The investigation of a persistent outbreak of bovine tuberculosis using a novel enhanced cattle testing programme and evaluation of environmental contamination

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Introduction

An intensive dairy herd suffered a chronic endemic infection of bovine tuberculosis for more than five years. The whole herd, comprising around 1100 animals, were tested using the Single Comparative Intradermal Tuberculin Test (SCITT) every 60 days or so in accordance with the UK statutory testing and control programme. Between October 2012 and December 2017 there were 107 reactors disclosed by this routine testing, with none showing visible lesions at post mortem examination (Table 1).

A comprehensive disease investigation in 2015 suggested that the low prevalence infection within the herd was being maintained by undisclosed infected and infectious animals being retained, with the possible reintroduction of infection from environmental contamination of grazing pastures from wildlife.

An enhanced testing programme was introduced in 2015 using novel organism based tests Actiphage®, (Swift 2016) and rd4 qPCR (Taylor 2007) to detect infected cattle that were not being detected by the routine SCITT, and to determine the environmental risks by testing badger latrines around the farm for the presence of *Mycobacterium bovis* (King 2015). In addition, many of the cattle were tested for antibodies against *M.bovis* using the Idexx bTB Elisa test.

Once infected cattle and environmental risks were identified, a robust bTB management programme was introduced to manage the disease and enhance the statutory bTB controls.

Method

The herd comprise 350 high yielding dairy cows, continuously housed in five separate herds and milked through milking robots, with about 700 young stock and beef cattle. The average yield per cow per year is around 12,500 litres, and excellent management, husbandry and disease control ensures the health and welfare of these intensively managed cows.

Because of the movement restrictions, all offspring, including beef cross calves, were kept on the farm and reared to a point where they could be sold either to slaughter, or through specially licensed markets for cattle from TB infected farms. This restriction on trade was a major economic burden for the farm, preventing the potential trade of productive breeding and milking cows from this valuable herd.

The routine statutory SCITT testing programme detected several bTB reactors at each routine short interval test, conducted every 60 days or so, in accordance with the statutory testing programme for infected herds. The reactors were invariably found in the adult dairy

herd (most of which had never been outside in their entire lives) and none had visible lesions at post mortem inspection, and so were classified as “unconfirmed”.

At each test it was noticed that several animals gave detectable reactions to the bovine tuberculin but were not classified as reactors because of an accompanying reaction to the avian tuberculin, and so were retained in the herd under the UK interpretation guidelines. Such reactions were carefully recorded at each test, and the identity of the cattle was noted. It was then observed that many of these “high risk” cattle did not show similar reactions at subsequent tests: many of these bovine reactions were transient.

Any animal that has shown any form of bovine reaction over 2mm increase in skin thickness but was not a reactor under the standard interpretation rules was classified as a “high risk” animal and then submitted for enhanced testing using a bacteriophage based method combined with PCR (phagePCR, Actiphage®) on whole bovine blood samples, and rd4 qPCR on bovine blood and faeces samples in an attempt to investigate if any of these animals were actually infected with *Mycobacterium bovis* and the diagnosis was being desensitised by reactions to the avian tuberculin.

In addition, as part of the comprehensive disease investigation, a biosecurity and biocontainment risk assessment was conducted on the farm, similar to the risk assessments used for the prevention and control of Johnes Disease (using myhealthyherd.com) as part of the comprehensive disease management system that was to be introduced following the established principles of managing all four pillars that support the disease status of the farm (Orpin 2014, Sibley 2010).

As part of the biosecurity risk assessment, the potential risks of wildlife infection contributing to the endemic disease in the cattle was assessed and quantified using survey and latrine testing of badger faeces (King 2015). A complete survey of badgers around the farm showed a significant population (although there was no evidence of badgers entering the farm buildings or feed stores). 273 badger faecal samples were taken from 26 latrines in the locality of the farm and the buildings and tested using the RD4 qPCR method to detect the presence of *Mycobacterium bovis*.

Once biosecurity and biocontainment risks had been established and quantified, and the enhanced surveillance had identified high risk cows that were shedding infective organisms, a series of management and husbandry procedures were introduced to prevent new infections from the reservoir of infection in the cattle, wildlife and environment, while the endemically infected cattle were removed when economically convenient.

In order to mitigate the environmental risk produced from infected badgers shedding high levels of infection, a comprehensive badger vaccination programme was started in 2017 using BCG vaccine (Chambers 2011).

Results

The overall objective of the project was to investigate and understand the endemic disease within the herd and then attempt to remove TB infection from the herd and achieve Official TB Free status, allowing the farm to trade normally and realise the true value of the livestock. Restrictions were lifted in June 2018, having had two clear short interval skin tests, thus achieving this objective.

