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

**Food Institutional Research Measure**

**Final Report**

An investigation of Verocytotoxigenic *E. coli* super-shedding in beef and dairy cattle and the factors underpinning human virulence potential and strain emergence as a result of vt phage transduction "VTEC-SUPVIRT"

**DAFM Project Reference No:** 11/F/051

**Start date:** 1st December 2012

**End Date**: 30th November 2016

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Basic/Fundamental → Applied → Pre Commercial

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

| **Priority Area (s)** | I - Sustainable Food Production and Processing |
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**Key words:** *(max 4)* Verocytotoxigenic *E. coli,* food, safety, sequencing

## 1. Rationale for Undertaking the Research

This project focused on Verocytotoxigenic *E.coli*, a significant microbial pathogen. Human infection with VTEC can be asymptomatic or cause a spectrum of illnesses ranging from mild non-bloody diarrhoea, through bloody diarrhoea and haemorrhagic colitis to Haemolytic Uraemic Syndrome (HUS), and can be potentially fatal. Since 2008, and excluding the outbreak linked with fenugreek seeds reported by Germany in 2011, Ireland has had the highest STEC notification rate in Europe with 12.92 cases per 100,000 in 2015 compared to an EU average of 1.27 per 100,000 in 2015. The potential public health and economic consequences from a VTEC outbreak are enormous and highlights the need to protect the Irish consumer and the agri-food sector from this emergent group of pathogens.

This project addressed the carriage and shedding of VTEC in beef and dairy cattle, and in particular its potential excretion in exceptionally high numbers (> $10^4$ CFU /g) by some animals. These so called "super-shedders" have a disproportionately high impact on risk of transmission in the farm and food chain and are thus a key target for control measures. To underpin such an approach an understanding was needed on how frequently this phenomenon occurs and on the potential risk factors which cause this in some animals but not in others.

A further issue to be addressed in this project was to gain an understanding of the genetic profile of VTEC beef isolates using whole genome sequencing, and to investigate if there was genetic difference/markers in isolates implicated in super shedding events in cattle as well their human virulence potential.

Verotoxin encoding genes are also mobile and have potential to be transduced into other *E. coli* pathotypes or indeed to *E. coli* which were previously non pathogenic. This study aimed to increase our understanding on how *vt* genes are persisting and transferring among *E. coli* bacteria in farm and food environs and whether any ongoing practices are promoting gene transfer.

This study aimed to extend knowledge which would support risk managers in addressing this pathogen.

## 2. Research Approach

A range of microbial and molecular tools were used to study the occurrence and basis of super shedding in beef and dairy cattle, the genetic profile and human virulence potential of VTEC cattle isolates and the potential for emergence of unusual virulent VTEC strains as a result of transduction of *vt* encoding bacteriophage in the farm and food chain environs.

To study super-shedding in beef and dairy cattle, accurate methods to count VTEC (O157 and O26) in the cattle recto-anal junction (RAJ) (the site of VTEC colonisation) were needed. Thus a real time real time polymerase chain reaction (PCR) protocol was developed (Lawal *et al*, 2015). This method involved a 5h enrichment followed by real time PCR targeting *rfb*E (O157) and *wzx* (O26). Counts (CFU $swab^{-1}$) were obtained from a standard calibration curve, relating the real time PCR cycle threshold ($C_t$) values against the initial concentration (CFU $g^{-1}$) of O157 or O26 in the Recto-anal junction (RAJ) sample. The method was employed to examine carriage and shedding of O157 and O26 in beef and dairy cattle.

In beef cattle, swab samples of the recto-anal junction of cattle (n=1317) were collected (2013 to 2015) at 3 large Irish commercial beef abattoirs. Metadata collected on the animals included farm/region of origin, age, gender, breed, sample date etc.

In dairy animals, study 1 was a longitudinal study undertaken where two dairy herds were visited up to seven times between August 2013 and July 2014. Forty cattle in each herd were selected as possible super-shedders following the criteria set out by Menrath et al., 2010. Rectal swabs from 520 lactating animals, two milk filters, two raw milks and two water samples (taken from the tap in the dairy) were screened. In study 2 a surveillance study was undertaken in which 13 dairy herds (40 – 184 cattle) were each visited on one occasion between August 2014 and July 2015. During each visit rectal swabs were procured from the animals by the private veterinary practitioner, plus a milk filter, raw milk and a water sample (where possible). Rectal swabs from 1,074 animals, 11 milk filters, 13 raw milks and 13 water samples were analysed.

For both beef and dairy studies, samples with counts $>10^4$ CFU $swab^{-1}$ of *E. coli* O157 or O26 were deemed to be super-shedders (SS).

