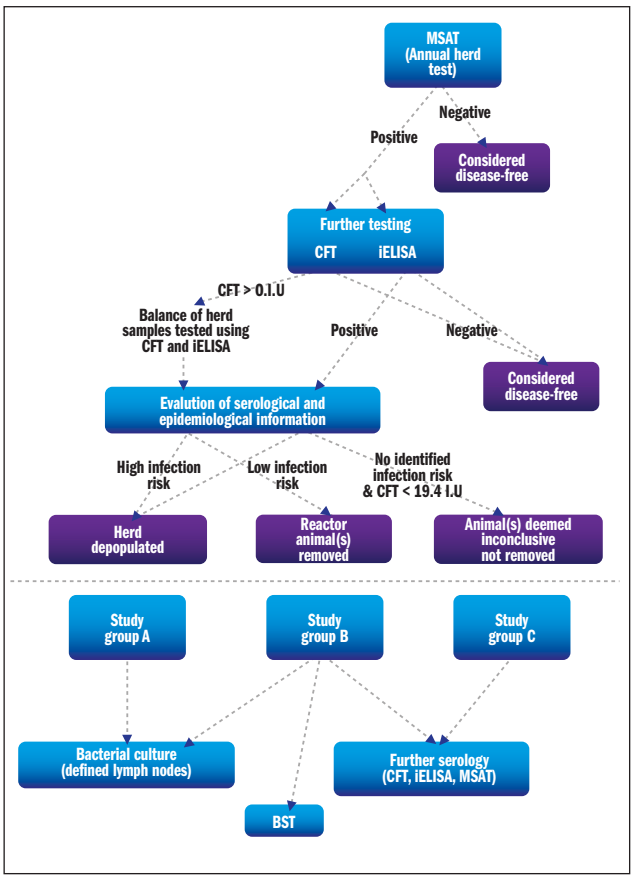

Figure 3: A flow diagramme highlighting the protocol for annual herd testing in Ireland, the process of herd categorisation in this study, and the diagnostic tests applied to the different study groups. A range of diagnostic tests are mentioned, including the microtitre serum agglutination test (MSAT), complement fixation test (CFT), the indirect enzyme-linked immunosorbent assay (iELISA) and the brucellin skin test (BST).

screened using the microtitre-serum agglutination test (MSAT), at a dilution of 1:14 (which provides increased sensitivity over the normal positive cut-off of 2:20). Samples failing the MSAT screening are re-tested using both the indirect enzyme-linked immunosorbent assay (iELISA) and complement fixation test (CFT). If a positive CFT result (considered 19.4 international units (IU) or greater; noting, at the time, that the titration methods did not allow an even result of 20 IU, the recognised positive cut-off) is obtained, the sera from all other eligible animals in the same herd are tested using both the CFT and iELISA (**Figure 3**). Serological testing is also conducted (in addition to the annual test) on herds that are contiguous to restricted herds, on animals pre- and post-movement, on post-abortion samples, as well as on animals that have been traced from diseased premises. A number of additional measures and obligations also form part of the programme. Approximately 75% of cull cows are blood sampled at the point of slaughter, and these samples are tested in the blood testing laboratory in Cork. A whey iELISA test is conducted monthly on bulk milk tank samples from all dairy herds. There is a legal obligation on an owner or person in charge of an animal that aborts to notify the Department of Agriculture, Fisheries and Food (who will arrange follow up testing) or to arrange for a registered veterinary surgeon to arrange for the relevant samples or specimens to be sent for analysis, including culture where relevant, in an approved laboratory. The MSAT, iELISA, CFT and culture are standard tests, conducted in accordance with both international (OIE 2004) and EU (Anon. 1964) guidelines. Movement restrictions are imposed on all test-positive herds, and rapid herd depopulation is undertaken when disease is considered to be present. Extended restrictions were used in high incidence areas to prevent re-infection. A range of decontamination procedures are undertaken, including cleansing and disinfection of housing, lime treatment of slurry (to raise pH to 12 or greater) or prolonged slurry storage if lime treatment is not feasible, and prolonged storage of farmyard manure prior to spreading onto land (Hahesy and Sheahan 2002).

