**FS101055: *E. coli* O157 super-shedding in cattle and mitigation of human risk**

**Project report 30/09/15**

**Objective 1:** To determine the excretion dynamics and transmission frequencies of wild type *E. coli* O157 strains under controlled experimental conditions

**Progress relating to Deliverables:**

Deliverables relating to this objective are detailed in Table 1.

Deliverables 1.1.1, 1.1.2, 1.1.3, 1.1.4, 1.1.5, 1.1.6, 1.1.7, 1.2.1, 1.2.2, 1.2.3, 1.2.4:

These deliverables involved the oral challenge of calves with a PT21/28 *E. coli* O157 strain associated with super-shedding and a PT32 *E. coli* O157 strain not associated with super-shedding. Excretion levels from colonized calves as well as transmission from colonized to naive in-contact calves were measured for each strain. Levels of environmental contamination were also determined. Data has collated in an appropriate format for statistical and mathematical modelling. Statistical analysis of the excretion and transmission data has been performed but, due to recruitment issues at BioSS, analysis of excretion data is yet to be completed. From all animals systemic and local (rectal) immune responses to key EHEC antigens have been quantified. Therefore these objectives have been met, with the exception of 1.1.6 which is currently underway.

Deliverable 1.3.1:

This deliverable aims to determine the contribution of the Stx2 prophage to excretion and transmission dynamics in colonized calves. We have now compared directly compared excretion dynamics of the wild-type PT21/28 E. coli O157 strain with isogenic mutants either lacking the Stx2a encoding prophage or with a repaired Stx2a gene. Data has collated in an appropriate format for statistical and mathematical modelling. Statistical analysis of the excretion data is currently underway.

**Table 1.** Objective 1 deliverables

|  |  |  |  |
| --- | --- | --- | --- |
| **DELIVERABLE NUMBER** | **TARGET**  **DATE** | **DELIVERABLE DETAILS** | **PROGRESS** |
| **1.1.1** | **01/10/2014** | Excretion data for animals that have naturally-acquired a wild type PT21/28  (super-shedding lineage III) *E. coli* O157 strain | Completed |
| **1.1.2** | **01/04/2015** | Excretion data of animals that have naturally-acquired a wild type PT32  (non super-shedding lineage IV) *E. coli* O157 strain | Completed |
| **1.1.3** | **01/04/2015** | Quantification of the extent of environmental contamination in the different  groups. | Completed |
| **1.1.4** | **01/06/2015** | Measures of cellular and humoral responses of animals to key EHEC  antigens during colonisation with two wild type *E. coli* O157s | Completed |
| **1.1.5** | **01/12/2015** | Measures of innate responses from rectal pinch biopsies and both rectal  follicles and local lymph nodes during colonisation with two wild type *E. coli* O157s | Completed |
| **1.1.6** | **01/09/2015** | Statistical analyses of excretion data from PT21/28 and PT32 strains | Ongoing |
| **1.1.7** | **01/05/2015** | To provide numerical data for modelling under Objective 3. | Completed |
| **1.2.1** | **01/10/2014** | Transmission frequencies from animals that have naturally-acquired wild  type PT21/28 *E. coli* O157 strains to in-contact naïve animals. | Completed |
| **1.2.2** | **01/04/2015** | Transmission frequencies from animals that have naturally-acquired wild  type PT32 *E. coli* O157 strains to in-contact naïve animals. | Completed |
| **1.2.3** | **01/09/2015** | Statistical analyses of transmission data. | Completed |
| **1.2.4** | **01/05/2015** | To provide numerical data for modelling under Objective 3 | Completed |
| **1.3.1** | **01/03/2015** | Excretion and transmission dynamics for a PT21/28 strain (super-shedding lineage III) *E. coli* O157 strain no longer containing the Stx2 prophage | Ongoing |

**Progress in relation to Sub-tasks:**

***01/01/01*** *Generation of bacterial excretion data for animals that have naturally-acquired a wild type PT21/28 (super-shedding) E. coli O157 strain* **(COMPLETED)**

