



**An Roinn Talmhaíochta,
Bia agus Mara**
Department of Agriculture,
Food and the Marine

Research Stimulus Fund

Final Report

*'Multidisciplinary programme to deliver improved diagnosis, on-farm strategies, and economic drivers for the control of Mycobacterium avium subsp. paratuberculosis on Irish farms.
ICONMAP: Improved Control of Mycobacterium avium subsp. Paratuberculosis'*

DAFM Project Reference No: 11/S/141

Start date: 01/11/2012

End Date: 31/01/2018

Principal Coordinator and Institution: Principal Investigator: Professor Stephen Gordon, UCD
Project Coordinator: Assoc. Professor Bryan Markey, UCD
Email: Stephen.Gordon@ucd.ie
Bryan.Markey@ucd.ie

Collaborating Research Institutions and Researchers:

University College Dublin (UCD): Stephen Gordon, Bryan Markey, Michael Doherty, Paul Whyte, Joseph Cassidy, Nola Leonard, John Browne, David MacHugh, Jarlath Nally
Teagasc: Laurence Shalloo, Aine Macken Walsh
Cork Institute of Technology (CIT): Jim O'Mahony, Aidan Coffey
Agri-Food and Biosciences Institute (AFBI): Lyanne McCallan, Jonathon Cunningham, Cathy Brooks
Queen's University Belfast (QUB): Irene Grant
Central Veterinary Research Laboratory (CVRL): Kevin Kenny, John Egan

Please place one "x" below in the appropriate area on the research continuum where you feel this project fits

Basic/Fundamental			→	Applied		→	Pre Commercial	
1	2	3	4	X	5	6	7	

Please specify priority area(s) of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report;

Priority Area (s)	Diagnostics; Sustainable Food Production and Processing
--------------------------	---

Key words: Cattle, Paratuberculosis, Diagnosis, Control

1. Rationale for Undertaking the Research

Johne's disease, aka paratuberculosis, is a serious disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). MAP infection presents in cattle as a spectrum from subclinical infection to chronic enteritis with persistent diarrhoea, weight loss, and ultimately death. Johne's disease (JD) has major consequences for productivity in the dairy and beef sectors. The prevalence of MAP has been increasing in Ireland over the last two decades and estimates of overall herd prevalence of MAP infection in Ireland are 21.4% (dairy 31.5% herd prevalence; beef 17.9%). However, the available diagnostics for MAP infection in cattle show sensitivities ranging from 10-30%, making it difficult to determine accurate prevalence data. In addition, diagnostic tools capable of detecting infection in animals less than 3 years of age are lacking. The project aligned with and provided an evidence base for Animal Health Ireland's (<http://www.animalhealthireland.ie/>) establishment of an industry-led national control programme for Johne's disease. It should be noted that Denmark and the Netherlands, two competitors in the dairy and infant milk formula markets, have established JD control programmes. The research carried out has addressed a range of knowledge gaps including the need to validate and optimize current MAP diagnostic tests for use in Ireland, to explore novel approaches to diagnosis, to develop a hazard analysis and critical control point (HACCP) handbook for control of Johne's disease, to define the cost effectiveness and cost dynamics of a national JD control programme, and to determine the sociological and economic drivers on Irish farms which influence the successful implementation of such a programme.

2. Research Approach

A multi-institutional, multi-disciplinary approach has been employed to deliver a wide range of research outputs and to capitalise on the synergies possible through the pooling of all-Ireland resources and expertise. Industry involvement in the form of Animal Health Ireland and Riverview Veterinary Group has ensured that the research is industry-relevant and appropriately balanced between innovative scientific inquiry and knowledge application. The project consisted of three interdependent work packages:

- (1) Evaluation and validation of the best diagnostic tests for MAP infection currently available;

Collection and testing of blood, milk and faecal samples collected from dairy cows in ten infected herds in the Republic and North of Ireland was performed. The tests used were direct PCR, serum ELISA, milk ELISA and liquid culture. In order to assess the risk of the environment as a source of MAP, 51 dust samples were collected from 5 herds with a history of JD. In line with the needs of the AHI control programme milestone 5.3 was modified to allow the comparison of 4 commercial serological assays for JD using a panel of sera from animals of known status. Genotyping of 126 MAP Irish isolates was carried out using mycobacterial interspersed repetitive unit and variable number tandem repeat (MIRU-VNTR). Novel MAP-specific monoclonal antibodies and phage display derived peptide binders were generated and evaluated for MAP capture by magnetic separation.

