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ENUMERATION OF *ESCHERICHIA COLI* OL57 IN CATTLE FECES USING IMMUNOMAGNETIC SEPARATION COMBINED WITH MOST PROBABLE NUMBER TECHNIQUES

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Introduction

Escherichia coli O157 is a food borne pathogen that causes hemorrhagic colitis and hemolytic uremic syndrome in humans *(1)*. In 1982 *E. coli* O157 was first identified as a human pathogen after the investigation of two outbreaks involving undercooked ground beef *(8)*. Since these outbreaks, investigation of pre and post-harvest interventions has been conducted to ensure food safety of the beef supply. Direct or indirect contamination with ruminant feces is the leading predecessor to infections caused by *E. coli* O157 *(6, 7)*. Most preventive measures involving the reduction of *E. coli* O157 in beef products have been at the post-harvest level. As new interventions have been investigated we have observed an increase in pre-harvest prevention of *E. coli* O157. Due to the direct correlation between positive animal samples (fecal and hide) and carcass contamination *(4)* pre-harvest intervention plays an important role in decreasing the pathogen load *(1)*.

To date, the detection of *E. coli* O157 in feces of cattle has mainly been qualitative. Quantitative analysis of bovine feces naturally infected with *E. coli* O157 would be a valuable asset to determine the impact of interventions in the pre-harvest environment and to determine the risk of transferring the pathogen to beef products. We have taken into account previously reported methods *(3, 5)* and have developed a new methodology that is more specific and sensitive to *E. coli* O157 enumeration in bovine feces.

Objectives

The objective of this study was to develop an enumeration protocol that is sensitive and accurate in determining the amount of *E. coli* O157 present in bovine feces.

Methodology

Experimentation

Five separate studies were conducted as follows to determine the accuracy and specificity at both low-level and high-level inoculations in feces:

Study 1: Manure was sterilized and inoculated with a 10^2 *E. coli* O157:H7/g and populations were determined by direct plating of the cocktail (prior to inoculation of the feces) and the newly developed MPN/IMS methodology.

Study 2: Non-sterile, freshly collected manure was inoculated with a cocktail mixture of 10^3 *E. coli* O157:H7/g and populations were determined by direct plating of the cocktail (prior to inoculation of the feces) and the newly developed MPN/IMS methodology.

Study 3: Non-sterile, freshly collected manure was inoculated with a cocktail mixture of a 10^1 *E. coli* O157:H7/g and populations were determined by direct plating of the cocktail (prior to inoculation of the feces) and the newly developed MPN/IMS methodology.

Study 4: Manure was sterilized and inoculated with a 10^4 streptomycin-resistant *E. coli* O157:H7/g cocktail and samples were directed-plated onto media containing streptomycin and subjected to the newly developed MPN/IMS methodology.

Study 5: Manure was sterilized and inoculated with a 10^2 streptomycin-resistant *E. coli* O157:H7/g cocktail and samples were directed-plated onto media containing streptomycin and subjected to the newly developed MPN/IMS methodology.

Sample collection

Approximately 1,000 g of bovine feces was collected for each of the three replications for all of the studies from the floor of a commercial feedlot pen. Each of the samples were stored in a cooler for transport from the feedlot to the microbiological laboratory (approximately 15 miles).

E. coli O157 Detection

Immunomagnetic separation (IMS) was initially performed on sample used in study 2 and 3 to qualitatively detect the presence of *E. coli* O157. The IMS detection methods used in these studies where adopted from Brashears et al.[2] *(2003)* and slightly modified. Instead of manually washing the beads, an automatic IMS machine was used (Dynal, Lake Success, N.Y.). Another modification was in the biochemical tests that were performed in these studies. Instead of using indole and Voges-Proskauer tests, we used MacConkey broth (MACb), trypticase soy broth (TSB), and triple sugar iron slants (TSI). The above tube media cultures were then incubated at 37^0C overnight. Colonies that fermented lactose (caused a purple to yellow media color change) in MACb, created turbidity in TSB, and were A/A (glucose and lactose and/or sucrose fermentation) or K/A (glucose fermentation only, peptone catabolized) plus gas in TSI were tested for O157 antigen with a latex agglutination kit (Remel, Lenexa, Kansas).