***01/01/02*** *Generation of excretion data of animals that have naturally-acquired a wild type PT32 (non super-shedding) E. coli O157 strain* **(COMPLETED)**

***01/01/03*** *Measures of innate and adaptive immune responses of animals in response to colonisation with wild type E. coli O157 strains* **(COMPLETED)**

***01/01/04*** *Quantification of the extent of environmental contamination by environmental sampling* **(COMPLETED)**

***01/01/05*** *Statistical analyses of shedding and environmental data for the PT21/28 vs. PT32 E. coli O157 strains* (**ONGOING)**

***01/01/06*** *Collation of numerical data for modelling under Objective 3* **(COMPLETED)**

***01/02/01*** *Transmission frequencies from animals that have naturally-acquired wild type PT21/28 and PT32 E. coli O157 strains to in-contact naive animals* **(COMPLETED)**

***01/02/02*** *Statistical analysis of transmission data to determine if differences exist between the PT21/28 and PT32 strains* **(COMPLETED)**

***01/02/03*** *Collation of numerical data for modelling under Objective 4* **(COMPLETED)**

***01/03/01*** *Excretion and transmission data for a PT21/28 (super-shedding Lineage III) E. coli O157 strain no longer containing the Stx2 prophage* **(ONGOING)**

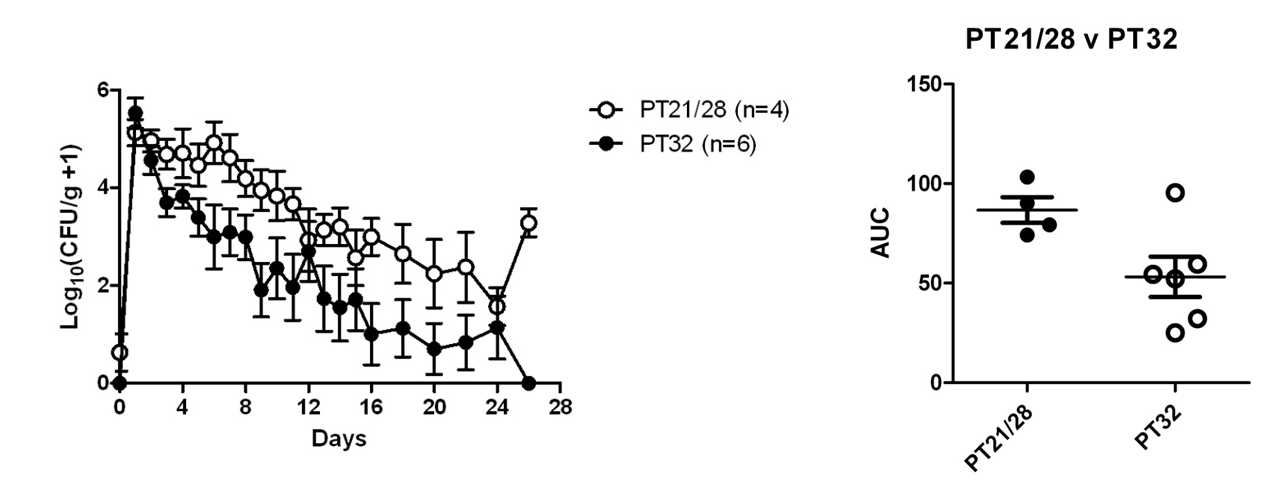
**Scientific progress**

Deliverables 1.1 and 1.2

We have now completed transmission and excretion studies for a wild-type PT21/28 strain (9000) and a wild-type PT32 *E. coli* O157 strain (10671). For each strain 16 Holstein-Friesian calves, screened as negative for *E. coli* O157 on 4 separate occasions (8, 9, 10, and 11 weeks of age) by both immunomagnetic separation (IMS) and quantitative PCR techniques entered the Moredun High Security Unit, and were assigned to three separate rooms (C1 (n=6), C2 (n=5), C3 (n=5)). Additional *E. coli* O157 negative spare calves (n=5 for the PT21/28 challenge study and n=4 for the PT32 challenge study) acted as unchallenged controls for immunological studies and were housed in the Moredun farm at CL2.

