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Sensitivity of an Immunomagnetic-Separation-Based Test for Detecting *Escherichia coli* O26 in Bovine Feces[▽]

L. M. Hall,[1]* J. Evans,[1] A. W. Smith,[1,2] M. C. Pearce,[1,2] H. I. Knight,[1,2] G. Foster,[3] J. C. Low,[1] and G. J. Gunn[1]

Animal Health Group, Scottish Agricultural College, West Mains Road, Edinburgh EH9 3JG,[1] Centre for Tropical Veterinary Medicine, University of Edinburgh, Roslin, Midlothian EH25 9RG,[2] and Veterinary Services, Scottish Agricultural College, Stratherrick Road, Inverness IV2 4JZ,[3] United Kingdom

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The sensitivity of a test for cattle shedding *Escherichia coli* serogroup O26 was estimated using several fecal pats artificially inoculated at a range of concentrations with different *E. coli* O26 strains. The test involves the enrichment of fecal microflora in buffered peptone water, the selective concentration of *E. coli* O26 using antibody-coated immunomagnetic-separation beads, the identification of *E. coli* colonies on Chromocult tryptone bile X-glucuronide agar, and confirmation of the serogroup with *E. coli* serogroup O26-specific antisera using slide agglutination. The effective dose of *E. coli* O26 for an 80% test sensitivity (ED_{80}) was 1.0×10^4 CFU g^{-1} feces (95% confidence interval, 4.7×10^3 to 2.4×10^4). Differences in test sensitivity between different *E. coli* O26 strains and fecal pats were also observed. Individual estimates of ED_{80} for each strain and fecal pat combination ranged from 4.2×10^2 to 4.8×10^5 CFU g^{-1}. These results suggest that the test is useful for identifying individuals shedding a large number of *E. coli* O26 organisms or, if an appropriate number of individuals in a herd are sampled, for identifying affected herds. The study also provides a benchmark estimate of sensitivity that can be used to compare alternative tests for *E. coli* O26 and a methodological approach that can be applied to tests for other pathogenic members of the *Enterobacteriaceae* and other sample types.

The recovery of *Escherichia coli* serogroup O26 from cattle has been reported for more than 50 years (2, 17) from a wide range of countries (5, 15, 18, 22, 23). In a human context, *E. coli* O26 has been associated with infantile diarrhea (21), and verocytotoxin-producing strains have been identified as a cause of hemolytic-uremic syndrome (13). *E. coli* O26 was the most common serogroup recovered from human verocytotoxin-producing *E. coli* (VTEC) infections in Italy between 1995 and 2001 (20), was the second most frequent serogroup among human VTEC isolates in Scotland during 2003 (14), and is responsible for 20% of human clinical enterohemorrhagic *E. coli* infections in Japan (11). Domestic ruminants, including cattle and sheep, are significant reservoirs of VTEC and are thought to be a major source of human non-O157 infection (24).

A reliable test for *E. coli* O26 has to detect an appropriate concentration of its target with high sensitivity and specificity in the presence of associated microflora. Several methods to achieve this end have been described (7, 10, 19), including the use of antibody-coated immunomagnetic-separation (IMS) beads (18, 23), though there has been only a limited evaluation of IMS-based tests for *E. coli* O26 (12, 18). We felt that it was desirable to evaluate a previously used IMS-based test for *E. coli* O26 (17, 18) more thoroughly to help validate cattle prevalence estimates. This report describes our approach to estimating the sensitivity of this test.

* Corresponding author. Mailing address: Scottish Agricultural College, Stratherrick Rd., Inverness IV2 4JZ, United Kingdom. Phone: 44 (0) 1463 243030. Fax: 44 (0) 1463 711103. E-mail: malcolm.hall@sac.ac.uk.

