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Investigation of verocytotoxigenic *E. coli* supershedding status in cattle at slaughter

Key external stakeholders:
Beef sector, Department of Agriculture, Food and the Marine, Food Safety Authority of Ireland

Practical implications for stakeholders:

The data generated in this project gives an understanding of the shedding dynamics of *E coli* O157 and O26 in cattle presented for slaughter. 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}), highlighting the risk that these animals pose in the agri-food chain. It is one of the first studies to show bovine super–shedding of VTEC O26 although at a very low level (0.22%, 3/1317). Such information can direct management practices and policy for addressing VTEC in cattle.

Main results:

This project investigated faecal shedding patterns and super-shedding of *Escherichia coli* O157 and O26 in Irish cattle (n =1317) presented for slaughter at three commercial beef abattoirs over an 18 month period. The animal site examined was the distal portion of the terminal recto-anal junction (RAJ) which is known to be the site of colonisation for *E.coli* O157 in bovines.

- A new quantitative real time PCR assay was developed to enable both detection and enumeration *E. coli* O157 and O26 serogroups in cattle RAJ samples and this method was then used to examine the RAJ of cattle presented for slaughter for shedding and super shedding (SS) of *E. coli* O157 and O26
- Overall, 4.18 % (55/1317) of RAJ samples were positive for VTEC O157, and 2.13 % (28/1317) were classified as VTEC O157 SS (Log_{10} 4- 7.7 CFU $swab^{-1}$). All of the VTEC O157 isolated encoded *vtx*2 and *eae*A genes.
- Overall, 0.76% (10/1317) of cattle were positive for VTEC O26 and 0.22% (3/1317) were classified as VTEC O26 SS (Log_{10} 4.1- 5.8 CFU $swab^{-1}$), with a variety of *vtx*1/*vtx*2 and *eae* virulence gene profiles.
- One 18 month old beef animal was super-shedding VTEC O157 (Log_{10} 5.3 CFU $swab^{-1}$) and also a high level of VTEC O26 (Log_{10} 3.56 CFU $swab^{-1}$).
- One farm had five positive animals over three separate visits in a five month period, showing the persistence of VTEC on that farm.
- The average age of animals super-shedding both serogroups was 26 m (range 15 to 37 m).
- A seasonal trend was observed for the super-shedding of O157, with 64% (18/28) recovered in March/April or September to November.

Opportunity / Benefit:

The data generated in this project gives a fundamental understanding on the shedding dynamics of *E. coli* O157 and O26 in cattle. It showed that almost 50% of animals which were shedding *E. coli* O157 were super-shedders, highlighting the risk that these animals pose. It is one the first studies to show bovine super–shedding of VTEC O26. Such information can direct management practices and policy for addressing VTEC in cattle.

Collaborating Institutions:

University College Dublin

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Teagasc project team: Dr Geraldine Duffy
Dr Kaye Burgess
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1. Project background

Cattle are considered to be the main reservoir of *E. coli* O157. The pathogen colonises the distal portion at the terminal recto-anal junction (RAJ) of infected animals and animals can typically shed the bacteria at 10 to 100 CFU/g of faeces, with some cattle termed 'super-shedders' (SS), known to excrete high numbers (>$\log_{10}$ 4 CFU g^{-1}). Such animals are a significant transmission source of *E. coli* O157 in the agri-food chain. Little is known about the shedding dynamics of non O157 VTEC, despite the fact that they are increasingly being associated with human illness..

2. Questions addressed by the project:

- What is prevalence and concentration of *E coli* O157 in *E.coli* O26 in the recto anal junction of Irish cattle presented for slaughter?
- What is the frequency of VTEC super-shedding among animals?
- Is shedding and super-shedding impacted by season or animal age
- What are the virulence profiles of *E.coli* O157 and O26 isolates and do they vary between low shedding and super shedding animals?

3. The experimental studies:

- A new quantitative real time PCR assay was developed for *E. coli* O157 and O26, incorporating an initial enrichment period and a real time PCR method. A standard calibration curve was developed, 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.
- At three large Irish commercial beef abattoirs, recto-anal mucosal swabs (RAMS) samples were collected from cattle (n=1317) over an 18 m period. The cattle included dairy and beef animals, and the average age was 26 m (range 11 to 66 m).
- RAMS samples were examined for the presence and numbers of *E. coli* O157 and O26 using the developed real time PCR protocol. Any 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 were characterised for *vtx1/stx1*: *vtx1a, vtx1c, vtx1d; vtx2/stx2*: *vtx2a, vtx2b, vtx2c, vtx2d, vtx2e, vtx2f* and *vtx2g eae* and *hlyA* and phylo-grouped.

Main results:

- Overall, 4.18% (55/1317) of RAJ samples were positive for VTEC O157, and 2.13% (28/1317) were classified as VTEC O157 SS (Log_{10} 4- 7.7 CFU $swab^{-1}$). All of the VTEC O157 isolated had *vtx*2 and *eae*A genes.
- Overall, 0.76% (10/1317) of cattle were positive for VTEC O26 and 0.22% (3/1317) were classified as VTEC O26 SS (Log_{10} 4.1- 5.8 CFU $swab^{-1}$), with a variety of *vtx*1/*vtx*2 and *eae* virulence profiles.
- One 18 month old beef animal was super-shedding VTEC O157 (Log_{10} 5.3 CFU $swab^{-1}$) and also a high level of VTEC O26 (Log_{10} 3.56 CFU $swab^{-1}$).
- One farm had five positive animals over three separate visits in a five month period showing that the persistence of VTEC on that farm.
- The average age of animals super-shedding both serogroups was 26 m (range 15 to 37 m).
- A seasonal trend was observed for the SS O157 (18/28) recovered in March/April or September to November.
- For *E.coli* O157 isolates, 50/55 (91%) were positive for *vtx2*, and 5/55 (11%) were positive for both

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vtx1 and *vtx2.* For the O26 isolates (n=10), three had *vt1* only, two had both *vtx1* and *vtx2*and four had *vtx2* only.

4. Opportunity/Benefit:

The data generated in this project provides a fundamental understanding on the shedding dynamics of *E. coli* O157 and O26 in cattle. It showed that almost 50% of animals which were shedding *E. coli* O157 were super-shedders, highlighting the risk that these animals pose. It is one the first studies to show bovine super–shedding of VTEC O26. Such information can direct management practices and policy for addressing VTEC in cattle.

5. Dissemination:

Main publications:

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

Popular publications:

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 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., Burgess, C., McCabe, E., Whyte, P. and Duffy, G. (2015). 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., Burgess, C., McCabe, E., Whyte, P. and Duffy, G. (2015). 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.

Lawal, D., Burgess, C., McCabe, E., Whyte, P. and Duffy, G. (2015). 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.

Lawal, D., Burgess, C., McCabe, E., Whyte, P. and Duffy, G. (2015). 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.

Lawal, D., Burgess, C., McCabe, E., Whyte, P. and Duffy, G. (2015). 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.

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