Cocktail preparation of E. coli *O157:H7*

For studies 1, 2 and 3, five different *E. coli* O157:H7 isolates originally isolated from bovine feces were cultivated in TSB broth at 37^0C for 24 h. For studies 4 and 5, three different streptomycin-resistant *E. coli* O157:H7 isolates from bovine manure were used (University of Nebraska Department of Food Sciences and Technology). Each culture was sub-cultured at least 2 times before experimental use. Buffered-peptone water was inoculated with a portion of each culture to obtain a cocktail mixture for experimental use. Serial dilutions of the cocktails were performed

to obtain 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions to be inoculated into the manure for all of the studies.

Sample preparation

Feces were sterilized for studies 1, 4 and 5 by autoclaving at 121^0C for 15 minutes. Sterile manure was used to facilitate both direct-plating of the *E. coli* O157 in the manure and MPN/IMS methodology analysis. An appropriate amount of the cocktail of *E. coli* O157:H7 was added to the manure to yield a 10^2 (study 1), 10^4 (study 4), and 10^2 (study 5) populations of *E. coli* O157:H7 in the sterile manure while a 10^3 (study 2) and 10^1 (study 3) target was obtained in the non-sterile manure. The pathogen cocktail was directly plated in the studies using non-sterile manure to verify the populations inoculated into the manure.

MPN/IMS methodology

Serial dilutions of the manure samples were made in 99 ml of BPW for each subsequent sample in all the studies. Based on the initial inoculation levels, 1 ml of the appropriate serial dilution was used to inoculate each of the first 3 MPN tubes, containing 9 ml of GN broth. The adjacent 3 MPN tubes were then inoculated with 1 ml of the next highest dilution and while the following 3 MPN tubes where then inoculated with 1 ml of the next highest dilution. These steps were then repeated for the next two highest dilutions providing a 3 X 5 MPN dilution scheme. The MPN tubes were incubated at 37^0C for 6 h.

IMS was performed as previously described on each of the 15 MPN tubes for each of the samples after the incubation. After IMS, 50 µl of the bead-bacteria and PBS Tween mixture was plated onto CT-SMAC (studies 1, 2 and 3) or *E. coli* agar with Methylumbelliferyl-β-glucuronide and streptomycin (EC_{MUG}) (studies 4 and 5). The sorbitol-negative colonies (if present) on the CT-SMAC and any colonies on the EC_{MUG} were tested for O157 antigen with a latex agglutination kit (Remel, Lenexa, Kansas). Once a colony was found to be positive on each plate that MPN tube was considered to be positive.

Direct plating methodology

The pathogen cocktails for all the studies were enumerated by direct plating dilutions onto CT-SMAC (studies 1, 2 and 3) or EC_{MUG} (studies 4 and 5). This information was an important benchmark to determine the original population that was added to the feces. Manure samples were also direct-plated onto EC_{MUG} (studies 4 and 5 only) to determine the populations in the manure itself providing us with a double verification of the populations in the manure.

Data calculations and statistical analysis

All MPN calculations were conducted by using the Bacteriological Analytical Manual Online at: http://www.cfsan.fda.gov/~ebam/bam-a2.html (FDA/CFSAN). The MPN/g data was calculated to remove dilution factors and were log base-10 transformed to control statistical variance. All statistical analysis was performed using SAS proc GLM procedures (SAS Institute Inc., Cary, N. C.).

Results & Discussion

In studies 1, 2, and 3 there was no detectible statistical variation between direct plating of the cocktail and the IMS/MPN methodology (Fig. 1). The amount of the pathogen in the cocktail was adjusted to determine the amount of the pathogen actually added to the manure samples. In study one, the amount of *E. coli* O157:H7 inoculated into the feces as determined by direct plating was 8.88 log cfu/g while the MPN analyses determined the population to be 8.54 log MPN/g. Similarly, in studies 2 and 3, the amount of *E. coli* O157 determined by direct plating was 8.88 log cfu/g and 8.88 log cfu/g, while the amount determined by direct plating was 8.25 log MPN/g and 8.59 log MPN/g, respectively.

