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Risk associated with animals moved from herds infected with brucellosis in Northern Ireland

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Abstract

The movement of cattle from herds infected with *Brucella abortus* was investigated in order to assess the control measures for eradication of brucellosis from the cattle population of Northern Ireland. Using recorded cattle movement data, a historical cohort study was designed and carried out to quantify the risk of seropositivity in bovine animals moved from herds infected with brucellosis. The study found that 3.1% of animals, moved in the 6-month period prior to disclosure of infection in the source herd and subsequently tested, were interpreted as seropositive in their destination herds. The odds of seropositivity were approximately 19 (95% confidence interval: 7.8–46.4) times higher in this cohort compared with animals from herds with no history of infection. A multivariate logistic regression model was constructed to examine factors influencing the risk of seropositivity in the exposed cohort of animals, identifying maternal status (whether the dam had been a brucellosis reactor) and age at leaving the infected herd as the main risk factors.

Keywords: Brucellosis; Bovine; *Brucella abortus*; Cattle; Livestock movement

1. Introduction

Bovine brucellosis presents animal and human health risks, as well as being of economic significance to the agri-food industry in Northern Ireland. In cattle, infection causes herd

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production losses as a result of abortions, reducing milk production, increasing calving intervals, the birth of weak calves and increased culling rates due to metritis, following retention of the placenta. Undulant fever in humans may be a consequence of direct or indirect contact with infected cattle (Blood and Radostits, 1989).

In the 1930s 60% of all Northern Ireland herds were infected with *Brucella abortus*. Calf vaccination with the strain 19 vaccine was started in the 1940s, and a voluntary eradication scheme was introduced in the 1960s, when a survey based on milk ring testing of milk supplied to dairies in 1962 showed that approximately 12% of dairy herds were infected. Compulsory annual on-farm testing of susceptible animals followed, and by July 1982 Northern Ireland was declared an Officially Brucellosis Free region. Annual herd incidence stayed below 0.2% for 4 years and biennial testing, in accordance with EU regulations was introduced in I988. Sporadic outbreaks occurred until 1997, when three primary outbreaks resulted in spread to more than 60 farms (Abernethy et al., 2006). Annual herd incidence increased to a peak of 1.4% in 2002, reducing to 0.56% in 2006 (Fig. 1).

Surveillance for bovine brucellosis in Northern Ireland involves annual serological screening of breeding animals, monthly bulk-tank milk testing by enzyme-linked immunosorbent assay (ELISA), and serological screening of all breeding animals over 30 months of age at slaughter. Herd owners are legally obliged to report all bovine abortions to the Divisional Veterinary Officer for investigation. Control of an outbreak involves slaughter of affected animals, possible herd depopulation, movement restriction and tracing and testing of animals or herds at risk from movements. Herds contiguous to an outbreak (‘‘inner ring’’ herds) have movement restrictions imposed (i.e. they may not sell any cattle) until they have undergone two negative serological herd tests at a 4-month interval. Herds in the ‘‘outer ring’’ (i.e. contiguous to the inner ring herds) are restricted until one clear herd test has been completed.

The risks associated with the progeny of reactor animals have been widely examined (Wilesmith, 1978; Catlin and Sheehan, 1986; Akhtar and Mirza, 1995), however a review of the