Selected isolates (n=78) including super shedder and low shedder strains from beef and dairy animals and historical strains were whole genome sequenced using the MiSeq platform. These data are securely stored at UCD-CFS. The sequencing data is available for subsequent trace back studies.

To ascertain the role of the microbiota at the recto-anal STEC colonisation site in shedding dynamics, swabs taken from three animals groups (STEC super shedder, low shedders and negative) were subjected to a 16SrRNA gene-based compositional metagenomics approach.

In a study to assess the potential for transmission of vtx genes, donor VTEC strains with marked verotoxin ($vt_2 24_B$ $kanamycin^R$) encoding bacteriophage were used to study the persistence of such phage in the farm environment and the potential for transduction of vt toxin into other *E. coli* pathotypes and non pathogenic *E. coli* in the farm and food environment.

## 3. Research Achievements/Results

**Development of a quantitative assay for the enumeration of *E. coli* O157 and O26 in bovine faeces (Task 1)**

A real time PCR protocol was developed to enumerate VTEC O157 or O26 in the RAJ sample (Lawal *et al*, 2015) and the method was employed to examine carriage and shedding of O157 and O26 in beef and dairy cattle.

**Shedding of VTEC/STEC in beef animals (Task 2)**

The study on beef cattle showed that overall, 4.18% (55/1317) of RAJ samples were positive for STEC O157, and 2.13% (28/1317) were classified as STEC O157 SS ($Log_{10}$ 4-7.7 CFU $swab^{-1}$). For STEC O26 0.53% (7/1317) of cattle were positive and 0.23% (2/1317) were classified as SS ($Log_{10}$4.1- 5.8 CFU/ $swab^{-1}$). Fewer STEC shedders and super–shedders were noted among older animals (>37 months). There was a seasonal trend observed with highest prevalence of shedding and super shedding events observed in the autumn (August to October). It was noted that some farms were persistently positive with animals being STEC positive on repeat occasions many months apart.

**Shedding of VTEC in dairy animals (Task 3)**

In study 1 longitudinal study on 2 dairy herds, from Farm A: 305 animals analyzed; 15 *E. coli* O157 (5%) were recovered, 13 were denoted VTEC encoding either *vtx1* and/or *vtx2* virulence genes and 5 (2%) VTEC O26 were recovered. One super-shedder was identified shedding VTEC O26 (*stx1*&2). Farm B: 224 animals were analyzed; eight *E. coli* O157 (3.5%) were recovered (seven were STEC) and 9 (4%) VTEC O26 were recovered. Three super-shedders were identified, one was shedding STEC O157 (*vtx2*) and two STEC O26 (*vtx2*). Three encoded the adhering and effacement gene (*eae)* and one isolate additionally encoded the haemolysin gene (*hlyA*). All four super-shedders were only super-shedding

once during the 1-year sampling period. The results of this study show, low numbers of super-shedders in the herds examined, with high numbers of low and medium shedding. Although four super-shedding animals were identified, no STEC O157 or O26 were recovered from any of the raw milk, milk filter, or water samples. The study highlights the need for further surveillance to assess the potential for environmental contamination and food chain security.

In study 2, the surveillance study on 13 dairy herds (40 – 184 cattle) were visited once each between August 2014 and July 2015. Fifty-five *E. coli* O157 were recovered; 49 from rectal swabs (48 were virulent); three from waters (2 were virulent); two from milk filters (both virulent) and one raw milk (non-virulent). Twenty-five *E. coli* O26 were recovered from animals (13 were virulent). Five *E. coli* O26 super-shedders were identified (four were non-virulent). The *E. coli* O157 recovered from the raw milk sample was non-virulent; no super-shedders were identified in this herd. Interestingly during the study three animals were found to be colonizing both *E. coli* O157 and O26 at the same time.

**Whole genome-scale analysis of a collection of pathogenic *Escherichia coli* of public health importance (Task 4)**

78 isolates were selected from Tasks-2 and -3 and dispatched for whole genome sequencing (WGS). This study collection comprised 36 *Escherichia coli* of serotype O157, along with 13 O26 serotype isolates and 29 other serotypes. Of the 36 *E. coli* O157, 32 were sequenced typed (ST) from the WGS data as ST11; 2 were ST4087; 1 ST10 and 1 ST112. Using the ResFinder database to search these genomes for antibiotic resistance genotypes - all *E. coli* O157 contained *bla*-encoding genes that can confer resistance to penicillin, along with *tet* genes, conferring resistance to tetracycline. No other resistance-associated genotypes were identified. Pan-genome analysis of the *E. coli* O157 strains found to have a core genome consisting of 3,244 genes, covering 4,880,849 bp with a GC content of 51%.

