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

Research Stimulus Fund

Final Report

'Predisposing factors for disease and immunocompetence in artificially-reared dairy and suckled beef calves from birth to weaning (€asyCalf)'

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Principal Coordinator and Institution: Dr. Bernadette Earley, Teagasc, AGRIC, Grange, Co. Meath

Email: Bernadette.earley@teagasc.ie

Collaborating Research Institutions and Researchers:

University College Dublin — Dr. Ingrid Lorenz

AFBI, Hillsborough, N. Ireland — Dr. Stephen Morrison, Dr. Michael McMenamamy, Dr. Catherine Duffy, Dr. Louise Cosby.

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)	Connected Health & Independent Living (Animal Health and Welfare)

Key words: Calf immunity; Disease; Antibiotic usage; Blood transcriptomics;

1. Rationale for Undertaking the Research

Calves for beef production in Ireland come from two sources, the suckler cow herd and the dairy cow herd, which comprise ca. 1.1 million cows each. In Ireland 5.783%, equivalent to ca. 133,684 calves, excluding stillborns, die in their first year of life (AIM, DAFM 2018). This mortality is a colossal cost and opportunity lost to the Irish beef industry and national economy. While information is available on mortality and the prevalence and control of statutory infectious diseases of cattle, this is not the case with non-regulatory endemic infectious diseases. Prevalence and incidence of infectious disease or morbidity in artificially-reared dairy and suckled beef calves is unknown and the associated cost unquantified. Likewise, environmental conditions and management practices associated with calf health status are unidentified for Irish herds. Health and performance of calves depends on minimising animals' exposure to disease and maximising their defence against disease. In this respect, information on the passive immune status of calves from modern genotypes within dairy and beef herds is urgently required. Calf health was prioritised as one of the most important animal health issues facing the Irish livestock industry in a recent expert policy Delphi study, and farm opinion survey, conducted on behalf of Animal Health Ireland (AHI). Abolition of milk quotas in 2015 resulted in a significant increase in the national dairy cow herd and consequently, an increase in dairy calf rearing was inevitable.

This project addressed research theme A1.2.1 focusing on artificially-reared dairy and suckled beef calves. The theme called for i) "*a better understanding of predisposing factors for disease*", and in this project proposal, this was addressed at an applied level through a large-scale observational farm survey and followed by more detailed, farm case studies and ii) some understanding of "*immune system function*", which was addressed via experiments designed to examine passive transfer of immunity, molecular predictors of immune function in colostrum, and development of immunocompetence. The theme also called for development of iii) "*objective measures of welfare*", which was an outcome from the farm survey, case studies and experimental data analysis. Under Food Harvest 2020, an ambitious development target was set for the Irish beef Industry, to increase its output value by 20% by the end of the decade. Devising targeted strategies and critical control points that minimise calf morbidity and death will be central to increasing farm output and achieving Food Harvest 2020 goals, while simultaneously reducing production costs, augmenting farm profitability and competitiveness and, enhancing the international image of Irish beef.

2. Research Approach

This project proposal was comprised of **7 inter-related Tasks.** The context, objectives, description, including a breakdown of experimental units and methods, responsibilities, milestones and deliverables are outlined for each Task. The **first 6 Tasks** each **investigated specific research objectives** and benefited from the complementary expertise of principal researchers that were integral to the entire project. The project pooled research resources available at Teagasc, UCD, and AFBI to enhance disease control programmes and reduce the incidence of infectious disease in artificially-reared dairy and beef suckled calves, and identify objective measures of welfare status. This project did build upon the successes of existing collaborations between Teagasc, AFBI and UCD. The participants of this project incorporated all required complementary skills, experience and infrastructure (state-of-the-art equipment, animals, animal facilities, veterinary skills base, laboratories, genomic, transcriptomic platforms, statistical support, and outreach). The multifaceted approaches employed in this project proposal spanned a variety of disciplines and were aimed at elucidating new layers of novel information about predisposing factors for infectious diseases, indicators of welfare status and development of immunocompetence in artificially reared dairy and suckled beef calves in a comprehensive manner.

The specific tasks and objectives of €asyCalf were:

Task 1

Large scale observational study of health management practices and environmental conditions on-farm and, passive immune status and prevalence of morbidity and mortality in artificially reared dairy and suckled beef calves from birth to weaning.

Objective(s):

i. Develop a questionnaire to assess on-farm environmental conditions and health management practices for artificially-reared dairy and suckled beef calves from birth to weaning (subtask 1.1).
ii. Characterise environmental conditions and health management practices on Irish dairy and beef farms and, the prevalence of morbidity and mortality for artificially-reared dairy and suckled beef calves from birth to weaning (subtask 1.2).
iii. Determine the passive immunity in artificially-reared dairy and suckled beef calves and if there is a particular concentration of maternally derived IgG below which calves will have a higher odds of contracting disease during the period from birth to weaning (subtask 1.2).

Research methodologies

Data were obtained from two studies: 1) a longitudinal study on herd-level factors associated with the health and survival of calves on Irish farms (hereafter referred to as the herd-level study) and 2), a longitudinal study on individual calf-level risk factors for morbidity in spring-born calves (hereafter referred to as the calf-level study. The herd-level study was conducted between July 1, 2014 – December 31, 2015 and the calf-level study was conducted between January 1 – December 31, 2016.

Farmer recruitment and participation

Recruitment of farmers for the herd-level study occurred throughout spring 2014. Farmers volunteered to participate after learning about the study while attending a national knowledge transfer (KT) event or they were contacted directly by their Teagasc

KT advisors. At the end of the recruitment efforts, 230 suckler beef and 103 dairy farmers expressed interest in participating in the herd-level study. Interested suckler beef farmers were stratified by location and a random number sequence was used to select 150 farms, proportional to the provincial distribution of Irish suckler beef herds (DAFM, 2013). All dairy farmers that expressed interest were selected to participate. Sample size calculations were completed for all studies; final sample size was determined based on logistical and financial resources.

A total of 9 suckler beef and 8 dairy farms from the herd-level study were selected to participate in the calf-level study. These farms were selected based on the following criteria: 1) herd had a spring calving pattern, 2) farmers had demonstrated their willingness and ability to maintain accurate project records during the herd-level study, and 3) some calf morbidity or mortality had been reported between 2012 and 2015. Sample size calculations were completed for the calf-level study in Stata ® 13.0 (StataCorp, College Station, Texas, USA) using preliminary data from the herd-level study to determine expected differences in morbidity. A final sample size of 450 suckler beef calves was determined, based on the following assumptions: occurrence of disease was expected to be 3-times greater among suckler beef calves with vs. those without the risk factor (54 vs. 18%), power of 80%, confidence of 95%, average of 50 suckler beef calves per herd, and adjustments for within-herd clustering (intra-class correlation) and confounding of 0.1 and 15%, respectively. A final sample size of 880 dairy calves was determined, based on the following assumptions: occurrence of disease was expected to be 3-times greater among dairy calves with vs. those without the risk factor (51 vs. 17%), power of 80%, confidence of 95%, average of 100 dairy calves per herd, and adjustments for within-herd clustering (intra-class correlation) and confounding of 0.1 and 15%, respectively. Four surveys were prepared in consultation with DAFM and the calfCare Technical working group of AHI. All surveys were piloted and validated prior to their administration at farm level.

Task 2

Title: Risk factors for infectious (enteric and respiratory) disease in dairy and beef calves from birth to weaning and, influence of disease on calf performance.

Objective(s): The overall objective of this task is to determine the risk factors for calf disease, and the impact on animal performance from birth to weaning in dairy and beef herds.

The specific objectives are:

1. To identify risk factors for calf diseases.
2. To determine the impact of disease on calf performance.

Research methodologies

Farm visits were completed over two time periods: July 1, 2014 – June 30, 2015 for the herd-level study and January 1 to June 30, 2016 for the calf-level study. Autumn and spring calving herds enrolled in the herd-level study were visited once between July 1 to December 31, 2014 and January 1 to June 30, 2015, respectively. Split calving herds enrolled in the herd-level study were visited once between July 1 – December 31, 2014 and then a second visit was arranged between January 1 – June 30, 2015. Each farm visit for the herd-level study was scheduled to coincide with a time when calves would be available for blood sample collection.

Female and male calves between 1 and 21 days of age were eligible for blood sampling. A maximum of 12 calves were blood sampled at the farm visit. In the event that more than 12 calves within the sampling age range were available, the youngest calves over 24 hours of age were blood sampled. Herds enrolled in the calf-level study were visited every 2 weeks during the 2016 spring calving season. At each farm visit, any calf that was at least 24 hours old, and had been born since the previous visit, was blood sampled.

