VTEC O157 & O26 Presence and Enumeration in Cattle Farm Waste

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METHODS

Eligible farms:- Cattle premises previously confirmed as VTEC O157-positive at a screening visit; within 2 hours travelling time of the testing laboratory; not a 'cattle dealer'.

6 enrolled farms were each visited 3 times (pre-turnout, cattle at pasture and post-housing) from 16/12/03 to 6/12/04 and 50 samples were collected (Table 1) [N.B On some farms fewer samples were collected due to lack of material].

Sample breakdown:-

- 12 samples from fresh farm waste sites (F)
- 20 stored waste samples (M)
- 12 dirty water samples (D)
- 6 surface of pasture samples (P)

Testing:- The presence of *E.coli* O157 was demonstrated by immunomagnetic separation (IMS) on buffered peptone water pre-enrichment broths following incubation at 37°C for 6 hours, and plating on sorbitol MacConkey agar containing cefixime and tellurite (CT-SMAC). Sorbitol non-fermenting colonies were then tested by latex agglutination for the O157 antigen. A selection of up to 8 representative *E.coli* O157 isolates per visit were further characterised by serology for the O157 antigen, and PCR for *vt1, vt2* and *eae* genes (1).

E.coli O157 enumeration was undertaken by spiral plating dilutions on Harlequin agar with O157-latex agglutination used to validate the count. Counts were recorded as cfu/g, although some IMS-positive samples had too few organisms to count and were recorded as "<200" (2).

2 farms also had a total of 5 of their visit samples tested for VTEC O26 by similar methods to those for O157 but substituting Rhamnose-MacConkey agar for Harlequin agar and using O26 instead of O157 latex beads, IMS beads and sera for confirmation. *E.coli* O26 counts were not taken at the first visit to either farm.

RESULTS

Of 878 samples tested for O157, 65 were found to be O157-presumptive positive (by IMS/ latex testing); 63 (7.0%) O157 samples were confirmed as VTEC O157 and 2 samples were non-VT producing and non-O157. (Tables 1 & 2)

Of 213 samples tested for O26, 20 were IMS +ve of which 18 O26 positive (2 not tested due to contamination) , 8 VTEC O26 positive (3.8%) and 10 were non-VT producing O26. (Tables 1 & 2)

Table 1: Visit Summary

Farm No.	Visit Date	VTEC O157 samples positive/ tested	VTEC O26 samples positive/ tested
Sampling visit 1 – pre-turnout			
8	16/12/2003	0/34	n/d
9	19/01/2004	0/50	n/d
15	01/03/2004	0/46	5/45
19	01/04/2004	4/50	n/d
20	15/03/2004	0/50	0*/18
25	23/03/2004	0/50	n/d
Sampling visit 2 – cattle at pasture			
8	11/05/2004	0/50	n/d
9	18/05/2004	0/48	n/d
15	26/07/2004	2/50	1/50
19	16/08/2004	2/50	n/d
20	20/09/2004	0/50	0*/50
25	06/09/2004	0/50	n/d
Sampling visit 3 – post-housing			
8	02/11/2004	1/50	n/d
9	08/11/2004	0/50	n/d
15	06/12/2004	3/50	2/50
19	29/11/2004	29/50	n/d
20	15/11/2004	1/50	n/d
25	23/11/2004	21/50	n/d

N/d = not done, *E.coli* O26 Non-VT

Table 2: Sample PCR results

Type	Antigen	VT1	VT2	eae	No. of samples
D	O157	-	+	+	2
F	O157	+	+	+	8
F	O157	-	+	+	16
M	O157	+	+	+	18
M	O157	-	+	+	16
P	O157	-	+	+	3
F	O26	+	-	+	3
F	O26	-	-	+	2
M	O26	-	-	+	1
P	O26	-	-	+	4