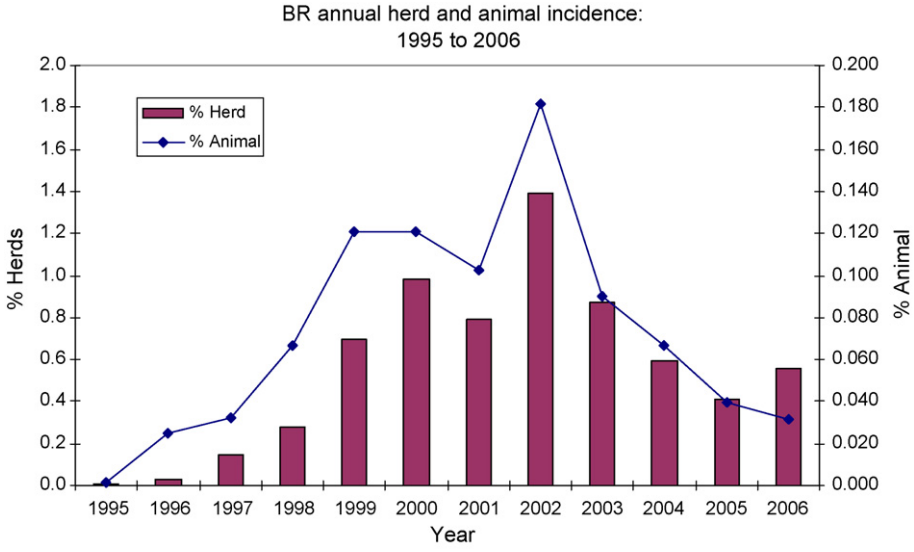

Fig. 1. Incidence of brucellosis in Northern Ireland cattle herds 1995–2006.

literature has not identified any studies directly quantifying the risk posed by animals sold from infected herds.

The aim of this study was to quantify the risk posed by susceptible animals sold from herds in the 6-month period prior to disclosure of brucellosis infection. A historical cohort design used recorded movement data, with the primary objective of quantifying the relative risk of seropositivity in animals that have moved from confirmed herds compared to animals moving from herds with no identified infection.

A secondary objective was to analyse the risk factors for seropositivity in the animals moved from infected herds.

2. Materials and methods

2.1. Reference population

Northern Ireland has a cattle population of 1.7 million, registered in around 25,000 active herds. Dairy cows and heifers represent 21% of the national herd, and beef cows and heifers 20%. During the 7-year study period, farm numbers fell from 27,107 to 24,329 while average herd size, based on cattle at herd tests, increased from 63.9 to 69.3. Farms are fragmented, with approximately 50% utilising multiple premises, and there is a high dependency on rented grazing. Traditionally, there has been substantial movement of cattle between herds and between premises within the same ownership. For example, 560,000 cattle moved to markets or other herds in 2000, equivalent to 33% of the national herd (Abernethy et al., 2006). Sixty five percent of the cattle were older than 1 year and most were sold to local herds or markets. Cattle density is among the highest in the United Kingdom (DEFRA, 2005) but within the country, herd and cattle population density is highest in the south, although herd size is larger in the north and east.

2.2. Study population

The unit of interest was a female or intact male animal. Although castrated males are susceptible, they play no significant role in transmission of the disease, and are not included in disease surveillance. Bulls used for natural service are also not considered to be important in disease transmission, although the risk is higher if semen is used for artificial insemination (AI) (Blood and Radostits, 1989). Data were not available on the possible use of individual bulls for AI, thus they were included in the study. The animals were identified from herds experiencing a brucellosis outbreak, confirmed by bacteriological culture, during the period 1997–2003 and herds within 1.5 km of these. Fig. 2 details the location of the outbreaks; the highest incidence of herd infection is in the southern region of the country, particularly around the border. Spatial clustering of disease in relation to the underlying population has been confirmed in analysis by Abernethy et al. (2006).

2.3. Data sources

Since 1988, the Veterinary Service in Northern Ireland has maintained a computerised record of cattle herd and animal registrations, as well as records of all between-herd movements and movements to markets and abattoirs. Herd owners, who are legally required to register cattle herds, animal births and deaths and to notify animal movements, supply the information.