(2) Identification and validation of biomarker(s) capable of indicating the future shedding status of infected animals;

An experimental challenge of calves with an Irish isolate of MAP was carried out with serial sacrifice and collection of a wide range of samples over 33 months. Circulating miRNA was analysed in serum from a panel of known infected and control cattle using RNA-seq. Due to the high abundance of contaminating RNA, RNA-seq could not be used with faecal samples. Instead NanoString nCounter® hybridization-based assays were used to investigate differential miRNA expression between faecal samples from clinical JD cattle and uninfected control animals.

(3) Analysis of the factors (economical, logistical, sociological) which affect the successful implementation of a JD control programme.

A data set comprising 92,854 cows across 1,700 herds enrolled in the JD control programme during 2013 to 2015 was used to evaluate the effect of MAP ELISA status on test-day milk performance. In addition, a disease sub model was developed and integrated within the Moorepark Dairy Systems Model in order to quantify the economic effect of MAP infection. The development a generic HACCP handbook for JD management in dairy herds served as the template for the participatory learning in action (PLA) and co-design process involving farmers, practicing veterinarians, farm advisers and other actors.

3. Research Achievements/Results

The main results from the work package tasks were:

(1) Evaluation and validation of the best diagnostic tests for MAP infection currently available.

Analysis indicates that when direct PCR was used to test faecal samples, 46.2% of the samples were positive. In comparison, faecal culture recorded 12.3% of animals as infected. Detection of antibodies to MAP by ELISA classed 3.3% of these animals as positive. The finding of MAP DNA by direct PCR in dust samples collected from feeding bins, barns and milking parlours on infected farms is a concern. However, culture results did not indicate that viable MAP was present in the samples. Four INMV (INRA, Nouzilly, MIRU-VNTR) genotypes were observed among Irish MAP isolates. INMV 1 was found in 67 MAP isolates (53.2%) and INMV 2 in 57 isolates (45.2%). INMV 3 and INMV 116 noted only 1 isolate each (0.8%). Furthermore, the coexistence of two genotypes within a single herd was also observed in two counties. Novel peptide-mediated magnetic separation (PMS)-phage assay and PMS-culture methods were successfully optimised and validated for milk testing.

(2) Identification and validation of biomarker(s) capable of indicating the future shedding status of infected animals.

A comprehensive, serial biobank of samples from animals of known status animals with regard to MAP infection has been established and is available for future and on-going collaborative research projects. Significant miRNA expression changes in serum were not detected in response to MAP infection. However, by switching the

focus to faecal miRNA, nine miRNAs were identified that have potential to serve as biomarkers of progressive MAP infection

(3) Analysis of the factors (economical, logistical, sociological) which affect the successful implementation of a JD control programme.

Analysis of the large database revealed that cows testing ELISA positive, low-positive and suspect for MAP infection produced slightly less milk than cows testing negative for the disease. Risk assessments have been completed on Irish farms with a history of MAP infection. A hazard analysis and critical control points (HACCP)-based handbook specifically for use on infected dairy farms has been developed and is available in both print format and an interactive digital format (<https://www.teagasc.ie/media/website/about/farm-advisory/JohneDisease.pdf>).

4. Impact of the Research

This body of work indicates the limitations of serology in identifying MAP infected animals, recognizing that ELISA does not have high throughput and can function as a whole herd test. Direct PCR is a very promising assay for both classifying herd and individual animal status. One concern is that it will test positive when MAP DNA, above a threshold value, is present in faeces regardless of the viability of the bacterium e.g. from ingestion of dead MAP organisms. These findings are relevant to the herd classification methods, design of control programmes and the implementation of control programmes in herds which have been shown to be infected. In addition, the direct PCR test is now accredited and offered as a diagnostic test.

The significance of the genetic typing data generated is that it can be used to assess genomic diversity across the island in terms of heterogeneity of disease. As a result of this work we now have for the first time a comprehensive overview of the types of MAP strains which exist nationally.