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MATERIALS AND METHODS

Outline. The recovery of *E. coli* O26 from six fecal pats artificially inoculated with six different *E. coli* O26 strains at concentrations ranging from 10^{-1} to 10^6 CFU g^{-1} feces was measured. *E. coli* was recovered using a test that involves the enrichment of fecal microflora in buffered peptone water (BPW), the selective concentration of *E. coli* O26 using IMS beads, the identification of *E. coli* colonies on Chromocult tryptone bile X-glucuronide (TBX) agar, and confirmation of the serogroup with *E. coli* serogroup O26-specific antisera using slide agglutination.

Origin and preparation of strains. Five of the six *E. coli* O26 strains used in this experiment had been recovered previously without using IMS from bovine feces sampled from around Scotland and had been stored at −80°C (laboratory strain codes C1414.1, C1991, C1528.4, C683.1, and 56C280/2). They are characterized by different pulse-field gel electrophoresis profiles, and while some change cannot be discounted, they are essentially unmodified field strains. The sixth strain (laboratory strain code UA3552NAR) is a nalidixic acid-resistant laboratory-adapted strain of unknown origin obtained from Iain Ogden, University of Aberdeen. Five of the strains possess the *vtx1* gene, one strain has an additional *vtx2* gene, three strains have *ehlyA*, and all six strains have *eae*. Each strain was individually cultured overnight in 20 ml BPW (Oxoid, Basingstoke, United Kingdom) at 37°C. An approximate concentration for each culture was estimated from its optical density at 570 nm, and a series of 10-fold dilutions ranging from approximately 10^0 to 10^6 CFU ml^{-1} was prepared for each strain in phosphate buffered saline, pH 7.4 (PBS; Sigma-Aldrich, Poole, United Kingdom), for inoculation.

Origin and preparation of fecal pats. A sample was taken from each of 11 fresh bovine fecal pats from separate farms near Inverness during November 2003. These samples were stored at 4°C. Six of the fecal pats were from enclosures of housed cattle and the remaining five from grazing cattle. The ages of the animals from which pats were collected are unknown. Ten 1-g subsamples were taken from each stirred fecal pat sample and were screened for endogenous *E. coli* O26 within 48 h of collection by using the procedure for recovery of *E. coli* O26 described below.

Interference with *E. coli* O26 growth by feces. Growth interference for each of the six *E. coli* O26 strains by each of the 11 fecal pats was evaluated. For each *E. coli* O26 strain, two 10- by 10-cm plates containing TBX agar (Merck, Darmstadt, Germany) were flooded with a 0.5 McFarland standard solution of the respective strain prepared in sterile saline. Excess solution was poured off and the plate dried. Six 8.5-mm-diameter wells were bored into each plate, and a subsample of

TABLE 1. Observed recovery of *E. coli* O26 by culture on TBX after IMS from inoculated bovine feces

Concn of inoculated *E. coli* O26 in feces (CFU g^{-1})	No. of positive subsamples/no. tested	% Positive subsamples
1.6×10^{-1} to 8.4×10^{-1}	5/36	14
1.6×10^{0} to 8.4×10^{0}	3/34	9
1.6×10^{1} to 8.4×10^{1}	7/36	19
1.6×10^{2} to 8.4×10^{2}	18/35	51
1.6×10^{3} to 8.4×10^{3}	21/35	60
1.6×10^{4} to 8.4×10^{4}	29/34	85
1.6×10^{5} to 8.4×10^{5}	33/36	92

TABLE 2. Predicted percent probability of recovering *E. coli* O26 from inoculated bovine feces

Concn of inoculated *E. coli* O26 in feces (CFU g^{-1})	% Probability of recovery	95% CI
1×10^{2}	28	21–37
1×10^{3}	56	46–65
1×10^{4}	80	73–86
1×10^{5}	93	90–95
1×10^{6}	98[a]	96–98

[a] The highest number of *E. coli* O26 inoculated into a subsample was 8.4×10^{5} CFU.

each fecal pat emulsified 1:2 (wt/vol) in maximum recovery diluent (Oxoid, Basingstoke, United Kingdom) was added to the appropriate well for each strain. A negative-control solution comprising maximum recovery diluent only was added to the remaining well for each strain. Plates were examined for the presence of clear zones around each well following overnight incubation at 37°C. The diameters of clear zones, corresponding to growth interference, were measured in millimeters and compared to the control wells.