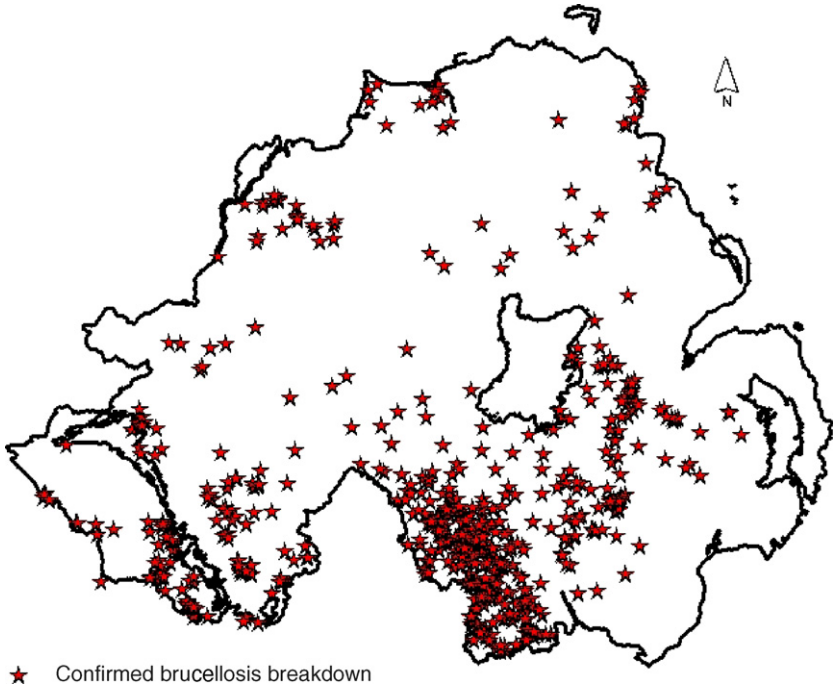

Fig. 2. Distribution of confirmed brucellosis outbreaks in Northern Ireland cattle herds 1997–2003.

Movements involving markets and abattoirs are recorded straight to the database by Department of Agriculture and Rural Development for Northern Ireland (DARDNI) staff using terminals at these locations. Tuberculosis and brucellosis diagnostic test results at the individual animal and herd level are recorded on the system, as is the record of interpretation of the tests by the DARDNI Veterinary Service local area or ''patch'' veterinarian. All animals over 6 weeks of age are tested annually for tuberculosis, and the record of this herd test effectively constitutes an annual validation of the animal location information. The original Animal Health System (AHS) was replaced by the Animal and Public Health Information System (APHIS) in 1999. This is the current national database and the main source of data for this study.

During the study period, APHIS did not hold a record of brucellosis herd status (i.e. whether infection was confirmed by culture) and a spreadsheet maintained by the reference laboratory, Veterinary Sciences Division, Stormont (now part of the Agri-Food and Biosciences Institute or AFBI) was used to identify all herds in which infection had been confirmed in the period 1997–2003. This was the period of highest incidence of disease, and allowed a minimum 18-month follow-up period for the cohorts (and thus a full calving cycle for female animals).

2.4. Selection criteria

2.4.1. Exposed cohort

Herds were selected on their first case of confirmed infection (by isolation of *B. abortus*) from 1997 to 2003. Any herds that experienced an unconfirmed breakdown less than 2 years prior to the confirmed breakdown were excluded, as it was feasible that management and trading behaviour may have been altered by the experience of the breakdown. All other herds with

confirmed infection were included. An exposed animal was defined as a female or bull of any age moved from a herd meeting these criteria in the 6-month period prior to disclosure of infection. Only those exposed animals with record of a brucellosis serological test in another herd after movement were included.

2.4.2. Unexposed cohort

Herds were eligible for selection if they contained test-eligible animals and had no history of brucellosis (confirmed or unconfirmed) in the previous 10 years. Geographical frequency matching was chosen to ensure that the unexposed cohort was as similar as possible to the exposed in geographically related factors. A Geographical Information System (ArcGIS 8.1 ESRI, 2000) was used to construct the sampling frame of candidate herds within a 1.5 km radius of exposed herds. Herd size is a known risk factor for brucellosis, and so eligible herds were stratified by herd size (based on numbers of test-eligible animals) prior to random selection within MS Excel (Microsoft Corporation, Redmond, WA, USA) An unexposed animal was defined as a female or bull of any age moved from the selected eligible herd in the 6-month period prior to disclosure of infection in the closest brucellosis-confirmed herd, and with a record of a serological test in another herd after movement.

Sample size determination was carried out using the statistical software package Stata 8.2 (StataCorp LP). Using a postulated relative risk of 2, a confidence level (CI) of 95% and power of 90%, a sample size of 2112 animals in each group was required, based on the assumption of simple random sampling. However, the study units were clustered within herds and it is known that there is a high intracluster correlation coefficient for brucellosis (Leech and Sellers, 1979). Twice as many herds were selected from the unexposed herd sample frame in an attempt to account for this.

2.5. Case ascertainment

The gold standard test for confirmation of brucellosis infection is isolation by bacteriological culture of *B. abortus* from reactors or aborted animals. However, samples are not cultured from every brucellosis reactor in Northern Ireland, as the objective of laboratory testing is to confirm infection at herd rather than individual level. Serological testing is carried out by the reference laboratory, in accordance with methods outlined in European Union council directive 64/432/EEC. Samples are screened using the serum agglutination test (SAT) and positives are serial tested with the complement fixation test (CFT). The final test interpretation is carried out by the patch veterinarian, and the case definition for follow-up was thus defined by a positive interpretation result of the serological test.

