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David R. Smith
University of Nebraska - Lincoln, dsmithr@unlnotes.unl.edu

J.T. Gray
University of Nebraska - Lincoln

Rodney A. Moxley
University of Nebraska - Lincoln, rmoxley1@unl.edu

S.M. Younts-Dahl
University of Nebraska - Lincoln

M.P. Blackford
University of Nebraska - Lincoln

See next page for additional authors

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Authors
David R. Smith, J.T. Gray, Rodney A. Moxley, S.M. Younts-Dahl, M.P. Blackford, S. Hinkley, L.L. Hungerford, C.T. Milton, and Terry J. Klopfenstein

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A diagnostic strategy to determine the Shiga toxin-producing *Escherichia coli* O157 status of pens of feedlot cattle

D. R. SMITH[1]*, J. T. GRAY[1]†, R. A. MOXLEY[1], S. M. YOUNTS-DAHL[1]‡, M. P. BLACKFORD[2], S. HINKLEY[1], L. L. HUNGERFORD[1]§, C. T. MILTON[2]‖ AND T. J. KLOPFENSTEIN[2]

[1] *Department of Veterinary and Biomedical Sciences, Institute of Agriculture and Natural Resources, University of Nebraska–Lincoln, USA*
[2] *Department of Animal Science, Institute of Agriculture and Natural Resources, University of Nebraska–Lincoln, USA*

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SUMMARY

Although cattle are reservoirs, no validated method exists to monitor Shiga toxin-producing *Escherichia coli* O157 (STEC O157) on farms. In 29 Midwestern United States feedlot pens we compared culturing faeces from the individual cattle to: (1) culturing rope devices that cattle rub or chew; and (2) culturing a composite of faecal pats. Eighty-six per cent (68–96%) of pens were classified correctly using rope devices to detect pens with at least 16% of the cattle shedding STEC O157 [sensitivity = 82% (57–96%); specificity = 92% (62–100%)]. Ninety per cent of pens (73–98%) were classified correctly using composite faeces to detect pens with at least 37% of the cattle shedding STEC O157 [sensitivity = 86% (42–100%); specificity = 91% (71–99%)]. Ranking pens into three risk levels based on parallel interpretation of the pen-test results correlated (Spearman's $r = 0{\cdot}76$, $P < 0{\cdot}0001$) with the pen's prevalence. This strategy could identify pens of cattle posing a higher risk to food safety.

INTRODUCTION

Cattle are an important reservoir of Shiga toxin-producing *Escherichia coli* (STEC) [1, 2]. Unfortunately, research and development of on-farm programmes to control STEC O157 (*E. coli* O157:H7 and O157:NM) in feedlot production systems has been hampered by the difficulty of determining the infection status of cattle at any point in time. Until now there have been no field-validated methods to monitor livestock for pathogens of food safety concern. The difficulty in diagnosis occurs because infection with STEC O157 in cattle does not result in clinical signs, except in neonatal calves [3]. Determining if individual live cattle are shedding STEC O157 is also expensive, logistically difficult, and may be injurious to the cattle because of the handling involved. Handling finished cattle for testing immediately prior to shipping is undesirable because of the loss in carcass quality and value due to stress and bruising.

Even though it is important to know the STEC O157 infection status of groups of feedlot cattle, it may not be necessary to know the infection status of individual cattle. Because feedlot cattle are managed

* Author for correspondence and reprint requests: D. R. Smith, University of Nebraska–Lincoln, P.O. Box 830907, Lincoln, NE 68583-0907, USA.
† Present address: USDA, ARS, Antimicrobial Resistance Research Unit, Athens, GA, USA.
‡ Present address: Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA.
§ Present address: Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, MD, USA.
‖ Present address: 10249 W. Loup River Road, Dannebrog, NE, USA.

as groups the control points or interventions for reducing human foodborne pathogens would most likely be directed towards pens of cattle rather than individuals [4]. If pens of cattle could be accurately and economically classified according to the level of faecal shedding of STEC O157, the research and development of risk-based feedlot food safety programmes might advance. A pen-level test for STEC O157 could serve as a monitoring tool in feedlot production food-safety programmes and it would allow researchers to test potential farm-level interventions, and/or identify feedlot production practices associated with the pens of cattle at greatest risk for contributing pathogens into the food supply. In this study we evaluated diagnostic strategies to more efficiently classify pens of feedlot cattle according to the percentage of cattle shedding STEC O157.