Three promising alternative diagnostic tests for Johne's disease (peptide-mediated magnetic separation (PMS)-phage assay and PMS-culture methods for milk, and a novel qPCR method for faeces) were evaluated and found to have significant potential. It was concluded that the combined application of the PMS-phage assay and PMS-culture to detect viable MAP in bovine milk samples may represent an alternative diagnostic approach to faecal culture and serum-ELISA as part of a Johne's disease control programme. The qPCR method could be a more sensitive method of detecting MAP in faeces than the current faecal culture approach.

A comprehensive biobank of faecal, blood and tissue samples representing 33 months of MAP experimental challenge study has been established, whose status has been examined using the following tests: MAP faecal culture, serum ELISA, Bovigam® Interferon- γ Assay and post-mortem examination.

Faecal miRNAs hold promise as they may originate from local regions of the intestinal that are infected with MAP. Human orthologs of seven of the identified miRNAs are known to be expressed in intestinal tissue. Potentially, changes in ileal miRNA release into the lumen as a result of Johne's Disease pathology may account for the differential faecal miRNA expression that was demonstrated by this research.

The actions contained in the handbook to implement HACCP to manage JD in dairy herds were co-designed by farmers, practicing veterinarians, farm advisers and other actors. This ensures that the actions contained in the handbook may be realistically and practicably implemented on farms

The design of the national voluntary control programme for JD established by AHI requires to be underpinned by reliable data relevant to Irish farming in order to command the confidence of all the participants. This project has been responsive to requests from AHI and has provided significant, useful data as well as a practice-ready tool that is for direct use by end-users (i.e. farmers, practicing veterinarians, farm advisers) to promote and aid implementation of HACCP to prevent/control Johne's disease at farm level. Successful implementation of the AHI JD Control Programme will have significant impact on the dairy sector in terms of control of this disease, herd assurance and market access. Scientifically the project has been an outstanding success providing novel alternative diagnostic assays for JD, potential new biomarkers of JD pathology and a HACCP-based handbook in print and interactive digital formats for use by farmers and professionals such as vets and advisers working with farmers.

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

A comprehensive, serial biobank of samples from animals of known status animals with regard to MAP infection has been established and is available for future and on-going collaborative research projects. Serum samples from the animals will be used in a collaborative project with Aberystwyth University funded under the Knowledge Economy Skills Scholarship (KESS) programme, funded by the Welsh Government.

Enfer group are currently in close collaboration with CIT in an effort to develop the "whole cell" internal control model into a commercially viable novel diagnostic kit. Validation of this approach is currently ongoing using primers and probe combinations developed as part of the ICONMAP programme.

The outputs of this project have been instrumental in opening up new research areas for scientists on the island of Ireland. Most noteworthy is the DAFM funded NEXUSMAP programme (project reference 15/S/651) which is predominantly centred on next generation genomics and allied computational approaches. The IconMAP network of researchers has extended the number of participants involved

in MAP research nationally and has secured the expertise of many other internationally recognised MAP experts both as part of the scientific team and the scientific advisory group.

The research programme has fostered new transdisciplinary collaborations in Irish animal health research, which have been furthered in newly funded research projects: a Teagasc Walsh Fellowship on *biosecurity in the context of contract rearing*; DAFM RSF projects (*Surveillance, Welfare and Biosecurity of Farmed Animals SWAB*); and *Next generation approaches to improved diagnostics and molecular epidemiology for control of Mycobacterium avium subsp. Paratuberculosis*, aka NexusMAP); and a Horizon 2020 proposal that has been shortlisted to the second stage (*Rethinking of antimicrobial decision-systems in the management of animal production ROADMAP*).

- (ii) Outcomes where new products, technologies and processes were developed and/or adopted

The direct PCR test is now accredited and offered as a diagnostic test for MAP detection. De Kruijf et al. (2017) showed that the insertion sequence MAP04 is a sensitive and specific target for detection of MAP by quantitative PCR. O'Brien et al. (2018) demonstrated that the combination of two novel peptide-mediated magnetic separation (PMS)-based tests (PMS-phage assay and PMS-culture) gave the most complete picture regarding the presence of viable MAP in bovine milk samples. Farrell et al. (2015) identified a range of novel miRNA in bovine serum, illustrating the utility of small RNA sequencing approaches to explore the potential of miRNA as novel biomarkers for infectious diseases of cattle. Farrell et al. (2015) and Shaughnessy et al. (2015) confirmed the remarkable stability of bovine circulating miRNAs and opened the way for increased miRNA analyses from biobanked samples for a range of cattle diseases. A novel technology, NanoString nCounter® hybridization-based assay, was used to successfully investigate differential miRNA expression between faecal samples from clinical JD cattle and uninfected control animals. This technology has significant potential. McAloon et al. (2015) described the development of a HACCP-based approach to the control of MAP in infected Irish dairy herds. Critical control points relating to the management of the calving area, calving, new-born calf management and colostrum management were identified and novel monitoring systems developed, along with targets and corrective actions. This novel approach is now available to farmers and veterinary practitioners for use in high prevalence herds, or farms where more robust monitoring of key control points would be beneficial.