Inoculation of feces with *E. coli* O26. To estimate the sensitivity of the test, six of the fecal pats containing no detectable *E. coli* O26 were selected. These included pats with weak and strong *E. coli* O26 growth interference profiles. Three of the pats were from housed cattle and three from grazing cattle. Forty-eight 1-g subsamples were taken from each fecal pat, and each subsample was placed in a separate 30-ml screw-cap universal. One hundred microliters of each of the seven dilutions of each *E. coli* O26 strain was added to a separate 1-g subsample in its universal. One hundred microliters of a negative-control solution, comprising PBS only, was added to the remaining 36 uninoculated subsamples. Inoculation took place 8 days after sample collection. Colony counts for each strain were performed on TBX agar plates following incubation at 37°C overnight using the 10^1, 10^2, and 10^3 CFU ml^{-1} dilutions in order to more accurately determine the concentration of bacteria. These counts were used for the subsequent analysis.

Recovery of *E. coli* O26. Fecal subsamples were suspended in 20 ml BPW, randomized, and incubated for 6 h at 37°C. These were then assigned to one of two laboratory operators, who tested for recovery "blinded" to the identity of each sample. One milliliter of each broth was added to 20 μl of *E. coli* O26-specific IMS beads (LAB M, Bury, United Kingdom) in 1.5-ml screw-cap microcentrifuge tubes, mixed on a blood tube rotator (Bibby Sterilin, Stone, United Kingdom) for 30 min, placed on an immunomagnetic separator (Dynal, Oslo, Norway) for 5 min, and the supernatant removed. The beads were washed three times using 1 ml of 10 mM PBS containing 0.05% (vol/vol) Tween (PBS-T; Sigma-Aldrich, Poole, United Kingdom) and resuspended in 50 μl PBS-T. Each suspension was then plated onto a separate TBX agar plate and incubated at 37°C overnight. All morphologically distinct colonies, up to 10 per plate, were tested against *E. coli* serogroup O26-specific antisera (Statens Serum Institut, Copenhagen, Denmark) using slide agglutination.

PCR amplification of recovered colonies. The possession of the *vtx1*, *vtx2*, *eae*, and *ehlyA* genes by recovered *E. coli* O26 was compared to that of the respective inoculated strain. A single bacterial colony from each *E. coli* O26-positive subsample was emulsified in 0.85% saline and heated at 100°C for 15 min. The *vtx1*, *vtx2*, *eae*, and *ehlyA* genes were amplified from 2 μl of the emulsified subsample using a previously published multiplex PCR procedure (16). Amplified DNA fragments were separated on 2% agarose alongside a molecular size marker comprising pUCBM21 DNA digested with HpaII, DraI, and HindIII (DNA MW-marker VIII; Roche, Basel, Switzerland), stained for 30 min with 7 μg ethidium bromide $liter^{-1}$ (Sigma-Aldrich, Poole, United Kingdom), and visualized at 254 nm. The pattern of amplified DNA fragments, corresponding to possession of the appropriate genes, was compared to that of the relevant inoculated strain.

Statistical analysis. Data were analyzed using SAS, version 9.1 (SAS Institute Inc., Cary, N.C.). Six subsamples for which the recovered *E. coli* O26 showed a PCR banding pattern different from that of the inoculated strain were excluded from analysis. The proportion of subsamples from which one or more *E. coli* O26 colonies were recovered was estimated for each inoculum dilution. Further analysis required a statistical model, because different *E. coli* O26 strains are associated with different inoculum concentrations. Further analysis was restricted to subsamples inoculated at a nominal concentration of no fewer than 7 CFU g^{-1}, comprising 74% of the laboratory data, to ensure that each subsample contained one or more target organisms. A generalized linear mixed model (3) with a binomial error distribution and using a second-order Taylor expansion was used to analyze the data, with fixed explanatory effects for inoculum concentration, laboratory operator, animal housing, and *E. coli* O26 growth interference, and random effects for strain and fecal pat. Statistical significance was declared when the probability of an effect occurring by chance was ≤0.05. The predicted percent probability of recovery at five inoculum concentrations, and the effective doses required for an 80% chance of recovering one or more *E. coli* O26 colonies from a sample (ED_{80}), were estimated using 1,000 simulations. Means were estimated from these, and approximate 95% confidence intervals (CI) were calculated by using a percentile bootstrap corrected for bias and acceleration (6) using a further 5,000 resamplings. ED_{80} were also estimated for individual strains and pats and for each strain-pat combination.