Fig. 1. Feedlot steer contacting the rope device. The device was prepared from manilla rope and was mounted over feedbunks and water-tanks. Curious cattle deposit microorganisms onto the ropes while rubbing, licking, or chewing.

MATERIALS AND METHODS

Twenty-nine pens of cattle located on five privately owned commercial Midwestern feedlots in the United States were each studied once during the period June to September, 1999 [5]. On evenings prior to sampling, seven manilla ropes of 1·3 cm diameter and measuring 80 cm were placed in the pen over feedbunks and water-tanks so that the cattle could rub, lick or chew the devices (Fig. 1). On mornings of sampling, approximately 30 g of faeces were collected from the rectum of each animal while they were restrained in a handling chute. The rope devices and a single 100 g composite sample of 20 fresh faecal pats from the pen surface were collected concurrently on the same morning (prior to 09:00 hours). All samples were tested for the presence of STEC O157 by bacteriological culture.

Culture methods were specific to the type of sample, but they included selective enrichment, immunomagnetic separation and agar plating. Identity of each isolate was confirmed by standard methods including PCR. Methods for recovery of STEC O157 from individual and composite fecal samples were modifications of those recently reported [6, 7]. A total of 10 g of faeces were incubated for 6 h at 37 °C in 90 ml GN broth containing 8 μg/ml vancomycin, 50 ng/ml cefixime and 10 μg/ml cefsulodin. Subsequently, 1 ml of this culture was subjected to O157 immunomagnetic separation (Dynal, Lake Success, NY, USA) and 50 μl of the bead-bacteria mixture was spread onto sorbitol–MacConkey plates containing cefixime (0·05 μg/ml) and potassium tellurite (2·5 μg/ml) (CT–SMAC). Individual sorbitol non-fermenting colonies were subcultured on MacConkey and Fluorocult agars (EM Science, Gibbstown, NJ, USA), and in MacConkey broth. Methylumbelliferyl-β-D-glucuronidase activity-negative, lactose-fermenting colonies were further tested for indole, fermentation pattern on triple sugar iron agar, and Voges–Proskauer reaction. Indole-positive isolates were tested for O157 antigen by latex agglutination, and O157-positive isolates were subcultured onto blood agar and tested for H7 antigen by latex agglutination (Remel, Lenexa, KS, USA). *E. coli* O157:NM or O157:H7 isolates were confirmed as *E. coli* by biochemical testing (API bioMérieux, Hazelwood, MO, USA), and a subset of these were tested by PCR for Shiga toxin (*stx*), intimin (*eae*), and the O157 cluster (*wbdN*) genes using published protocols [8, 9]. A positive PCR test result was indicated by the detection of the *wbdN* gene and one or both of the *stx* and *eae* genes. Rope devices were added to Brilliant Green bile broth containing 2% bile and 0·00133% Brilliant Green as inhibitory agents while maintaining an approximate ratio of 10 ml media/g of sample. The samples were incubated at 37 °C for 6 h. Subsequently, 1 ml of each sample was removed and processed according to the anti-O157 immunomagnetic

separation protocol (Dynal). Then 50 μl of the final resuspension was plated on CT–SMAC medium and incubated for 18 h at 37 °C. Sorbitol-negative suspect colonies were picked and subjected to STEC O157 confirmatory testing as described above.

The prevalence of cattle shedding STEC O157 was determined by culture of faeces from individual cattle in the respective pens. In addition, each pen was classified as being positive or negative based on the pen-test results of culture for (1) the rope devices, and (2) the composite faecal sample. A given pen was classified as rope-device positive if STEC O157 was recovered from at least one of the seven rope devices. The pen was classified as composite-faeces positive if the organism was recovered from the single composite faecal sample. The results of bacteriological culture of the rope devices and composite faeces were evaluated separately as tests to differentiate pens with a higher proportion of cattle shedding STEC O157 (higher risk groups) from pens with lower proportions (lower risk groups).

Pens were classified dichotomously as high or low prevalence at different cut-off points for the proportion of cattle within the pen that was culture-positive for STEC O157. Test sensitivity, specificity, and the per cent of pens classified correctly for the dichotomous pen classifications from testing ropes or composite faeces (positive or negative pen-test status) were evaluated compared to the differing prevalence cut-off points from the tests of individual cattle.

Pens were also classified into three ordinal levels as high risk, medium risk, or low risk based on the pen-test results from both the culture of rope devices and composite faeces. In this scheme pens were classified as high risk if STEC O157 was recovered from the composite-faeces sample, medium risk if the organism was recovered from the rope device but not the composite faeces, and low risk if the organism was not recovered from either the rope device or the composite faeces.