Blood samples were collected by jugular venipuncture into 8.5 ml vacutainers (BD Vacutainer Serum Separator Tube II Advance 367958 no anticoagulant, Unitech, Dublin, Ireland) using an 18-gauge needle. Samples were allowed to clot and stored at 4°C for 24 hours. Serum was harvested following centrifugation (1600 × g for 10 minutes at 4°C) and then frozen at -20°C.

Collection of health and growth data

Farmers enrolled in the herd-level and calf-level studies recorded birth, disease, health treatment, and death information on their calves using standardised recording sheets. Case definitions were provided to the farmers to assist with the classification of disease. Farmers were responsible for detecting, diagnosing, and administering treatment to any calf exhibiting clinical signs of disease, and encouraged to consult with their veterinarian when making health treatment decisions. The research team contacted the farmers every 2 to 4 weeks and reminded them to complete the project recording sheets. Nonetheless, despite this regular follow-up, health data were only available for calves on 84 suckler beef and 55 dairy farms from the herd-level study. All farmers for the calf-level study provided health data on their calves. Calves enrolled in the calf-level study were weighed twice using an electronic scale. The first body weight (BW1) was obtained at the first farm visit after birth. The second body weight (BW2) was obtained during a farm visit in autumn 2016. Animals were on pasture during this time. All BW2 measurements for suckler beef and dairy calves were collected before and after weaning, respectively.

<u>Task 3</u>

Title: HACCP-based health management blueprint

Objective:

Develop a HACCP-based health management blueprint for artificially-reared dairy and suckled beef calves from birth to weaning to prevent infectious diseases and welfare problems by monitoring and controlling the risk factors

Research methodologies

With the data collected in Task 1 and Task 2, guidelines were prepared with a focus on calf health and welfare problems, e.g. high pre- or post-weaning mortality, or high incidence of clinical health problems such as diarrhoea and pneumonia.

Task 4

Title: Economic evaluation of herd health costs for artificially reared dairy and suckled beef calves from birth to weaning.

Objective(s):

1. Evaluate herd health costs for artificially reared dairy and suckled beef calves from birth to weaning and to conduct cost benefit analysis of alternative herd health strategies.
2. Using the data generated identify least cost strategies for optimum herd level economic performance by integrating this data into bio-economic systems models.

Research methodologies

Health-control costs were evaluated on the farms visited in Tasks 1 and 2 to include costs for pre-occurrence treatments, post-occurrence treatments, veterinary fees, laboratory analysis for disease diagnosis (but not for feed or water), control programs for infectious diseases, equipment (e.g., syringes), and nutritional products specifically given in addition to feeding. The proportion of health-control costs related to preventive actions will be defined as pre-occurrence costs, including costs for disinfection, vaccines, systematic control programs, treatments administered prior to disease occurrence, and laboratory analyses. The herd health control costs described above will be coupled to the husbandry and management practices as documented on the farms visited, as part of Tasks 1 and 2 and data from the published literature.

Task 5

Title: Immunocompetence from birth to weaning in artificially reared dairy and suckled beef calves differing in neonatal passive immunity.

Objective(s):

Evaluate the effect of divergence in passive immunity on the development of an adaptive immune response within and between artificially reared dairy and suckled beef calves.

Research methodologies

Serum sample analyses

Serum samples were analysed using direct and indirect tests for assessment of passive immunity. Total IgG concentration was directly measured in the serum samples using a commercial ELISA (BIO K165 test kit, BioX Diagnostics, Jemelle, Belgium), as described by Dunn et al. (2018). A clinical chemistry analyser (Olympus AU400, Tokyo, Japan) and test reagent kits (OSR6132 and OSR6102, Beckman Coulter Ireland Inc., Lismeehan, Co.

Clare, Ireland) were used to quantitatively determine serum total protein (TP – CA) and albumin concentrations, as described by Earley et al. (2015). Globulin concentration was calculated for each serum sample as the difference between TP – CA and albumin concentration. Serum samples were analysed for ZST units, as described by McEwan et al. (1970). An optical Brix refractometer with automatic temperature compensation (RSG-100ATC, Grand Index Solution Enterprise Limited, Hong Kong, China) was used to determine total solids percentage by Brix refractometer (TS – BRIX). A digital hand held refractometer with automatic temperature compensation (DR-303, Index Instruments Ltd, Cambridgeshire, UK) was used to determine total protein concentration by digital refractometer (TP – DR).

Task 6

Title: Molecular predictors of immunity in bovine colostrum from dairy and beef cows and effect of colostral somatic cells on development of immunity in dairy and beef calves.

Objectives

1. This task aims to characterize the transcriptome and immune components of colostrum from beef and dairy cows and following consumption of varying quantities of colostrum immunoglobulin the transcriptome and immune variables will be determined in leukocytes of beef and dairy calves.
2. Data will be integrated with immune, physiological and production data using bioinformatics and network analysis, which will identify the critical molecular mechanisms regulating passive immunity in beef and dairy cattle.
3. Key master regulator genes will be interrogated for novel polymorphisms by sequencing these genes and their regulatory regions in DNA from animals varying in passive immunity collected in Task 1.
4. Novel and published SNPs will be genotyped across a large population (>1000) of animals and thus, allow the identification of putative molecular predictors of passive immunity in cattle.

Research methodologies

The animal model that was used was designed to fully elucidate the development of immune-competence in i), Holstein dairy calves (artificially fed 5% colostrum body weight (BW) at birth) and ii), naturally suckled beef calves, the progenies of Limousin × Friesian and Charolais × Limousin cows.

The birth of each dairy calf was vigilantly observed and calves were separated from their dam approximately 15 to 20 min post-birth to prevent suckling occurring. Calves were weighed and placed in a straw-bedded pen where they were blood sampled and received their first feed of colostrum, 5% total body weight (BW) colostrum via oesophageal tube within 2.5 h post birth. 12 h later, calves were fed 5% body weight, second milk colostrum, via a teat feeder. Calves were fed 12.5% of their BW of colostrum from their own dam for the first 4 days of life. Calves were individually penned for the first 4 days of life, and on day 5 were placed in a group pen where they were fed 'Calf Gold milk replacer'(Britmilk, Dumfries, UK) and offered ad libitum concentrate and clean water from an automatic milk feeder (Forster Technik vario, Germany). Beef crossbreeds consisted of thirty

multiparous (MP) beef suckler, spring-calving cows. Two cow breeds were included: Limousin × Friesian (LF) (n = 7) and Charolais × Limousin (CL) (n = 7). Mean parity (SD) for LF and CL cows was 5.5 (0.94) and 5.3 (1.03), respectively. The mean calving date was 28th February 2015 (26th January to 27th April). Cows were bred using an Aberdeen Angus (AA) and Charolais (CH) sires (natural mating). Births were supervised by farm personnel to ensure that calves did not suckle the cow prior to implementing the experimental protocol for collection of the first blood sample from the calf. Immediately post-partum a tincture of 5% iodine was applied to the umbilical cord of each calf, and calf birth weight was recorded. All calves were closely observed to ensure suckling of the dam and any calf not suckling within one h of birth or showing signs of weakness was assisted to suckle the cow. After parturition, animals remained in the straw pens for a minimum of 4 days with the calf having free access to the dam. The calves had free access to their dams for suckling.
Blood samples were collected from the calf via the jugular vein using a 10 mL serum vacutainer tube (BD, Oxford, UK), at 0, 48, 72, and 168 hour (h) post birth. A blood sample was taken from calves before colostrum intake at 0 h. All blood samples were stored at room temperature for a minimum of 1 h to allow clotting to occur; samples were then centrifuged at 1764 × *g* (rcf) for 15 min, serum was decanted into three aliquots and stored at -20°C until IgG analysis.
Bloods for RNA extraction were collected via jugular venipuncture from all calves at 0 (n = 7), 48 (n = 7), 72 (n = 7) and 168 h (n = 8) post birth. The blood (3 mL) was collected using tempus blood RNA tubes (Life technologies, Paisley, Scotland). Immediately after blood collection, the tubes were shaken vigorously for 10 s until the blood turned from blue to black, lysis occurs almost immediately once the blood is mixed with the stabilizing reagent in the tube. The purpose of this stabilizing reagent is to inactivate cellular RNases and it selectively precipitates RNA, and the genomic DNA and proteins remain in solution. Blood was stored in a -80°C freezer until RNA isolation, library preparation and sequencing.

