RISK FACTORS FOR SHEDDING OF SHIGATOXIGENIC *E. COLI* O157 AND O26 IN CATTLE AT SLAUGHTER

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Abstract

This project investigated risk factor for super-shedding of *E. coli* O157 and O26 in cattle (n =1317) presented for slaughter at three commercial beef abattoirs. The animal site examined was the recto anal junction which is known to be the site of colonisation for *E. coli* O157 in bovines. It showed that almost 50% of the animals shedding *E. coli* O157 (4.18 %, 55/1317) were super-shedding *E. coli* O157 (counts > 10^4 CFU/g^{-1}). It is one of the first studies to show bovine super–shedding of STEC O26, although at a very low level (0.23%, 2/1317). No risk factors for super shedding were identified though whole genome sequencing showed some clustering of super shedding and low shedding isolates which requires further investigation.

Keywords: Beef, pathogens, metagenomics

I. INTRODUCTION

In cattle colonised with Shigatoxigenic *E. coli* (STEC) O157 shedding patterns are known to be very variable, in terms of both shedding events and the numbers of pathogen shed. Some cattle, described as "super-shedders", are reported to excrete exceptionally high number of *E. coli* O157 (>10^4 CFU/g) in their faeces and continue to shed these levels over long periods. Such super shedding animals are likely to have significant impact on transmission of STEC on farm, transport, lairage and at slaughter contributing up to 80% of all STEC transmitted (Matthews *et al*. 2006). There is little data available on the shedding patterns of non O157 which are now a significant cause of human STEC illness.

II. MATERIALS AND METHODS

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, sample date etc. Samples were examined for the presence and numbers of *E. coli* O157 and O26. 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 (Lawal *et al*, 2015). Samples with counts >10^4 CFU $swab^{-1}$ of *E. coli* O157 or O26 were deemed to be super-shedders (SS). Samples positive by PCR were culturally examined and isolates examined for virulence genes; *stx1, stx2* and subtypes thereof, *eae* and *hly*A. Selected super shedder and low shedder strains were whole genome sequenced using Illumina MiSeq technology. To

ascertain the role of the microbiota at the recto-anal colonisation site in STEC 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.

III. RESULTS AND DISCUSSION

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 herds were persistently positive, with animals STEC positive on repeat occasions many months apart. WGS showed some clustering of SS and LS groups with genes related to virulence (*plc, toxB, senB, sta1, itc*A, *per*A and *sub*A) missing in clusters of low shedder isolates. 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 were *Ruminococcoae* (30%), *Prevotella* (10%) with 60% a variety of genera. Overall, the results indicated a high level of variability in operational taxonomic units between the three animal groups. Principle Co-ordinate Analysis showed no significant difference in microbiota composition at the RAJ based on shedding status, animal age, etc.

IV. CONCLUSION

The overall conclusion is that STEC super shedding appears to be a transient event, linked to colonisation at the RAJ site, with likely repeated intermittent sloughing of STEC when high levels of the pathogen are reached, leading to supershedding events. Persistently positive STEC herds are likely to both contain both super shedders and low shedders animals.

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