The *E. coli* O26 studied had a core genome of 3,552 genes, covering 3,837,429 bp with a GC content of 52%. Of the 13 *E. coli* O26 identified and sequenced, 3 were ST21; 3 ST396; 1 ST29; 1 ST187 and 3 ST types that are unknown. All *E. coli* O26 contained *bla* genes. Six of the *E. coli* O26 were positive for *tet* genes, and as before, no other additional resistance genes were identified.

18 of the isolates sequenced were cultured from animals defined as being *super-shedders* and 15 isolates were from animals shedding smaller numbers of these bacteria. All the *E. coli* O157 *super-shedder* strains possessed the virulence genes *nleB, espA, nleC, iss, Iha, espP, stx2B, ehxA, stx2A, katP, etpD, gad, espB, espF, tir, espJ, eae, astA, hlyE, capU, espC, stx1B, iroN.* Interestingly, only these supper shedder (SS) isolates contained the *pic, toxB, senB, sta1, ltcA, perA* and *subA* virulence genes. The low-shedder strains contained the *sigA, pet, sat, lpfA, tccP, stx1A* and *nleA* virulence genes which were not present in in the SS cohort The *super-shedder* group contained three *E. coli* O26 isolates that formed a subgroup when clustered according to virulence gene content. The major difference observed here was the absence of *stx* genes found in the *E. coli* O157 super-shedders. A combined core genome of all shedder isolates was generated. This resulted in a combined core of 4,022,190bp, with 3,728 genes and a GC content of 51%. Finally, the *super-shedder* genomes were mapped onto the *E. coli* O157 Sakai reference for phylogenomic analysis. No separation of *super-* and *low-shedders* was observed in the core genome SNP tree suggesting the differences in these groups might occur within the accessory genomic regions.

**Assessment of virulence potential of methylation on selected pathogenic *Escherichia coli* (Task 5)**

In total 33 isolates were selected from **Tasks 2** and **3** and dispatched for whole genome sequencing (WGS). This study collection comprised 22 *Escherichia coli* of serotype O157, along with 11 O26 serotypes isolates cultured from animals defined as being *super-shedders*. These were compared with 45 isolates (11 O26; 34 O157) collected from animals defined as *low-shedders* in Tasks 2 and 3. After undertaking a thorough pangenomic and core genome analysis we found that while some minor gene differences exist, overall there were no clear genetic differences separating the super and low shedder

isolates. Similarly, phylogenomic analysis did not separate the super- and low- shedders on the basis of core genome SNPs.

**Metagenomic compositional analysis** (**Task 2, 3**)
The composition of the bacterial community was assessed at the recto anal junction (RAJ) of beef and dairy cattle with the RAJ samples (n =119) selected based on metadata including STEC O157 positive animals (LS and SS) (n= 55); STEC O26 positive animals (LS and SS) (n= 7); STEC negative animals (n = 54) within each group animals were selected of differing age and mix of seasonal positive.

Metagenomic analysis of RAJ samples showed the principle phyla across three animals groups (STEC SS, LS and negative animals) were *Bacteroides* (~40%) and *Firmicutes* (~50%) and the principle genera in all RAJ samples were *Ruminococcoae* (30%), *Prevotella* (10%) and 60% a variety of genera. Overall the results indicated a high level of variability in operational taxonomic units between the animal groups. Principle Co-ordinate Analysis showed no significant difference in microbiota composition at the RAJ based on shedding status, animal age, or seasonality.

**Persistence and transduction of $vt_1$ and $vt_2$ bacteriophage in the beef farm environment (Task 6)**
Studies investigating the survival of a temperate vtx bacteriophage ($24_B$ ::kanamycin$^R$ ) in water (raw farm, pasteurized farm, laboratory tap and autoclaved purified water) and soil (sandy loam and loam soil), bovine faeces and slurry showed the phages survived albeit with some reductions observed, regardless of matrix or storage temperature. Moreover, $24_B$ :kanamycin$^R$ was able to transduce its host *E. coli* strain.

## 4. Impact of the Research

### 4(a) Summary of Research Outcomes

This study showed in both beef and dairy cattle that there was an overall low prevalence of *E. coli* O157 /O26 shedding but in positive animals shedding of high numbers of the pathogen was frequent. Some farms were persistently positive for *E.coli* O157 or O26 with both super shedding and low shedding animals detected. Shedding of *E. coli* O157 was generally highest in autumn. This showed some possible genetic differences in SS and LS strains but further analysis and phenotypic studies on SS and LS strains are required. Such whole genome sequencing data provide an overview of the STEC circulating among healthy Irish cattle providing an indication of the potential risks to public health linked to STEC cultured from cattle. The WGS data can also be further analysed to identify novel biomarkers for detection assays and monitoring for emerging pathogens. The project has provided new information on the survival and potential for *vtx* gene transfer in farming environments. With the ongoing emergence of new VTEC strains, impacting on public health, such information provides an important start in developing VTEC control strategies. The scientific knowledge and information generated in this project is helping to direct management practices and policy for addressing VTEC by food industry (meat and dairy), FSAI and DAFM, and is supporting export market access, including US beef markets.