1) Biosecurity and biocontainment risks.

The initial risk assessment of biosecurity and biocontainment risks was carried out in conjunction with a routine Johnes Disease Risk Assessment, which is conducted annually as part of the herd Johnes Management Plan using the standard risk assessment tool in myhealthyherd.com.

The overall biosecurity of the herd was good, apart from risks of contact with stock of unknown disease status at a contract calf rearing unit where replacement breeding stock were sent for rearing as weaned calves and returned to the farm as down-calving heifers. The contract young-stock rearer occasionally introduced other cattle, which could have direct contact with the replacement heifers, and shared the same feeders and air space. This contravened the agreement, but had been overlooked on some occasions.

Other biosecurity risks were minimal: the herd was completed closed at the time (although later purchased cattle in accordance with a biosecurity purchase plan to minimise the risk of TB and other diseases) was geographically isolated and has no shared watercourses.

Biocontainment risks were high and significant, with close contact between animals in a totally housed environment. At the time of the start of the project, no dairy cattle (young-stock or adults) ever went outside. Only beef cattle were grazed. As part of Johnes control, known MAP infected cattle were segregated prior to calving, but all other cattle were calved in three deep litter calving boxes, and fed colostrum and fresh milk which may not have been derived from their own mothers nor been pasteurised. There were other minor biocontainment risks which were highlighted and management procedures introduced to tighten the controls of Johnes Disease.

Environmental risks from wildlife were completely unknown, and so an effort was made to quantify potential biosecurity risks from wildlife. A survey of badger activity was completed by Warwick University, and latrine testing arranged to ascertain the level of infection in the population of badgers around the farm.

Figure 1 shows the latrines that were tested: a total of 273 latrine samples were taken and tested for the presence of *M.bovis* using the RD4 qPCR. 31% of all the samples were positive for the presence of detectable *M.bovis*, and over 70% of the latrines had at least one positive sample. This indicated a significant risk of wildlife infection potentially acting as a reservoir of infection.

Wildlife cameras were stationed around the farm to ascertain if the badger population was entering the farm buildings or feed stores and over a 3 month period, no incursions were observed, although there was considerable activity in the woods, pastures and maze fields.

Resilience

The farm has robust disease management plans to predict and prevent infectious and metabolic diseases and avoid production stress. Nutrition is key to the resilience of the adult cows, with great attention and effort made to avoid early lactation weight loss, not least to prevent infertility, lameness and mastitis.

The herd is BVD vaccinated, and partially IBR vaccinated to minimise shedding of BHV1. Regular surveillance of young-stock and sentinels demonstrates the lack of any active disease.

A comprehensive and robust Johnes Management plan keeps the prevalence of Johnes below 2%, and there are mastitis control programmes and lameness management procedures to keep the bulk milk somatic cell count below 120,000, the herd mobility score below 10% score 2 (with a zero tolerance of score 3 cows) and the herd calving index below 380 days despite average yields being around 12,500 litres per cow per year. There are weekly herd health clinics to ensure the health and productivity of the herd is maintained, and the farmer has enthusiastically engaged in the philosophy of investing in health rather than paying for disease.

Enhanced TB surveillance

At the routine statutory short interval test of October 2015, 34 adult dairy cows (approximately 10% of the adult dairy cows tested) were observed to have a positive reaction to the bovine tuberculin (as defined by an increase in skin thickness of 2mm or more) but were not defined as reactors or inconclusive reactors in the standard interpretation of the SCITT. There was one reactor, which was culled and had no visible lesions at post mortem.

The 34 cows with reactions to bovine tuberculin were classified as High Risk TB Cows and selected for enhanced testing using the Actiphage® test for the selective detection of *M.bovis* in blood (Swift 2016). Of the 34 cows tested some 4 weeks after the SCITT, 30 were positive for Actiphage® (88%).

In the light of these results, a comprehensive enhanced testing programme was devised, not least to determine if the high risk cows that were Actiphage® positive were infectious, through shedding *M.bovis* in their saliva or faeces. The testing programme was formulated so that all high risk cows (those that had ever had a positive reaction to bovine tuberculin) that were retained in the herd would be tested after each short interval statutory SCITT for the presence of *M.bovis* using Actiphage®, and qPCR on faeces and saliva. These animals would also be tested for antibodies for *M.bovis* using the approved Idexx Elisa.