2.6. Data analysis

Descriptive statistics were obtained for the exposed and unexposed cohorts and the relative risk was calculated as a measure of strength of association between being moved from a brucellosis-infected herd and subsequent seroconversion. Cluster-adjusted odds ratios were obtained by using robust standard errors in a univariate logistic regression model.

The population attributable fraction (PAF) was calculated as described by Elwood (1980):

$$\mathrm{PAF} = \frac{P(\mathrm{RR}-1)}{P(\mathrm{RR}-1)+1}$$

where P is the prevalence of exposure in the population and RR the risk ratio.

Exploration of the risk factors for seropositivity was limited to the exposed cohort and involved preliminary univariate analysis using the chi-squared test for association between the outcome (seropositivity) and the independent variables. The Mantel–Haenszel stratification technique was used to examine potential confounding or interaction between variables. A multivariate logistic regression model was developed using a forward stepwise selection procedure. Improvement in the model by addition of variables was assessed using the likelihood ratio test, with the criteria for retention set at $p < 0.05$.

All the analysis was carried out using Stata 8.2 (© StataCorp LP).

3. Results

The cohorts comprised 4213 exposed and 4449 unexposed animals traced from 632 and 1264 source herds, respectively. Table 1 details the structure of the exposed and unexposed cohorts.

One hundred and thirty cases (114 females and 16 bulls), were detected in the exposed cohort, six (all female) in the unexposed. The detection of cases occurred most frequently at a herd test. Only 8 of the 136 cases (6%) were identified as a result of an abortion, all of these from the exposed cohort. High-risk herd tests, in herds with an exposure risk, such as proximity to a breakdown, detected 62 (50%) of the positives, while tracing tests, which had been allocated to

Table 1
Characteristics of cohorts of animals moved from brucellosis-infected and control herds in Northern Ireland 1997–2003

	Exposed cohort		Unexposed cohort	
	n	%	*n*	%
Sex				
Bull	559	13	197	4
Female	3654	87	4252	96
Age when left herd				
Less than 1 year	1835	44	1786	40
1–2 years old	1103	26	1242	27
2–3 years old	505	12	607	14
3–4 years old	231	5	295	7
4–5 years old	175	4	161	4
Over 5 years	364	9	358	8
Source herd type				
Dairy	1223	29	2275	51
Beef	2990	71	2174	49
Source herd size				
1–23	428	10	483	11
24–46	617	15	906	20
47–94	1218	29	1441	32
95+	1950	46	1619	36
Dam status				
Reactor	223	5	3	0.1
Non-reactor	3065	73	3608	81
Dam unknown	925	2	838	19
Follow-up period (until last recorded test)	6716 years		9054 years	
Average number of tests per animal	4		3.2	

Fig. 3. Box plot of time to detection of *B. abortus* seropositivity in cattle moved from herds infected with brucellosis in Northern Ireland 1997–2003.

the animal once the diseased status of the source herd of the exposed cohort had been discovered, identified 40 (31%) of the exposed positives. The remainder were detected at routine or other herd tests.

Time from leaving the source herd until detection of seropositivity is illustrated in Fig. 3. This ranged from 13 to 1522 days (median 118).

Age at detection of seropositivity ranged from 330 days to 17 years (Fig. 4).

Although detection of seropositivity in animals from the exposed cohort was generally a short time after entering the destination herd, it was not always at the first test. Ten of the sixteen

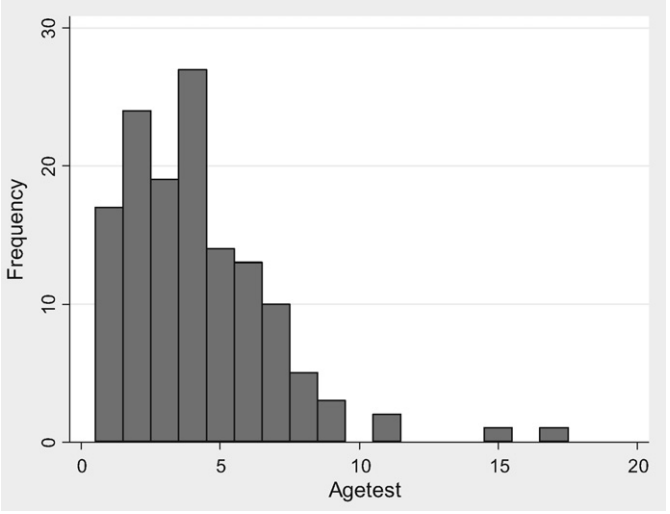

Fig. 4. Age distribution of cattle seropositive for *B. abortus* after movement from infected herds in Northern Ireland 1997–2003.

positive bulls failed to show a titre at the first test, while 34 of the114 positive females were not interpreted as seropositive at the first test in the destination herd.