(i) Collaborative links developed during this research

Links were developed with:

United States Department of Agriculture (USDA), Dr James Bono who collaborated in the project on whole genome sequencing of VTEC strains.

Dr Claire Jenkins, PHL, Colindale, UK who collaborated in the project on whole genome sequencing of VTEC strains.

Dr Rob Barlow and Dr Glen Mellor, CSIRO, Brisbane, Australia  Zealand who are new collaborators on shedding and carriage of VTEC in cattle and beef.

Dr Adrian Cookson, AgResearch, Palmerston North, New Zealand who is a new collaborators on shedding and carriage of VTEC in cattle and beef.

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

A real time PCR protocol was developed (Lawal *et al*, 2015). The method involved a 5h enrichment followed by real time PCR targeting *rfb*E (O157) and *wzx* (O26). Counts (CFU swab$^{-1}$) were obtained from a standard calibration curve, relating the real time PCR cycle threshold ($C_t$) values against the initial concentration (CFU g$^{-1}$) of O157 or O26 in the RAJ sample. The method was employed by the research consortia (Teagasc Cork county Council) to examine carriage and shedding of O157 and O26 in beef and dairy cattle. It is now being adopted in follow on research study to examined VTEC shedding by sheep.

(iii) Outcomes with economic potential

This was a predominantly public good, knowledge project, however Foodwise 2025 highlights that a foodborne outbreak is a key threat across all commodities which could impact on growth opportunities for Irish food. Market access to new global markets will require scientific evidence of the highest standards of safety in Irish foods and may also require the Irish Food Industry to meet different sets of food regulations in the new market. A key pathogen of concern in this regard is Verocytoxigenic/ Shigatoxigenic *E.coli*, which can cause serious illness in humans and is also important in terms of market access regulations for beef being exported to the USA.  As such this project is providing the science to support stakeholders in the meat sector in the risk based control of this pathogen and in securing market access.

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

This project is providing the science to support stakeholders in the public health and regulatory sector on dynamics and types of VTEC shed by Irish cattle and is thus supporting the risk based control of this pathogen and in protecting consumer health.

**4 (b) Summary of Research Outputs**

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

Lawal, D., Burgess, C., McCabe, E., Whyte, P. and Duffy, G. (2015). Development of a quantitative real time PCR assay to detect and enumerate *Escherichia coli* O157 and O26 serogroups in bovine recto-anal swabs *J. Micro methods* 114:9-15.

McCabe, E., Lawal, D., Burgess, C Whyte, P. and Duffy, G. (2018). An investigation of shedding and super-shedding of Shigatoxigenic *E. coli* O157 and *E. coli* O26 in cattle presented for slaughter in the Republic of Ireland (*in press, Zoonoses and Public Health*)

Murphy, B.P., McCabe, E., Murphy, M., Buckley, J.F., Crowley, D., Fanning, S., and Duffy, G. (2016). Longitudinal Study of Two Irish Dairy Herds: Low Numbers of Shiga Toxin-Producing *Escherichia coli* O157 and O26 Super-Shedders Identified. *Front Microbiol*. 18; 7:1850

Nyambe, S., Burgess, C., Whyte, P. and Bolton D. J. (2016). The survival of a temperate vtx bacteriophage and an anti-verocytotoxigenic *Escherichia coli* O157 lytic phage in water and soil samples. Zoonoses and Public Health, 63(8), 632-640.

Nyambe, S., Burgess, C., Whyte, P. and Bolton D. J. (2016). Survival studies of a temperate and lytic bacteriophage in bovine faeces and slurry. *Journal of Applied Microbiology*, 121(4):1144-1151.

Nyambe S, Burgess C, Whyte P, Bolton D. (2017) An investigation of $vtx_2$ bacteriophage transduction to different Escherichia coli patho-groups in food matrices and nutrient broth. *Food Microbiol*. 68:1-6.

Nyambe, S., Burgess, C., Whyte, P., O'Kiely, P. and Bolton D. J. (2016). The fate of Verocytotoxigenic *Escherichia coli* C600 and its' $vtx_2$ prophage during grass silage preparation. *J Appl Microbiol.* 122(5):1197-1206.

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

Duffy G, McCabe E., an Burgess, C (2017).Risk factors for shedding of shigatoxigenic *E. coli* O157 and O26 in cattle at slaughter. *International Congress of Meat Scientists* (ICoMst). Cork, August 13 to 17th 2017.

