Short Communication

Absence of *E coli* O157:H7 in sheep and cattle faeces in North Wales

N. A. Alhelfi, H. Adam, D. L. Jones, A. P. Williams

Escherichia coli O157:H7 is a human pathogen associated with haemolytic uraemic syndrome and haemorrhagic colitis cases throughout the developed world (García and others 2010). The faeces of ruminant animals, in particular cattle and sheep, are regarded as the primary reservoir of *E coli* O157:H7. Contact with ruminant faeces either directly (eg, in the environment) or indirectly (eg, ingestion of contaminated meat products) plays a central epidemiological role in human *E coli* O157 infections. As a result, there has been considerable effort to increase our understanding of colonisation patterns in ruminants. Studies have shown that livestock infection and colonisation is transient and seasonal (Paiba and others 2002), and that it is affected by multiple factors including stress and diet (Jones 1999). A comparison of different studies also implies that there are substantial regional differences in carriage rates. Spatial variation is also known to occur in the frequency of human *E coli* O157 infection; for instance, the rate of infection is considerably greater (4–10 times) in Scotland than in Wales even though both countries appear to be similar in terms of population demographics, geography and diet (Jones and others 2011). Attempts to explain this phenomenon have looked at possible social and epidemiological factors (Jones and others 2011, Strachan and others 2011, Rotariu and others 2012), but there remains more fundamental research to be done on prevalence rates within the animal reservoir. The aim of this study was to examine fresh cattle and sheep faeces from farms in Gwynedd, North Wales, for total coliforms, *E coli* and *E coli* O157 bacteria, over a 12-month period.

Samples of freshly excreted sheep and cattle faecal material were collected monthly from September 2010 to August 2011 (total n=150 for sheep and 150 for cattle) from 25 lowland or upland farms, all within a 10-mile radius of Bangor, Gwynedd, UK. Farms were chosen randomly, but had to be commercial ventures rather than 'hobby' farms (as verified by discussion with the owners), and reflect typical livestock agricultural systems in the locality (eg, in terms of breeds and management). No dairy farms were selected so as to focus on livestock intended for the meat sector. Some farms reared sheep only (n=10); others reared cattle only (n=10); others reared sheep and cattle (n=5). All samples were from adult animals (>5 months old for sheep and >18 months for cattle) and were representative of all cattle and sheep groups on the farm. Depending on logistics, 10–15 samples were collected per farm per sampling event, which were then pooled and kept at 4°C. Upon return to the laboratory, 5 g of each faecal sample was placed in 45 ml of 1/4 strength Ringer's solution, and the mixture then homogenised in a stomacher machine (for 30 seconds). Serial dilutions were prepared from the homogenised samples; 1 ml of the dilution was then plated on a Petrifilm *E coli* and coliform Count Plates (3M, St Paul, Minnesota, USA) and incubated (24 hours, 37°C). To determine the presence or absence of *E coli* O157, samples were analysed by enrichment, where 5 g of sample was shaken (6 hours at 37°C, 150 rev min^{-1}) in 25 ml of modified Tryptone Soy Broth (Oxoid, Basingstoke, UK), then 1 ml of the enriched sample was subject to immunomagnetic separation (Dynamag 2; Life Technologies, Paisley, UK) then spread on a sorbitol MacConkey agar with Cefixime and tellurite (CT-SMAC) plate (Oxoid) and incubated at 37°C for 24 hours (Ogden and others 2001). Presumptive *E coli* O157 colonies (non-sorbitol fermenting) were confirmed by latex agglutination (Oxoid). To validate the detection protocol, faeces which tested negative for *E coli* O157 were subsequently inoculated with an environmental isolate of *E coli* O157 to confirm that if the pathogen was present it would have been detected. Data were analysed using SPSS Statistics (IBM V.19.0 for Windows). All plate count data were $\log_{10}(x+1)$ transformed prior to analysis to meet the assumptions of analysis of variance.