The dam of 72 of the 130 exposed cases was recorded, 15 of these being reactors themselves (21%). The dam was known and not recorded as a reactor for 4 of the unexposed cases.

3.1. Risk of seropositivity

The primary objective of the study was to estimate the risk of seropositivity in animals moved from a brucellosis-infected herd, and the relative risk compared with animals moved from a herd where there was no history of infection.

The risk in exposed animals was 0.031. Thus, 3.1% of animals moved from a herd in the 6 months prior to disclosure of infection and tested after movement were interpreted as positive in their destination herd.

The crude risk ratio for the association between movement from an infected herd and seropositivity was estimated to be 22.9 (95% CI: 10.1–51.8, $p < 0.0001$). To adjust for clustering of the subjects within the herd of origin, the robust standard errors method was used, giving a cluster-adjusted odds ratio of 23.6 (95% CI: 9.2–60.4, $p < 0.0001$). However, the subjects were also clustered in the destination herds, closer analysis of the dataset revealing one large cluster of 65 animals in one particular destination herd, 26 of which became seropositive. To assess the effect of this on the statistical analysis, the intraclass correlation coefficient (ICC) for the effect of destination herd on the outcome was calculated using a one-way ANOVA, which confirmed a moderate clustering effect (ICC = 0.18). When the observations relating to this one herd were removed, the ICC was re-assessed as low at 0.019. The revised dataset summary results are presented in Table 2, the cluster-adjusted odds ratio of the revised dataset being 19 (95% CI: 7.8–46.4). This is the crude estimate for animals moving from a confirmed brucellosis-infected herd in the period 1997–2003 compared to animals moved from a herd with no history of infection determined by this study. Exposed animals thus have approximately 19 times the odds of seropositivity compared to unexposed animals. The small number of cases ($n = 6$) in the unexposed cohort precluded further multivariate analysis.

3.2. Population impact

All of the confirmed herds occurring between 1997 and 2003, with the exception of 12, were included as source herds in the study, and the exposed cohort included all animals leaving these herds and subsequently tested. The proportion of herds receiving these animals thus represents the prevalence of herds exposed in the population. The total number of herds in Northern Ireland with test-eligible animals during the study period was 28,547. The number of herds receiving exposed animals ($n = 1754$) and which also experienced a brucellosis breakdown was 223, and

Table 2
Association between movement of animals from brucellosis-infected and control herds in Northern Ireland 1997–2003 and subsequent seropositivity

	Exposed cohort	Unexposed cohort	Total
Seropositive	104	6	110
Not seropositive	4046	4441	8487
Total	4150	4447	8597

Table 3
Association between cattle herds in Northern Ireland receiving animals from brucellosis-infected (exposed) and control herds (unexposed) and experiencing a brucellosis breakdown

	Exposed	Unexposed	Total
Brucellosis breakdown	223	648	871
No brucellosis breakdown	1531	26,145	27,676
Total	1754	26,793	28,547

there were 871 brucellosis breakdowns in the reference population in the period 1997–2004 (Table 3).

The risk ratio for brucellosis breakdown in exposed compared to unexposed herds is 5.26 (95% CI 4.55–6.07, $p < 0.0001$) and the proportion of the population exposed was 0.06. This represents a population attributable fraction of 0.2.

3.3. Risk factors for seropositivity

The secondary objective of the study, to analyse risk factors for seropositivity in animals moved from brucellosis-infected herds, was confined to the cases in the exposed cohort, using the revised dataset.

Univariable analysis found statistically significant associations between seropositivity and age at leaving the exposed herd, and having a positive dam (Table 4).

A logistic regression model was constructed, using a forward stepwise method, with the final model (Table 5) including the variables dam positive, and age at leaving the herd as a linear effect.