Duffy, G. (2016). Verocytoxigenic *E.coli* carriage in food production animals and transmission risks in meat slaughter. Environmental Health Forum. *Climate for Change*, Radisson Blu Hotel Galway. May 26th to 27th 2016

McCabe, E, Burgess, C, Whyte, P and Duffy, G (2016). An assessment of *E. coli* O157 and O26 shedding dynamics and super-shedding status in Irish cattle at slaughter. 25th International Committee on Food Microbiology and Hygiene (ICFMH) Conference, Food Micro 2016, 19th-22nd July 2016, University College Dublin, Ireland.

McCabe, E, Burgess, C, Whyte, P and Duffy, G (2016). An assessment of *E. coli* O157 and O26 shedding dynamics and super-shedding status in Irish cattle at slaughter. IuFost VTEC. IUFoST 18th World Congress of Food Science and Technology. 21st – 25th August 2016, Dublin, Ireland

Nyambe, S. K. Burgess, P. Whyte, D.J. Bolton (2016) A study assessing transduction and persistence of a verocytotoxin bacteriophage during the ensiling of grass. Poster presentation at the FOOD MICRO International Conference, University College Dublin, 19th to 22nd July 2016, Abstract book, page 376.

Nyambe, S., Catherine Burgess, Paul Whyte and Declan Bolton (2016). A study assessing transduction and persistence of a verocytotoxin bacteriophage during the ensiling of grass. Poster presentation at the IUFoST International Conference, RDS Dublin, 21st to 25th August, 2016, Abstract Book, page 681.

Duffy, G., (2015). Investigation of E. coli O157 and O26 shedding dynamics and super-shedding status in Irish cattle at slaughter. VTEC Knowledge Network Annual Conference 2015.

Murphy. B, (2015). Investigation into VTEC O157 and O26 super-shedding in dairy herds in Ireland, and its impact on raw milk contamination. VTEC Knowledge Network Annual Conference 2015.

Lawal, D., K. Burgess, E. McCabe, P. Whyte, G. Duffy, Development of a quantitative real time PCR assay to detect and enumerate *Escherichia coli* O157 and O26 serogroups in recto-anal junction (RAJ) samples and rapidly identify(Poster). 9th International Symposium on Shiga Toxin (Verocytotoxin)-producing Escherichia coli Infections (VTEC 2015), Boston, September 13, 2015 - September 16, 2015.

Lawal, D., Evonne McCabe, Catherine Burgess, Paul Whyte and Geraldine Duffy. Investigation of the shedding dynamics of E. coli (O157 and O26) in Irish cattle at slaughter (oral). 9th International Symposium on Shiga Toxin (Verocytotoxin)-producing *Escherichia coli* Infections (VTEC 2015), Boston, September 13, 2015 - September 16, 2015.

Lawal, D., Catherine Burgess, Evonne McCabe, Paul Whyte and Geraldine Duffy. Validation of quantitative PCR method for VTEC O157 and O26 in Bovine faeces. Oral presentation at the Safe food knowledge on VTEC conference, on the 18th of October 2013 at Teagasc Food Research Centre Ashtown. Dublin 15.

Lawal, D., Catherine Burgess, Evonne McCabe, Paul Whyte and Geraldine Duffy. Development of a rapid PCR method to quantify verocytotoxigenic *E. coli* (O157 and O26) in bovine faeces. Poster presentation at the Veterinary Officers Association (VOA), on the 4th and 5th of April 2014 at Dublin Carlton Airport Hotel.

Burgess, C., Dolapo Lawal, Evonne McCabe, Paul Whyte and Geraldine Duffy. Development of a rapid PCR method to quantify verocytotoxigenic E. coli (O157 and O26) in bovine faeces. Poster presentation at the 17th World Congress of Food Science & Technology from August 17th - 21st, 2014, in Montreal, Canada.

Duffy, G., Dolapo Lawal, Catherine Burgess, Evonne McCabe and Paul Whyte. Development of a rapid PCR method to quantify *E. coli* (O157 AND O26) in bovine faeces. Poster presentation at the 60th International Congress of Meat Science and Technology, on 17-23rd August 2014, at Punta del Este, Uruguay.

McCabe, E., Dolapo Lawal, Catherine Burgess, Paul Whyte and Geraldine Duffy. Development of a rapid PCR method to quantify verocytotoxigenic *E. coli* (O157 and O26) in bovine faecal swabs. Poster presentation at the 24th International ICFMH Conference, from 1st-4th September in Nantes France.

McCabe, E., Dolapo Lawal, Catherine Burgess, Paul Whyte and Geraldine Duffy. Application of quantitative PCR method for VTEC O157 and O26 in bovine faeces at slaughter. Oral presentation at the Safe food knowledge network on VTEC conference, on the 21st of October 2014 at Crowne Plaza hotel Blanchardstown. Dublin 15.