Serum samples were thawed at 4°C overnight. IgG concentration was then analysed using an ELISA kit for bovine IgG from Bio-X Diagnostics (Jemelle, Belgium). All kit components were brought to 21oC before use. The wash buffer was diluted 20 fold with distilled water. A calibration curve was developed and samples were diluted in PBS as per the manufacturer's instructions. Diluted samples were added to the test plate in duplicate and incubated at 21oC for 1 h. The test plate was then washed 3 times with the wash buffer; chromogen solution (100 μL) was added to each well and incubated away from light for approximately 10 min. Finally, stop solution (50 μL) was added to each well and the optical densities were recorded using a microplate spectrophotometer with a 450 nm filter. An inter-assay CV of < 0.15 was observed. The concentration of IgG in samples was calculated from the standard reference curve containing known concentrations of IgG provided in the test kit. Any sample that resulted in an IgG concentration that fell above or below the range of the standard reference curve was retested after further dilution according to the test kit recommendations.

RNA Isolation and Purification

RNA was isolated from whole blood collected from dairy and beef calves using the tempus spin RNA isolation reagent kit following manufacturers guidelines (Applied Biosystems, USA). The quantity of the RNA isolated was determined by measuring the absorbance at 260 nm using a Nanodrop spectrophotometer ND-1000 (Nanodrop Technologies, DE, USA). RNA quality was assessed on the Agilent Bioanalyser 2100 using the RNA 6000 Nano Lab Chip kit (Agilent Technologies Ireland Ltd., Dublin, Ireland. RNA samples with 28S/18S ratios ranging from 1.8 to 2.0 and RIN (RNA integrity number) values of between 8 and 10 were deemed to be of high quality. RNA was stored at -80°C until subsequent cDNA library preparation.

cDNA library preparation and sequencing

cDNA libraries were prepared using the Illumina TruSeq RNA sample prep kit following the manufacturer's instructions (Illumina, San Diego, CA, USA). For each sample, 0.7 µg of RNA was used for cDNA library preparation. Briefly, mRNA was purified from total RNA and then fragmented. First strand cDNA synthesis was performed using SuperScript II Reverse Transcriptase (Applied Biosystems Ltd., LifeTechnologies) subsequently synthesising the second strand using components of the Illumina TruSeq RNA sample prep kit. Adaptors were ligated to the cDNA which was then enriched by PCR. Final individual cDNA libraries were validated on the Agilent Bioanalyser 2100 using the DNA 1000 Nano Lab Chip kit (Agilent Technologies Ireland Ltd., Dublin, Ireland), ensuring that library fragment size was ~100 base pairs (bp) and library concentration was >30 ng/µl. Subsequently, individual RNAseq libraries were pooled based on their respective sample-specific-6 bp adaptors and sequenced at 100 bp/sequence single-end read using an Illumina HiSeq 2000 sequencer (Macrogen Europe, The Netherlands). Approximately 38.4 million sequences per sample (Mean ± SD = 38,432,764 ± 7,169,473) were generated and bioinformatics conducted in-house.

<u>Task 7</u>

Title: Knowledge dissemination and technology transfer

Objective(s).

To raise awareness of the research project and its outcomes, disseminate new knowledge, and transfer of technical knowledge to end-users.

<u>Research methodologies</u>

Dissemination was carried out at two main levels:

Technical

- Through the conduit of the Teagasc, Knowledge Transfer Directorate, applied research findings from the project were disseminated to a wide farmer audience via education and training courses, Teagasc farmer meetings and one-to-one interactions with farmer clients.
- Discussion group activities with Teagasc advisors and lead beef farmers are a very successful avenue for technology uptake. The launch of the Beef Technology

Adaptation Programme (BTAP) programme provides a means of scaling up this dissemination pathway.
- Publication of project results in Technical publications (e.g. Todays Farm, Teagasc, Beef Advisory newsletter, Technology updates), the Farming press and targeted websites e.g. Teagasc Grange beef page, DAFM (FAWAC web site), Teagasc, UCD, AHI (The CalfCare™ Technical Working Group web site on calf health), ICBF and an Bord Bia.
- Presentations of project results at farmer/advisor meetings workshops and open days.

Scientific

- Presentations of project results at scientific conferences (PD 1 and 12 month PD)
- Publication of project results in international peer-reviewed research journals (e.g. Journal of Animal Science; Welfare; Animal; BMC Veterinary Research; BMC Genomics; Physiological Genomics, Veterinary Immunology and Immunopathology (PD and PG)
- Results from Task 1 and Task 5 of the project will be presented in one M.Sc. thesis (PG)

The milestones and deliverables in this Task focused on reports, manuals and guidelines on principles for development of animal health and welfare plans, and international peer-reviewed papers, one M.Sc.thesis and one PhD.

3. Research Achievements/Results

A total of 1,392 suckler beef calves (n = 111 farms) and 2,090 dairy calves (84 farms) were included in the observational study (Task 1 and Task 2). Blood samples were collected by jugular venipuncture. Serum samples were analysed for total IgG concentration using an ELISA assay, total protein concentration by clinical analyser (TP – CA), globulin concentration, zinc sulphate turbidity (ZST) units, total solids percentage by Brix refractometer (TS – BRIX), and total protein concentration by digital refractometer (TP – DR). Crude and cause-specific morbidity, all-cause mortality, and standardised 205-days body weight (BW) were determined. Generalised linear mixed models were used to evaluate associations between suckler beef and dairy calves for morbidity, mortality, growth and passive immunity. Receiver operating characteristic (ROC) curves were constructed to determine optimal test cut-offs for classification of health and growth outcomes. Overall, 20% of suckler beef and 30% of dairy calves were treated for at least one disease event by 6 mo. of age. Suckler beef calves had greater odds of bovine respiratory disease (BRD; odds ratio (OR), 95% confidence interval (CI): 2.8, 1.2 – 6.5, $P = 0.01$), navel infection (5.1, 1.9 – 13.2, $P < 0.001$), and joint infection/lameness (3.2, 1.3 – 7.8, $P = 0.01$) during the first 6 mo. of life than dairy calves. In addition, from birth to 6 mo. of age, suckler beef calves had greater rates of navel infection (incidence rate ratio (IRR), 95% CI: 3.3, 1.3 – 8.4, $P = 0.01$), but decreased rates of diarrhoea (0.9, 0.2 – 0.9, $P = 0.03$) compared to dairy calves. Optimal test cut-offs for classification of morbidity and mortality outcomes in suckler beef calves ranged from 8 to 9 mg/ml ELISA, 56 to 61 g/l TP – CA, 26 to 40 g/l globulin, 12 to 18 ZST units, 8.4% TS – BRIX, and 5.3 to 6.3 g/dl TP – DR. Optimal test cut-offs for classification of morbidity and growth outcomes in dairy calves ranged from 10 to 12 mg/ml ELISA, 57 to 60 g/l TP – CA, 29 to 34 g/l globulin, 19 ZST units, 7.8 to 8.4% TS – BRIX, and 5.7 to 5.9 g/dl TP – DR.

Detailed information pertaining to on-farm usage of antimicrobials in suckler beef and artificially-reared dairy calves from birth-to-6 months (mo) of age, in Ireland was analysed. A total of 123 farms (79 beef and 44 dairy), comprising of 3,204 suckler beef calves and 5,358 dairy calves, representing 540,953 and 579,997 calf-days at risk, respectively, were included in the study. Suckler beef calves were more frequently treated for respiratory disease, navel infection, and joint infection/lameness in the first 6 months of life, as compared to dairy calves. Suckler beef calves had greater standardised 205-day body weight than dairy calves. Relationships between passive immunity test results and morbidity, mortality and growth were examined and optimal test values for classifying calves for passive immune status were determined. Suckler beef and dairy calves with lower passive immunity test results were more likely to experience a negative health outcome or poor growth. Herd and animal-level factors associated with passive immunity are being examined.

Antimicrobial treatment records for calves born between July 1, 2014 and June 30, 2015 on 79 suckler beef and 44 dairy farms were analysed. Calves were followed from birth (day 0) until 6 months of age. According to standard farm protocol, calves exhibiting clinical signs of any disease were identified and antimicrobial treatment was administered. Farmers recorded the following information for each treatment administered: calf identification, age at treatment, disease event, drug name, number of treatment days, and amount of drug administered. In total, 3,204 suckler beef calves and 5,358 dairy calves, representing 540,953 and 579,997 calf-days at risk, respectively, were included in the study. A total of 1,770 antimicrobial treatments were administered to suckler beef (n = 841) and dairy calves (n = 929) between birth and 6 months of age. There was large variation in TI_{DDDvet} and TI_{DCDvet} by farm. It was concluded that beef and dairy calves in the study population were treated with antimicrobial substances for diseases at a relatively low frequency (mainly individual treatments).

