



# A longitudinal study of *Escherichia coli* O157 contamination of a watercourse by farm ruminants at pasture

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## Introduction

*Escherichia coli* O157 is a human pathogen capable of causing severe disease such as haemorrhagic colitis and haemolytic uremic syndrome, particularly in the young and the elderly population. Ruminants are regarded as the main reservoir of *E. coli* O157 and transmission to humans occur through faecal contamination of food, water and the environment, or through direct contact with carrier animals. There is an increasing concern regarding environmental infection by *E. coli* O157, particularly since the minimum infectious dose to humans is thought to be low.

The aims of the present study were to investigate contamination of a watercourse with *E. coli* O157 by farm ruminants at pasture and to determine if the contamination was dependent on rainfall.



Figure 1. Map of *E. coli* O157 positive farm. Fields 1 to 5 and water sampling places A to D are marked.

## Material and methods

### Farm information

Figure 1 shows a map of an *E. coli* O157 positive farm with a burn running through the middle. Sheep and cattle grazed in fields 1 through 5, with the number of animals per field varying extensively during the study.

### Faecal samples

Pooled faecal samples (3 single samples together) were taken to identify and track fields with *E. coli* O157 positive animals. *E. coli* O157 was identified and quantified by IMS and plating onto CT-SMAC and CT-SMAC-BCIG. Enumeration of *E. coli* O157 was done by direct plating of single samples. Presumptive *E. coli* O157 were confirmed by latex agglutination and biochemical reactions.

### Water samples

The amount of rainfall was registered daily at the farm. Two automatic water samplers sampled 250 ml once an hour for 48 hours (at sampling places A and B, Figure 1). Water samples (4 – 5 l) were also taken manually at sampling places C and D. From each sampling day and place, a total water volume of 3 l (500 ml per filter) was filtered through sterile 0.45 µm membrane filters and further analysed for *E. coli* O157 by IMS and plating onto CT-SMAC and CT-SMAC-BCIG. Enumeration of *E. coli* was performed on diluted samples using the Colilert®-18 method and a MPN table.

### Statistics

Statistical analysis was performed using STATA. Descriptive analysis of a few characteristics was performed by the Kruskal Wallis test for continuous variables and Fisher's Exact test for categorical variables. Some variables were also tested in linear regressions with log *E. coli* per ml water as the dependent variable. Model assumptions were tested by graphical examination of the residuals.

## Results

### Faecal samples

Week	No. <i>E. coli</i> O157 positive (No. sampled)				Total
	Sheep faecal samples		Cattle faecal samples		
	Pooled samples	Single samples	Pooled samples	Single samples	
2		5 (20)			5 (20)
3	2 (20)	2 (20)	0 (10)		4 (50)
4	0 (15)			0 (12)	0 (27)
5	0 (15)			0 (15)	0 (30)
6	2 (16)		0 (6)		2 (22)
7	3 (20)		0 (10)		3 (30)
8	4 (10)	6 (30)	0 (10)		10 (50)
9	14 (30)	0 (60)			3 (30)
Total	25 (126)	13 (130)	0 (36)	0 (27)	38 (319)

Table 1. Number of *E. coli* O157 positive sheep and cattle faecal samples. No "high shedders" were found.

### Water samples

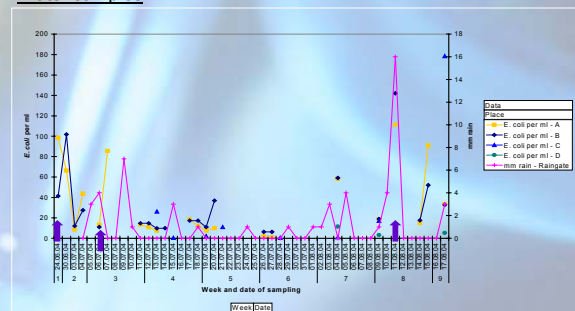
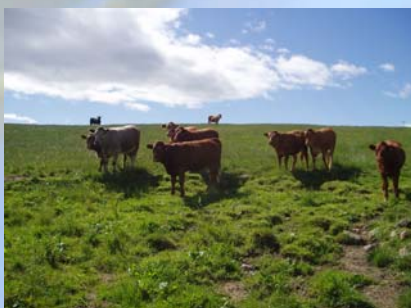


Figure 2. *E. coli* per ml water per time at sampling points A – D and mm rain per day. Arrows show date of *E. coli* O157 detection from water. Only 4 of the 53 water samples were found to be positive for *E. coli* O157.

There was no difference in *E. coli* per ml water at the four sampling points ( $p = 0.09$ , Kruskal-Wallis test). *E. coli* per ml water (sampling place A and B) =  $0.039$  (95% CI 0.012-0.066)  $\times$  "mm rain last 3 days" +  $1.125$  ( $R^2 = 0.34$ ).



## Concluding remarks

Faecal sheep samples were positive for *E. coli* O157 throughout the study. Although there was close contact between sheep and cattle, *E. coli* O157 was not detected from faecal cattle samples.

*E. coli* runoff from pasture increased with rainfall. Figure 2 also indicates that detection of *E. coli* O157 in the watercourse is associated with increased rainfall.

*E. coli* O157 carrying animals at pasture may contaminate watercourses and thereby contribute to the risk of human environmental infection with *E. coli* O157.