- (iii) Outcomes with economic potential

The review by Garcia and Shalloo (2015) and the meta-analysis by McAloon et al. (2016) highlighted the effects that MAP infection can cause including decreased milk yield, premature culling, replacement costs, reduced feed conversion

efficiency, fertility problems, reduced slaughter values and increased susceptibility to other disease. Such adverse effects on animal productivity are the key drivers in attempts to control MAP infection at the farm level. The analysis by Botaro et al. (2017) of the test-day records from a relevant proportion of Irish dairy herds (92,854 cows across 1,700 herds) indicated that cows with an antibody response to MAP produce less milk than their test-negative herd mates. McAloon et al. (2017) investigated the relative importance of risk factors for an increased probability of herd MAP infection. Their findings supported a number of key management interventions to reduce the prevalence of MAP in Irish dairy herds including avoiding the use of the calving pen to house sick or lame cows, reducing the length of time calves spend in the calving pen to less than 15 minutes and avoiding the use of pooled colostrum feeding.

(iv) Outcomes with national/ policy/social/environmental potential

Bayesian methodology was used by McAloon et al. (2016) to estimate the prevalence of paratuberculosis in Irish dairy herds. The median posterior estimate for herd level true prevalence among dairy herds was 0.28. This is higher than previous Irish estimates but lower than estimates from other major dairy producing countries. There is clearly still an opportunity for a national JD control programme to be established to prevent further increases in the prevalence of paratuberculosis, to avoid the high prevalence rates that are the norm in the dairy herd of many of our competitors.

The publication by McAloon et al. (2017) clearly demonstrated the value of using qualitative research methods to explore the values and knowledge of Irish cattle farmers relative to the control of JD at farm level. The analysis revealed that cultural and social capital informed farmers' decisions on whether to introduce control and preventive measures. Furthermore the findings highlighted important barriers and aids to prevention and control in both MAP infected and uninfected herds. This work has significant implications for the communication, implementation and adoption of the Animal Health Ireland national control programme for Johne's disease.

One significant and tangible outcome arising from the use of participatory action research has been the production of a HACCP-based handbook specifically for use on infected dairy farms. This handbook is available in both print and an interactive digital format (<https://www.teagasc.ie/media/website/about/farm-advisory/JohnesDisease.pdf>). It is disseminated through the Farm Advisory section of the Teagasc website under Advisory Methodologies. The co-design approach used has ensured that the actions contained in the handbook are both realistic and practical, greatly improving the likelihood of their implementation on farms. The success of this approach will lead to increased adoption of this methodology in future research programmes where alteration of farmer behaviour and implementation of control recommendations are key priorities.