Neonatal calves possess a very immature and naïve immune system and are reliant on the intake of maternal colostrum for passive transfer of immunoglobulins. Variation in colostrum management of beef and dairy calves is thought to affect early immune development. We examined changes in gene expression and investigated molecular pathways involved in the immune-competence development of neonatal Holstein dairy calves and naturally suckled beef calves using next generation RNA-sequencing during the first week of life. Jugular whole blood samples were collected from Holstein (H) dairy calves (n = 8) artificially fed 5% B.W. colostrum, and from beef calves which were the progenies of Charolais-Limousin (CL; n = 7) and Limousin-Friesian beef suckler cows (LF; n = 7), for subsequent RNA isolation. In dairy calves, there was a surge in pro-inflammatory cytokine gene expression possibly due to the stress of separation from the dam. LF calves exhibited early signs of humoral immune development with observed increases in the expression genes coding for Ig receptors, which was not evident in the other breeds by 7 days of age. Immune and health related DEGs identified as upregulated in beef calves are prospective contender genes for the classification of biomarkers for immune-competence development, and will contribute towards a greater understanding of the development of an immune response in neonatal calves.

High mortality (5.83%) in Irish dairy and beef calves, results in economic losses. The diseases responsible for the majority of the morbidity and mortality in young calves are scour and pneumonia. Calves which have lower levels of immunoglobulin G (IgG) in their blood, or failure of passive transfer, are at a greater risk of developing disease.
As there is a genetic component to the success of passive transfer in calves and in their ability to resist diseases, it is possible to select and breed cattle with a genetic makeup associated with superior immunity and disease resistance. Therefore, the objective of this study was to perform GWAS for passive immunity and disease related traits in Irish commercial beef and dairy calves in order to identify the SNPs associated with superior immunity and disease resistance. Blood samples were collected from beef (n=698) and dairy (n=1178) calves, aged between 1 and 21 days, from commercial beef and dairy farms across Ireland. Passive immunity traits were obtained from several analyses performed on the calves' serum samples (total IgG, total protein, albumin, specific gravity, globulin, brix refractometer, zinc sulphate turbidity). Incidents of pneumonia, scour, and any other illnesses during the calves' first 6 months of life observed by the enrolled farmers were recorded using standardised recording sheets. DNA was extracted from blood samples and genotyping was performed using the IBD*v3* chip which contains 50,855 markers. Quality control was carried out on genotypes using PLINK. SNPs were removed from the analyses if they had a call rate of less than 0.95, a minor allele frequency of less than 0.05 or a Hardy-Weinberg P-value of less than $1 \times e^{-4}$. Phenotypes were corrected for significant fixed effects of population structure principal components, sex, age and season of birth, and for the random effect of farm, by obtaining the residuals of the optimal model for each phenotype and carrying these values forward for the GWAS. Genetic relationship matrixes were generated and heritability estimates for each phenotype were performed using GCTA. GWAS were carried out using a mixed linear model method in GCTA. The heritability of the passive immunity associated traits and the disease traits were low to moderate (range 0.00-0.16). Several SNPs approached genome wide significance in the pneumonia, scour, crude illness, total IgG, total protein, albumin, specific gravity, globulin, brix refractometer and zinc sulphate turbidity GWAS analyses ($P<5\times10^{-5}$). One SNP "ARS-BFGL-BAC-27914" reached Bonferroni genome wide significance ($P<1.15\times10^{-6}$) for an association with total circulating IgG concentration in beef calves. This SNP is located within the intron of the PARP8 gene. This SNP and the suggestive SNPs associated with enhanced passive immunity and disease resistance can be added to future SNP chips and, following validation, may contribute to Ireland's national genomic selection breeding programme. Inclusion of the SNPs discovered in this study may lead to the breeding of more robust animals which display resistance to disease.

A number of peer-reviewed publications, reports and presentations were prepared and presented at National and International conferences. In Addition, a PhD thesis and an MSc thesis were delivered. All milestones were completed by the end of the project. The research outcomes of this project have been communicated widely to stakeholders. In addition, this project has contributed to further successful grant applications, including internal and external research calls for funding.

4. Impact of the Research

A summary of the tangible impact of the research project should be provided under the outcomes' and 'outputs' heading below. In addition, please provide a short narrative synopsis of the benefits / improvements the research has made to the area under investigation particularly as regards end users, e.g. industry, consumers, regulatory authorities, policymakers, the scientific community, etc

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

During this project we strengthened our collaborative linkages with UCD, AFBI and with staff at DAFM Backweston. In addition, due to the nature of the work on calf health and on immune transcriptomics in neonatal calves we consulted with other research groups in the US, namely, Prof Jerry Taylor at University of Missouri and with Dr. Tim Smith at US MARC, Clay Center, Newbraska, USA. These discussions led to the submission of two successful US-Ireland tri-partite grants on bovine respiratory disease, and other Teagasc core funded projects.

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

We have highlighted our research findings on *"Predisposing factors for disease and immunocompetence in artificially-reared dairy and suckled beef calves from birth to weaning"* at several National events (BEEF open days 2016 and 2018), at AHI-Teagasc events over the past 4.5 years and at international meetings. We have worked closely and informed AHI, ICBF and the Teagasc KT Advisory service of new developments with the project. The farm survey was designed and subsequently evaluated in conjunction with AHI's CalfCare™ Technical Working Group. This ensured relevance and dissemination in a rapid manner. It was also essential that the Teagasc KT advisory service were informed of project outcomes as they disseminated the information rapidly at farm level.

Neonatal passive immunity

Particular emphasis was placed on the study of neonatal passive immunity in suckler beef calves and in dairy calves. Bovine neonates are agammaglobulinemic at birth because the structure of the synepitheliochorial placenta prevents the *in-utero* transfer of immunoglobulins (Ig) to the foetus. Neonates obtain passive immunity by consuming colostrum and absorbing maternal Ig into circulation, and this provides immunologic protection against exposure to pathogens in first weeks of life. We have reported that neonates that do not acquire adequate levels of maternal Ig are classified as having failure of passive transfer (FPT) of immunity are at greater risk of illness and death. Colostrum management factors are important determinants in the acquisition of passive immunity. The neonate's small intestine is permeable to Ig and other macromolecules during the first 24 hours after birth. The rate of Ig absorption is greatest within the first hours of life, progressively declines by 12 hours, and absorption ceases at approximately 24 hours after birth. Delays in colostrum ingestion are associated with increased risk of FPT in dairy calves. The quality and amount of colostrum consumed can also impact the transfer of passive immunity. Thus, given the absorption dynamics and variations in colostrum quality, ensuring adequate intakes of colostrum immediately after birth is an important management practice. Feeding large volumes of colostrum increases the likelihood of neonates ingesting sufficient total Ig mass and achieving greater passive immunity. In Ireland, dairy farmers are now routinely advised to hand feed a minimum of 3 L of colostrum to calves within 2 hours of birth (CalfCare Technical Working Group - Animal Health Ireland, 2011). In contrast, since suckler beef calves remain with their dams, Irish beef farmers are recommended to ensure that every calf consumes colostrum by either suckling or hand-feeding within 2 hours after birth, and to closely monitor situations where suckling may be delayed (e.g. weak calf, difficult birth, twins, dam prevents calf from suckling, etc).

Immunocompetence development

The use of a systems approach such as RNA sequencing offers advantages over other molecular based techniques such as microarray, enabling unbiased opportunities towards the profiling of developing immunocompetence using a global unbiased view of relative transcriptomic alterations. Peripheral whole blood samples are commonly used for immunological studies as they are easily obtained and may provide an insight into immune development, particularly when combined with a transcriptomics approach. Previous studies from our group have successfully investigated the immune response of two dairy breeds to gradual weaning using whole blood to analyse alterations in the relative abundance of key immune genes. Here, utilizing the whole blood transcriptome of dairy calves in addition to two beef breeds, we aim to elucidate the molecular mechanisms involved in the development of immunocompetence, from birth through the first 7 days of life. We observed for the first time, the development of an immune response in calves of different breeds over the first seven days of life, using whole blood and RNA sequencing technologies. The molecular variations in key genes such as cytokines and B cell receptors across all breeds gives a tremendous insight into possible reasons as to why certain cattle breeds are more susceptible than others to illness during the neonatal period. It is clear from these data, that dairy calves undergo a systemic stress response following separation from the dam at birth and following artificial feeding of colostrum. Key genes increased in LF calves at 168 h including the immunoglobulin receptors *FCER1A* and *FCRLA*, and *IL-12r*, an immune regulator consistently down regulated in beef CL and in dairy calves, offer possible targets for biomarker discovery and a greater understanding into the development of immunocompetence.

