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. Gunn1

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

Received 23 December 2005/Accepted 4 September 2006

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 (ED80) was 1.0 × 10^4 CFU g⁻¹ feces (95% confidence interval, 4.7 × 10³ to 2.4 × 10⁴). Differences in test sensitivity between different *E. coli* O26 strains and fecal pats were also observed. Individual estimates of ED₉₀ for each strain and fecal pat combination ranged from 4.2 × 10² to 4.8 × 10⁴ 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.

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 10⁶ 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, C668.1, and S6293.2). 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. Of the five strains, the vteC gene, one strain has an additional vtx2 gene, three strains have eaeA, 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 10⁶ to 10⁰ 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 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 10⁻¹- by 10⁻⁰ 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

<table>
<thead>
<tr>
<th>Conc of inoculated <em>E. coli</em> O26 in feces (CFU g⁻¹)</th>
<th>No. of positive subsamples/no. tested</th>
<th>% Positive subsamples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6 × 10⁻¹ to 8.4 × 10⁻¹</td>
<td>5/36</td>
<td>14</td>
</tr>
<tr>
<td>1.6 × 10⁻¹ to 8.4 × 10⁻¹</td>
<td>3/34</td>
<td>9</td>
</tr>
<tr>
<td>1.6 × 10⁻¹ to 8.4 × 10⁻¹</td>
<td>7/36</td>
<td>19</td>
</tr>
<tr>
<td>1.6 × 10⁻¹ to 8.4 × 10⁻¹</td>
<td>18/35</td>
<td>51</td>
</tr>
<tr>
<td>1.6 × 10⁻¹ to 8.4 × 10⁻¹</td>
<td>21/35</td>
<td>60</td>
</tr>
<tr>
<td>1.6 × 10⁻¹ to 8.4 × 10⁻¹</td>
<td>29/34</td>
<td>85</td>
</tr>
<tr>
<td>1.6 × 10⁻¹ to 8.4 × 10⁻¹</td>
<td>33/36</td>
<td>92</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Conc of inoculated <em>E. coli</em> O26 in feces (CFU g⁻¹)</th>
<th>% Probability of recovery 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 × 10⁴</td>
<td>28</td>
</tr>
<tr>
<td>1 × 10³</td>
<td>56</td>
</tr>
<tr>
<td>1 × 10²</td>
<td>80</td>
</tr>
<tr>
<td>1 × 10¹</td>
<td>93</td>
</tr>
<tr>
<td>1 × 10⁰</td>
<td>98*</td>
</tr>
</tbody>
</table>

* The highest number of *E. coli* O26 inoculated into a subsample was 8.4 × 10⁻¹ CFU.

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 × 10³ and 8.4 × 10⁵ 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 × 10³ and 1 × 10⁵ CFU of *E. coli* O26 g⁻¹, respectively. The mean ED₈₀, averaged across strains and fecal pats, was 1.0 × 10⁴ CFU of inoculated *E. coli* O26 g⁻¹ feces (CI, 4.7 × 10³ to 2.4 × 10⁴). Estimates of ED₈₀ for each strain and fecal pat combination range from 4.2 × 10⁴ to 4.8 × 10⁵ CFU g⁻¹, with first and third quartiles of 1.1 × 10⁴ and 1.0 × 10⁵ 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 (χ² = 122.45; P < 0.001). Estimates of ED₈₀ for individual strains range from 4.7 × 10³ to 2.8 × 10⁴ 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 (χ² = 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 (F₁,₅ = 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 \((x^2_1 = 30.75; P \leq 0.001)\). Estimates of \(ED_{90}\) for individual fecal pats range from \(9.1 \times 10^2\) to \(1.7 \times 10^3\) 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 \(1.6 \times 10^9\) to \(3.4 \times 10^7\) CFU g\(^{-1}\). The PCR banding patterns of isolates from four of the sub-samples 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 \(ED_{90}\). 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 \(ED_{90}\) would be more desirable. The \(ED_{90}\) of \(1.0 \times 10^6\) 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 \(ED_{90}\) for each strain and fecal pat combination provide some guidance as to how the test performs on naturally infected samples. The range of \(ED_{90}\) estimates for fecal pat–strain combinations from \(4.2 \times 10^2\) to \(4.8 \times 10^5\) CFU g\(^{-1}\) compares to published estimates of the concentration of VTEC O26 in the feces of naturally infected cattle, ranging from less than \(1 \times 10^2\) to \(1 \times 10^5\) 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 \(1.6 \times 10^{-3}\) to \(8.4 \times 10^{-3}\) 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 \((\approx 0.001)\) 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 \textit{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 \textit{Enterobacteriaceae} and other sample types.

ACKNOWLEDGMENTS

Valuable discussions with Norval Strachan (University of Aberdeen), Graham Horgan, Iain McKenzie (Biometrics and Statistics Scotland), and Roger Humphry (Scottish Agricultural College) and the helpful comments of three anonymous referees are gratefully acknowledged.

This work was supported by the Food Standards Agency, Scotland, and a Wellcome Trust International Partnership Research Award in Veterinary Epidemiology, \textit{Epidemiology and Evolution of Enterobacteriaceae Infections in Humans and Domestic Animals}. The Scottish Agricultural College also receives financial support from the Scottish Executive Environment and Rural Affairs Department.

REFERENCES