4 (b) Summary of Research Outputs

- (i) Peer-reviewed publications, International Journal/Book chapters.
- McAloon C., Whyte P., More S.J., O'Grady L. and Doherty M.L. (2015). Development of a HACCP-based approach to control paratuberculosis in infected Irish dairy herds. *Preventive Veterinary Medicine*, 120:152-161.
- Garcia A.B. and Shalloo L. (2015). The economic impact and control of paratuberculosis in cattle. *Journal of Dairy Science*, 98:5019-5039.
- Farrell D., Shaughnessy R.G., Britton L., MacHugh D.E., Markey B. and Gordon S.V. (2015). The identification of circulating miRNA in bovine serum and their potential as novel biomarkers of early *Mycobacterium avium* subsp. *paratuberculosis* infection. *PLOS One*, 10:e0134310.
- Shaughnessy R., Farrell D., Riepema K., Bakker D. and Gordon S.V. (2015). Analysis of biobanked serum from a *Mycobacterium avium* subsp. *paratuberculosis* bovine infection model confirms the remarkable stability of circulating miRNA profiles and defines a bovine serum miRNA repertoire. *PLOS One*, 10:e0145089.
- McAloon C., Whyte P., More S.J., Green M.J., O'Grady L., Garcia A.B. and Doherty M.L. (2016). The effect of paratuberculosis on milk yield - a systematic review and meta analysis. *Journal of Dairy Science*, 99:1449-1460.
- McAloon, C.G., Whyte, P., O'Grady, L., Lorenz, I., Green, M., Hogan, I., Johnson, A., Doherty, M.L. (2016). Relationship between selected perinatal paratuberculosis management interventions and passive transfer of immunity in dairy calves. *Veterinary Record*, 179:47.
- McAloon, C. G., Doherty, M. L., Donlon, J., Lorenz, I., Meade, J., O'Grady, L., & Whyte, P. (2016). Microbiological contamination of colostrum on Irish dairy farms. *Veterinary Record*, 178:474.
- McAloon, C.G., Doherty, M.L., Whyte, P., O'Grady, L., More, S.J., Messam, L.L.M., Good, M., Mullowney, P., Strain, S. and Green, M.J. (2016). Bayesian estimation of prevalence of paratuberculosis in dairy herds enrolled in a voluntary Johne's Disease Control Programme in Ireland. *Preventive Veterinary Medicine*, 128:95-100.
- Britton L., Cassidy J., O'Donovan J., Gordon S. and Markey B. (2016). Potential application of emerging diagnostic techniques to the diagnosis of Johne's disease (paratuberculosis). *Veterinary Journal*, 209: 32-39.
- O'Brien L., Stewart L.D., Strain S. and Grant I.R. (2016). Novel monoclonal antibody and peptide binders for *Mycobacterium avium* subsp. *paratuberculosis* and their application for magnetic separation. *PLOS One*, 11:e0147870.

de Kruijf, M, Coffey, A, O'Mahony, J. (2017). The investigation of the truncated mbtA gene within the mycobactin cluster of *Mycobacterium avium* subspecies *paratuberculosis* as a novel diagnostic marker for real-time PCR. *Journal of Microbiological Methods*, 136:40-48.

de Kruijf, M, Govender, R, Yearsley, D, Coffey, A, O'Mahony, J. (2017). A comparative study evaluating the efficacy of IS_MAP04 with IS900 and IS_MAP02 as a new diagnostic target for the detection of *Mycobacterium avium* subspecies *paratuberculosis* from bovine faeces. *Veterinary Microbiology*, 204:104-109.

McAloon, C.G., Doherty, M.L., Whyte, P., More, S.J., O'Grady, L., Citer, L., and Green, M.J. (2016). Relative importance of herd-level risk factors for probability of infection with paratuberculosis in Irish dairy herds. *Journal of Dairy Science*, 100:9245-9257.

McAloon C.G., Walsh, A.M.W., Moran, L., Whyte, P., Doherty, M.L. (2017). Johne's disease in the eyes of Irish cattle farmers - A qualitative narrative approach to understanding implications for disease management. *Preventive Veterinary Medicine*, 141:7-13.

Botaro B., Ruelle E., More S., Strain S., Graham D., O'Flaherty J. and Shalloo L. (2017). Associations between paratuberculosis ELISA results and test-day records of cows enrolled in the Irish Johne's Disease Control Program. *Journal of Dairy Science*, 100:7468-7477.

De Kruijf, M., Lesniak O., Yearsley D., Ramovic E., Coffey A. and O'Mahony A. (2017). Low genetic diversity of bovine *Mycobacterium avium* subspecies *paratuberculosis* isoaltes detected by MIRU-VNTR genotyping. *Veterinary Microbiology* 203: 280-285.

O'Brien, L.M., McAloon, C.G., Stewart, L.D., Strain, S.A.J., Grant, I.R. (2018). Diagnostic potential of the peptide mediated magnetic separation (PMS)-phage assay and PMS-culture to detect *Mycobacterium avium* subsp. *paratuberculosis* in bovine milk samples. *Transboundary and Emerging Diseases*, 65:719-726.

- (ii) Popular non-scientific publications and abstracts including those presented at conferences

Macken-Walsh A., Moran L., McAloon C., Doherty M. and Whyte P. Applying social science to the development of HACCP-based tools for the control of paratuberculosis. 12th International Colloquium on Paratuberculosis, Parma, Italy, June 2014.

