

Project number: 6325

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Development of diagnostic and disease prevention strategies to decrease the level of *Mycobacterium avium*, subspecies *paratuberculosis* (Johne's Disease), in Irish dairy herds



Key external stakeholders:

Animal Health Ireland (AHI); Department of Agriculture, Food & the Marine (DAFM); The Irish Cattle Breeding Federation (ICBF); Dairy Farmers; Veterinarians; Dairy Co-operatives; Consumers

Practical implications for stakeholders:

This study has

- Highlighted sub-optimal management practices on Irish dairy farms with regard to *Mycobacterium avium* subsp. *Paratuberculosis* (MAP)
- Highlighted minimal economic impact on the majority of Irish dairy farms as a result of MAP infection
- Outlined the optimal time, with regard to annual bovine tuberculosis (bTB) testing, to test individual animals by ELISA for MAP using either blood or milk samples
- Outlined a longitudinal study of a MAP-infected herd to assist in designing a test and control protocol for MAP appropriate to Irish dairy herds
- Highlighted a significant increase in serum IFN- γ production post-TB which may indicate a beneficial effect of bTB testing in controlling MAP.

Main results:

- ELISA testing for MAP should not be conducted within 43 days post-TB testing, if using a milk sample and 71 days if using a blood sample.
- In contrast to many international studies, we were unable to identify any significant economic impact associated with MAP infection in a sub-set of Irish dairy herds
- Based on a five-year longitudinal study in a MAP infected herd, a strategy involving regular individual blood testing, faecal culture, and culling of persistently ELISA-positive individuals, control of MAP infection can be achieved
- The extensive testing procedures that exist in Ireland to achieve eradication of bTB may result in beneficial effects in the control of MAP

Opportunity / Benefit:

Offering farmers tools with which to gain increased efficiencies and a competitive advantage is of considerable value in increasing farm profitability and viability. Should these tools also improve the quality of the product being produced (e.g. MAP-free milk), the consumer also benefits. The results of this project will assist farmers and their service providers greatly in implementing best-practice with regard to future MAP prevention and control. The project also generated considerable expertise across a range of stakeholders and generated valuable on-farm data which has contributed to AHI's dissemination aims.

Collaborating Institutions:

Cork Institute of Technology (CIT)
University College Dublin (UCD)

Teagasc project team: Riona Sayers (PI);
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External collaborators: Jim O'Mahony (CIT);
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1. Project background:

Diseased animals perform sub-optimally and decrease on-farm efficiency and profitability through reduced feed efficiency, higher mortality, higher culling, reduced production, reduced product quality, higher labour and veterinary costs. A number of studies internationally (Raizman et al., 2006 and 2007; Kudahl et al., 2007) have quantified the biological and economic consequences of MAP infection. A MAP control programme is, therefore, critical to sustainable dairying. While a link to Crohn's Disease remains has been postulated (Chacon et al., 2004) but is not yet proven, the possibility that MAP may have zoonotic implications could also seriously impact the Irish dairy industry. In that regard, many EU and non-EU countries have established national and regional herd health strategies in order to deal with the impact of MAP. At the time of project set up, Ireland did not employ a coordinated approach to MAP control. With the establishment of Animal Health Ireland, however, a vehicle now exists through which national disease control programmes can be rolled out. However, in order to do so, a detailed investigation into the design of an appropriate Irish MAP strategy is required. For example, the Danish MAP control strategy relies on repeat ELISA testing of animals using milk samples. However, direct application of such a programme to Ireland may not be appropriate due to the level of testing for bovine tuberculosis that is carried out annually in Irish herds (TB test may have a negative impact on the specificity of MAP ELISAs). Generation of Irish data using a variety of MAP diagnostic techniques was therefore critical to the design of an Irish MAP programme.

