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Molecular epidemiology of Shiga toxin-producing *Escherichia coli* (STEC) O157 and non-O157 STEC in calves in the North Island of New Zealand

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by

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Abstract

Shiga toxin-producing *Escherichia coli* (STEC) are common inhabitants of the ruminant gastrointestinal tract and have emerged as important zoonotic pathogens of global public health concern. Four studies were carried out in the North Island of New Zealand to understand the epidemiology of STEC O157 and non-O157 STEC in calves.

The aim of the first study was to determine population structure, transmission dynamics and spatial relationship between genotypes of two zoonotic pathogens, STEC and *Campylobacter jejuni*, found on farms in a defined catchment area in the Waikato region. Pooled faecal samples (n=72) obtained from calves grazing in the catchment were analysed by RT-PCR for *E. coli* O26, O103, O111, O145 and O157. The number of samples positive for O26 (30/72) was high compared to O103 (7/72), O145 (3/72) and O157 (2/72) while no samples were positive for O111. Using immuno-magnetic separation 18 O26, two O103 and a single O145 isolate were recovered from RT-PCR positive samples. Fifty-three *C. jejuni* isolates were recovered from 72 pooled faecal samples. *E. coli* O26 and *C. jejuni* isolates were genotyped using pulsed field gel electrophoresis (PFGE) and multilocus sequence typing respectively. All O26 isolates could be divided into three clusters on the basis of PFGE using *XbaI*. These results indicated that *E. coli* O26 isolates recovered from calves on the same farm were generally more similar than isolates recovered from different farms in the catchment. There were a total of 13 different sequence types (STs) of *C. jejuni* isolated from the cattle and most of the molecular variation (approximately 75%) resided between animals within farms. This study suggested a high level of within farm transmission, and limited between-farm transmission indicating direct contact between animals or sharing of personnel or equipment could be important routes of transmission and good biosecurity measures may be helpful in reducing localised transmission.

The aim of the second study was to determine the distribution of virulence genes (*stx1*, *stx2*, *eae* and *ehxA*) in *E. coli* isolates from dairy calves less than a week of age (bobby calves). Sampling was carried out by systematic collection of recto-anal mucosal swab (RAMS) samples from bobby calves slaughtered at two abattoirs in the North Island of New Zealand. The samples were inoculated onto tryptone bile X-glucuronide (TBX) and sorbitol MacConkey agar (CT-SMAC). Blue and white colonies (one each) and purple and grey colonies (one each) were selected at random from TBX and CT-SMAC plates respectively. In total 975 *E. coli* isolates obtained from the two media were analysed by multiplex PCR to detect *stx1*, *stx2*, *eae* and *ehxA* genes. The most common combination of virulence markers were *eae*, *ehxA* (n=35) followed by *eae* (n=9). Only eight STEC were identified of which four were *stx2*, *eae*, *ehxA*-positive (3 O157:H7 and 1 O68:H24), three were *stx1*, *eae*, *ehxA* (2 O26 and 1 O71:HR) and there was one *stx2*-only isolate (ONT:HNM). All the isolates could be divided into 15 clusters with >70% similarity using *XbaI* by PFGE. These findings indicate that STEC of public health significance such as O157 and O26 are present in bobby calves and may represent an important source of human infection in New Zealand.

In the third study samples obtained in the second study were processed to determine the occurrence and spatial distribution of *E. coli* O26, O103, O111 and O145 in bobby calves in the North Island of New Zealand. The association of IgG concentration, weight, sex and breed with occurrence of O26, O103, O111 and O145 as determined by direct RT-PCR on RAMS enrichments was also investigated. Using RT-PCR 134/299 (44.8%) of RAMS were positive for O26, 68/299 (22.7%) for O103 and 47/299 (15.7%) for O145. No RAMS samples were RT-PCR positive with O111-specific primers. The success of isolation by culture of *E.*

coli O26 (49/134 isolates) was higher from RT-PCR positive samples as compared to O103 (4/68 isolates) and O145 (5/47 isolates). Using a logistic regression model no association was observed between PCR prevalence and the variables IgG, sex or breed of the calves. However, calves positive for O26 were more likely to be positive for O103 and vice versa (P=0.01) and similar association was found between calves positive for O145 and O103 (P=0.03). O26 isolates could be grouped into four clusters (A-D) of $>70\%$ similarity using *XbaI* PFGE. K function analysis did not indicate any evidence of spatial clustering of farms positive for O26, O103 or O145. This study indicted that O26 is more prevalent in bobby calves as compared to O103 and O145 and colostrum feeding may not be helpful in reducing the carriage of *E. coli* O26, O103 and O145.

In the fourth study *E. coli* isolates (n=137) obtained from previous studies were genotyped using PFGE and allelic profiling (based on 8 genes). The *eae* and *ehxA* genes of *E. coli* isolates were subtyped using PCR-restriction fragment length polymorphism (RFLP) analysis. Endonuclease digestion of *ehxA* PCR products with *TaqI* from 121 *ehxA* positive *E. coli* isolates resulted in six *ehxA* subtypes (A-F). Endonuclease digestion with *HhaI* or *HaeIII* enzymes of the 129 *eae* positive isolates indicated that 82 were β followed by ϵ (n=34), γ (n=11) and δ/κ (n=2). An association between *eae* subtype and *E. coli* serogroup was also observed. All O26 isolates were subtype β, all O103 isolates were subtype ϵ and all O157 isolates were γ. *E. coli* isolates were also analysed for plasmid-associated allels *espP*, *etpD*, *katP* and α-*hly* genes. Of 137 isolates 93 (67.8%) were positive for *espP*, 32 for *etpD* (23.3%), 76 for *katP* (55.4%) and nine for the α-*hly* (6.5%) gene. The genotyping ability of allelic profiling was compared with PFGE profiling using PERMANOVA and multidimensional scaling and the results indicated that isolates having similar allelic profiles had similar PFGE profiles and tended to cluster together. The results also indicted that *eae* and *ehxA* profiles could explain a high population of the variance in PFGE profiles. These results may provide basis for the development of new genotyping method for *E. coli* isolates, having high discriminatory power and being easier to perform. The *E. coli* serogroups and *eae* and *ehxA* subtypes observed in these serogroups have also been observed from human *E. coli* isolates indicating the public health significance of these isolates.

These studies provide useful information about epidemiology of O157 and non-O157 in calves in the North Island of New Zealand and indicate that calves may be an asymptomatic reservoir of STEC and a possible source of infection for humans. Better understanding of the population structure and transmission of STEC would help in devising appropriate control strategies. The implementation of these control measures could reduce the prevalence of STEC in calves and thus reduce transmission to humans.

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