All six calves in room C1 were orally challenged with ~3 × 109 CFU *E. coli* O157 marked with nalidixic acid resistance and levels of bacteria within the faeces monitored on a daily basis using nalidixic acid containing Sorbitol MacConkey agar (NAL-SMAC) plates. At 5 days post-challenge, one colonized calf each from C1 was moved into pens C2 and C3 to act as a source of infection for the naive in-contact sentinel calves, and levels of *E. coli* O157 challenge strain within faeces and the environment were monitored on a daily basis.

We observed higher levels of shedding in calves orally challenged with the PT21/28 strain compared to the PT32 strain, consistent with previous epidemiological evidence (Fig.1).



**Figure 1: Levels of *E. coli* O157 within faeces of calves either orally challenged with either a PT21/28 or PT32 strain of *E. coli* O157.** (A) Mean daily bacterial shedding from calves orally challenged with ~3 x 109 CFU of either a PT21/28 (9000) or a PT32 (10671) *E. coli* O157 strain. (B) Total bacterial shedding over the study period, as determined by the area under the curve (AUC) for calves challenged with the PT21/28 or a PT32 *E. coli* O157 strain.

For the PT21/28 strain, all (10/10) sentinel calves became positive for the challenge strain on at least one occasion, and one of the sentinel calves shed high (>104) levels of the challenge strain for a three day period (Fig. 2A). In contrast, for the PT32 strain the challenge strain was not detected in any of the sentinel calves (Fig. 2B). Therefore, a highly significant difference in transmission was observed between the PT21/28 and PT32 wild-type strains (Table 2). Furthermore, environmental levels of each strain mirrored the mean shedding level of calves within the pen suggesting little replication of the bacteria within the environment (Fig. 2).

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**Figure 2: Levels of *E. coli* O157 within faeces of naive sentinel calves in contact with calves super-shedding either a PT21/28 or PT32 strain of *E. coli* O157.** Calves 103479C and 301098C were orally challenged with either (A) wild-type PT21/28 *E. coli* O157 strain 9000 (calves 103479C and 301098C) or (B) wild-type PT32 *E. coli* O157 strain 10671 (101883C and 401879C). Five days later experimentally infected calves were moved into rooms containing five naive sentinel calves each (one experimentally infected calf per pen). Levels of the challenge strain within the faeces of sentinel calves and the environment were determined over a 21 day period. PT21/28 challenge strain was detected in the faeces of all sentinel calves on at least on occasion with one sentinel calf (201205S) shedding high (>104 CFU/g faeces) levels of bacteria for a three day period. PT32 challenge strain was not detected in any of the sentinel calves during the study period.

Finally, we have evaluated systemic and local humoral and cellular immune responses to key EHEC antigens in these studies. No significant cellular immune response against type-three secretion system proteins within the circulating peripheral lymphocyte population. However, challenge with both PT21/28 and PT32 strains resulted in the generation of IgA responses against H7 flagellin and Tir both within serum (data not shown). These results are consistent with previous studies suggesting that shigatoxigenic *E. coli*’s are more able to suppress cellular rather than humoral adaptive immune responses (Hoffman *et al.*, 2006).

**Table 2**: Transmission of PT21/28 vs. PT32 E. coli O157 to in-contact sentinel calves

|  |  |  |  |
| --- | --- | --- | --- |
| ***E. coli* O157 strain** | **Positive sentinels** | **Negative sentinels** | **P value (Fishers Exact test)** |
| PT21/28 (9000) | 10 | 0 | *P* < 0.0001 |
| PT32 (10671) | 0 | 10 |

Deliverable 1.3

We have now performed an experimental challenge study to compare excretion dynamics of the wild-type PT21/28 strain with isogenic strains where either the Stx2a-encoding prophage has been removed, or where the Stx2a gene, which contains an inactivating insertion sequence element (ISEc8) in the wild-type strain, has been removed to allow functional Stx2a to be produced. Calves (n=6) were challenged with ~3 × 109 CFU of each strain and daily bacterial excretion monitored over a four week period. Data is now being analysed but it appears that either genetic modification has little effect on bacterial excretion (Figure 3).