(iii) Outcomes with economic potential

In this project basic and applied research approaches were carried out to comprehensively examine the *Predisposing factors for disease and immunocompetence in artificially-reared dairy and suckler beef calves from birth to weaning.* Exploitation activities of this project proposal were addressed in detail, being structured along several dimensions, i.e.: the research partners (i.e., academic and stakeholder linkages), the type (strategic health management guidelines and technical improvements), and the end-users (internally to the consortium and externally to a wider community). We have listed (below) the peer-reviewed and non-peer reviewed publications that were prepared from the data collected during the €asyCalf project.

Overall, 20.4% of suckler beef calves and 14.8% of dairy calves were treated with antibiotics for disease by 6 months of age. The leading cause of morbidity from birth to 6 mo. of age in the present study was diarrhoea, accounting for 44 and 77% of the disease events in suckler beef and dairy calves, respectively. The second and third most frequent causes of morbidity in calves during the first 6 mo. of life were BRD and navel infection, respectively. Suckler beef calves had greater odds of BRD, navel infection, and joint infection/lameness, as well as increased rate of navel infections during the first 6 months of life compared to dairy calves. Conversely, the incidence rate of diarrhoea from birth to 6 months of age was greater in dairy calves than suckler beef calves. Incidence rates of crude morbidity for suckler beef and dairy calves from birth to 6 mo. of age were 4.1 and 8.7 disease events per 100 calf-mo. at risk, respectively. Colostrum-derived passive immunity is central to the health, performance and welfare of neonatal calves. Calves with inadequate passive immunity are at greater risk of calfhood disease. The first step in evaluating a colostrum management programme is to assess the effectiveness of passive transfer of immunity to the calf. Passive immunity test results can be categorised for failure of passive transfer (FPT) using test-specific cut-off values. Farmers should consider implementing a testing programme to monitor calf passive immune status. There are still opportunities for improvement in colostrum management on Irish suckler and dairy farms.

The incorporation of genomic information into breeding programmes has the potential to significantly increase the rate of genetic gain in a number of traits, including health traits such as BRD resistance, and is much faster and more reliable than traditional selective breeding methods. Variation exists between individual animals in the DNA sequence of genes controlling health traits. This variation is responsible for differences in susceptibility to diseases such as bovine respiratory disease (BRD), commonly known as pneumonia, and scour. Genomic research aimed to identify the differences in cattle DNA sequences that contribute to resistance to BRD. Identified DNA variants, following validation, will be added to the Irish single nucleotide polymorphism (SNP) chip and integrated into the national Genomic Selection breeding programme to evaluate their association with BRD. This will facilitate the selection of robust animals with superior genetic merit for resistance to BRD. Increasing the abundance of certain favourable immune traits through the analysis of gene expression and the occurrence of SNPs linked with resistance to BRD, has massive potential benefits for both the beef and dairy industries. In the present project, a total of 413 significant SNPs were identified across 7 breeds, associated with overall disease occurrence, scour, pneumonia, joint disease, navel infection and immunoglobulin G (IgG) serum concentration. A SNP in the *CNTN1* gene was associated with BRD in Hereford calves, a SNP in the *PAX3* gene was associated with diarrhoea and pneumonia in Belgian Blue and Hereford calves, respectively, and a SNP in the *CAB39* gene was associated with an increased incidence of diarrhoea in Belgian Blue calves. Following further investigation and validation, these SNPs may be applied in future genomic breeding programmes to enhance the health status of cattle.

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

This research project provided the first detailed information pertaining to on-farm usage of antimicrobials in suckler beef and artificially-reared dairy calves from birth-to-6 months (mo) of age, in Ireland. A total of 123 farms (79 beef and 44 dairy), comprising of 3,204 suckler beef calves and 5,358 dairy calves, representing 540,953 and 579,997 calf-days at risk, respectively, were included in the study. All calves were raised on farm of origin and most of the studied herds were closed herds. In this study, only animals showing signs of disease were treated with antimicrobials and no mass administration of antibiotics was practiced. On beef farms overall, 12.7%, 5.7%, 2.9% and 20.4% of suckler beef calves were treated with antimicrobials for disease from birth-to-1 mo, 1-to-3 mo, 3-to-6 mo, and birth-to-6 mo of age, respectively. The corresponding values on dairy farms overall for calves treated with antimicrobials were 10.2%, 5.3%, 1.9% and 14.8%. The highest risk period for disease in the present study was between birth and 1 mo. of age, with approximately two-thirds of all disease events occurring during this time period. This is reflected in the proportion of antimicrobials administered to calves at this time

Concern about the use of antimicrobials in food producing animals is increasing. The present study offers a benchmark for antimicrobial use in Ireland. The following guidelines are recommended to maintain acceptable levels of antimicrobial usage on beef and dairy farms;

- Develop a herd health plan in consultation with your veterinarian and Teagasc advisor.
- Pay attention to colostrum feeding, animal nutrition and animal purchasing policies.
- Vaccinate animals to reduce the need for antimicrobials and use alternatives to antimicrobials when available.
- Only give antimicrobials to animals under veterinary supervision.
- Do not use antimicrobials for growth promotion or to 'prevent' diseases in healthy animals.
- Improve biosecurity on farms and prevent infections through improved hygiene and animal welfare.

4 (b) Summary of Research Outputs

(i) Peer-reviewed publications (A1), International Journal/Book chapters.

1. **Earley**, B., Arguello, A., O'Riordan, E, Crosson, P., Mcgee, M. Quantifying antimicrobial drug usage from birth to 6 months of age in artificially reared dairy calves and in suckler beef calves. Journal of Applied Animal Research, 47:1, 474-485, DOI: 10.1080/09712119.2019.1665525 **A1**
2. McCabe, M., Esnault, G., Murray, G., **Earley,** B., Cormican, P. **2019**. Complete Genome Sequence of Mannheimia varigena isolated from bovine milk" Genome Sequences. Microbiology Resource Announcements, DOI: 10.1128/MRA.01377-18. **A1**
3. McGee, M., **Earley**, B. 2019. Review: Passive immunity in beef suckler calves. Animal. 2019 Apr;13(4):810-825. doi: 10.1017/S1751731118003026. Epub 2018 Nov 21. Review **A1**
4. Castro, N., Gómez-González, L., **Earley**, B., Argüello, A. 2018. "Use of clinic refractometer at farm, as a tool to estimate the IgG content in goat colostrum". Journal of Applied Animal Research 2018;46(1):1505-1508; doi http://dx.doi.org/10.1080/09712119.2018.1546585. **A1**
5. Surlis, C., **Earley**, B., McGee, M., Keogh, K., Cormican, P., Blackshields, G., Tiernan, K., Dunn, A., Morrison, A. Arguello, A., Waters, S.M. 2018. Blood immune transcriptome analysis of artificially fed dairy calves and naturally suckled beef calves from birth to 7 days of age. Scientific reports 8:15461 | DOI:10.1038/s41598-018-33627-0. **A1**
6. Todd, C.G., McGee, M., Tiernan, K., Crosson, P., O'Riordan, E., McClure, J., Lorenz, I., **Earley**, B. 2018. An observational study on passive immunity in Irish suckler beef and dairy calves: Tests for failure of passive transfer of immunity and associations with health and performance. Preventive Veterinary Medicine. 2018 Nov 1;159:182-195. **A1**
7. Murray, G.M., More, SJ, Clegg, TA, **Earley**, B., O'Neill, R.G., Johnston, D., Gilmore, J., Nosov, M., McElroy, M.C., Inzana, T.J., Cassidy, J.P. Risk factors associated with exposure to bovine respiratory disease pathogens during the peri-weaning period in dairy bull calves *BMC Veterinary Research* 2018 14:53 10.1186/s12917-018-1372-9 **A1**
8. Dunn, A., Duffy, C., Gordon, A., Morrison, S.J., Argűello, A., Welsh, M., **Earley**, B. 2018. Comparison of single radial immunodiffusion and ELISA for the quantification of immunoglobulin G in bovine colostrum, milk and calf sera. Journal of Applied Animal Research, Journal of Applied Animal Research, 46:1, 758-765, DOI: 10.1080/09712119.2017.1394860 **A1**
9. **Earley**, B., Tiernan, K., Duffy, C., Dunn, A., Waters, S.M., Morrison, S., McGee, M. 2018. Effect of suckler cow vaccination against glycoprotein E (gE)-negative bovine herpesvirus type 1 (BoHV-1) on passive immunity and physiological response to subsequent bovine respiratory disease vaccination of their progeny. Research in Veterinary Science, 118, Pages 43-51. **A1**
10. Dunn, A., Duffy, C., Gordon, A., Morrison, S.J., Argűello, A., Welsh, M., **Earley**, B. 2018. Effect of passive transfer status on response to a glycoprotein E (gE)-negative bovine herpesvirus type 1 (BHV-1) and bovine respiratory syncytial virus (BRSV) vaccine and weaning stress in pre-weaned dairy calves (JAAR). Journal of Applied Animal Research, 46 (1) 907-914 **A1**