No *E coli* O157 was detected in either cattle or sheep faecal samples within the target area. The literature shows considerable variation in the degree of carriage and excretion of the organism by livestock. In their comprehensive review of the literature reporting prevalence rates of *E coli* O157 in cattle faeces, Rhoades and others (2009) report a mean incidence rate of 6.2 per cent, but a range of 0–57 per cent. In their recent Scottish study, Solecki and others (2009) found 10.5 per cent (41/390) sheep faecal samples were positive for *E coli* O157. Variability in the accuracy and sensitivities of the methods used will affect detection of *E coli* O157 in environmental samples (Quilliam and others 2011); however, using the same methods as those employed in the current study, Chapman and others (1997) found that 15.7 per cent of cattle and 2.2 per cent of sheep faecal samples were positive for the organism. Although this study was comparatively small in terms of sample size, the failure to detect any positive samples suggests that its incidence within the livestock population in Gwynedd is low. Although animal husbandry and age, diet and climatic effects are all known to affect prevalence rates within livestock (Jones 1999, Rhoades and others 2009), much larger studies, or a meta-analysis approach, are needed to elucidate the relative importance of each factor singularly and as interactions. In the current study, it is unknown whether all livestock from which faeces were collected had been born and raised in the locality. Transport of livestock between geographical areas could inevitably lead to the introduction and cycling of bacterial pathogens, such as *E coli* O157 in the agricultural environment, and ultimately the animal reservoir (Williams and others 2008b). However, of the livestock that is transported to Wales, a considerable proportion are sent direct for slaughter purposes (HCC, 2013); which may be another explanatory factor in the absence of O157-positive faecal samples in the present study. The importance of livestock transport could be assessed in future studies through combining livestock movement data with identification measures (eg, ear tag numbers) on farms or at point of slaughter. This study did not screen faecal samples for other potentially pathogenic serogroups of *E coli* (eg, O26 and O115), although these can be prevalent within sheep (Evans and others 2011) and cattle (Lynch and others 2012) faeces. Neither did the study screen for other bacterial pathogens that may be present in ruminant faeces (eg, *Salmonella* species and *Campylobacter* species). Although serovar O157 is the *E coli* most often linked with human disease, its absence from the faecal samples tested in this study should of course not be over-interpreted so that O157-negative livestock faeces is perceived to present zero risk.

Fig 1 shows the $\log_{10}$ of the mean faeces coliform and *E coli* counts for sheep and cattle from all samples taken in 2010 and 2011. Significantly greater ($P<0.01$) populations of coliform and generic (non-O157) *E coli* were seen in sheep faeces compared with cattle;

Veterinary Record (2013) doi: 10.1136/vr.101660

N. A. Alhelfi, BSc, PhD
Department of Food Science, Agriculture College, University of Basra, Basra, Iraq
H. Adam, BSc, PhD
D. L. Jones, BSc, PhD
A. P. Williams, BSc, PhD
School of Environment, Natural Resources & Geography, College of Natural Sciences, Bangor University, Gwynedd, UK