Britton L., Brady C., Cassidy J., O'Donovan J., Leonard F., Gormley E., Shaughnessy R., Gordon S.V. and Markey B. Long term experimental infection of cattle with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) : preliminary findings. 48th Annual Scientific Meeting of Association of Veterinary Teachers and Research Workers, Backweston, DFAM, October 2014.

Macken-Walsh, A., Moran, L., McAloon, C., Doherty, M., Whyte, P. Applying Social Science to the Development of HACCP-based tools for the Control of Paratuberculosis. Sociological Association of Ireland (SAI) Conference, October , 2014.

O'Brien L., Strain S. and Grant I. Generation of novel peptide binders against *Mycobacterium avium* subsp. *paratuberculosis* by phage display biopanning. 12th International Colloquium on Paratuberculosis, Parma, Italy, June 2014.

Ramovic E. A comparison of laboratory tests for the diagnosis of bovine paratuberculosis. Teagasc Agricultural Forum, Tullamore, March 2015.

Shaughnessy R., Farrell D., Riepema K., Britton L., MacHugh D., Markey B., Bakker D. and Gordon S. Characterisation of remarkably stable circulating miRNA in bovine serum and their potential as novel biomarkers of early *Mycobacterium avium* subsp. *paratuberculosis* infection. 49th Annual Scientific Meeting of Association of Veterinary Teachers and Research Workers, Grange, Teagasc, October 2015.

Britton L., Brady C., Cassidy J., O'Donovan J., Leonard F., Gormley E., Shaughnessy R., Gordon S.V. and Markey B. Long term experimental infection of cattle with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) : the first 20 months. 49th Annual Scientific Meeting of Association of Veterinary Teachers and Research Workers, Grange, Teagasc, October 2015.

Gordon S.V. and Markey B.K. At the hub of veterinary research: the ICONMAP Programme. Veterinary Ireland Journal, June, 2015.

McAloon, C.G., Whyte, P. and Doherty, M.L. Application of HACCP principles in dairy herd health and production management. Veterinary Ireland Journal, September, 2015.

Britton L. IconMAP project: infection model. MycobactDiagnosis ERA-NET Meeting, Dublin, June 2015.

Britton L., Brady C., Cassidy J., O'Donovan J., Leonard F., Gormley E., Shaughnessy R., Gordon S.V. and Markey B. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) bovine experimental infection model: disease progression over twenty-four months. Sixth Annual Dublin Academy of Pathogenomics and Infection Biology (DAPI) Symposium. Trinity College Dublin, 15th January 2016.

Grant I. and O'Brien L. Diagnostic potential of a peptide-mediated magnetic separation (PMS) - phage assay applied to milk relative to faecal- and blood-based tests. 13th International Colloquium on Paratuberculosis. Nantes, France. 20-24th June, 2016.

Shaughnessy R., Farrell D., Ripema K., Britton L., MacHugh D.E., Markey B., Bakker D. and Gordon S.V. Circulating miRNA in bovine serum and their potential use as novel bio

markers of early *Mycobacterium avium* subsp. *paratuberculosis* infection. 13th International Colloquium on Paratuberculosis. Nantes, France. 20-24th June, 2016.

Britton L., Brady C., Cassidy J., O'Donovan J., Leonard F., Gormley E., Shaughnessy R., Gordon S.V. and Markey B. Experimental infection of cattle with *Mycobacterium avium* subsp. *paratuberculosis*: results from the first two years. 13th International Colloquium on Paratuberculosis. Nantes, France. 20-24th June, 2016.

de Kruijf M., Lesniak O., Yearsley D., Ramovic E., Coffey A. and O'Mahony J. MIRU-VNTR molecular genotyping of *Mycobacterium avium* subspecies *paratuberculosis* isolates from bovine herds across the Republic of Ireland. 13th International Colloquium on Paratuberculosis. Nantes, France. 20-24th June, 2016.

Britton L., McAloon C., Kennedy A., Sayers R., Cassidy J., O'Donovan J., Leonard F., Gordon S.V. and Markey B. The challenge of confirming Johne's disease: an evaluation of 14 cattle with suspected *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection based on herd status and/or suggestive clinical signs. 13th International Colloquium on Paratuberculosis. Nantes, France. 20-24th June, 2016.