In studies 4 and 5 there was no detectible statistical variation between direct plating of the inoculated manure and the IMS/MPN methodology (Fig. 2). Direct plating of the manure in study 4 indicated that the population of *E. coli* O157 was 4.44 log cfu/g while MPN analysis indicated that it was 4.37 log MPN/g. In study 5, no differences were observed with direct plating yielding populations of 2.57 log cfu/g and MPN analysis indicating the populations were 2.42 log MPN/g.

Conclusions

The IMS/MPN methodology used in these studies is useful in enumeration of *E. coli* O157 in inoculated bovine fecal samples. Enumeration of *E. coli* O157 in bovine feces has been and will continue to be an important tool to the beef industry. In order to provide a safe and wholesome product to the consumer it is useful to know the quantity of *E. coli* O157 present in feces of cattle at the feedlot. If producers can provide cattle to the slaughter plants with lower fecal shedding and quantity of *E. coli* O157 then preventative measures at the plant will be more effective. *E. coli* O157 enumeration of bovine feces is imperative to the producer in order to investigate and monitor preventative measures at the feedlot.

References

Brashears, M. M., D. Jaroni, and J. Trimble. 2003. Isolation, selection, and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of Escherichia coli O157:H7 in cattle. J. Food Prot. 66:355–363.

Brashears, M. M., M. L. Galyean, G. H. Loneragan, J. E. Mann, and K. Killinger-Mann. 2003. Prevalence of Escherichia coli O157:H7 and performance by beef feedlot cattle given Lactobacillus direct-fed microbials. J. Food Prot. 66:748–754.

Chapman, P. A., A. T. Cerdan-Malo, M. Ellin, R. Ashton, and M. A. Harkin. 2001. Escherichia coli O157 in cattle and sheep at slaughter, on beef and lamb carcasses and in raw beef and lamb products in South Yorkshire, UK. Int. J. Food Microbiol. 64:139–150.

Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic Escherichia coli O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci. USA. 97:2999–3003.

Fegan, N., G. Higgs, P. Vanderlinde, and P. Desmarchelier. 2004. Enumeration of Escherichia coli O157 in cattle faeces using most probable number (MPN) technique and automated immunomagnetic separation (AIMS). Lett. Appl. Microbiol. 38:56–59.

Locking, M. E., S. J. O'Brien, W. J. Reilly, E. M. Wright, D. M. Campbell, J. E. Coia, L. M Browning, and C. N. Ramsay. 2001. Risk factors for sporadic cases of Escherichia coli O157 infection: the importance of contact with animal excreta. Epidemio. Infect. 127:215–220.

O'Brien, S. J., G. K. Adak, and C. Gilham. 2001. Contact with farming environment as a major risk factor for shiga toxin (vero cytotoxin)-producing Escherichia coli O157 infection in humans. Emerg. Infect. Dis. 7:1049–1051.

Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare Escherichia coli serotype. N. Eng. J. Med. 308:681–685.