At the next short interval SCITT in January 2016, 37 animals were classified as High Risk and subjected to enhanced testing. This test disclosed 13 reactors. 24 high risk cows were positive to Actiphage® (65%). None had detectable *M.bovis* in either faeces or saliva as determined by the qPCR test, and only one was positive for Elisa. It was concluded that many animals were infected, but none were shedding.

By April 2016, the third enhanced test after the disclosure of just 2 reactors in the routine SI SCITT, 44 animals were now classified as High Risk, of which 14 (33%) were positive to Actiphage®, and 13 were positive to faecal qPCR, indicating shedding. Several cows that had previously tested positive using the Actiphage® test were now negative to that test, but were positive to the qPCR. None had detectable *M.bovis* in their saliva. 13 were positive to Elisa, and although 8 of these were not shedding at the time.

The enhanced testing programme continued in accordance with the system of identifying high risk cows from the repeated short interval SCITT, and once identified, the High Risk TB Cows were repeatedly tested with Actiphage® and faecal qPCR. As resources permitted, many were also tested with Idexx Elisa, but this was not completed for all cows on every occasion.

Due to issues of compliance with TB regulations and the controls on using unapproved tests, all enhanced testing ceased in July 2016. Testing was resumed in July 2017, under the

conditions and guidance of the “Exceptional private use of non-validated tests for Tb on cattle in England”, issued by APHA in 2017.

Overall, 192 cattle were identified as High Risk TB Cows during the control period from October 2015 to June 2018. Of these 161 were tested as least once with Actiphage® (the others were culled before Actiphage® testing was performed). Of these, 129 had at least one positive phage test (80% of all High Risk TB Cows have been positive to Actiphage®) suggesting that there is a hidden reservoir of infection in cows that are not classified as reactors under the standard interpretation of the SCITT.

156 High Risk TB Cows have had at least one faecal qPCR test, of which 33 have had a positive result. This suggests that 21% of the High Risk TB Cows are shedding enough organisms in their faeces to be detectable by the qPCR at the time of testing.

134 High TB Cows were tested for Idexx Elisa, of which 18 (13.5%) had at least one positive result. Of the 33 cattle that were shedding detectable *M.bovis* in their faeces, 8 had a positive Elisa test (24%) either previous to the PCR or at the same time. Conversely, of the 18 high risk cows had a positive Elisa,13 (72%) became faecal shedders as defined by having a positive faecal qPCR result at the same time as the positive elisa, or in subsequent tests. However, Elisa testing was not done on many of the samples submitted over the period of investigation, due to regulatory and resource constraints.

In the statutory short interval SCITT of January 2016, of the 934 animals tested, there were 11 official reactors under standard interpretation, 6 inconclusive reactors and a further 33 animals that had a detectable reaction to the bovine tuberculin but were not classified as reactors and hence classified as high risk cows. In the routine short interval test of May 2018, 1070 animals were tested: there were no reactors, no inconclusive and no animals with a detectable reaction to the bovine tuberculin. As this was the second clear short interval test, the herd was declared Officially TB Free in May 2018.

Wildlife surveillance and risks

A comprehensive survey of badger setts and latrines was conducted between January and April 2016, with latrine sampling in accordance with the methods described by King (King 2015). 273 faecal samples were collected from latrines around the farm, of which 31% were positive to qPCR, indicating the presence of *M. bovis*. 69% of the latrines had at least one positive sample, indicating a significant potential environmental risk to cattle with access to the latrines and the contaminated environment. Interestingly, the only grazing cattle that had access to the positive latrines prior to 2017 were beef cattle; no dairy cattle were ever grazed. There had never been any reactors in any beef cattle that had grazed these pastures (Figure 1).

Because of the potential risk from an environment contaminated with infectious badger faeces, a comprehensive badger vaccination programme was started in the autumn of 2017 using human BCG vaccine sourced from Canada and used under a Special Treatment Authority (STA) issued by the Veterinary Medicines Directorate and a trapping and vaccination licence issued by English Nature. It is hoped that this vaccination programme will continue for at least three years, and the success will be measured by monitoring latrine samples over that period to determine any fall in the environmental risk.

Risk management programme

Once it was realised that the most likely source of new infections was contaminated faeces, the risk management programme was focussed on preventing faecal contamination of environments in which susceptible cattle had contacts. Fortunately, this matched well with the risk management programme for the control of Johnes disease that was already being implemented on the farm. Enhancements to husbandry to reduce the risk of faecal transmission of *M.bovis* included:

- Pasteurisation of all colostrum
- Milk powder feeding for all calves
- Total separation of calves from cows at birth
- Creation and maintenance of rubber floored maternity pens which are cleansed and disinfected after each calving
- Introduction of automatic scraper systems to improve cow housing hygiene
- Care over potential faecal contamination of feeds
- Regular cleansing of all water troughs and raising adult cow troughs to prevent faecal contamination.
- Subsurface injection of the 7000 tonnes of slurry produced on the farm each year, or use only on arable land with immediate ploughing after spreading to avoid environmental contamination and potential contamination of water courses.
- Early culling of known high risk cows that are known to be infected and shedding.