11. Johnston, D., **Earley**, B., Cormican, P, Murray, G., Kenny, D.A., Waters, S.M., McGee, M., Kelly, A.K., McCabe, M. 2017. Illumina MiSeq 16S amplicon sequence analysis of bovine respiratory disease associated bacteria in lung and mediastinal lymph node tissue. BMC Veterinary Research (DOI: 10.1186/s12917-017-1035-2, December 2017 (13:118). **A1**
12. Murray, G.M., O'Neill, R.G., Lee, A. McElroy, M., More, S.J., Monagle, A., **Earley**, B., Cassidy, J.P. 2017. The Bovine Paranasal Sinuses: Bacterial Flora, the Presence of Inducible Nitric Oxide Synthase and the Potential Role of the Paranasal Sinuses in the Persistence of BRSV and BPI-3. (PlosOne Published: March 10, 2017 http://dx.doi.org/10.1371/journal.pone.0173845. **A1**
13. Dunn, A., Ashfield, A., **Earley**, B. , Welsh, M., Gordon, A., McGee, M., Morrison, S. 2017. Effect of concentrate supplementation during the dry period on colostrum quality and impact of colostrum feeding regime on passive transfer of immunity, calf health and performance J Dairy Sci. 2017 Jan;100 (1):357-370. doi: 10.3168/jds.2016-11334. **A1**
14. Dunn, A., Ashfield, A., **Earley**, B., Welsh, M., Gordon, A., Morrison, S. 2017. Evaluation of factors associated with IgG, fat, protein, and lactose concentration in bovine colostrum, and colostrum management practices within grassland based dairy systems in Northern Ireland. Journal of dairy Science, volume 100 (3) 1-12. **A1**
15. Murray, G.M., More, S.J., Sammin, D., Casey, M.J., McElroy,M.C., O'Neill, R.G., **Earley**, B., Ball, H., Cassidy, J.P. 2017. Pathogens, patterns of pneumonia, and epidemiologic risk factors associated with respiratory disease in recently weaned cattle. Journal of Veterinary Diagnostic Investigation, 29 (1) 20-34. **A1**
16. Johnston, D., Kenny, D.A., McGee, M., Waters, S.M., Kelly, A.K., **Earley**, B. 2016. Electronic feeding behavioural data as indicators of health status in dairy calves (Irish Journal of Agricultural and Food Research) (DOI:10.1515/ijafr-2016-0016 IJAFR 55(2)). **A1**
17. Murray, G., O' Neill, R.G., More, S.J., McElroy, M.C., **Earley**, B., Cassidy, J.P. 2016. **Review:** Evolving views on bovine respiratory disease Part 1: An appraisal of selected key pathogens. The Veterinary Journal. 217: 95-102 **A1**
18. Murray, G., O' Neill, R.G., More, S.J., McElroy, M.C., **Earley**, B., Cassidy, J.P. 2016. **Review:** Evolving views on bovine respiratory disease Part 2: An appraisal of selected control measures. The Veterinary Journal. 217; 78-82. **A1**
19. Murray, G.M., Cassidy, J.P., Clegg, T.A., Tratalos, J.A., McClure, J., O Neill, R.G., Casey, M.J., McElroy, M., **Earley**, B., Bourke, N., More, S.J. 2016. A retrospective epidemiological analysis of fatal cases of bovine respiratory disease diagnosed by the Irish Regional Veterinary Laboratories (RVLs) between 2005 and 2012. Preventive Veterinary Medicine: Preventive Veterinary Medicine 132:49-56. **A1**
20. Johnston, D., **Earley**, B. Cormican, P., Kenny, D.A., McCabe, M.S., Kelly, A.K., McGee, M., Waters, S.M. Characterisation of the whole blood mRNA transcriptome in Holstein-Friesian and Jersey calves in response to gradual weaning. PlosOne; doi.org/10.1371/journal.pone.0159707). 11(8):e0159707. PLoS One, 11(8), e0159707. **A1**
21. Johnston, D., Kenny, D.A., Kelly, A.K., McCabe, M., McGee, M., Waters, S.M., **Earley**, B. 2016. Characterisation of haematological profiles and leukocyte relative gene

expression levels in artificially reared Holstein-Friesian and Jersey bull calves undergoing gradual weaning. Animal, 10(9), 1547-1556. **A1**

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

Dissemination Recording System (Teagasc) Publications by Project - GE - 6476 €asyCalf
The descriptors with codes, for the various contributions by authors are listed below for Popular non-scientific publications and abstracts including those presented at conferences.

	Scientific Contributions
A1	Peer-reviewed scientific publication
A2*	Invited paper to an international conference which is published in full in edited proceedings.
A3*	Scientific book (or chapter), monograph, etc.
A4*	Patent/Standard
A5*	Thesis
A6*	Resource/soil survey, specialised map/atlas
A7*	Contributed paper (*not invited*) to international conference which is published in full in edited proceedings
A8*	Abstract in refereed journal or in proceedings of international conference
A9*	Editor of proceedings, book etc. (Scientific). This means 'one-off' publications. Excludes regular serial publications
	Technical contributions
B1*	Article in technical non-referred journal (e.g. TResearch, Food Ireland, Technology Ireland)
B2*	Paper/summary in proceedings of technical conference/workshop e.g. Teagasc conferences, IGAPA, BSAP, Cheese Symposia etc.
B3*	Manual/handbook which is bound and published.
B4	Scientific book review/letter to editor/comment
B5	Non-confidential contract research report – *full copy must be attached*. Exclude ad-hoc reports on samples, problems, queries, diagnosis etc. Technology update.
B6	In-house report, including literature review, report on visits abroad, research notes for advisory staff. *Full copy must be attached.*
B7	Other – specify (*must be accompanied by text*)
R1	National report (special once-off report on a subject of national interest).
	Practical/Popular (written output)
C1*	Article in newspapers, farming press, popular serials. Open day brochures, (e.g. Farmers Journal, CoOp Ireland, Farmers Monthly)

1. Earley, B. (2019). Oral presentation - Antibiotic consumption study in Irish beef and dairy calves from birth to 6 months of age and calf health & immunity. IFA Animal Health Committee, Meeting in Teagasc, Grange, 26th July 2019, ***C1*.**
2. Earley, B. (2019). Oral presentation - Antibiotic consumption study in Irish beef and dairy calves from birth to 6 months of age and animal welfare research updates. Teagasc in-service training, to Teagasc Teachers, (8th July, 2019). ***C1*.**
3. Earley, B. (2019). Oral presentation - Antibiotic consumption study in Irish beef and dairy calves from birth to 6 months of age and calf health & immunity. IFA Dairy group meeting IFA National Council Meeting in Teagasc Grange on 20th June 2019, ***C1*.**
4. Earley, B. (2018). Oral presentation - Antibiotic consumption study in Irish beef and dairy calves from birth to 6 months of age. IFA – Animal Health Committee meeting Teagasc Grange 11th December 2018. ***C1***

5. Earley, B. (2018). Oral presentation - Antibiotic consumption study in Irish beef and dairy calves from birth to 6 months of age. DAFM conference - One Health Symposium, Convention Centre, Dublin. (20th November, 2018). ***C1*.**

6. Earley, B. (2018). What is antimicrobial resistance. Open days/handouts/Teagasc literature Fact Sheet BEEF2018 Open Day 36924 ***C1***

7. Earley, B. (2017). Stress and immunologic responses of beef cattle (I). In: The National Animal Disease Center (NADC) AFBI Symposium, Madison, Wisconsin, USA, 07-Nov-2017, Invited Talk ***A8***