The crude odds ratio for positive dam, estimated at 3.3 (95% CI: 1.63–6.6), increased to 4.1 (95% CI: 1.98–8.33) on the addition of ''age at leaving herd'' to the model, and remained close to this value as the other variables were considered. This was the only possible confounding effect observed. Interaction between variables in the model was assessed; no statistically significant

Table 4
Univariate associations between predictor variables and seropositivity in animals ($n = 4150$) moved from brucellosis-infected herds in Northern Ireland 1997–2003

Variable	Odds ratio	χ^2	p	95% CI
Positive dam				
Not positive = reference				
Positive = 1	3.3	12.49	<0.001	1.63–6.61
Herd type				
Dairy = reference				
Beef = 1	0.9	0.18	0.67	0.60–1.40
Age at leaving herd (years)				
Youngest = reference (score test for trend of odds)	1.68	79.32	<0.001	1.50–1.89
Sex				
Bull = reference				
Female = 1	0.89	0.17	0.68	0.51–1.55
Herd size				
Smallest = reference (score test for trend of odds)	1.17	2.39	0.12	0.96–1.42

Table 5
Results of a logistic regression model for seropositivity in animals moved from brucellosis-infected herds ($n = 4150$ animals) in Northern Ireland 1997–2003 using robust standard errors

Variable	Odds ratio	Wald test p-value	Confidence interval
Dam positive	4.06	<0.001	2.10–7.86
Age at leaving herd	1.63	<0.001	1.36–1.96

associations were found, although it was not possible to test interaction between herd size and maternal status due to a limited amount of data in some categories.

4. Discussion

The annual incidence of brucellosis in Northern Ireland has fallen from a peak of 227 herds in 2002 to 88 in 2005. The control measures have been enhanced during the interim to include compulsory slaughter of female offspring of reactor animals and pre-movement testing of susceptible livestock, introduced in accordance with European Union council directive 64/432/EEC (as amended).

Although purchased animals have been identified as a risk factor for brucellosis, this is the first recorded quantification of the risk specifically from animals known to originate in infected herds.

Classic cohort study analysis normally includes a comparison of the rates of disease in each cohort or a comparison of time-to-event. However in this case, the outcome was determined not by development but by detection of disease, which was constrained by the timing of serological tests. Only 6% of the cases were detected following clinical signs (abortion). An analysis emphasising time to event was thus considered not to be biologically justified.

Study limitations included the classic problems of historical cohort studies. Recording of data on APHIS is done with the objective of administering the tuberculosis and brucellosis eradication schemes and providing public health and marketing assurance through the traceability function. There is reliance on correct and complete notification and input of movement and disease data, and of geographical location data. The use of point data to geo-reference farm premises has limitations in that, even if correctly recorded, it locates only the main farm premises. Farms in Northern Ireland are often fragmented, with parcels of land at a number of locations, so that certain groups of livestock may not be located at the point reference.

While the case definition (bacteriological culture of the organism) of infection in the source herd was considered to be 100% specific, there are issues of sensitivity and specificity of serological tests for case ascertainment during the follow-up period. Sensitivity of the diagnostic test can depend on factors such as age, sex, stage of gestation, immune status and infective dose (Nielsen and Duncan, 1990). The relative sensitivity of the screening test, the SAT, in the Northern Ireland cattle population is estimated at 79–91%, so that there may be misclassification of infected animals (Abernethy et al., unpublished data). The relative specificity of the confirmatory test, the CFT, has been estimated at 100%, thus false positives are not considered to be a major problem. The lower sensitivity of the SAT test at the screening level cut-off may account, at least in part, for the finding that 34% of the exposed cohort case animals were not interpreted seropositive at the first test in the destination herd. This finding warrants further, more detailed, investigation to eliminate other possible sources of infection for these animals and to consider the effect of age and stage of pregnancy in particular.

There is the possibility of observer bias due to the patch veterinarian applying a more severe interpretation to animals known to come from breakdown herds, and thus an overestimation of risk in the exposed group. However, a basic visual scan of actual titres for the exposed cases, which had been allocated tracing tests, showed that only five were not seropositive on standard interpretation, so it is unlikely that this had an impact.

Animals with no record of a test after leaving the source herd were excluded from the cohorts, as their status could not be ascertained. The animal exclusion criteria were the same in both cohorts, thus any bias this introduced is likely to be non-differential, biasing the measure of effect towards null (Dohoo et al., 2003). However, the possibility remains that these animals were infected but not detected, and could have been a source of infection to their destination herd.