Lawal, D., Catherine Burgess, Evonne McCabe, Paul Whyte and Geraldine Duffy Investigating Shedding dynamics of *E. coli* (O157 AND O26) in Irish cattle at slaughter. Oral presentation at the 43rd Annual Food Research Conference on 10th of December 2014 at University College Dublin. Belfield, Dublin 4.

Nyambe, S., C. Burgess, Paul Whyte and D. Bolton (2015). Survival of a temperate vtx and a lytic bacteriophage in water and soil. Poster presentation at the Oxford Bacteriophage Conference, Phages 2015, held at St Hilda's College, Oxford, United Kingdom on the 1st-2nd September 2015.

Nyambe, S., C. Burgess, Paul Whyte and D. Bolton (2015). A study assessing transduction and persistence of a verocytotoxin (vtx) bacteriophage during the ensiling of grass. Oral presentation at the Safefood VTEC Conference, held at the Plaza hotel Dublin 15 on the 10st November 2015.

Nyambe,S., C. Burgess, Paul Whyte and D. Bolton (2015). A study assessing transduction and persistence of a verocytotoxin (vtx) bacteriophage during the ensiling of grass. Poster presentation at the 44th Annual Food Research Conference, Teagasc Food Research Centre, Moorepark, 14th December 2015.

Nyambe, S., K. Burgess, P. Whyte and D. Bolton (2013). Potential for the emergence of new VTEC strains with human virulence potential as a result of horizontal gene transfer. Oral presentation (A5.2) at the 42nd Annual Food Research Conference, Teagasc Food Research Centre, Ashtown, 27th and 28th June 2013.

Nyambe S., and D. Bolton (2014) The survival of vtx2 encoding bacteriophage in water. Poster 2, page 2 of the Abstract Book, at the All-Island State Veterinarians Conference 2014, held at the Dublin Carlton Airport Hotel, 4-5th April, 2014.

Nyambe. S., and D. Bolton (2014). Survival of a temperate and lytic bacteriophage in water. Poster presentation at the Safefood VTEC Conference, held at the Plaza hotel Dublin 15 on the 21st October 2014.

Nyambe, S., Paul Whyte and D. Bolton (2014). Survival of a temperate and lytic bacteriophage in beef ecological niches. Oral presentation at the Safefood VTEC Conference, Annual food safety conference, held at the University College Dublin on the 10-1th December 2014.

Rogers, L., S. Fanning and P. Ó Gaora (2014). Optimisation of Genome Assembly Strategies. Poster presentation at the PhD Symposium in Computational Biology & Innovation, on the 4-5th December 2014 at University College Dublin. Belfield, Dublin 4.

Rogers, L., S. Fanning and P. Ó Gaora (2015). A Genomic Analysis of Verotoxigenic Escherichia coli. Poster presentation at the Wellcome Trust Final Years meeting, on the 16th October 2015 at Wellcome Trust. Gibbs Building. 215 Euston Road. London NW1 2BE. UK .

Rogers, L., S. Fanning and P. Ó Gaora (2015). A Genomic Analysis of Verotoxigenic Escherichia coli. Oral presentation at the Safefood knowledge on VTEC conference, on the 10th of November 2015 at Crowne Plaza hotel Blanchardstown. Dublin 15.

Rogers, L., S. Fanning and P. Ó Gaora (2015). A Genomic Analysis of Verotoxigenic Escherichia coli. Poster presentation at the PhD Symposium in Computational Biology & Innovation, on the 3rd-4th December 2015 at University College Dublin. Belfield, Dublin 4.

(iii) National Report

Not relevant

(iv) Workshops/seminars at which results were presented

*International Congress of Meat Scientists* (ICoMst). Cork, August 13 to 17th 2017.
Risk factors for shedding of shigatoxigenic *E. coli* O157 and O26 in cattle at slaughter.

Environmental Health Forum. *Climate for Change*, Radisson Blu Hotel Galway. May 26th to 27th 2016
Verocytoxigenic *E.coli* carriage in food production animals and transmission risks in meat slaughter.

International Committee on Food Microbiology and Hygiene (ICFMH) Conference, Food Micro 2016, 19th-22nd July 2016, University College Dublin, Ireland.
An assessment of *E. coli* O157 and O26 shedding dynamics and super-shedding status in Irish cattle at slaughter. 25th

IUFoST 18th World Congress of Food Science and Technology. 21st – 25th August 2016, Dublin, Ireland.
An assessment of *E. coli* O157 and O26 shedding dynamics and super-shedding status in Irish cattle at slaughter. IuFost VTEC.

FOOD MICRO International Conference, University College Dublin, 19th to 22nd July 2016,
A study assessing transduction and persistence of a verocytotoxin bacteriophage during the ensiling of grass.