8. Earley, B. (2018). Safely handling animals on a beef farm (2). Open days/handouts/Teagasc literature Fact Sheet BEEF2018 Open Day 36920 ***C1***

9. Earley, B. (2018). Safely handling animals on a beef farm (1). Open days/handouts/Teagasc literature Fact Sheet BEEF2018 Open Day 36921 ***C1***

10. Earley, B. (2018). Management of purchased calves (1). Open days/handouts/Teagasc literature Fact Sheet BEEF2018 Open Day 36923 ***C1***

11. Earley, B. (2016). Stress and immunologic responses of beef cattle (I). In: 2016 International Symposium on Beef Cattle Welfare, Manhattan, KS, 08-Jun-2016, 2 pages 34731 **A2**

12. Earley, B. (2018). Management of purchased calves (2). Open days/handouts/Teagasc literature Fact Sheet BEEF2018 Open Day 36922 ***C1***

13. Earley, B. (2016). Beef cattle facilities - housing. In: 2016 International Symposium on Beef Cattle Welfare, Manhattan, KS,, 08-Jun-2016, 8 pages 34730 **A2**

14. Earley, B. (2016). Teagasc response to the Scientific Advisory Committee on Animal Health and Welfare (SACAHW) Public Consultation on SACAHW Opinion on animal husbandry practices. Scientific Advisory Committee on Animal Health and Welfare 37 pages 34743 **B4**

15. Earley, B. (2016). Stress and immunologic responses of beef cattle (II). In: 2016 International Symposium on Beef Cattle Welfare, Manhattan, KS, 08-Jun-2016, 7 pages 34732 **A2**

16. Earley, B. (2018). Tests for failure of passive transfer of immunity and associations with health and performance in calves. Teagasc 6476 4 page Technology Update 36928 **B5**

17. Earley, B. (2018). Heat stress. Open days/handouts/Teagasc literature Fact Sheet for Advisers on Tnet 36927 ***C1***

18. Earley, B. (2018). Antimicrobial drug usage in calves on commercial beef and dairy farms in Ireland. Identification of SNP's associated with enhanced immunity in Irish dairy and beef calves. Open days/handouts/Teagasc literature Press Release x 2 for BEEF2018 36925 ***C1***

19. Earley, B. and McGee, M. (2018). Tests for failure of passive immunity and associations with health and performance in calves. Beef 2018 Enhancing Knowledge p114-117 ISSN 9781841706467 ***C1***

20. Earley, B. and McGee, M. (2019). Passive immunity in Irish calves. Open days/handouts/Teagasc literature Feb 2019 Teagasc Beef Newsletter 37475 ***C1***

21. Earley, B. and McGee, M. (2016). Minimising stress of weaning of beef calves. Veterinary Ireland Journal Vol 6, No. 11: 628-631 ISSN 2009-3942 35360 ***C1***

22. Earley, B. and McGee, M. (2017). Colostrum management (Colostrum management for a healthy suckler calf). Teagasc Advisory Newsletter Research Update February 2017 36028 ***C1***

23. Earley, B. and Murray, A. (2018). Dairy calf to beef -calf management on farm. Teagasc/AHI BHC Events Leaflet. Animal Health Ireland 3 pages - Spring 2018 36792 ***C1***

24. Earley, B. and Welsh, M. (2014). Preventing animal diseases - novel approach for the control of bovine respiratory disease (BRD) in calves. Open days/handouts/Teagasc literature Grange Open Day Book, p110-111 31525 ***C1***

25. Earley, B., Argue, D. and Murray, A. (2019). Once-a-day feeding of milk replacer - Teagasc Factsheet - Green Acres Programme. Open days/handouts/Teagasc literature April 2019, 2 pages 37476 ***C1***

26. Earley, B., Arguello, A., Murray, A. and McGee, M. (2018). Antimicrobial drug usage in calves on commercial beef and dairy farms in Ireland - implications for antimicrobial resistance. Beef 2018 Enhancing Knowledge p122-125 ISSN 9781841706467 36913 *C1*

27. Earley, B., Arguello, A., Murray, A. and McGee, M. (2018). AMR - three deadly letters.Drug resistance is a global problem in human and animal health.A Teagasc team has studied antimicrobial drug usage in calves on commercial beef and dairy farms in Ireland. Tresearch Vol 13, No 3, p22-23, Autumn ISSN 1649-8917 37026 **B1**

28. Earley, B., Prendiville, R., Kelly, Pearse, Keane, J., Peppard, G. and Dukelow, K. (2017). Buying dairy calves for beef production. Open days/handouts/Teagasc literature Spring 2017, 4 pages ***C1***

29. Earley, B., Tiernan, K., Dunn, A., Morrison, S., Waters, S. and McGee, M. (2017). Immune response of suckled beef calves to dam vaccination against bovine respiratory disease. (Abstract) Proceedings of the 7th International WAFL Conference pp280 doi.org/10.3920/9789086868629 ISSN ***A8***

30. Earley, B., Todd, C., Arguello, A., Capelleri, A. and McGee, M. (2018). Quantifying antimicrobial drug usage in calves from birth to 6 months of age on Irish suckler beef and dairy farms. (Abstract) British Society of Animal Science p117 36850 ***A8***

31. Earley, B., Todd, C., Kennedy, E. and Lorenz, I. (2016). Technical notes on calf milk

replacer for rearing dairy heifer calves. CalfCare Publication. Animal Health Ireland 4 pages 34168 **C1**

32. Earley, B., Todd, C., Kennedy, E. and Lorenz, I. (2016). Technical notes on calf milk replacer for rearing dairy heifer calves. Animal Health Ireland CalfCare Publications, 4 pages 34740 **C1**

33. Johnston, D., Earley, B., Cormican, P., Murray, G., Kenny, D.A., Waters, S., McGee, M., Kelly, A.K. and McCabe, M. (2018). Leptotrichiaceae identified in post-mortem cranial lung lobe lesions and mediastinal lymph node tissue obtained from calves with respiratory disease. (Abstract) British Society of Animal Science p116, Oral Presentation 36816 **A8**

34. Johnston, D., Earley, B., McCabe, M. and Cormican, P. (2017). Novel bacteria potentially associated with pneumonia. Tresearch 12, 1:18-19 ISSN 1649-8917 35576 **B1**

35. Johnston, D., Earley, B., Surlis, C., McGee, M. and Waters, S. (2018). Genomics of health traits in cattle. Beef 2018 Enhancing Knowledge p110-113 ISSN 9781841706467 36915 **C1**

36. Johnston, D., McCabe, M., Murray, G., Cormican, P., Waters, S. and Earley, B. (2018). Bovine respiratory disease (BRD) diagnostics and vaccine responses. Beef 2018 Enhancing Knowledge p118-121 ISSN 9781841706467 36914 **C1**

37. McGee, M. and Earley, B. (2018). Suckler cow feeding and calf health. Animal Health Ireland Bulletin Newsletter Winter Ed. Page 5 - 6 37152 **C1**

38. McGee, M. and Earley, B. (2019). Review: passive immunity in beef-suckler calves. Animal 2019:13 (4): 810-825 ISSN 1751-7311 37229 **A1**

39. McGee, M. and Earley, B. (2017). Feeding the suckler cow for a healthy calf. Animal Health Ireland Newsletter Winter Edition: pages 9-10 35435 **C1**

40. Mee, J, Earley, B. (2019) Responsible use of antimicrobials in farm animals, Today's Farm – Jan/Feb , p18-19. **C1**

41. Dunn, A., Keogh, K., Waters, S., McGee, M., Welsh, M., Morrison, S. and Earley, B. (2016). "Immune related transcriptional responses to colostrum feeding in neonatal dairy calves". Abstract) Conference of research workers in animal disease (CRWAD), www.crwad.org Chicago, USA. Proceedings Presentation No. 70 ISSN Pesentation No. 70 **A2**

42. Surlis, C., Earley, B., McClure, M., McClure, J., Higgins, M. and Waters, S. (2018). Genome wide association study of health related traits in dairy and beef cattle on Irish commercial farms. (Abstract) British Society of Animal Science p83 36855 **A8**

43. Surlis, C., Earley, B., McGee, M., Keogh, K., Cormican, P., Blackshields, G., Dunn, A., Tiernan, K., Morrison, S., Arguello, A. and Waters, S. (2018). Blood immune transcriptome analysis of artificially fed dairy calves and naturally suckled beef calves

from birth to 7 days of age. (Abstract) British Society of Animal Science p141 36861 **A8**