The study herds

a. Selection criteria and study period

All herds in the Republic of Ireland were eligible for inclusion in this study. We enrolled all herds into the study that showed a serological response in either the CFT or serum iELISA at any test during the 12 months from September 1, 2004. Each study herd was observed from enrolment until the end of April 2007.

b. Herd categorisation

Each study herd was categorised on the basis of results from the initial test(s) conducted at the time of enrolment ('the initial assessment'), as follows:

- At the initial assessment, Group A herds had either conclusive evidence of brucellosis (culture-positive abortion, or a high CFT following a suspected abortion but a foetus was not available for culture) or at least two animals with a CFT result of 111 IU or more (chosen arbitrarily; in the Irish programme, this figure is considered indicative of infection, regardless of the number positive);
- At the initial assessment, Group B herds had one or more animals with a CFT reading greater than zero; however, Group A herd criteria were not met; and,
- At the initial assessment, Group C herds had one or more animals with a positive iELISA result; however, Group A and/or B herd criteria were not met.

In cases where the initial assessment was a part-herd test, the result of the complete herd test was taken into account before the herd was categorised. Herds remained in their initial herd category, despite later events during the study period.

c. Herd management

Each of the study herds was managed as presented in **Figure 3**. Briefly:

- Group A herds were immediately depopulated. Therefore, follow-up sampling from CFT-positive animals was not conducted. When feasible, however, the retropharyngeal and/or supramammary lymph nodes from these animals were submitted for bacterial

culture. These farms were considered disease-free when they were subsequently re-populated;
- In Group B herds, all CFT-positive animals were re-bled for testing using the MSAT, CFT and iELISA. Where feasible, these animals were also tested using the brucellin skin test (BST; Brucellergene OCB, Synbiotics Europe, Lyon, France), in accordance with internationally recognised methods (OIE 2004). Animals with a positive CFT result at the initial and/or a subsequent assessment were slaughtered and retropharyngeal and/or supramammary lymph nodes were collected for bacterial culture, where feasible; and,
- In Group C herds, all cattle positive to the iELISA were re-bled for testing using the MSAT, CFT and iELISA.

Data collection

Data were collected from a range of data sources about each study herd, including:
- The National Brucellosis Laboratory (results from the initial test and all relevant testing conducted subsequently);
- The Animal Health Computer System (AHCS) and Animal Identification and Movement (AIM) System (a central national database of animal movement and health information, including herd depopulations); and,
- The Land Parcel Identification System (LPIS; geographic information on land owned by farmers).

Furthermore, field veterinary inspectors were asked to collect epidemiological data from each Group B, and where possible Group C, herd (see below) using an investigative template that focused on potential linkages with any previously restricted herd. These linkages related to the history of the CFT and/or iELISA positive animals, the infection history of the herd and of the locality, and the potential for mechanical transmission to the herd, either by people or equipment.

Data management and analysis

Herd and animal-level data was managed in a Microsoft Access 2003 database. Data analyses were conducted in Microsoft Access, and results were presented using Microsoft Excel 2003 (Microsoft Corporation, Redmond, USA). Using StatXact (Cytel Inc. Cambridge, USA), we calculated McNemar's chi square test and kappa values to assess the level of inter-test agreement.

RESULTS

General results

During the 12 months from September 1, 2004, 639 herds were enrolled in the study, including 10 Group A herds (117 CFT- and/or iELISA-positive animals), 247 Group B herds (451 CFT- and/or iELISA-positive animals) and 382 Group C herds (398 iELISA-positive animals). The Group B and C herds were located throughout Ireland, whereas the Group A herds were found in counties Cavan, Clare, Cork, Kerry, Meath and Tipperary (**Figure 4**).