Email for correspondence: prysor.williams@bangor.ac.uk

Provenance: Not commissioned; externally peer reviewed

Accepted June 15, 2013

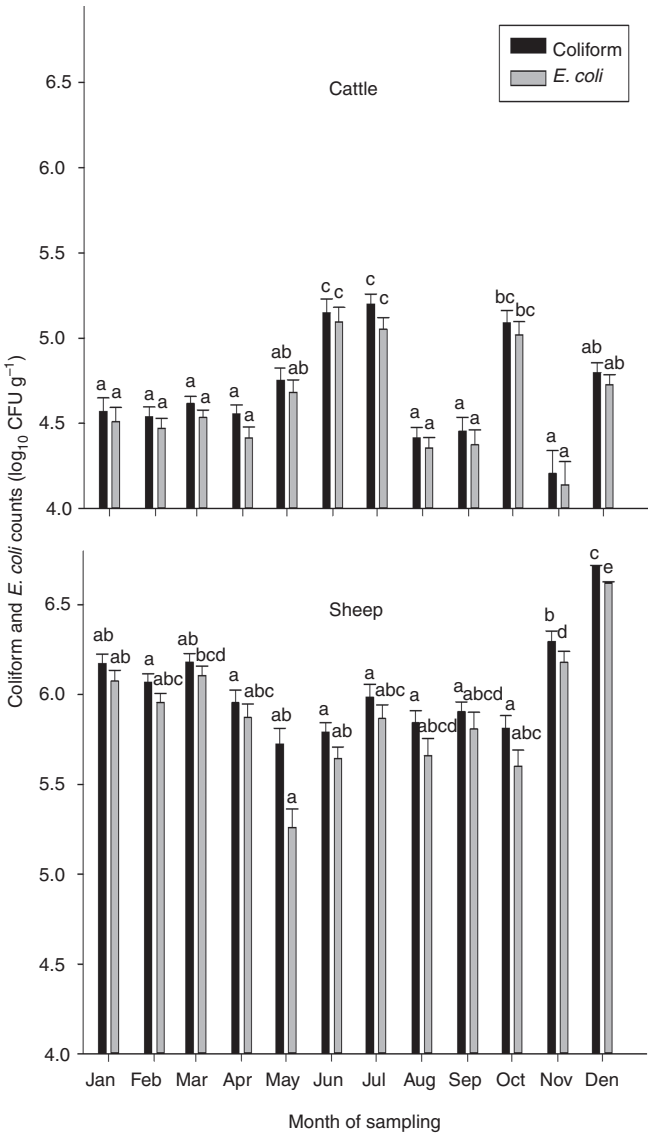

FIG 1: Number of coliform and *Escherichia coli* (log_{10} CFU g^{-1}±sem) in faecal samples of cattle and sheep in North Wales over a 12 month sampling period. Samples were collected from 25 farms (10–15 samples per farm, per sampling point). Different lowercase letters within each graph denote significant differences ($P<0.05$) between months for the same subgroup (ie, coliforms or *E coli*) for that livestock species, as determined by analysis of variance

being approximately tenfold greater in sheep faeces. Our findings of elevated counts in sheep faeces are consistent with the results of Williams and others (2008a) and Weaver and others (2005). It is unclear as to why coliform counts are higher in sheep than in cattle, but this may be due to physiological differences as well as the management and dietary factors mentioned previously. As coliforms are used as a proxy for pathogens, it reiterates the potential role that sheep have in the aetiology of pathogen infections (Rotariu and others 2012); although no direct correlations or predictions about O157 prevalence rates can be made from coliform values, therefore, extrapolation of results requires caution. Although no *E coli* O157 was detected, the significant ($P<0.05$) increase in generic *E coli* concentrations in summer is analogous with reports of elevated summer excretion of pathogenic (O157) excretion in cattle (Jones 1999). Coliform and *E coli* excretion within sheep, however, did not show any patterns of seasonal peaks.

In conclusion, the present study indicates that sheep and cattle from Gwynedd have a low prevalence of *E coli* O157 in their faeces; consistent with the comparatively low numbers of human infection in the region (Jones and others 2011). Given their importance in the epidemiology of *E coli* O157 infections, knowing the prevalence rates within ruminant livestock is important to design risk-based mitigation strategies.

Acknowledgements

This research was undertaken as part of the project 'Reducing *E coli* O157 Risk in Rural Communities' (RES-229-25-0012) funded under the UK Research Councils' Rural Economy and Land Use (RELU) programme. We thank James Gibbons for statistical advice.

References

CHAPMAN, P. A., SIDDONS, C. A., CERDAN-MALO, A. T. & HARKIN, M. A. (1997) A 1-year study of *E. coli* O157 in cattle, sheep, pigs and poultry. *Epidemiology and Infection* **119**, 245–250

EVANS, J., KNIGHT, H., MCKENDRICK, I. J., STEVENSON, H., BARBUDO, A. V., GUNN, G. J., LOW, J. C. (2011) Prevalence of *Escherichia coli* O157:H7 and serogroups O26, O103, O111 and O145 in sheep presented for slaughter in Scotland. *Journal of Medical Microbiology* **60**, 653–660

GARCÍA, A., FOX, J. C., & BESSER, T. E. (2010) Zoonotic Enterohemorrhagic *Escherichia coli*: A one health perspective. *ILAR Journal* **51**, 221–232

HCC (2013) Movement of Cattle into and Out of Wales, 2012. Aberystwyth, Wales: Hybu Cig Cymru.