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**Figure 3: Levels of *E. coli* O157 within faeces of naive sentinel calves in contact with calves orally challenged with either wild-type PT21/28, an isogenic strain with a repaired Stx2a gene or an isogenic strain with the Stx2a-encoding prophage removed.** Calves (n=6 per strain) were orally challenged with ~3 × 109 CFU of either wild-type PT21/28 strain 9000, an isogenic strain with a functional Stx2a gene (Stx2a repaired) or an isogenic strain with the St2a encoding prophage removed (PT21/28 ΔStx2a prophage). Levels of each challenge strain were monitored for 25 days.

**(II) PhD Studentship 1 (Sub-task 01/01/03)**

This studentship aims to analyse the relationship between cattle innate and adaptive immune responses and shedding levels of *E. coli* O157 PT21/28 and PT32. To date, the student has demonstrated that antibody responses to certain *E. coli* O157 antigens are depressed in cattle shedding high levels of *E. coli* O157 in the field. This suggests that infections with these bacteria are capable of suppressing adaptive immune responses. Subsequent to this, the student has analysed EHEC antigen-specific immune responses in experimental challenge studies performed in Objective 1, as well as determining the effects of colonization with either the PT21/28 or the PT32 *E. coli* O157 strain on the ability of cattle to respond to concurrent immunization with the model antigen ovalbumin. This will determine whether any immunomodulatory effects of shigatoxigenic *E. coli*’sare limited to *E. coli* O157-specific immune responses or can affect immune responses to unrelated antigens, so called bystander effects.

To date, it appears that challenge of cattle with either E. coli O157 strain elicits only weak *E. coli* specific cellular immune responses, consistent with previous studies, but does induce IgA responses to particular *E. coli* O157 antigens including H7 flagellin and Tir. Interestingly, colonization with *E. coli* O157 appears to affect responses to systemically administered ovalbumin, resulting in suppressed levels of circulating ovalbumin-specific T cells but an enhancement of ovalbumin-specific IgG1 and CD8 responses and IgG1 in the lymph node draining the site of immunization, particularly in PT21/28 challenged calves (Fig. 4). When calves are challenged with the PT21/28 strain with the repaired Stx2a gene, this enhancement effect in the draining lymph node is lost (data not shown), indicating that possession of a functional Stx2a gene suppresses this immune-stimulatory effect. Together, these suggest that colonization with Shigatoxigenic *E. coli* O157 can modulate immune responses to unrelated antigens, and that these effects appear to be related to the strain and/or shigatoxin repertoire of the bacteria. Work is currently underway to determine the underlying mechanisms for this immune modulation.

**References**

Hoffman MA, Menge C, Casey TA, et al. Bovine immune response to Shiga-toxigenic *Escherichia coli* O157 : H7. *Clinical and Vaccine Immunology* **2006**; 13:1322-1327.

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**Figure 4: Ovalbumin-specific cellular and humoral immune responses in the lymph node draining the site of immunization.** Calves experimentally challenged with PT21/28 or PT32 *E. coli* O157, or left unchallenged, were subsequently immunized on two occasions 14 days apart with 60 µg ovalbumin plus 5mg Quil A adjuvant in the left cervical region, with the first immunization occurring at peak colonization (5 days post-challenge). Lymph node cells isolated from the prescapular lymph node draining the site of immunization one week after the final immunization and cells were subsequently analysed for the presence of ovalbumin-specific immune responses (A) Proliferation of lymph node cells in response to ovalbumin showing a general enhanced proliferative response in immunized and colonized calves. (B) Levels of ovalbumin-specific IgG1 secreted from lymph node plasma cells showing enhanced IgG1 repsonses in PT21/28 colonized calves; (C) levels of ovalbumin-specific CD4+ T cell activation showing no significant differences in antigen-specific CD4+ T cell responses; (D) levels of ovalbumin-specific CD8+ T cell activation demonstrating enhanced antigen-specific CD8+ responses in PT21/28 colonized calves. \* *P*<0.05 (Kriskall-Wallis ANOVA followed by a Dunn’s multiple comparisons test).