Group A herds

During the study period, 10 Group A herds were identified. Tissue samples were submitted for culture from three of these herds, and all were positive. At the initial assessment, CFT and iELISA serological data were available for 116 study animals from these herds. Serum from one study animal was haemolysed; therefore, a CFT result was not available. Ninty-four (81.0% of 116) study animals were CFT-positive (an average of 9.4 CFT-positive animals/herd) and 97 (82.9% of 117) were iELISA-positive. The distribution of the initial CFT result is presented in **Table 1**. Samples containing 356 IU in the CFT were not further diluted and so this reading represents a reading of 356 or greater. There was moderate agreement between the 116 CFT and iELISA results (kappa = 0.24, 95% confidence interval -0.03 to 0.46; McNemar's chi-square P = 0.70; **Table 2**). Following the initial assessment, all Group A herds were rapidly depopulated. In the two years since study end, there has been no evidence of infection in any Group A herd following re-population.

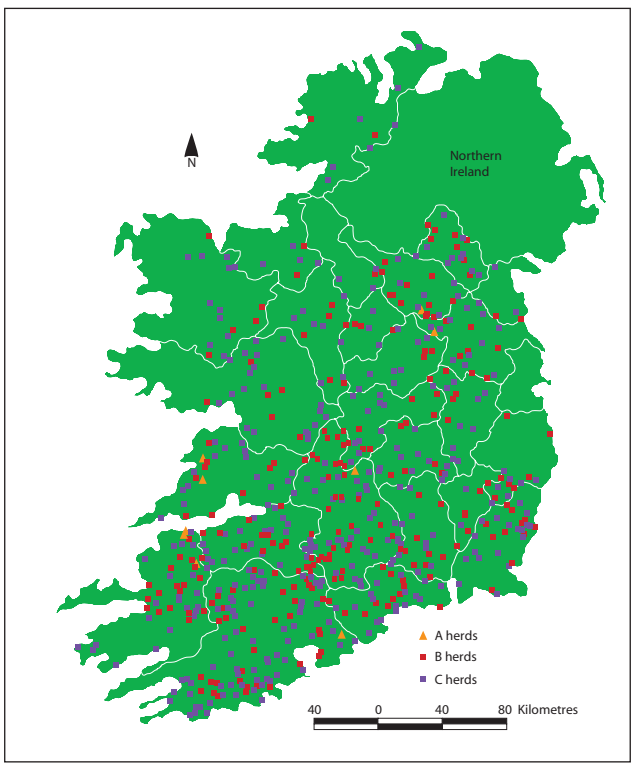

Figure 4: The location of the study herds.

Group B herds

At the initial assessment, CFT and iELISA serological data are available for 441 animals from 246 Group B herds. Sera from a further 10 animals were haemolysed, and CFT results are not available. At this assessment, 225 (51.0%) samples had a positive CFT (an average of 0.9 CFT-positive animals/herd), and 331 (75.1%) samples were iELISA-positive (**Table 3**). There was a poor correlation between the initial CFT and iELISA results (kappa = -0.38, 95% confidence interval -0.46 to -0.31; McNemar's chi square test P < 0.001).

Table 1: The initial CFT results (international CFT units, IU) from 116 animals that were CFT- and/or iELISA-positive at the initial assessment in 10 Group A herds. A CFT result for one animal was not available

CFT result (IU)	No. of animals
0	3
13.9	4
16.7	15
19.4	4
28	8
33	1
39	8
44	1
56	5
67	7
78	3
111	4
133	3
156	2
178	4
222	6
266	1
311	3
356	34
Total	116

Table 2: The initial complement CFT (complement fixation test) and iELISA (indirect enzyme-linked immunosorbent assay) results from 116 animals that were CFT- and/or iELISA-positive at the initial assessment in 10 Group A herds. A CFT result for one additional animal was not available

CFT result[a]	iELISA result		
	Positive	Negative	Total
Positive	82	12	94
Negative	14	8	22
Total	96	20	116

[a] A CFT result of 19.4 international CFT units or greater was considered positive, and negative otherwise.