JONES, C. D. R., HUNTER, C., WILLIAMS, A. P., STRACHAN, N. J. C., & CROSS, P. (2011) *Escherichia coli* O157: comparing awareness of rural residents and visitors in livestock farming areas. *Epidemiology and Infection* **139**, 1522–1530

JONES, D. L. (1999) Potential health risks associated with the persistence of *Escherichia coli* O157 in agricultural environments. *Soil Use and Management* **15**, 76–83

LYNCH, M. J., FOX, E. M., O'CONNOR, L., JORDAN, K., MURPHY, M. (2012) Surveillance of verocytotoxigenic *Escherichia coli* in Irish bovine dairy herds. *Zoonoses and Public Health* **59**, 264–271

OGDEN, I. D., HEPBURN, N. F., & MACRAE, M. (2001) The optimisation of isolation media used in immunomagnetic separation methods for the detection of *E. coli* O157 in foods. *Journal of Applied Microbiology* **91**, 373–379

PAIBA, G. A., GIBBENS, J. C., PASCOE, S. J., WILESMITH, J. W., KIDD, S. A., BYRNE, C., RYAN, J. B., SMITH, R. P., MCLAREN, M., FUTTER, R. J., KAY, A. C., JONES, Y. E., CHAPPELL, S. A., WILLSHAW, G. A., & CHEASTY, T. (2002) Faecal carriage of verocytotoxin-producing *Escherichia coli* O157 in cattle and sheep at slaughter in Great Britain. *Veterinary Record* **150**, 593–598

QUILLIAM, R. S., WILLIAMS, A. P., AVERY, L. M., MALHAM, S. K., JONES, D. L. (2011) Unearthing human pathogens at the agricultural-environment interface: a review of current methods for the detection of *E. coli* O157 in freshwater ecosystems. *Agriculture, Ecosystems & Environment* **140**, 354–360.

RHOADES, J. R., DUFFY, G., & KOUTSOUMANIS, K. (2009) Prevalence and concentration of verocytotoxigenic *Escherichia coli, Salmonella enterica* and *Listeria monocytogenes* in the beef production chain: a review. *Food Microbiology* **26**, 357–376

ROTARIU, O., OGDEN, I. D., MACRITCHIE, L., FORBES, K. J., WILLIAMS, A. P., CROSS, P., HUNTER, C. J., TEUNIS, P. F. M., & STRACHAN, N. J. C. (2012) Combining risk assessment and epidemiological risk factors to elucidate the sources of human *E. coli* O157 infection. *Epidemiology and Infection* **140**, 1414–1429

SOLECKI, O., MACRAE, M., STRACHAN, N., LINDSTEDT, B. A., & OGDEN, I. (2009) *E. coli* O157 from sheep in Northeast Scotland: prevalence, concentration shed, and molecular characterization by multilocus variable tandem repeat analysis. *Foodborne Pathogens and Disease* **6**, 849–854

STRACHAN, N. J. C., HUNTER, C. J., JONES, C. D. R., WILSON, R. S., ETHELBERG, S., CROSS, P., WILLIAMS, A. P., MACRITCHIE, L., ROTARIU, O., CHADWICK, D. (2011) The relationship between lay and technical views of *Escherichia coli* O157 risk. *Philosophical Transactions of the Royal Society B: Biological Sciences* **366**, 1999–2009

WEAVER, R. W., ENTRY, J. A. & GRAVES, A. (2005) Numbers of fecal streptococci and *Escherichia coli* in fresh and dry cattle, horse, and sheep manure. *Canadian Journal of Microbiology* **51**, 847–851

WILLIAMS, A. P., GORDON, H., JONES, D. L., STRACHAN, N. J. C., AVERY, L. M., KILLHAM, K. (2008a) Leaching of bioluminescent *Escherichia coli* O157:H7 from sheep and cattle faeces during simulated rainstorm events. *Journal of Applied Microbiology* **105**, 1452–1460

WILLIAMS, A. P., MCGREGOR, K. A., KILLHAM, K. & JONES, D. L. (2008b) Persistence and metabolic activity of *Escherichia coli* O157:H7 in farm animal faeces. *FEMS Microbiology Letters* **287**, 168–173

Veterinary Record

Absence of *E coli* O157:H7 in sheep and cattle faeces in North Wales

N. A. Alhelfi, H. Adam, D. L. Jones, et al.

Veterinary Record 2013 173: 143 originally published online July 6, 2013
doi: 10.1136/vr.101660