Conclusions and discussion

The enhanced testing programme for bovine tuberculosis used unvalidated tests and was done in conjunction with the statutory testing programme. The use of unvalidated tests is unlawful in the UK, without the written permission of the Secretary of State.

The enhanced surveillance used in the herd demonstrated an undisclosed reservoir of infection in a herd that was repeatedly tested using the statutory SCITT programme. As can be expected with infection with a mycobacterium, the testing programme suggested that many cattle within this endemically infected herd were latently infected and would never succumb to disease. However, a significant proportion of these latently infected animals (as detected by the Actiphage® test) went on to shed significant numbers of organisms in their faeces (as detected by the qPCR test on faeces), and so became infectious and a significant risk to further new infections.

It is not known if these shedding cows produce infectious organisms continuously or intermittently, but, in any case, it can be assumed that the faeces produced from this group of animals would contain enough viable organisms to be of infection risk to susceptible animals, including cows in contact, and wildlife. The potential for environmental contamination from infected dung, slurry and farm yard manure is significant. Whilst farm yard manure can be composted to minimise the survival of the organism, slurry is spread

untreated and in a form that *M.bovis* can survive for several months, posing a risk to wildlife, grazing cattle and animals drinking from potentially contaminated watercourses.

The detection of infected and infectious cows within the herd requires repeated testing using a variety of tests: single samples at one time point are insensitive. Many of the cows that eventually shed organisms had commonly tested negative for both Actiphage® and Elisa tests in previous testing periods. A robust surveillance programme to detect infected and infectious animals and which can be used as part of an effective control programme will require repeated, serial testing to be reliable. To maximise both sensitivity and specificity of surveillance testing for pathogens such as the mycobacteria which have the ability to evade traditional immune based testing systems and can exist latently in the host, it may be necessary to use several tests and repeat regularly.

Testing is not enough to control and eradicate a disease: in this programme the test results have to be interpreted and used to manage the disease within the herd. The test results were used to identify infectious animals to reduce the risk of them infecting susceptible animals around them. Culling was minimised in order to make the control programme affordable and economically sustainable. It may be that the Idexx Elisa test (which is validated and approved for use in the EU) may be a reasonable predictor of shedding when used frequently on high risk cows, similar to the use of MAP antibody elisa to determine the risk of shedding of *Mycobacterium Avium Paratuberculosis* (MAP, causing Johnes Disease) in Johnes management programmes.

The wildlife risk was determined and quantified by measuring the environmental contamination that the wildlife were creating using faecal samples taken from latrines. Despite a significant environmental contamination being detected, the risk to the grazing cattle appeared to be minimal, as no SCITT reactors were discovered in the cattle that were exposed to the greatest environmental risk from infected badger latrines. That is not to say that the risk was not significant, and many of these cattle at risk may have been infected but would have been slaughtered at a young age (as beef cattle) before disease may have become established and the statutory testing programme detected them.

This clinical investigation demonstrated the challenges that face large herds with high risks of disease spread attempting to eradicate a disease that can exists in latent form and is difficult to detect using insensitive tests. The system is a potential method to control and eradicate disease in the chronic, persistently infected herds which may be acting as the true reservoir of infection in bTB endemically infected areas such as the South West. Interestingly, these herds are relatively small in number (although they tend to be large herds) with only 67 herds of a total of over 4700 cattle herds in Devon (<1.5%) being persistently infected in June 2018, defined as being under continuous restriction for more than 18 months (APHA personal communication 2018).

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Table 1

Reactors at each statutory Single Comparative Intradermal Tuberculin Test (SCITT) between October 2012 and May 2018

Date of Test	Number of reactors
10.12	9
01.13	6
04.13	3
06.13	1
09.13	2
11.13	1
02.14	5
09.14	25
12.14	9
02.15	4
05.15	1
07.15	10
10.15	1
01.16	11
02.16	2
04.16	2
09.16	1
11.16	3
02.17	0
05.17	3
08.17	6
10.17	2
01.18	0
05.18	0
Total since 10.12	107

Figure 1

Map of badger latrines and sampling points, with percentage positive samples at each latrine. With thanks to Sian Powell, University of Warwick.