Ireland must become a consumer responsive sector and FH2020 and FoodWise 2025 have highlighted the need for roadmaps to guide optimal production at all stages of the production process in order to not only increase efficiencies but also improve product quality. The primary producer must operate with, i) reduced costs, ii) higher productivity, and iii) produce higher value outputs. This proposal aimed to impact on primary producers in all three areas through development of an Irish-appropriate MAP control programme. It aimed to provide data to support strategies for improving on-farm MAP status, thereby increasing productivity and production of higher value outputs in terms of low-risk MAP milk.

In conclusion, accurate identification of preclinical MAP-infected individuals is of paramount importance in establishing herd MAP status. It is essential in allowing infected farms to move towards low-risk status through strategic management practices and culling.

2. Questions addressed by the project:

This project has contributed to sustainable dairying by providing data relating to MAP disease risk analyses at farm level allowing design of a comprehensive risk analysis protocol i.e. AHI's VRAMP. It has generated MAP within herd-prevalence, risk factor, and production data to allow informed decision making with regard to disease prioritization and control. It provides baseline data which can ultimately be used to improve the health profile of the Irish dairy herd and investigates a potential protective link between Ireland's extensive bTB testing regimen and the clinical manifestations of MAP infection.

3. The experimental studies:

- Study 1: The aim of study 1 was to document JD-related management practices employed on Irish dairy farms
- Study 2: The aim of study 2 was to examine the impact of the single intradermal cervical comparative test (SICCT) for bTB, on both serum and milk MAP ELISA tests, in a herd containing both seropositive and seronegative cows pre-SICCT. A secondary objective was to provide appropriate timing of JD ELISA tests in relation to the SICCT.
- Study 3: The aim of study 3 was to highlight the production losses associated with testing MAP ELISA positive in Irish dairy cows. Secondary objectives included investigation of risk factors associated with testing MAP ELISA positive. A survey of management practices on study farms was also conducted, with examination of associations between management practices and herd MAP status.
- Study 4: The aim of study 4 was to investigate if animals showing DTH reactions to PPD had an

associated increase in MAP ELISA response, thereby identifying potential cases of sub-clinical JD.

- Study 5: The aim of study 5 was to investigate the hypothesis that administration of purified protein derivative (PPD), as part of the bTB test, is associated with a systemic increase in IFN- γ production, which may potentially limit clinical progression of Johne's disease.

4. Main results:

- Management practices implemented by Irish dairy farmers pose a high risk of JD transmission. Approximately 97% of surveyed farmers used calving areas for more than one calving, 73.5% and 87.8% pooled colostrum and milk respectively, 33.7% never cleaned the CA between calving's, and 56.6% used the CA for isolating sick cows. We also highlighted that larger herds were more likely to engage in high risk practices for JD transmission and were also less likely to clean calving areas (OR 0.28) a high risk practice in the transmission of JD.
- MAP ELISA values remained significantly elevated until day 43 post-bTB testing in milk samples ($P=0.850$), and day 71 in serum samples ($P=0.602$) compared to pre-bTB test results. If the "new" positives detected post-bTB testing are deemed false positives due to generation of cross-reacting antibodies by administration of PPD, milk would appear a more suitable sample for JD ELISA testing within 2 months of SICCT. Sampling for JD utilizing milk ELISA should be avoided in the 43-day period following PPD administration, with serum ELISA sampling avoided for an additional 28 days.
- No statistically significant association was highlighted between testing positive on MAP ELISA and dairy cow production variables. Risk factors associated with testing MAP ELISA positive included herd size with larger herds being over twice more likely to test positive than smaller herds (OR 2.4 $P < 0.001$). In terms of breed investigations, Holstein-Friesian cows were less likely to test MAP ELISA positive than other dairy breeds.
- Generalised estimating equations were performed to identify associations between the delayed-type hypersensitivity (DTH) response post-bTB testing and MAP ELISA results. Significant associations were identified between DTH responses and MAP ELISA readings with animals yielding a DTH response at both avian and bovine PPD injection sites most likely to test ELISA positive in the post-PPD period. Further research is required to identify whether DTH skin thickness increases post-bTB testing and the associated increase in MAP ELISA response identifies animals previously exposed to MAP, or if results are due to serological cross reactivity.
- Interferon gamma (IFN- γ) is an inflammatory cytokine integral to both innate and adaptive immune responses. Detection of IFN- γ is used in the diagnosis of mycobacterial diseases such as bTB, MAP and human tuberculosis. We highlighted that serum IFN- γ production significantly increased post-bTB testing ($P < 0.001$) which may indicate a beneficial effect in controlling the clinical manifestations of MAP.