Non-parametric statistical methods were used to test for association between the pen-test classifications based on tests of the rope devices or composite faecal samples and the prevalence of the pen as determined by testing the individual cattle. Differences in rank-order of categorical variables were tested using the Kruskal–Wallis test. Correlation between the ranks of ordinal variables was tested using the Spearman rank correlation test. Exact binomial 95% confidence intervals (CIs) were calculated for proportions using epidemiological software (Epi-Info 6.04, Jan. 2001, Centers for Disease Control and Prevention, Atlanta, GA, USA).

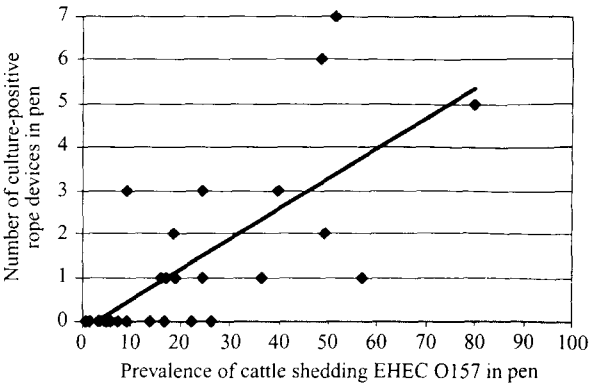

Fig. 2. Relationship between the prevalence of cattle shedding STEC O157 and the concurrent number of culture-positive rope devices from seven placed within the pen. The solid line represents the least-squares linear regression line.

RESULTS

The total number of cattle tested was 3162 from 29 feedyard pens. The number of cattle in each pen ranged from 36 to 231 (median 107). Rope devices were placed in pens between 17:42 and 20:30 hours and recovered the following morning between 05:30 and 08:00 hours. STEC O157 was isolated from at least one animal in each of the 29 pens. The percentage of cattle shedding detectable numbers of the organism within a pen ranged from 0·7 to 79·8% (median 17·1%). The number of rope devices culture-positive for STEC O157 correlated with the prevalence of cattle shedding the organism within the pen (Spearman's $r=0{\cdot}72$, $P<0{\cdot}0001$, Fig. 2). STEC O157 was recovered from at least one rope device in 15 pens (Fig. 3*a*) and from the composite faecal sample of 8 pens (Fig. 3*b*). Both the rope devices and composite faecal sample were culture negative in 14 pens. All pens classified as composite-faeces positive were rope-device positive.

Pens classified as rope-device positive had a greater median prevalence of cattle shedding STEC O157 than did pens that were rope-device negative ($P=0{\cdot}001$). A maximum 86% (95% CI 68–96) of the pens were classified correctly by the culture results from the rope devices if pens with at least 16% of the cattle shedding STEC O157 were defined as high prevalence (Fig. 4). At that cut-off point for defining high prevalence, the probability of a positive pen-test