IUFoST International Conference, RDS Dublin, 21st to 25th August, 2016,
A study assessing transduction and persistence of a verocytotoxin bacteriophage during the ensiling of grass. Poster presentation at the Abstract Book, page 681.

VTEC Knowledge Network Annual Conference 2015.
Investigation of E. coli O157 and O26 shedding dynamics and super-shedding status in Irish cattle at slaughter.

VTEC Knowledge Network Annual Conference 2015.
Investigation into VTEC O157 and O26 super-shedding in dairy herds in Ireland, and its impact on raw milk contamination.

9th International Symposium on Shiga Toxin (Verocytotoxin)-producing Escherichia coli Infections (VTEC 2015), Boston, September 13, 2015 - September 16, 2015.
Development of a quantitative real time PCR assay to detect and enumerate *Escherichia coli* O157 and O26 serogroups in recto-anal junction (RAJ) samples and rapidly identify.

9th International Symposium on Shiga Toxin (Verocytotoxin)-producing *Escherichia coli* Infections (VTEC 2015), Boston, September 13, 2015 - September 16, 2015
Investigation of the shedding dynamics of E. coli (O157 and O26) in Irish cattle at slaughter.

Safe food knowledge on VTEC conference, on the 18th of October 2013 at Teagasc Food Research Centre Ashtown. Dublin 15.
Validation of quantitative PCR method for VTEC O157 and O26 in Bovine faeces.

Veterinary Officers Association (VOA), on the 4th and 5th of April 2014 at Dublin Carlton Airport Hotel.
Development of a rapid PCR method to quantify verocytotoxigenic *E. coli* (O157 and O26) in bovine faeces.

17th World Congress of Food Science & Technology from August 17th - 21st, 2014, in Montreal, Canada.
Development of a rapid PCR method to quantify verocytotoxigenic E. coli (O157 and O26) in bovine faeces.

60th International Congress of Meat Science and Technology, on 17-23rd August 2014, at Punta del Este, Uruguay.
Development of a rapid PCR method to quantify *E. coli* (O157 AND O26) in bovine faeces.

24th International ICFMH Conference, from 1st-4th September in Nantes France.

Development of a rapid PCR method to quantify verocytotoxigenic *E. coli* (O157 and O26) in bovine faecal swabs.

Safe food knowledge network on VTEC conference, on the 21st of October 2014 at Crowne Plaza hotel Blanchardstown. Dublin 15.
Application of quantitative PCR method for VTEC O157 and O26 in bovine faeces at slaughter.

43rd Annual Food Research Conference on 10th of December 2014 at University College Dublin. Belfield, Dublin 4.
Investigating Shedding dynamics of *E. coli* (O157 AND O26) in Irish cattle at slaughter.

Oxford Bacteriophage Conference, Phages 2015, held at St Hilda's College, Oxford, United Kingdom on the 1st-2nd September 2015.
Survival of a temperate vtx and a lytic bacteriophage in water and soil.

Safefood VTEC Conference, held at the Plaza hotel Dublin 15 on the 10st November 2015.
A study assessing transduction and persistence of a verocytotoxin (vtx) bacteriophage during the ensiling of grass

44th Annual Food Research Conference, Teagasc Food Research Centre, Moorepark, 14th December 2015.
A study assessing transduction and persistence of a verocytotoxin (vtx) bacteriophage during the ensiling of grass.

42nd Annual Food Research Conference, Teagasc Food Research Centre, Ashtown, 27th and 28th June 2013.
Potential for the emergence of new VTEC strains with human virulence potential as a result of horizontal gene transfer.
All-Island State Veterinarians Conference 2014, held at the Dublin Carlton Airport Hotel, 4-5th April, 2014.
The survival of vtx2 encoding bacteriophage in water.

Safefood VTEC Conference, held at the Plaza hotel Dublin 15 on the 21st October 2014. Survival of a temperate and lytic bacteriophage in water.

Safefood VTEC Conference, Annual food safety conference, held at the University College Dublin on the 10-1th December 2014.
Survival of a temperate and lytic bacteriophage in beef ecological niches.
Symposium on Computational Biology & Innovation, on the 4-5th December 2014 at University College Dublin. Belfield, Dublin 4.
Optimisation of Genome Assembly Strategies.

Wellcome Trust Final Years meeting, on the 16th October 2015 at Wellcome Trust. Gibbs Building. 215 Euston Road. London NW1 2BE. UK.
A Genomic Analysis of Verotoxigenic *Escherichia coli*.

Safefood knowledge on VTEC conference, on the 10th of November 2015 at Crowne Plaza hotel Blanchardstown. Dublin 15.
A Genomic Analysis of Verotoxigenic Escherichia coli.

Symposium in Computational Biology & Innovation, on the 3rd-4th December 2015 at University College Dublin. Belfield, Dublin 4.