Kennedy A.E., O'Mahony J., O'Donovan J., Markey B., Britton L., Kenny K., Byrne N. and Sayers R. Control of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in an Irish context. 29th World Buiatrics Congress, Dublin. 3rd - 8th July, 2016.

Britton L., Brady C., Cassidy J., O'Donovan J., Leonard F., Gormley E., Shaughnessy R., Gordon S.V. and Markey B. Long term experimental infection of cattle with *Mycobacterium avium* subsp. *paratuberculosis* (MAP): the first two years. 29th World Buiatrics Congress, Dublin. 3rd - 8th July, 2016.

Shaughnessy R., Farrell D., Ripema K., Britton L., MacHugh D.E., Markey B., Bakker D. and Gordon S.V. Circulating miRNA in bovine serum and their potential use as novel biomarkers of early *Mycobacterium avium* subsp. *paratuberculosis* infection. 29th World Buiatrics Congress, Dublin. 3rd - 8th July, 2016.

de Kruijf M., Coffey A. and O'Mahony J. The use of *Mycobacterium smegmatis* mc2 155 as a DNA extraction and RT-PCR internal control for the detection of *Mycobacterium avium* subspecies *paratuberculosis* from a bovine faecal environment. 29th World Buiatrics Congress, Dublin. 3rd - 8th July, 2016.

Macken-Walsh A., McAloon C., Moran L., Byrne A., Doherty M. and Whyte P. Applying sociology to the control and prevention of paratuberculosis. 29th World Buiatrics Congress, Dublin. 3rd - 8th July, 2016.

McAloon C., Doherty M., Whyte P., O'Grady L., Messam L., More S., Good M., Mullaney P., Strain S. and Green M. Bayesian estimation of prevalence of paratuberculosis in dairy

herds enrolled in a voluntary Johne's disease control programme in Ireland. 29th World Buiatrics Congress, Dublin. 3rd - 8th July, 2016.

Hyland S., Nally J., Meade K. and Gordon S. Proteomic analysis of *Mycobacterium avium* subspecies *paratuberculosis* in vitro and the identification of seroreactive antigens. 29th World Buiatrics Congress, Dublin. 3rd - 8th July, 2016.

de Kruijf M., Coffey A. and O'Mahony J. Use of *Mycobacterium smegmatis* as a DNA extraction and RT-PCR internal control for the detection of *Mycobacterium avium* subspecies *paratuberculosis* from bovine faeces. 44th Annual Food Research Conference, Teagasc, Moorepark, 2016.

Britton L., McAloon C., Kennedy A., Sayers R., Cassidy J., O'Donovan J., Leonard F., Gordon S.V. and Markey B. The challenge of confirming Johne's disease: an evaluation of 14 cattle with suspected *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection based on herd status and/or suggestive clinical signs. Association of Veterinary Teachers and Research Workers, Annual Scientific Meeting, University College Dublin. 7th October, 2016.

Markey B. The IconMAP Project 2012-2017. The Paratuberculosis Newsletter, March 2017.

(iii) National Report
N/A

(iv) Workshops/seminars at which results were presented
Markey B. Improved Control of *Mycobacterium avium* subsp. *paratuberculosis* (ICONMAP). Report to Johne's Disease Technical Working Group of Animal Health Ireland, February, 2013.

Markey B. Improved Control of *Mycobacterium avium* subsp. *paratuberculosis* (ICONMAP): Update. Report to Johne's Disease Technical Working Group of Animal Health Ireland, October, 2015.

Macken Walsh A., McAloon C. and Moran L. Bridging the gap between science and practice: a focus on animal health. Animal Health Ireland Technical Working Groups meeting, November 2015.

(v) Intellectual Property applications/licences/patents
N/A

(vi) Other
N/A

5. Scientists trained by Project

Total Number of PhD theses: 5

Lorna O'Brien, Queen's University Belfast, January 2016. Novel detection methods for *Mycobacterium avium* subsp. *paratuberculosis*: development, optimisation and field validation.

Conor McAloon, University College Dublin, January 2017. Epidemiology and control of paratuberculosis in Irish cattle.

Louise Britton, University College Dublin, May 2016. The immunopathology of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection in cattle as a basis for novel diagnostics.

Marcel de Kruijf, Cork Institute of Technology, July, 2017. The development of new molecular tools to detect *Mycobacterium avium* subsp. *paratuberculosis* isolated in the Republic of Ireland.