Table 3: The CFT (complement fixation test) and iELISA (indirect enzyme-linked immunosorbent assay) results from 441 animals that were CFT- and/or iELISA-positive at the initial assessment in 246 Group B herds. CFT results for a further 10 such animals were not available

CFT result[a]	iELISA result		
	Positive	Negative	Total
Positive	127	98	225
Negative	204	12	216
Total	331	110	441

[a] A CFT result of 19.4 international CFT units or greater was considered positive, and negative otherwise.

A subsequent assessment, which included iELISA and CFT testing and the collection of herd-level risk factor data, was carried out, on average 25 days later, on 334 animals in 198 herds (**Figure 5**). The CFT results, summarised and in detail, at the initial and subsequent assessments for these 334 animals from 198 Group B herds are presented in **Table 4** and **Table 5**, respectively. The correlation between the initial and subsequent CFT result was poor (kappa = 0.26; 0.18, 0.33; McNemar's chi square test $P < 0.001$; **Table 4**), mainly due to a falling CFT titre at the subsequent test. Of 173 animals with a positive (19.4 or greater) CFT reading at the initial assessment, 122 (70.5%) subsequently tested negative (**Table 4**). The iELISA results at the initial and subsequent assessments for these 334 animals from 198 Group B herds are presented in **Table 6**. There was poor correlation between the initial and subsequent iELISA results (kappa = 0.39; 0.31, 0.47; McNemar's chi square test $P < 0.001$). There was also poor correlation between the CFT and iELISA results at the subsequent assessment (kappa = -0.17; -0.25, -0.09; McNemar's chi square test $P < 0.001$) (**Table 7**). At this test, there were 56 animals in Group B herds with a positive CFT reading, including 40 (71.4%) that were also iELISA-positive.

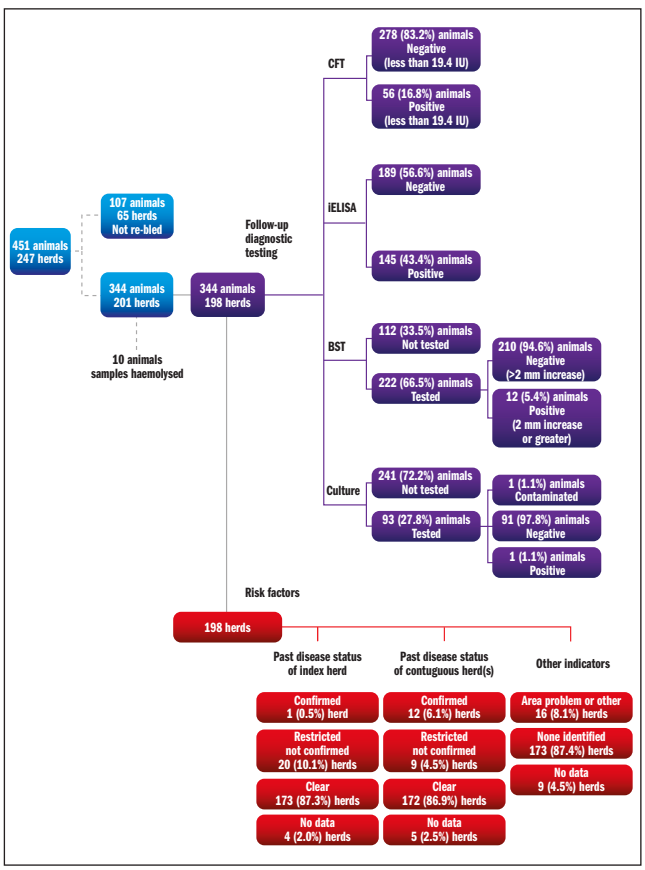

Figure 5: Risk factors and diagnostic test results at the subsequent assessment for 334 study animals in 198 Group B herds. A range of diagnostic tests are mentioned, including the complement fixation test (CFT), the indirect enzyme-linked immunosorbent assay (iELISA) and the brucellin skin test (BST).

At the subsequent assessment, the BST was conducted on 222 animals, including 12 (in 11 herds) that were positive (skin increase of at least 2 mm) (**Table 8, Figure 5**).