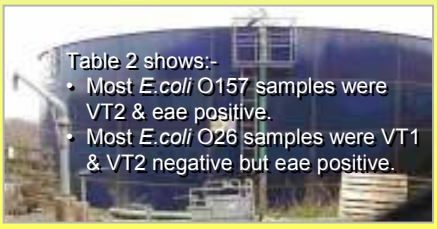

Table 3: Phagetyping results

Farm	Visit	'Phagetype	Sample type	Number of samples
15	2	8	Fresh	2
15	3	8	Fresh	3
19	1	21/28	Fresh	1
19	1	21/28	Stored	2
19	1	21/28	Dirty water	1
19	2	21/28	Fresh	2
19	3	21/28	Fresh	2
19	3	21/28	Stored	1
19	3	51	Fresh	1
19	3	51	Pasture	1
20	3	34	Fresh	1
25	3	2	Stored	4

Table 3 shows:
The most common 'phagetype was 21/28, same as that found in recent GB abattoir survey (3)

Table 4: *E.coli* O157 enumeration results (cfu/g)

Type	Visit	negative counts	positive counts	% of total	No. of <200 samples	% of total	mean countable	Range
F	1	59	0	0.0	1	1.7	0	-
F	2	68	3	4.4	1	1.5	4,475	300-12,300
F	3	55	2	3.6	15	27.3	14,450	500-28,400
F		182	5	2.7	17	9.3	8,465	300-28,400
M	1	115	0	0.0	1	0.9	0	-
M	2	120	0	0.0	0	0.0	0	-
M	3	87	3	3.4	30	34.5	8,375	485-22,500
M		322	3	0.9	31	9.6	8,375	485-22,500
D	1	57	0	0.0	1	1.8	0	-
D	2	72	0	0.0	0	0.0	0	-
D	3	71	1	1.4	0	0.0	419	-
D		200	1	0.5	1	0.5	419	-
P	1	30	0	0.0	0	0.0	0	-
P	2	32	0	0.0	0	0.0	0	-
P	3	33	0	0.0	3	9.1	0	-
P		95	0	0.0	3	3.2	0	-

Table 5: *E.coli* O26 enumeration results (cfu/g)

Type	Visit	No. of negatives	No. of positives	% of total	No. of <200 samples	% of total	mean countable	Range
F	2	20	4	20.0	1	5.0	1,103	650-1,410
F	3	10	2	20.0	2	20.0	0	-
F		30	6	20.0	3	10.0	1,103	650-1,410
M	2	39	0	0.0	0	0.0	0	-
M	3	20	0	0.0	0	0.0	0	-
M		59	0	0.0	0	0.0	0	-
D	2	24	0	0.0	0	0.0	0	-
D	3	12	0	0.0	0	0.0	0	-
D		36	0	0.0	0	0.0	0	-
P	2	8	4	50.0	4	50.0	0	-
P	3	6	0	0.0	0	0.0	0	-
P		14	4	28.6	4	28.6	0	-

Tables 4 and 5 show:

- O157 enumeration results were highest in fresh waste and stored waste, especially at visit 2 (cattle at pasture) and visit 3 (post-housing).
- IMS-positive samples were extracted from the dirty water and pasture but, bar one dirty water sample at visit 3, O157 levels were too low to count (Table 4)
- O26 enumeration results show a higher percentage of samples were positive than for O157, but most were at levels too low to count (Table 5)

CONCLUSION

- *E. coli* O157 counts and the proportion of samples positive were higher in faeces and stored waste samples than in dirty water or pasture samples indicating a reduction in bacterial load in more dilute types of waste and from storage and spreading.
- The same 'phagetypes were found in several sample types at the same visit, indicating possible spread from the fresh waste around the farm.
- A Chi-squared test showed a significant difference in O157 bacterial load between the seasons (Chi-sq score = 53.38, P-value >0.000). Autumn had the most IMS O157-positive samples, then the summer, winter and spring respectively.

FURTHER WORK

- The results of a risk factor analysis are being prepared for publication.
- Results from the longitudinal study have been used to populate mathematical and risk models to evaluate the rôle of different farm wastes in the maintenance and epidemiology of VTEC on cattle farms.

References

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