Elvira Ramovic, University College Dublin, anticipated submission date - May 2019. Optimising John's disease control with particular focus on diagnostic tests and sampling strategies for the detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infected animals and herds.

Total Number of Masters theses: 1

Stephen Hyland, University College Dublin, September 2016. Host and pathogen proteomics for improved diagnosis of Johne's disease in Irish cattle.

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
University College Dublin	9	4.04
Teagasc	2	1.13
Cork Institute of Technology	2	0.56
AgriFood and Biosciences Institute	3	0.58
Queen's University Belfast	1	0.41
Total	17	6.72

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers	5	8.48
PhD students	4	14.25
Masters students	1	0.3
Temporary researchers	3	4.17
Other		
Total	13	27.2

8. Involvement in Agri Food Graduate Development Programme

N/A

9. Project Expenditure

Total expenditure of the project: €1,399,151.49

Total Award by DAFM: €1,496,738.90

Other sources of funding including benefit in kind and/or cash contribution(specify): None

Breakdown of Total Expenditure

Category	UCD	Teagasc	CIT	AFBI	QUB	Total
Contract staff						
Temporary staff	105,852					105,852
Post doctorates	183,742	197,952				381,694
Post graduates	164,670		65,626		66,299	296,625
Consumables	145,341		33,587	49,723	30,537	259,188
Travel and subsistence	32,647	6,315	2,287	296	1,411	46,433
Sub total	632,282	204,267	101,450	50,019	98,247	1,089,792
Durable equipment	678					678
Other	7,136					7,136
Overheads	189,685	51,936	30,450		29,474	301,545
Total	829,781	259,681	131,950	50,019	127,721	1,399,151

10. Leveraging

Four post graduate students have been funded by Teagasc through the Walsh Fellowship Scheme were associated with and benefitted from this project. Each of these students was awarded €88K (i.e. 4 x €88K = €240K).

Funding of £5,000 was awarded to Dr Irene Grant, QUB in February 2015 as a result of an internal Dragons' Den competition to permit early stage development of a lateral flow immunochromatographic assay for detection of MAP in faeces incorporating antibody or peptide binders generated during the IconMAP project. The development work is a collaboration between Dr Grant and Abingdon Health Limited, York.

Dr Irene Grant has secured InvestNI funding (£ 175,000) via the AgriFood Quest Competence Centre at Queen's University Belfast for a two year project to develop the PMS-phage assay into a more streamlined, phage-based one-day test for viable MAP in milk. The project began on 1 May 2018 and is being carried out in collaboration with three industry partners - AgriSearch NI, Biorex Food Diagnostics and Dale Farm.

Several of the participants of IconMAP, led by Prof. Stephen Gordon, have successfully applied and received funding of €1,161,864 from DAFM for the NEXUSMAP programme (project reference 15/S/651) which started in June 2017 and carries forward work from the IconMAP programme.

Dr Irene Grant and Dr Conor McAloon in collaboration with Dr Rebecca Smith, University of Illinois, and Prof. Yrgo Grohn, Cornell University, USA, have a funding application submitted to US-Ireland R&D Partnership Call in Agriculture for a follow-on project entitled: TRIPARTITE; Decision-making for paratuberculosis control in dairy herds (Acronym DecisionMAP). A decision on this application is expected in early 2019.

11. Future Strategies

The IconMAP programme has provided significant resources, research data and collaborative links that will be built on and expanded upon in the NEXUSMAP programme (project reference 15/S/651). This research project started in June 2017 and is being funded by DAFM. It will be centred around next generation sequencing, bioinformatics and computational approaches to MAP strains and sample biobanks, allied with disease modelling and interrogation of national datasets, to provide an early warning surveillance system as well as invaluable epidemiological information on disease transmission dynamics between and within Irish farms. In addition, the application of these technologies to defined clinical sample collections will provide insights into the role of the microbiota and their potential as biomarkers as infection progresses from subclinical to faecal shedding to clinical disease. A multiplex panel of novel mRNA and miRNA biomarkers for improved diagnosis and monitoring of disease progression will be identified. Finally, computational approaches and experimental validation to identify new MAP antigens will be used to feed into vaccine and diagnostic test development.

Funding has also been secured by Dr Irene Grant to refine and bring closer to market a number of novel diagnostic assays for MAP, utilising the peptide-mediated magnetic separation assays that she has successfully developed.