RESULTS

Observed percentages of inoculated fecal subsamples from which *E. coli* O26 was recovered are presented in Table 1. These range from 9% of subsamples containing between 1.6 and 8.4 CFU of *E. coli* O26 g^{-1} of feces to 92% of those containing between 1.6×10^5 and 8.4×10^5 CFU g^{-1}.

Predicted percent probabilities of recovering *E. coli* O26 from inoculated fecal subsamples are presented in Table 2. These range from 28% (CI, 21% to 37%) to 93% (CI, 90% to 95%) for subsamples containing 1×10^2 and 1×10^5 CFU of inoculated *E. coli* O26 g^{-1} of feces, respectively. The mean ED_{80}, averaged across strains and fecal pats, was 1.0×10^4 CFU of inoculated *E. coli* O26 g^{-1} feces (CI, 4.7×10^3 to 2.4×10^4). Estimates of ED_{80} for each strain and fecal pat combination range from 4.2×10^2 to 4.8×10^5 CFU g^{-1}, with first and third quartiles of 1.1×10^3 and 1.0×10^5 CFU g^{-1}.

E. coli O26 was recovered from a minimum of 12 and a maximum of 21 of the 29 fecal pat-concentration combinations tested for each strain. Differences in recovery between strains are statistically very highly significant ($\chi^2_1 = 29.79$; $P < 0.001$). Estimates of ED_{80} for individual strains range from 4.7×10^3 to 2.8×10^4 CFU g^{-1}.

E. coli O26 was recovered from a minimum of 8 out of 31 and a maximum of 24 of the 30 strain-concentration combinations tested for each fecal pat. A preliminary statistical analysis, omitting an animal housing effect, suggested that differences in recovery between fecal pats were statistically very highly significant ($\chi^2_1 = 122.45$; $P < 0.001$). Further analysis suggests that differences in recovery from fecal pats from housed and grazing animals may partly explain this. *E. coli* O26 was recovered from 68 of 92 (74%) and 41 of 90 (46%) inoculated subsamples from grazing and housed animals, respectively, a difference that approaches statistical significance ($F_{1,5} = 6.21$; $P = 0.067$). This possible systematic effect does

not account for all the variation, however, and differences between fecal pats from the same management system remain statistically very highly significant ($\chi^2_1 = 30.75$; $P < 0.001$). Estimates of ED_{80} for individual fecal pats range from 9.1×10^2 to 1.7×10^5 CFU g^{-1}.

Control results for the experiment are as follows. First, *E. coli* O26 was not recovered from any of the negative-control subsamples. Second, although interference with the growth of *E. coli* O26 by feces was observed for 7 of the 36 strain–fecal pat combinations, involving 4 strains and 3 fecal pats (data not shown), there was no evidence of an association between the recovery of *E. coli* O26 from inoculated fecal subsamples and the pattern of growth-interference ($F_{1,178} = 1.24$; $P > 0.10$). Third, *E. coli* O26 strains with PCR banding patterns different from those inoculated were recovered from six subsamples. These involved five fecal pats, three strains, and inoculum concentrations ranging from 1.6×10^0 to 3.4×10^4 CFU g^{-1}. The PCR banding patterns of isolates from four of the subsamples were consistent with other inoculated strains, while the other two isolates were different. As mentioned above, these were excluded from the statistical analysis. Finally, there was no evidence of a difference in recovery between laboratory operators ($F_{1,178} = 1.12$; $P > 0.10$).