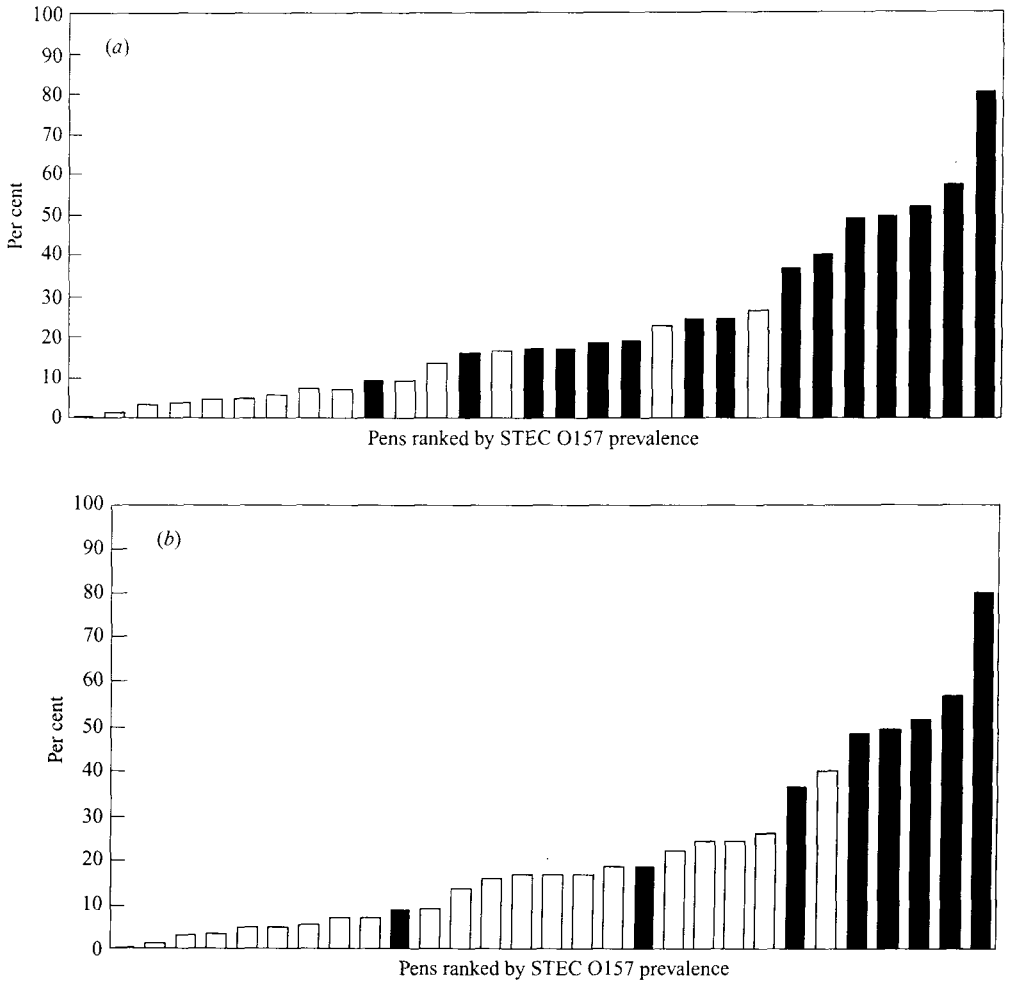

Fig. 3. The percentage of cattle shedding STEC O157 from each of 29 feedlot pens (bars arranged in order of prevalence). Culture of STEC O157 from (*a*) at least one of seven rope devices, or (*b*) from a composite of 20 faecal pats, was compared to culture of the organism from faeces collected from each animal in the pen. The height of the bars represents the percentage of cattle shedding the organism in faeces. (*a*) ■, Rope-device positive; □, rope-device negative. (*b*) ■, Composite-faeces positive; □, composite-faeces negative.

result correctly classifying a high-prevalence pen using the test results from rope devices (i.e. pen-level sensitivity) was 82% (95% CI 57–96) and the probability of correctly identifying a low-prevalence pen (i.e. pen-level specificity) was 92% (95% CI 62–100).

Pens which were composite-faeces positive had a greater median prevalence of cattle shedding STEC O157 than did those pens that were composite-faeces negative ($P=0{\cdot}001$). A maximum 90% of the pens (95% CI 73–98) were classified correctly by the culture results from composite faeces if pens with at least 37% of the cattle shedding STEC O157 were defined as high prevalence (Fig. 4). At that cut-off point for defining high prevalence, the probability of a positive pen-test result correctly classifying a high-prevalence pen using the test results from composite faeces (i.e. pen-level sensitivity) was 86% (95% CI 42–100) and that of correctly identifying a low-prevalence pen (i.e. pen-level specificity) was 91% (95% CI 71–99).

The classification of pens into three levels of risk based on the pen-test results from rope devices and composite faecal samples correlated (Spearman's $r=0{\cdot}76$, $P<0{\cdot}0001$) with the pen prevalence measured from tests of individual cattle. Pens classified by this scheme as high risk had significantly higher proportions of cattle shedding STEC O157 (based on the tests of individuals) than pens classified as medium risk ($P=0{\cdot}05$) or low risk ($P=0{\cdot}0006$), and pens classified as medium risk had significantly greater proportions of cattle shedding STEC O157 than pens classified as low risk ($P=0{\cdot}005$).

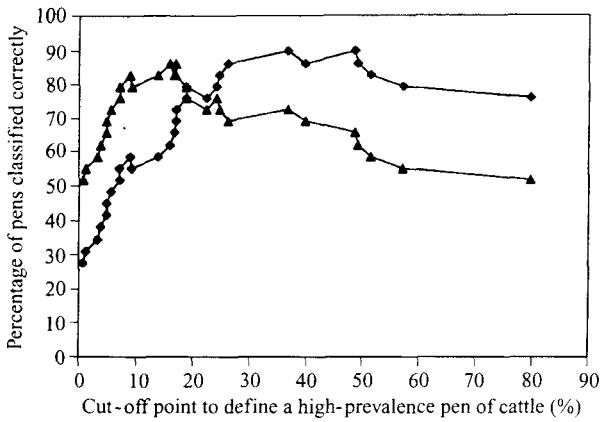

Fig. 4. The percentage of pens classified correctly by testing rope devices (▲) or a composite of faeces (◆) at different cut-off values for the prevalence to define pens at high risk for STEC O157.