Table 4: The summarised CFT (complement fixation test) results at the initial and subsequent assessment for 334 animals in 198 Group B herds. Each of these animals was CFT- and/or iELISA-positive at the initial assessment

Initial CFT result[a]	Subsequent CFT result[a]		
	Positive	Negative	Total
Positive	51	122	173
Negative	5	156	161
Total	56	278	334

[a] A CFT result of 19.4 international CFT units or greater was considered positive, and negative otherwise.

There was a poor correlation between the CFT and the BST results (kappa = 0.03; -0.09, 0.14; McNemar's chi square test P < 0.001; **Table 9**), and between the iELISA and BST results (kappa = 0.03; -0.05, 0.10; McNemar's chi square test P < 0.001; **Table 10**). Bacterial culture was conducted on 93 animals from Group B herds, including one animal that tested positive (**Figure 5**).

Epidemiological information about 198 Group B herds is presented in **Figure 5**. On 21 farms, brucellosis had previously been reported on either the index and/or contiguous herd(s).

A decision was taken by headquarters staff, in consulation with local staff, to depopulate 14 of the Group B herds, based on epidemiological and laboratory evidence, and after a qualitative risk assessment had been completed examining the potential risk of infection, and of spread, if infection were present. In four of these herds, there was an increase in CFT titre in at least one animal between the initial and subsequent assessment.

In the two years since study end, only one of the 246 Group B herds disclosed any CFT reactors. Six animals were removed as reactors, and cultures of lymphatic tissues for *B. abortus* were negative. The herd was not depopulated and no further reactors were detected.

Group C herds

In the Group C herds, 398 animals from 382 herds were iELISA-positive at the initial serological assessment. Follow-up data, including herd-level risk factor information and serological results were collected on 252 of these animals in 244 herds (**Figure 6**). At the subsequent assessment, there were two (0.8%) animals with a positive CFT reading and 94 (37.3%) iELISA-positive animals (**Figure 6**). Epidemiological information about 198 Group C herds is presented in **Figure 6**. On five and 25 farms, respectively, brucellosis had previously been reported on the index and contiguous herd(s). Three Group C herds were depopulated. In the two years since study end, one Group C herd disclosed one positive CFT reactor. This herd was not depopulated (although this animal was removed), and no further reactors were detected.

DISCUSSION

In this study, we have sought to evaluate the infection status of Irish herds and animals with inconclusive serological evidence of bovine brucellosis. This assessment was essentially observational, relying on field and laboratory data collected as part of (and, in some cases, additional

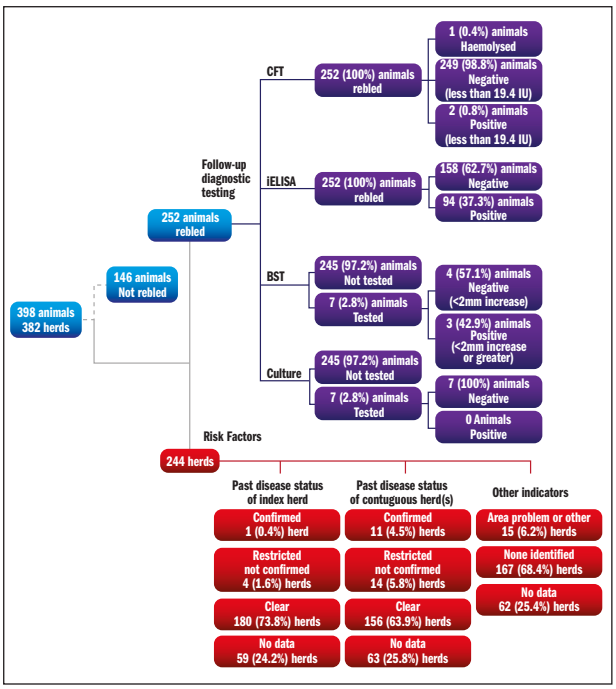

Figure 6: Risk factors and diagnostic test results for 252 animals in 244 Group C herds. A range of diagnostic tests are mentioned, including the complement fixation test (CFT), the indirect enzyme-linked immunosorbent assay (iELISA) and the brucellin skin test (BST).