There were twice as many unexposed herds selected during the construction of the unexposed cohort. This was designed to improve the power of the study, particularly with regard to the clustering of animals within herds (McDermott et al., 1994). However, the resultant number of eligible animals was similar to that of the exposed cohort. A possible explanation is that the infected herds exhibited more risky behaviour – buying and selling more animals – while those which were unexposed were more likely to be self-contained, retaining rather than selling breeding animals. Another possibility is the effect of movement restrictions on herds within an infected area, as previously described. However, this would be expected to affect herds from which each cohort was established similarly—it was not possible to assess this with the available data.

Attempts were made during construction of the unexposed cohort to minimise confounding effects of herd size by carrying out the random selection by herd size strata, with the intention of performing a stratified analysis. However, it could not be foreseen how many eligible animals would result from these herds until the data extraction and cleaning had been performed. It can be seen from the structure of the cohorts that they are broadly similar although not perfectly matched for herd size. This is not thought to present a major source of bias, particularly as the results of the multivariate analysis confined to the exposed cohort failed to identify herd size as a significant independent risk factor in this study, after controlling for the other risk factors.

The population attributable fraction (0.2), is presented, though gives only an approximate value, and needs to be interpreted with care. This is because the exposed cohort of animals came only from confirmed brucellosis breakdowns meeting the selection criteria, while all brucellosis breakdowns were included in the risk ratio calculation. Confining the exposed cohort to herds with confirmed disease may have underestimated the proportion of herds exposed in the population, as the sensitivity of bacteriological culture methods is estimated at 94–97% (S. McDowell, AFBI, personal communication). It does, however, give a broad indication of the impact of the primary exposure in the reference population and suggests that approximately 20% of brucellosis breakdowns in Northern Ireland are attributable to animals moved from infected herds.

The outcome of the multivariate analysis of the exposed cohort for other risk factors found maternal status to be a significant risk factor, with the adjusted odds ratio estimated at 4.1 (95% CI: 1.98–8.33, $p < 0.001$). This is consistent with previous descriptive studies (Wilesmith, 1978), and supports the policy decision to purchase female offspring of brucellosis reactors. A linear relationship between age at exposure and seropositivity was established, indicating an increasing odds ratio of 1.6 (95% CI: 1.40–1.9, $p < 0.001$) for each year increase in age. Again this is consistent with knowledge of the disease (Crawford et al., 1990).

The information relating to herd type was not reliable. It was not possible to establish the herd type at the time of the cohort trace date, nor was it possible to take into account herds which were

mixed (i.e. with both dairy and beef cattle). This limits the conclusions that can be drawn from failure to find a statistically significant association.

The enhancement of control measures after 2002 to include purchase of female offspring of reactor animals is a possible source of bias. This would underestimate risk in the exposed cohort, but occurred late in the study period when the annual herd incidence was lower and thus is not expected to have a major effect.

The limitations of the study are all recognised, and the small number of cases in the unexposed cohort prevented the use of stratification in the analysis, thus the relative risk estimate presented is very much a crude one. However, the ratio estimated is so large, even taking the wide confidence interval into account, that any undetected biases would need to be very large to negate the results.

The use of animal movement information in veterinary epidemiology has immense potential as an epidemiological tool. Movement data in APHIS is already used routinely in Northern Ireland to automatically trace, apply movement restrictions and allocate tests to animals that have moved, during veterinarian-defined tracing windows, from herds infected with tuberculosis or brucellosis. Since the database also records results of individual animal tests, there is potential for continued detailed analysis of the impact of animal movements on disease spread.

5. Conclusion

This study has used recorded movement data to provide an estimate of the risk of brucellosis seropositivity for animals moved from an infected herd in the period before disclosure of infection, and an estimate of relative risk compared to animals moving from a herd with no identified brucellosis infection. The crude estimate of effect indicates that animals in the exposed cohort are 19 times more likely to be seropositive than those in the unexposed, and we estimate that between 1997 and 2003 this had an approximate impact of 20% on disease in the reference population. The findings of this study will provide important inputs to cost–benefit analyses on the compulsory slaughter of animals moved from brucellosis-infected herds, and contribute to the development of national brucellosis control policies in Northern Ireland

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