DISCUSSION

This report describes differences in the recovery of inoculated *E. coli* O26 from bovine feces for different strains and fecal pats. Differences can be explained as being due either to variation in test sensitivity or to interactions between inoculated *E. coli* O26 strains and fecal growth inhibitors. The lack of an association between the recovery of *E. coli* O26 and the pattern of growth interference suggests that the differences are due to variation in test sensitivity. Previously published work suggesting variation between strains for an *E. coli* O157 IMS-based test (4) is consistent with these observations, although variation in recovery between fecal pats has not been previously reported. The results also raise the possibility of a difference in test sensitivity associated with fecal pats obtained from grazing and housed animals. Although the difference only approaches statistical significance, the observation is potentially important and, if confirmed, would have implications for the interpretation of statistical associations with factors such as season and diet.

The sensitivity of this test has been defined using the ED_{80}. This is the inoculated dose of *E. coli* O26 required for an 80% chance of recovering one or more *E. coli* O26 colonies from a sample using the test procedure. We use this definition because it represents a reasonable chance that a test will identify samples containing the target organism, although an ED_{95} would be more desirable. The ED_{80} of 1.0×10^4 CFU g^{-1} for this test is the average value for all the strains and fecal pats used in the validation. We believe that the distribution of estimates of ED_{80} for each strain and fecal pat combination provide some guidance as to how the test performs on naturally infected samples. The range of ED_{80} estimates for fecal pat–strain combinations from 4.2×10^2 to 4.8×10^5 CFU g^{-1} compares to published estimates of the concentration of VTEC O26 in the feces of naturally infected cattle, ranging from less than 1×10^2 to 1×10^9 CFU g^{-1} (7, 8, 9, 23). The utility of this test is therefore restricted to identifying individuals shedding a large number of *E. coli* O26 organisms or, if an appropriate number of individuals in a herd are sampled, to identifying affected herds.

Two observations surprised us, and it is important for future validation work to describe these. The first is the recovery from six subsamples of *E. coli* O26 strains with PCR banding patterns different from those inoculated. The second is the recovery from five subsamples of *E. coli* O26 inoculated with between 1.6×10^{-1} to 8.4×10^{-1} CFU, particularly given that we would expect the test to have a low sensitivity at these levels. Possible explanations for these observations include crossover contamination and/or the presence of undetected populations of endogenous *E. coli* O26 in the samples, although the recovery of colonies with different PCR banding patterns may be a consequence of rapid chromosomal change, perhaps similar to that observed for *E. coli* O157 (1). We are reassured by the failure to recover *E. coli* O26 from any of the 36 negative controls, which suggests that these unexpected observations do not have a major impact on the results.

An advantage of this validation procedure is that it evaluates the performance of a test from the individual performances of a range of strains in different fecal pats. However, there are also limitations with this approach. First, care has to be taken in selecting the range of inoculum concentrations. Attempts to inoculate samples at a concentration of less than 7 CFU g^{-1} feces will result in a proportion of samples ($\geq$0.001) receiving only carrier solution and no target organism. Care also has to be taken not to inoculate samples at too many concentrations where the test sensitivity approaches 100%, since this will result in a poor model fit and unreliable error estimates. Second, we used 6 strains and fecal pats, and while this is more than previously used on an individual rather than a pooled basis, it would be desirable to increase these to at least 10 of each for future validations. Finally there is likely to be some concern about the use of artificially inoculated samples to estimate test sensitivity. While we cannot contest the possible validity of this, we note that a major advantage of artificial inoculation is that the number of target organisms added is known. This is not the case with alternative approaches in which fecal samples from naturally or artificially infected animals are used and where the number of target organisms has to be estimated using a less-than-perfect test procedure.

This validation experiment has not estimated the test specificity. The 36 samples inoculated with 0 CFU of *E. coli* O26 g^{-1} could have been used for this purpose rather than as negative controls. Given our current experimental design, where the recovery of an *E. coli* O26 isolate from any one of the negative controls would have invalidated the experiment, we have not provided a quantitative estimate of specificity. However, we note that the failure to recover an isolate from any of the negative controls is an indication that the specificity of the test is high.