5. Opportunity/Benefit:

Primary producers must operate with, i) reduced costs, ii) higher productivity, and iii) produce higher value outputs. Generating scientific evidence to allow stakeholders in the Irish dairy industry to apply appropriate and effective disease control is essential. This project presents an innovative response to both consumer and dairy industry requirements with targeted and commercially-focused research. It will lead to improved on-farm skills but also significantly contribute to generating research expertise across veterinary, agriculture and food sectors. It will align strongly with Animal Health Ireland's mission statement of "creating value for livestock farmers through superior animal health" and will generate data of immeasurable value to supporting AHI's technical working groups.

6. Dissemination:

International conferences

- Kennedy, A., Da Silva, A., Byrne, N., Sayers, R., (2014). Influence of the SICCT on Johnes ELISA testing. Proceedings of the first DairyCare COST international conference, Copenhagen Denmark.
- Kennedy (2014). The single intradermal cervical comparative test and Johnes disease ELISA diagnostics. 4th International Conference on Vaccines & Vaccination, September 24-26, Valencia, Spain.

National Conference

- da Silva, A. Kennedy, N. Byrne and R. Sayers. (2014). Preliminary results examining the impact of the single intradermal comparative test for bovine tuberculosis on Johne's disease ELISA diagnostics. In: Agricultural Research Forum, Tullamore Court Hotel.

Open Days & seminars

Results presented at all Teagasc, Moorepark Open Days between 2013 and 2017 and frequently at AHI Johnes Technical Working Group meetings

Industry consultation days, farmer discussion groups

Presented and discussed at several industry meeting days and at numerous farmer discussion groups, farm walks and seminars.

Theses

This project generated a PhD thesis which included five experimental chapters.

Main publications:

- A.E. Kennedy, A.T. Da Silva, N. Byrne, R. Govender, J. MacSharry, J. O'Mahony, R.G. Sayers. (2014). The single intradermal cervical comparative test interferes with Johne's disease ELISA diagnostics. *Frontiers in Immunology* 5..
- E. Kennedy, N. Byrne, A. B. Garcia, J. O'Mahony, R. G. Sayers, (2016). Analysis of Johne's disease ELISA status and associated performance parameters in Irish dairy cows. *BMC Veterinary Research* 12, 43.
- Kennedy, N. Byrne, J. O'Mahony, R.G. Sayers. (2017). Investigations and implications of associations between mycobacterial purified protein derivative hypersensitivity and MAP-antibody ELISA in Irish dairy cows. *Research in Veterinary Science* 115, Pages 13–16.

Popular publications:

- Kennedy, R. Sayers, N. Byrne, J. O'Mahony. (2017) Special Feature: Walsh Fellowships seminar winners. Johne's disease and bovine tuberculosis testing *TRResearch* 12, 14.
- Kennedy, A., Sayers, R., Byrne, N., (2016). Control of Johne's disease in Ireland. *TRResearch* 11, 20.
- Kennedy, A., Sayers, R. Johnes disease in Irish dairy herds. *Irish Farmers Journal*, 2015.
- Kennedy, A., Sayers, R. A bug's tale: Johne's. *Today's Farm*, May-June 2017, 18.

7. Compiled by: Dr. Riona Sayers
