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Risk factors for failure to detect bovine tuberculosis in cattle from infected herds across Northern Ireland (2004–2010)

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ABSTRACT

Correctly identifying animals that are truly infected with a pathogen using ante-mortem tests is the cornerstone of any disease eradication programme. Failure to identify all infected animals will impede the progress towards controlling and eradicating disease and may also have unforeseen consequences when specific prevention measures are in place to avoid animal-to-animal transmission. In the case of bovine tuberculosis (bTB), the screening ante-mortem test, the Single Comparative Intradermal Tuberculin Test (SCITT), can exhibit moderate sensitivity which can result in a "hidden burden" of infection residing within the population. Using an animal-level dataset relating to the disclosure of infected cattle with *Mycobacterium bovis*, the causative agent of bTB within infected herds in Northern Ireland, we investigated what factors influenced the probability of an animal being a false-negative when truly infected (using post-mortem (PM) microbiological culture confirmation results to assess infection status).

We found that different risk factors affected the probability of a test-negative outcome on infected animals depending on the ante-mortem test or their combination (SICTT and/or interferon gamma (IFN-ɣ) testing). Using multivariable models, SCITT disclosure performance varied significantly by age, location (region), and production type. The IFN-ɣ tests were significantly affected by region or season, but these effects depended on the cut-off used during interpretation of the test which affected the tests characteristics. Parallel use of SCITT and IFN-ɣ tests resulted in the least number of false-negatives, and their disclosure was affected by season and age-class. Understanding the factors that lead to the non-disclosure of infected animals is essential to optimise large-scale bTB disease eradication programmes.

1. Introduction

Bovine tuberculosis (bTB) is a chronic zoonotic infectious disease of cattle caused by any mycobacterial species belonging to the *Mycobacterium tuberculosis* complex, this includes *Mycobacterium bovis* of which bovine animals are the main host (Allen et al., 2010; Anonymous, 2010; de la Rua-Domenech et al., 2006; EU-Commission, 2013). BTB is a serious animal health problem that can incur severe economic losses through international trading restrictions imposed by the many countries that are now officially free of bTB (DARD, 2014a; Good and Duignan, 2011; Zinsstag et al., 2006). In Northern Ireland (NI), as in other countries where bTB is still endemic, an approved but costly bTB eradication programme has to be maintained to ensure international trading can be retained in support of its meat and dairy industries. The early and accurate detection of infection plays a major role in the delivery of an effective control and eradication strategy (de la Rua-Domenech et al., 2006; Salman, 2004; Schiller et al., 2010). Thus, it is essential to have reliable ante-mortem diagnostic methods in place that can be employed during the screening phase of eradication programmes (Gormley et al., 2013; Salman, 2004; Schiller et al., 2010). At present, there are no viable alternative interventions available, such as cattle vaccination (OIE, 2015). Thus, the bTB control and eradication programmes are based on "test and cull schemes" alone.

In Europe, the ante-mortem screening tests for bTB are officially specified in the Directive EU/64/432 (as amended). The recognised

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screening test is either the single intradermal test (SIT) or the single comparative intradermal tuberculin test (SCITT), while the gamma-interferon test (IFN-ɣ) is accepted as an ancillary test (EEC, 1964). Both types of test are imperfect in terms of performance (de la Rua-Domenech et al., 2006) but it is well documented that they can complement each other, especially when used in parallel; SCITT is considered a good screening test with high specificity and moderate sensitivity, whereas the IFN-ɣ test has higher sensitivity but lower specificity (Alvarez et al., 2012a; Clegg et al., 2011; de la Rua-Domenech et al., 2006; Downs et al., 2011; Gormley et al., 2013). Consequently both tests might fail to identify a proportion of truly infected animals resulting in residual infection remaining in the herd (Brooks-Pollock et al., 2014; Conlan et al., 2012; Gallagher et al., 2013). This undesirable characteristic is of particular concern when testing in herds with a persistent bTB infection problem. Failure to detect truly bTB infected animals has profound implications for disease maintenance, as well as herd owner profitability and animal welfare. The risk factors influencing sub-optimal test sensitivity are not well characterised. A better knowledge of them could improve active surveillance and enhance the eradication process.

The cattle farming industry is an important contributor and valued sector of the economy of Northern Ireland (NI). There were approximately 23,000 registered cattle herds with a total of 1.6 million animals tested for bTB in 2013 (DARD, 2013). BTB is considered endemic in cattle in NI with an annual herd incidence of 6.4% and an associated economic cost of £26 million in 2013 (DARD, 2014a).

There is a statutory bTB eradication programme for NI, consisting of every herd undergoing SCITT at least once a year. Moreover, problematic bTB herds, or herds that recently have had a large outbreak, are given the opportunity to undergo SCITT and an IFN-ɣ testing in parallel to maximize the detection of potentially infected animals (DARD, 2014b). The veterinary officer (VO), on behalf of the Department of Agriculture and Rural Development (DARD), offers this possibility after careful epidemiological consideration in selected herds.