This study provides a benchmark estimate of sensitivity for an IMS-based test for a member of the *Enterobacteriaceae*. Although the test appears to be relatively better at detecting *E. coli* O26 than a PCR/DNA probe technique (12, 18), the results of the experiment suggest that its utility is restricted to reliably identifying individuals shedding high numbers of *E. coli* O26 organisms or, if an appropriate number of individuals

in a herd are sampled, providing estimates of herd prevalence. Both applications are useful, the first because high-shedding animals are likely to contribute disproportionately to environmental contamination and the persistence of pathogenic *E. coli* in a herd and the second because the test identifies herds where there is a current outbreak. We believe that it would be useful to apply the methodological approach described in this report to estimating the sensitivity of tests for other pathogenic members of the *Enterobacteriaceae* and other sample types.

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REFERENCES

1. **Bielaszewska, M., R. Prager, W. Zhang, A. W. Friedrich, A. Mellmann, H. Tschäpe, and H. Karch.** 2006. Chromosomal dynamism in progeny of outbreak-related sorbitol-fermenting enterohemmorrhagic *Escherichia coli* O157:NM. Appl. Environ. Microbiol. **72:**1900–1909.
2. **Bokhari, S. M. H., and F. Ørskov.** 1952. O-grouping of *E. coli* strains isolated from cases of white scours. Acta Pathol. Microbiol. Scand. **30:**87–89.
3. **Breslow, N. E., and D. G. Clayton.** 1993. Approximate inference in generalized linear mixed models. J. Am. Statist. Assoc. **88:**9–25.
4. **Chapman, P. A., A. T. Cerdan-Malo, C. A. Siddons, and M. Harkin.** 1997. Use of commercial enzyme immunoassays and immunomagnetic separation systems for detecting *Escherichia coli* O157 in bovine fecal samples. Appl. Environ. Microbiol. **63:**2549–2553.
5. **Cobbold, R., and P. Desmarchelier.** 2000. A longitudinal study of Shiga-toxigenic *Escherichia coli* (STEC) prevalence in three Australian dairy herds. Vet. Microbiol. **71:**125–137.
6. **Efron, B.** 1987. Better bootstrap confidence intervals. J. Am. Statist. Assoc. **82:**171–185.
7. **Fukushima, H., and M. Gomyoda.** 1999. An effective, rapid and simple method for isolation of Shiga toxin-producing *Escherichia coli* O26, O111 and O157 from faeces and food samples. Zentbl. Bakteriol. **289:**415–428.
8. **Fukushima, H., K. Hoshina, and M. Gomyoda.** 1999. Long-term survival of Shiga toxin-producing *Escherichia coli* O26, O111, and O157 in bovine feces. Appl. Environ. Microbiol. **65:**5177–5181.
9. **Fukushima, H., and R. Seki.** 2004. High numbers of Shiga toxin-producing *Escherichia coli* found in bovine faeces collected at slaughter in Japan. FEMS Microbiol. Lett. **238:**189–197.
10. **Hiramatsu, R., M. Matsumoto, Y. Miwa, Y. Suzuki, M. Saito, and Y. Miyazaki.** 2002. Characterization of Shiga toxin-producing *Escherichia coli* O26 strains and establishment of selective isolation media for these strains. J. Clin. Microbiol. **40:**922–925.
11. **Infectious Disease Surveillance Center.** 2004. Enterohemorrhagic *Escherichia coli* infection as of May 2004, Japan. Infect. Agents Surveillance Rep. **25:**138–139.