44. Tiernan, K. (2016). Passive immune status and the development of immunocompetence in artificially reared dairy and naturally suckled beef calves from birth to three months of age. M.Sc. Thesis University College Dublin 35223 **A5**

45. Todd, C. (2014) Benchmarking calf health. Teagasc/ Irish Farmers Journal BETTER Farm Beef Open Day – T. Haplin Farm, Kells, Co. Meath, Ireland. July 2014. *(Oral presentation at a national open day event for suckler farmers)* **C1**

46. Todd, C. (2015) Herd health planning. Teagasc/ Irish Farmers Journal BETTER Farm Beef Open Day – P. Grennan Farm, New Ross, Co. Wexford, Ireland. May 2015. *(Oral presentation at a national open day event for suckler farmers)* **C1**

47. Todd, C., McGee, M., Crosson, P. and Earley, B. (2016). Passive immunity and health of suckler beef and dairy calves. Beef 2016 Profitable Technologies p74-77 ISSN 9781841706276 34738 **C1**

48. Todd, C., McGee, M., Crosson, P. and Earley, B. (2017). Associations between passive immunity and health status for Irish dairy and suckler beef calves. Teagasc Research Impact Highlights 2015 page 5 **C1**

49. Todd, C., McGee, M., Crosson, P. and Earley, B. (2017). Passive immunity and health of suckler calves. It's your Field Animal Health & Agri Trade Journal page 26-27 35529 **C1**

50. Todd, C., McGee, M., Crosson, P., Earley, B. and Tiernan, K. (2016). Passive immunity and health of Irish dairy and suckler calves. Tresearch Vol 11, No. 2, p32-33 ISSN 1649-8917 34742 **B1**

51. Todd, C., McGee, M., O'Riordan, E.G., Crosson, P. and Lorenz, I. (2018). Epidemiology of morbidity and mortality in Irish suckler beef and dairy calves from birth to 6 months of age. (Abstract) British Society of Animal Science p234 36860 **A8**

52. Todd, C., Tiernan, K., McGee, M., Crosson, P., Lorenz, I. and Earley, B. (2015). Passive immune status of Irish dairy and suckler beef calves. In: Proceedings of the AVTRW (Irish Region) 49th Scientific Meeting, Teagasc, Grange Research Centr, 02-Oct-2015, p4 33542 **B2**

53. Todd, C., Tiernan, K., McGee, M., Lorenz, I. and Earley, B. (2016). Assessment of passive immunity in Irish dairy and beef calves. (Abstract) Book of Abstracts of the 67th Annual meeting of the EAAP p293, Abstract No. 28.13 ISSN 9789086862849 35002 **A8**

54. Todd, C., Tiernan, K., McGee, M., O'Riordan, E.G. and Crosson, P. (2018). Passive immunity in Irish suckler beef and dairy calves: Associations between test cut-offs for failure of passive transfer classification and calfhood morbidity, mortality and growth. (Abstract) British Society of Animal Science. (BSAS) 9-11th of April, Croke Park, Dublin, Ireland. P 122 **A8**

(iii) National Report & International guidance document

Earley, B. (2018) Technology Report (Teagasc) End of Teagasc Report for RMIS 6476 (€asyCalf)
Earley, B. (2016). Teagasc response to the Scientific Advisory Committee on Animal Health and Welfare (SACAHW) Public Consultation on SACAHW Opinion on animal husbandry practices. Scientific Advisory Committee on Animal Health and Welfare 37 pages 34743 **B4**
Teagasc research on calf husbandry, and on castration, weaning, transport and housing of beef cattle by Dr. Bernadette Earley contributed to the **OIE 2017 © OIE - Terrestrial Animal Health Code -** 25/07/2017.

(iv) Workshops/seminars at which results were presented

Research and post-graduate seminars at Teagasc, Grange, Co. Meath.
at meetings with stakeholders, and at Teagasc open days (Teagasc BEEF Open days (2016 and 2018 Teagasc, Grange);
Animal health Ireland Farmer events;
In-service training for Teagasc Advisors and Teagasc Education staff (2015-2019);
One Health Conference – Convention Centre Dublin, 20th October 2018. Talk on antibiotic usage on beef and dairy farms nationally with implications for antimicrobial resistance (AMR)

(v) International conferences with invited talks (Dr. B. Earley) where results from €asyCalf were presented

1. SIVAR (Italian association for large animal practitioners) that was held in Cremona, Italy 8th -10th May 2014; Topic: Immunological responses to stress in beef cattle
2. Iowa State University, hosting the 4th International Symposium on Beef Cattle Welfare in Ames, IA on 16th-18th July 2014. Topic: An overview of the latest research examining the impact of stress on the health and welfare of beef cattle.
3. European Association for Animal Production (EAAP): The conference was held in Copenhagen in Denmark from the 25th-28th of August. Topic: Interactions between stress and immune reactions.
4. British Society for Animal Science (BSAS) Conference April 2015; Invited speaker. Precision calf management for optimal health and welfare.
5. Kansas state university, delivered three invited talks at the 5th International beef welfare symposium, Kansas State University, USA, 8th to 10th June 2016.
6. Veterinary faculty, University of Gran Canaria (March 28-30th 2017). Delivered talk on animal welfare & biomarkers of stress.
7. ICoMST (August 2017) Pre-Congress Course, PhD School, 11th August 2017, delivered lecture on Animal welfare
8. International Beef Working Group (Teagasc, Grange) September 20th to 22nd Talk on floor type and space for winter finishing beef cattle.

9. Madison - The Teagasc/NADC workshop on November 7th 2017 in Madison, Wisconsin, USA prior to Teagasc-AFBI Fall forum. The National Animal Disease Center (NADC) AFBI Symposium, Madison, Wisconsin, USA, 07-Nov-2017. Talk on Bovine respiratory disease talk.

(v) Intellectual Property applications/licences/patents

None

(vi) Other

One PhD
One MSc

5. Scientists trained by Project

Total Number of PhD theses: **1**

Title of PhD: Colostrum quality and immunocompetence development in artifically reared dairy calves. Ph.D. Thesis. Awarded. December 2016. ***Institute for Global Food Security, Queen's University Belfast, Agri-Food and Biosciences Institute, Hillsborough, Co. Down.***

Total Number of Masters theses: **1**

Title of M.Sc.: Passive immune status and the development of immunocompetence in artificially reared dairy and naturally suckled beef calves from birth to three months of age. Awarded December 2016. ***The Vet School, UCD, Belfield, Dublin 4, Ireland.***

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
Teagasc	5	3.307
UCD	1	1.600
Total	**6**	**4.907**

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers	2	4.646
Technician	1	2
Masters students	1	2
Total	**4**	**8.646**

8. Involvement in Agri Food Graduate Development Programme

N/A

9. Project Expenditure

Total expenditure of the project: € 699,890.78
Total Award by DAFM: € 754,719.2
Other sources of funding including benefit in kind and/or cash contribution(specify): € 0

Breakdown of Total Expenditure

Category	Name Institution 1 **Teagasc**	Name Institution 2 **UCD**	Name Institution 3 **AFBI**	Name Institution 4	Total
Contract staff					
Temporary staff					
Post doctorates	288,571.62				**288,571.62**
Post graduates		43,741.86			**43,741.86**
Consumables	87,963.54		21,895.62		**109,859.16**
Travel and subsistence	24,495.91				**24,495.91**
Sub total	**401,031.07**	**43,741.86**	**21,895.62**		**466,668.55**
Durable equipment					
Other	93,221.66				**93,221.66**
Overheads	120,309.32	13,122.56	6,568.69		**140,000.57**
Total	**614,562.05**	**56,864.42**	**28,464.31**		**699,890.78**

10. Leveraging

N/A

11. Future Strategies

Most recently, we have presented the finding of the project on antimicrobial usage on-farm at the recent iNAP conference which was held in the convention centre Dublin, in 2018 (ONE HEALTH 2018 – November 20th 2018). Work is on-going on disseminating further guidelines on antimicrobial usage at farm level, and on providing input to curriculum development for the Teagasc teachers on husbandry management practices, including calf immunity, calf health, disbudding and castration practices.

12. Consent to Publish Final Report on the DAFM Website and/or Through Other Dissemination channels

I consent to this report being made available to the public, through the Department's website and other dissemination channels.

Yes x☐ No ☐

13. Declaration

I declare that the information contained in this final report is complete and true to the best of my knowledge and belief.

Signed: Bernadette Earley Project Coordinator

Bernadette Earley

Date: 30th July 2019