At present, the significance of potential risk factors to the outcome of ante-mortem screening tests has not been properly evaluated in cattle in NI. Thus, the aim of this paper was to determine possible risk factors for the probability of finding an animal positive for bTB at post-mortem (culture confirmation) for animals with a negative result to one or both ante-mortem tests combined. The ante-mortem tests included the SCITT, the IFN-ɣ test using a NI cut-off (IFN-ɣ NI), or IFN-ɣ with a commercially recommended cut-off (IFN-ɣ Commercial). Thus, five different ante-mortem test scenarios were explored within our study population in Northern Ireland from 2004 to 2010 (SCITT; IFN-ɣ NI; IFN-ɣ Commercial; parallel SCITT/IFN-ɣ NI; parallel SCITT/IFN-ɣ Commercial).

The main objective of the study was to determine the risks factors in each of five scenarios that would allow a better understanding of the failure of the ante-mortem tests to detect truly infected animals under field conditions.

2. Materials and methods

2.1. Ante-mortem testing

Throughout this study, SCITT was performed by DARD Veterinary Officers (VO), or Private Veterinary Practitioners (PVPs) on behalf of DARD, according to the specifications described in the Directive EC 64/432/EEC in the field. In essence, all SCITT tested animals with a bovine bias (PPD-B – PPD-A) of >4 mm were automatically declared as bTB reactors while a more severe interpretation could be applied within bTB breakdown herds where a bovine bias of >2 mm was used at the discretion of the VO to define bTB reactors.

The IFN-ɣ test was performed as described elsewhere (Welsh et al., 2008). Essentially, whole blood samples were collected immediately prior to the commencement of the SCITT and delivered to the laboratory within 8 h of collection. Blood cultures were stimulated using tuberculins (PPD-B and PPD-A) overnight at 37 °C. Supernatant fluids were harvested and clarified by centrifugation and then tested for the presence of bovine interferon gamma by ELISA using the Bovigam test kit (Thermo Fisher Scientific Company, USA). Two cut-off values were used to interpret the IFN-ɣ results – the Northern Ireland (NI) cut-off and the commercially recommended cut-off. The NI cut-off is more stringent interpretation using a lower optical density threshold (PPDB-PPDA OD = 0.05 if PPDB OD ≥ 0.1), increasing the sensitivity of the test, but reducing the specificity of the test in comparison to the commercial test cut-off (OD = 0.1).

2.2. Post-mortem testing

All animals sent to slaughter were inspected by Meat Hygiene Inspectors for the presence of macroscopic lesions consistent with bTB (visible lesions [VL]). Where VLs were found in animals sent for routine slaughter (LRSs; i.e. animals not removed under the TB programme), these were sent for bacteriological and/or histological examination confirmation. For SCITT reactor animals, up to five animals from each herd were sampled (selected lymph nodes) and bTB confirmation sought through bacteriological and/or histological examination. For IFN-ɣ reactors, all VL samples were subjected to bacteriological examination while up to three non-VL samples were sent for laboratory examination when no VL were observed. Mycobacterial culture was carried out as follows. Clinical samples recovered from animals at slaughter were examined for the presence of macroscopic lesions during laboratory processing. Tissue structure was disrupted using either ribolysation or grinding with sterile sand in a pestle and mortar. Prior to inoculation, clinical samples were decontaminated using 5% oxalic acid for a maximum of 30 min and washed twice with sterile PBS. Samples were then inoculated onto Lowenstein-Jensen and Stonebrink slopes as well as into MGIT culture vessels containing PANTA. All cultures were examined for mycobacterial growth and at 56 days post inoculation cultures were stained by Ziehl-Neelsen for the presence of acid fast bacteria. The isolation of *M. bovis* was confirmed by spoligotype as described elsewhere (Roring et al., 2000; Skuce et al., 2010).

2.3. Study population, case definition and epidemiological analysis

Only herds with chronic, recurrent or very large recent breakdowns ("problem" herds) were eligible for this study as part of the Northern Ireland IFN-ɣ scheme. Herd participation within the scheme, however, was voluntary (Lahuerta-Marin et al., 2015). Specifically, the herd selection criteria for participation in the IFN-ɣ scheme were based on the following DARD requirements: 1. Herds with five or more SCITT reactors identified at the previous test or 2. Herds with SCITT reactors identified on at least three separate occasions within the preceding two calendar years with bTB confirmed in the herd during the same period or 3. Current bTB breakdown herds having had confirmed lesions at routine slaughter (LRS) within 60 days preceding selection, following a SCITT herd test in the 90 days prior to selection. Logistical and financial constraints censored the inclusion of pedigree as herds as part of the IFN-ɣ scheme.

The outcome variable throughout was whether or not an ante-mortem test negative animal (or combination of tests), slaughtered within the first two months after testing, was found to have microbiological culture confirmation of *M. bovis*. Selected animals were ante-mortem test negative to single tests (e.g. SCITT negative or and IFN-ɣ NI negative) or a combination of parallel tests (e.g. parallel SCITT/IFN-ɣ NI negative). Laboratory confirmation was restricted to only animals that were either a SCITT reactor, animals that had a lesion found at routine slaughter (LRS) and NVLs reactors from risk herds included in the gamma-interferon testing scheme that did not have any SCITT reactors confirmed by culture or "in-contact" risk animals (contact with bTB reactors within the herd etc). We restricted our case definition to animals with an ante-mortem test within two-months of slaughter to reduce the