12. **Jenkins, C., M. C. Pearce, A. W. Smith, H. I. Knight, D. J. Shaw, T. Cheasty, G. Foster, G. J. Gunn, G. Dougan, H. R. Smith, and G. Frankel.** 2003. Detection of *Escherichia coli* serogroups O26, O103, O111 and O145 from bovine feces using immunomagnetic separation and PCR/DNA probe techniques. Lett. Appl. Microbiol. **37:**207–212.
13. **Karmali, M. A., M. Petric, C. Lim, P. C. Fleming, G. S. Arbus, and H. Lior.** 1985. The association between idiopathic hemolytic uremic syndrome and infection by Verocytotoxin-producing *Escherichia coli.* J. Infect. Dis. **151:** 775–782.
14. **Locking, M., L. Allison, L. Rae, and M. Hanson.** 2004. VTEC in Scotland 2003: enhanced surveillance and reference laboratory data. SCIEH Wkly. Rep. **38:**294–297.
15. **Parma, A. E., M. E. Sanz, J. E. Blanco, J. Blanco, M. R. Vinas, M. Blanco, N. L. Padola, and A. I. Etcheverria.** 2000. Virulence genotypes and serotypes of verotoxigenic *Escherichia coli* isolated from cattle and foods in Argentina. Importance in public health. Eur. J. Epidemiol. **16:**757–762.
16. **Paton, A. W., and J. C. Paton.** 1998. Detection and characterization of Shiga toxigenic *Escherichia coli* using multiplex PCR assays for stx_1, stx_2, *eaeA*, enterohemorrhagic *E. coli hlyA*, rfb_{O111}, and rfb_{O157}. J. Clin. Microbiol. **36:**598–602.
17. **Pearce, M. C., J. Evans, I. J. McKendrick, A. W. Smith, H. I. Knight, D. J. Mellor, M. E. J. Woolhouse, G. J. Gunn, and J. C. Low.** 2006. Prevalence and virulence factors of *Escherichia coli* serogroups O26, O103, O111, and O145 shed by cattle in Scotland. Appl. Environ. Microbiol. **72:**653–659.
18. **Pearce, M. C., C. Jenkins, L. Vali, A. W. Smith, H. I. Knight, T. Cheasty, H. R. Smith, G. J. Gunn, M. E. J. Woolhouse, S. G. B. Amyes, and G. Frankel.** 2004. Temporal shedding patterns and virulence factors of *Escherichia coli* serogroups O26, O103, O111, O145, and O157 in a cohort of beef calves and their dams. Appl. Environ. Microbiol. **70:**1708–1716.
19. **Sharma, V. K.** 2002. Detection and quantitation of enterohemorrhagic *Escherichia coli* O157, O111, and O26 in beef and bovine feces by real-time polymerase chain reaction. J. Food Prot. **65:**1371–1380.
20. **Smith, H. R., T. Cheasty, G. A. Willshaw, A. Caprioli, A. E. Tozzi, E. Coia, I. S. T. Fisher, S. J. O'Brien, A. Fruth, H. Tschäpe, and W. J. Reilly.** 2002. Changing patterns of VTEC infection in Britain and continental Europe. Notiziario ISS **15:**S1.
21. **Taylor, J.** 1961. Host specificity and enteropathogenicity of *Escherichia coli.* J. Appl. Bacteriol. **24:**316–325.
22. **Wells, J. G., L. D. Shipman, K. D. Greene, E. G. Sowers, J. H. Green, D. N. Cameron, F. P. Downes, M. L. Martin, P. M. Griffin, S. M. Ostroff, M. E. Potter, R. V. Tauxe, and I. K. Wachsmuth.** 1991. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J. Clin. Microbiol. **29:**985–989.
23. **Widiasih, D. A., N. Ido, K. Omoe, S. Sugii, and K. Shinagawa.** 2004. Duration and magnitude of faecal shedding of Shiga toxin-producing *Escherichia coli* from naturally infected cattle. Epidemiol. Infect. **132:**67–75.
24. **World Health Organization.** 1998. Zoonotic non-O157 Shiga toxin-producing *Escherichia coli* (STEC). Report of a WHO scientific working group meeting, Berlin, Germany. Department of Communicable Disease Surveillance and Response, World Health Organization. [Online.] http://whqlibdoc.who.int/hq/1998/WHO_CSR_APH_98.8.pdf.