DISCUSSION

The premise of the pen-test strategy as implemented in this study was to culture a few samples from which many cattle in a pen could have contributed organisms. The use of ropes as a pen-testing device was a novel sampling strategy that capitalized on the behavioural characteristic of cattle to rub, lick or chew objects in their environment which pique their curiosity.

It is likely that recovery of STEC O157 from the ropes resulted from the transfer of the organism from the mouth or hide as the cattle rubbed, licked, or chewed the devices. The source of the organism may be regurgitated rumen fluid or environmental, acquired during grooming or ingestion of contaminated feed or water [10, 11]. Regardless of the source, recovery of STEC O157 from the rope devices was correlated with the prevalence of cattle shedding the organism from within the same pen.

We have observed and reported a greater proportion of cattle sampled in pens with seven ropes rather than pens with three, and their rate of contact with the ropes is greatest in the first hour of placement [12]. We empirically chose to place the rope devices into the pens 1–2 h prior to dusk because that is the period when cattle have shown the most interest in the devices.

Even though STEC O157 was recovered from the faeces of cattle of each pen the proportion of cattle shedding the organism varied widely. The number of culture-positive ropes was positively correlated with the proportion of cattle within the pen shedding STEC O157; however, the number of culture-positive ropes was not a sufficiently useful diagnostic criterion. Recovery of STEC O157 from one or more of the rope devices or from the composite faecal sample was useful to accurately classify the pens with the greater proportion of cattle shedding STEC O157.

Testing the rope devices accurately classified the pens to a lower level of prevalence than culture of the composite faecal sample. The cut-off values of 16% prevalence and 37% prevalence for defining the sensitivity and specificity of culture from the rope devices and the composite faeces respectively, were based on maximizing the proportion of herds that were classified correctly using each method. The information from both tests used in parallel was useful for ranking the pens in order of risk to food safety. The proportion of cattle carrying STEC O157 has been positively correlated with the rate of contamination on carcasses [13]. Therefore, culture of the rope devices alone or in combination with culture of a composite faecal sample should prove useful for identifying high-prevalence pens of cattle, presumably posing greater risk to food safety (testing near marketing) or environmental contamination.

As demonstrated here, bacteriological culture of the pen-test rope devices alone or in parallel with culture of a composite faecal sample was a diagnostically efficient strategy to characterize the STEC O157 faecal shedding in feedlot pens during the summer months. For example, in the population of cattle studied we classified with reasonable accuracy pens of cattle above or below the median prevalence of shedding using information from 203 rope devices compared to 3162 faecal samples from individual cattle. Similarly, the pens of cattle were reasonably accurately classified as above or below near the upper 25th percentile using information from only 29 composite faecal samples. This diagnostic strategy was more efficient than sampling individual cattle because small numbers of tests were necessary and cattle performance was not altered by injury or stress that could occur during sampling. To our knowledge this is the only pen-level strategy for detecting human food safety pathogens in live cattle that has been evaluated for test performance.

The principles of hazard-analysis critical-control points (HACCP) were developed to minimize the likelihood that food might be contaminated with potentially dangerous pathogens [14]. Ideally, the safety of food would be maximized if HACCP principles were applied at all levels of food production and processing, employing the 'microbial hurdles'

approach to food safety [15]. The first hurdle for STEC O157 should logically begin with live cattle. Unfortunately, there is not enough known about the epidemiology and ecology of STEC O157 to design and implement HACCP-based food safety programmes in cattle feedyards [14].

This novel diagnostic approach may be useful to the development and implementation of animal production food safety programmes as a monitoring tool within a HACCP approach to control human food safety pathogens on the farm. For example, researchers could use this testing strategy to characterize the STEC O157 status of pens of cattle enrolled in large observational studies or in clinical trials to test interventions. Also, this testing strategy might be used to monitor pens of cattle for their risk to food safety relative to STEC O157 as part of an on-farm food safety programme. In the future this approach may provide a method to selectively target certain high-risk pens for corrective action either prior to or after harvest.

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