to) the nationally-directed disease eradication programme. Rapid herd depopulation has been an important component of the national programme for some years in situations where infection is likely. This approach was continued throughout the current study period, with CFT-positive animals being rapidly removed following detection. In addition, a decision to conduct full herd depopulations was undertaken centrally in several Group B and C herds, in consultation with local staff. These decisions were based on serological and other laboratory results and epidemiological information, and after a qualitative risk assessment had been completed examining the potential risk of infection, and of spread if infection were present. In the latter stages of the national programme (from 2003 onwards), it was considered prudent to rapidly depopulate some herds with less robust evidence of infection but where management practices (such as outdoor calvings) were considered to pose an unacceptable risk of infection spread. Consequently, during this study it was often not possible to conduct exhaustive laboratory tests to directly determine the infection status of these herds and animals. Rather, we have measured this indirectly, by considering the disease status of the index, contiguous and associated herds, for at least two years from the time of the initial test. In this way, we are evaluating the infection status of these study herds for at least two post-calving tests after disclosure of serological evidence of infection.

Although microbiological confirmation is available for only three of the ten Group A herds, it is more likely than not that each of these herds was infected, based on compelling serological and/or epidemiological evidence of infection. In the initial assessment of these herds, there were an average of 9.4 CFT-positive animals/herd. These

Table 5: The detailed CFT (complement fixation test) results at the initial and subsequent assessment for 334 animals in 198 Group B herds. Each of these animals was CFT- and/or iELISA-positive at the initial assessment

Initial CFT result (international units)	Subsequent CFT result (international units)																
	0	13.9	16.7	19.4	22.2	28	33	39	44	56	67	111	133	178	311	356	Total
0	84	4		2	1												91
13.9	36	8	2														46
16.7	16	5	1	1					1								24
19.4	20	7	3	1		1											32
22.2	4	1	2														7
28	7	4	3					1									15
33	11	3	1			2	1		1								19
39	6	2	4	1		1	1	1								1	17
44		1	1	1		2		1									6
56	6	6	2	2		1	2			1			1			1	22
67	5	3	2				1		1		1	1		1	1		16
78	2	3					2		1								8
89		2	1					1			1						5
111	3	2	1				1	2									9
133	2		1				2					2					7
156								1									1
178												1				1	2
222																1	1
266								1									1
311	1																1
356											1					3	4
Total	203	51	24	8	1	7	10	8	4	1	3	4	1	1	1	7	334

Table 6: The iELISA (indirect enzyme-linked immunosorbent assay) results at the initial and subsequent assessment for 334 animals in 198 Group B herds. Each of these animals was CFT- and/or iELISA-positive at the initial assessment

Initial iELISA result	Subsequent iELISA result		
	Positive	Negative	Total
Positive	141	104	245
Negative	4	85	89
Total	145	189	334

Table 7: The CFT (complement fixation test) and iELISA (indirect enzyme-linked immunosorbent assay) results from 334 animals in 198 Group B herds at the subsequent assessment. Each of these animals was CFT- and/or iELISA-positive at the initial assessment

CFT result[a]	iELISA result		
	Positive	Negative	Total
Positive	16	40	56
Negative	173	105	278
Total	189	145	334

[a] A CFT result of 19.4 international CFT units or greater was considered positive, and negative otherwise.

