EPIDEMIOLOGY OF VEROCYTOTOXIGENIC E. COLI INFECTION IN ONTARIO DAIRY CATTLE

WILSON, J.B.[1], GYLES, C.L.[1], MCEWEN, S.A.[1], CLARKE, R.C.[2], LESLIE, K.[1]

Verocytotoxigenic *E. coli* (VTEC) infection in humans has been associated with a spectrum of disease including haemorrhagic colitis and haemolytic uraemic syndrome (HUS). VTEC infection has been associated with the consumption of beef and unpasteurized milk. VTEC have also been isolated from beef, suggesting the possibility of a bovine reservoir for human VTEC infection (Karmali, 1989). Several authors have reported the isolation of VTEC from the faeces of cattle (Karmali, 1989; Wells *et al*, 1991). However, with the exception of an abattoir survey conducted in Ontario (Clarke *et al*, 1988) none of these studies involved a formal random sample of animals from a defined target population.

If cattle are an important source of VTEC infection in humans, an understanding of the factors which promote bovine VTEC infection could prove valuable in reducing the incidence of human disease caused by these agents. This aspect of the epidemiology of VTEC infection has so far received little attention. The objectives of this study were thus to describe the distribution of bovine VTEC infection in a well-defined population of dairy cattle, and to identify risk factors for bovine VTEC infection in this population, operating both at the herd and individual animal levels.

MATERIALS AND METHODS

A simple random sample of 120 dairy farms was obtained from a list of all milk producers in four contiguous southern Ontario counties; one hundred producers agreed to participate. Each farm was visited once between January and October, 1988. On farms with 40 or more dairy cows milking on the day of the visit, a formal random sample of 25% of these cows was selected for testing. On farms with fewer than 40 cows milking on that day, a random sample of 10 was selected. A single faecal swab was obtained from each of the cows selected and from all dairy calves three months of age or younger on each farm. In addition, producers were interviewed to obtain information regarding farm and individual animal characteristics potentially associated with bovine VTEC infection. Supernatants from broth cultures of swabs were tested using a vero cell assay. Five isolates from each positive culture were tested similarly. Positive isolates were subjected to a test sequence consisting of 1) hybridization with DNA probes for Shiga-like toxin

[1] Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[2] Agriculture Canada, Health of Animals Laboratory, 110 Stone Road West, Guelph, Ontario, Canada N1G 3W4

Proceedings of the 6th International Symposium on Veterinary Epidemiology and Economics, 1991
Available at www.sciquest.org.nz

I (SLT-I), SLT-II and SLT-IIv sequences, 2) neutralization of verocytotoxicity using a mixture of anti-SLT-I and anti-SLT-IIv antisera 3) amplification of VT genes by a polymerase chain reaction procedure. Colonies positive on any test in this sequence were confirmed to be *E. coli* biochemically, and serotyped. Neutralization assays were performed on positive crude faecal supernatants from which no positive isolates were obtained. Animals were defined as VTEC-positive if their crude faecal cultures demonstrated a positive neutralization response, or if a confirmed VTEC isolate (based on the test sequence described above) was identified in their faeces.

Simple associations between VTEC infection status of individual cows and potential risk factors for infection were tested using logistic regression. Potential risk factors for infection of individual calves were examined in a similar manner. To identify risk factors for herd-level VTEC infection status, a logistic transformation of the proportion of cows infected on each farm was used. Simple associations between this transformed proportion and potential risk factors were then tested using weighted least squares regression. Variables with significant crude associations (alpha = 0.10) were then offered to a forward stepwise regression model. Risk factors for herd-level calf infection were studied in a similar manner.

RESULTS

Ten per cent of cows and 25 per cent of calves were classified VTEC-positive (95% confidence intervals [7.1,11.8] and [20.2,29.3], respectively). The difference in the prevalence of infection between cows and calves was statistically significant (95% confidence interval [10.2,20.3]). The estimated proportion of animals infected on each farm ranged from zero to 60% for cows and zero to 100% for calves. Two hundred and six VTEC isolates were identified. Few were of serotypes reported in humans and no *E. coli* O157:H7 (the VTEC serotype most strongly linked to human illness) were found.

Calves greater than two weeks of age were at significantly greater risk of infection than those under two weeks (p=0.004, OR=2.04). In both simple and multivariate analyses, farm-level cow infection was negatively associated with herd size, the use of loose housing, the maintenance of a relatively open herd, and the use of milking parlours or pipelines as opposed to bucket type milking machines (alpha = 0.05). Similarly, farm-level calf infection was negatively associated with herd size, the use of nipple bottles for feeding calves, the use of traditional tie-stall housing as opposed to loose housing or other methods, and maintenance of a closed herd. Herd-level calf infection had a negative unconditional association with registration with the Ontario Dairy Herd Improvement Corporation (ODHIC), a provincial organization which provides monthly summaries of individual animal production and health parameters to participating producers.

Proceedings of the 6th International Symposium on Veterinary Epidemiology and Economics, 1991
Available at www.sciquest.org.nz

DISCUSSION

The results of this study indicate that VTEC infection is widespread among dairy cattle in the four counties examined. Interestingly, no *E. coli* O157:H7 were identified in this study. Other authors have reported the isolation of *E. coli* O157:H7 from apparently healthy cattle, particularly on farms linked epidemiologically to cases of O157:H7-associated illness in humans (Karmali, 1989; Wells *et al*, 1991). The results of the present study suggest that farms linked to such incidents of human illness may have a higher prevalence of bovine O157:H7 infection than the general dairy cattle population. Furthermore, the majority of VTEC identified in the present study were not of serotypes previously reported in humans (Karmali, 1989). Statements regarding the pathogenicity of bovine VTEC isolates for humans should be made with caution, however. Other than for *E. coli* O157:H7, the pathogenicity of specific VTEC serotypes in humans remains unclear.

The mechanisms underlying the observed relationship between bovine VTEC infection and age are unknown, though possible contributing factors could include differences in diet, immunity, or rates of exposure to VTEC in the environment. Several variables were found to have a significant association with herd-level VTEC infection of cows or calves. Rather than attempt to propose specific causal mechanisms for these associations, these variables are probably most appropriately viewed as potential predictors, or markers of herd-level VTEC infection in the target population. Thus, our results suggest, for example, that dairy farms in Ontario with a high prevalence of VTEC infection among cows tend to be small, and are more likely to use traditional tie stall housing systems, than are herds with a low rate of VTEC infection. Similarly, farms with a high prevalence of VTEC infection in calves tend to be relatively small, tend to have relatively open herds, and tend not to be registered with ODHIC. Further research will be necessary to verify the herd-level associations observed in this study, and to investigate possible underlying causal mechanisms.

REFERENCES

Clarke, R., McEwen, S., Harnett, N., Lior, H. and Gyles, C., 1988. The prevalence of verotoxin-producing *Escherichia coli* (VTEC) in bovines at slaughter. Abstr. Annu. Meet. Am. Soc. Microbiol.

Karmali, M.A., 1989. Infection by Verocytotoxin-producing *Escherichia coli*. Clin. Microbiol. Rev., 2:15-38.

Wells, J.G., Shipman, L.D., Greene, K.D. *et al, 1991.* Isolation of *Escherichia coli* O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J. Clin. Microbiol., 29:985-989.