(v) Intellectual Property applications/licences/patents

Not relevant

(vi) Other

Not relevant

## 5. Scientists trained by Project

Total Number of PhD theses: 2

Sepo Nyambe, University College Dublin
The potential for the emergence of new strains of verocytotoxigenic *E. coli* as a result of horizontal gene transfer, 23rd May 2017

Lisa Rogers (2017) University College Dublin
Genomic characterisation of environmental verocytotoxigenic *Escherichia coli*
17th July 2017

Total Number of Masters theses: None

## 6. Permanent Researchers

| Institution Name | Number of Permanent staff contributing to project | Total Time contribution (person years) |
|---|---|---|
| Teagasc | 3 | 1.192 |
| UCD | 1 | 0.400 |
| CCC | 2 | 0.800 |
| **Total** | **6** | **2.392** |

## 7. Researchers Funded by DAFM

| Type of Researcher | Number | Total Time contribution (person years) |
|---|---|---|
| Post Doctorates/Contract | 2 | 5.376 |
| Researchers | 1 | 0.524 |
| PhD students | 2 | 6.999 |
| Masters students | | |
| Temporary researchers | | |
| Other | | |
| **Total** | **5** | **12.899** |

## 8. Involvement in Agri Food Graduate Development Programme

| Name of Postgraduate / contract researcher | Names and Dates of modules attended |
|---|---|
| Sepo Nyambe | Agri-food Career management module UCC (23-25 November 2015). |
| Sepo Nyambe | Science writing and presentation skills for the Agri-food researcher UCC (22-24th October 2013). |
| Sepo Nyambe | Media and Communication skills training programme, Teagasc (4-5 March 2014). |
| Sepo Nymbe | AFGDP/FHI Summer school, UCD (11-13 June 2014). |

## 9. Project Expenditure

Total expenditure of the project: €811,647.35

Total Award by DAFM: €943,566.40

Other sources of funding including benefit in kind and/or cash contribution(specify): €0

Breakdown of Total Expenditure

| Category | Teagasc | UCD | Cork County Council | Name Institution 4 | Total |
|---|---|---|---|---|---|
| Contract staff | 0 | 21927.43 | 82466.10 | 0 | 104393.53 |
| Temporary staff | 0 | 0 | 0 | 0 | 0 |
| Post doctorates | 152770.08 | 0 | 0 | 0 | 152770.08 |
| Post graduates | 87750 | 65999.88 | 0 | 0 | 153749.88 |
| Consumables | 68340.10 | 47781.44 | 80584.42 | 0 | 196705.96 |
| Travel and subsistence | 5904.46 | 0 | 3688.25 | 0 | 9592.71 |
| Sub total | 314764.64 | 135708.75 | 166738.77 | 0 | 617212.16 |
| Durable equipment | 0 | 0 | 0 | 0 | 0 |
| Other | 0 | 0 | 9271.54 | 0 | 9271.54 |
| Overheads | 94429.39 | 40712.63 | 50021.63 | - | 185163.65 |
| **Total** | **409194.03** | **176421.38** | **226031.94** | **0** | **811647.35** |

## 10. Leveraging

This project has leveraged the group to obtain funding in:

Project from FIRM on "Surveillance of Verocytotoxigenic *E. coli* in Ireland: A One Health Approach" (Ref 15F629). It has helped build up capacity on both VTEC and Whole Genome Sequencing and maintained Ireland's international profile in these areas. This project is funded to € 1,243,523.20

The capacity building in metagenomics at Teagasc has leveraged the institute and researchers (K Burgess and G Duffy) to host a recently funded EU Marie Curie Career fit Post-doctoral researcher (Amélie Rouger) to work on *Utilisation of next generation sequencing technology to support meat safety and shelf life* extension: Funding Source: Enterprise Ireland (EI) Career-Fit

The capacity building has supported Teagasc role in newly funded H2020 COFUND-EJP
Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards.

Teagasc through its Walsh fellowships Programme has funded an MSc (Jennifer Gray) to carry out further phenotypic and genotypic studies on VTEC isolates recovered in the current study from super shedding and low shedding beef and dairy cattle.

## 11. Future Strategies

This project builds on research expertise on VTEC by this consortia, addressing issues related to detection, characterisation, virulence profiling, transmission, behaviour and control of this group of these emergent pathogens. This project has also built on the national capacity on Whole Genome Sequencing forfood pathogens. Such capacity building and scientific knowledge on VTEC provides essential risk assessment information in an Irish context on this key pathogen to underpin national stakeholder risk management strategies. Strengthening the research expertise has also enabling the group to successfully compete for further research funding both national and European in the key areas of VTEC and in application of next generation sequencing technologies to food safety applications.