Table 8: The detailed CFT (complement fixation test) and BST (brucellin skin test) results from 222 animals in 146 Group B herds at the subsequent assessment. Each of these animals was CFT- and/or iELISA positive at the initial assessment

CFT result[a]	BST result[b]								
	0	0.5	1	2	3	4	5	>5	Total
Positive	34		6	1	0	0	2	0	43
Negative	162	1	7	5	2	0	1	1	179
Total	196	1	13	6	2	0	3	1	222

[a] A CFT result of 19.4 international CFT units or greater was considered positive, and negative otherwise; and,

[b] A measurement of 2 mm or greater was considered positive, and negative otherwise.

herds were rapidly depopulated following initial diagnosis, in line with national policy. A rigorous programme of post-infection decontamination was then conducted in each of these herds, including the addition of lime into the slurry tank. In contrast, it is more likely than not that most of the Group B and C herds were not infected. Certainly, there was no evidence of within-herd transmission from any animals with CFT- and/or iELISA-positive results. To illustrate, based on ongoing serological monitoring over the intervening two years, there has been no evidence of

Table 9: The summarised CFT (complement fixation test) and BST (brucellin skin test) results from 222 animals in 146 Group B herds at the subsequent assessment. Each of these animals was CFT- and/or iELISA-positive at the initial assessment

CFT result[a]	BST result[b]		
	Positive	Negative	Total
Positive	3	40	43
Negative	9	170	179
Total	12	210	222

[a] A CFT result of 19.4 international CFT units or greater was considered positive, and negative otherwise; and,

[b] A measurement of 2 mm or greater was considered positive, and negative otherwise.

Table 10: The iELISA (indirect enzyme-linked immunosorbent assay) and BST (brucellin skin test) results from 222 animals in 146 Group B herds at the subsequent assessment. Each of these animals was CFT- and/or iELISA-positive at the initial assessment

iELISA result	BST result[a]		
	Positive	Negative	Total
Positive	6	83	89
Negative	6	127	133
Total	12	210	222

[a] A measurement of 2 mm or greater was considered positive, and negative otherwise.

infection in 231 (93.9% of 246) Group B herds and 378 (99.0% of 382) Group C herds. We accept that infection may have been present in the other Group B and C herds, noting that 14 Group B and three Group C herds were rapidly depopulated at the time of initial investigation. CFT-positive animals were disclosed in one Group B herd and one Group C herd, in this two year period, however, drawing on all available evidence, these two herds were not depopulated, and have disclosed no further reactors.

In Ireland, the CFT is currently used as the primary confirmatory diagnostic test for brucellosis, with the iELISA and the BST providing supplementary information. The CFT and iELISA are each considered prescribed tests for trade internationally (OIE 2004) and within the European Union (Anon. 1964), whereas the BST is considered either an 'other' (OIE 2004) or complementary (Anon. 1964) test (EFSA 2006). Other tests, such as the competitive enzyme-linked immunosorbent assay (cELISA), the fluorescence polarisation assay (FPA) and the rose Bengal plate test (RBT) were not considered in this paper, as they are not part of the decision making process in the Irish brucellosis eradication programme. The serological diagnosis of brucellosis has been an area of intensive study over many years (for example: Neilson 2002; Special Edition of *Veterinary Microbiology* vol. 90, no. 3-4, 2002; EFSA 2006). This study presents useful insights into the relative performance of those tests that are currently in use in the Irish programme. Any interpretation of the absolute performance (and particularly the specificity) of these tests must be undertaken with care, noting that we are not able to definitively distinguish true from false positive results. As noted previously, most (but not all) of the test-positive animals in Group B and C herds were believed to be false-positive results.

In this study, a negative or falling CFT titre on the second or subsequent test was highly suggestive that the result was a false-positive. Serological responses generally followed this pattern in both Group B and Group C herds. In the Group B herds, 173 animals were CFT-positive at the initial, but only 56 (32.4%) at the subsequent assessment (**Table 4, Figure 5**). In general, the CFT titre tended to persist on the second or subsequent test if a high titre was recorded initially (**Figure 5**). In the Group C herds, only two (0.8%) of the 252 study animals were CFT-positive on the subsequent test (**Figure 6**). We accept that reliance should not be placed on serology alone, noting that non-pregnant heifers may give a weak and transient response to a challenge with *Brucella* organisms (Cunningham 1968; Wilkinson *et al.* 1988). Pregnant heifers may present a similar serological picture (Cunningham 1968). False-positive results, which often present as a positive, but falling titre in one or a small proportion of the herd, have traditionally been attributed to infection with cross-reacting organisms (Corbel *et al.* 1984). The specificity of the CFT test may be compromised when animal populations are infected with *Yersinia enterocolitica* 0:9 (Godfroid and Käsbohrer 2002; Godfroid *et al.* 2002). Godfroid *et al.* (2002) have found that the different brucellosis serological tests could not differentiate brucellosis from infection with *Y. enterocolitica 0:9,* whereas the BST could. Recent findings (as yet unpublished) confirm the presence of infection with *Y. enterocolitica* 0:9 in some Irish herds, which may be leading to false-positive CFT reactions. Further work is underway to quantify this problem.

In this study, there was variable, but often poor, correlation between each of the three diagnostic tests under investigation. In the Group B herds, there was poor correlation between CFT and iELISA results at both the initial (kappa = -0.38, **Table 3**) and subsequent assessment (kappa = -0.25, **Table 7**). In contrast, there was moderate agreement between these tests in animals from Group A herds (kappa = 0.24, **Table 2**). Also, there was poor correlation between the BST, and both the CFT (kappa = 0.03, **Table 8** and **Table 9**) and iELISA (kappa = 0.03, **Table 10**) results from Group B herds. These results confirm our experience with the iELISA, noting that iELISA-positive but CFT-negative results are not uncommon. These results are consistent with those from a recent detailed meta-analysis, where the iELISA had a generally higher sensitivity but lower specificity that the CFT (EFSA, 2006).

Within Annex C of EU Directive 64/432/EEC (Anon. 1964), the BST is classified a 'complementary test', to assist in distinguishing false and true positive reactors following testing with prescribed 'standard tests' (iELISA, CFT, MSAT, Rose Bengal test). It has been reported that the BST, depending on the source of the brucellin, is highly specific (greater than 99%), and with moderate to high sensitivity (64-93%) (Pouillot *et al.* 1997; Saegerman *et al.* 1999; Bercovich and Muskens 1999) that decreases with increasing time following infection (Saegerman *et al.* 1999). In the meta-analysis above-mentioned, however, there were

insufficient data to estimate sensitivity and specificity. In the current study, it has not been possible to estimate the absolute performance of the BST. Brucellergene OCB is currently not available from Synbiotics Europe, however, we understand that attempts are being made to re-establish commercial production.

As part of this study, data on a range of epidemiological risk factors was collected from each of the Group B and C herds, including the past disease status of the index and contiguous herds, and other potential indicators of infection including the presence (or otherwise) of infection in the locality. The distribution of these risk factors between Group B and C herds is not dissimilar, and may reflect – at least in part – the historically widespread nature of infection in Ireland. For example, in 6.1% and 4.6% of Group B and C herds, respectively, there is a past history of confirmed disease in one or more contiguous herds. Nonetheless, among the Group A herds, and those Group B herds where disease was subsequently suspected, a combined understanding of all relevant information (including epidemiological risk factors) contributed to effective decision-making.

CONCLUSION

Current serological tests for brucellosis have imperfect specificity; therefore, seropositive results during brucellosis testing will continue after *Brucella abortus* has been successfully eradicated (Godfroid *et al.* 2002). During the latter stages of the Irish eradication programme, there has been a need for science-based information, both serological and epidemiological, relevant to a managed, risk-based move away from full-herd depopulation. Knowledge of the CFT reading at the initial and a subsequent blood test proved useful in distinguishing false positive and true positive brucellosis results. In addition, the iELISA was more sensitive, but less specific than the CFT test. As a result of these findings, the national policy has been modified to include re-sampling of all animals with CFT readings of 20 IU or greater. The BST may help to clarify true- and false-positive results, noting the potential for serological cross-reaction with *Yersinia enterocolitica* serotype 0:9. This project has also led to a reduction in the number of herds restricted, as well as restriction duration. It has also contributed to a reduction in the number of herds listed for contiguous tests, and therefore the potential for contiguity testing of false positive results.

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