Tables and Figures

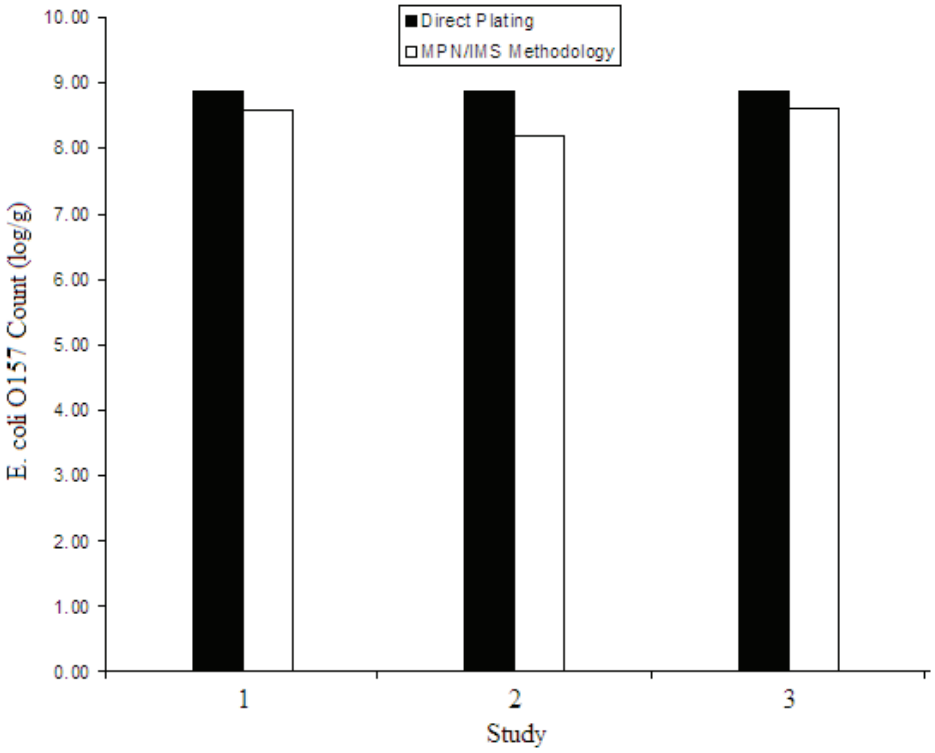

Fig. 1. *E. coli* O157 count in the manure adjusted from direct plating of the cocktail (Log cfu/g) and MPN/IMS methodology (Log MPN/g) for studies 1, 2, and 3. There was no detectible statistical variation between direct plating of the cocktail and the IMS/MPN methodology in each of these studies. Study 1 involved sterilized manure inoculated with a 10^2 *E. coli* O157:H7/g cocktail. Studies 2 and 3 involved non-sterilized manure inoculated with a 10^3 and 10^1 *E. coli* O157:H7/g cocktail, respectively.

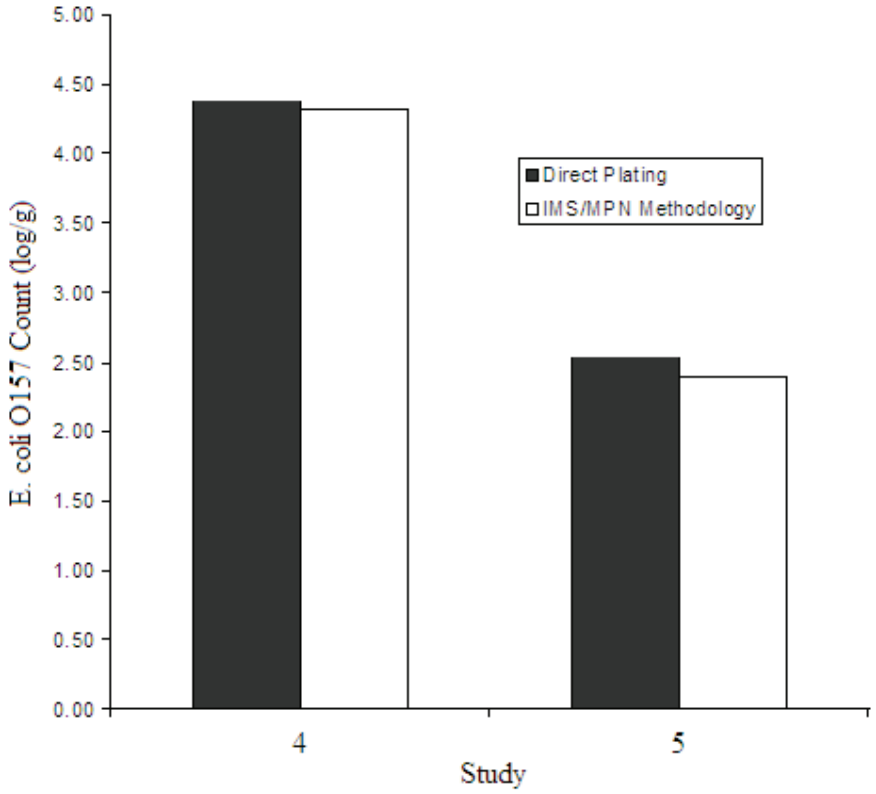

Fig. 2. *E. coli* count from direct plating of the inoculated manure (Log cfu/g) and MPN/IMS methodology (Log MPN/g) for studies 4 and 5. There was no detectible statistical variation between direct plating of the inoculated manure and the IMS/MPN methodology in both of these studies. Studies 4 and 5 involved sterilized manure inoculated with 10^4 and 10^2 streptomycin resistant *E. coli* O157:H7/g cocktail, respectively.

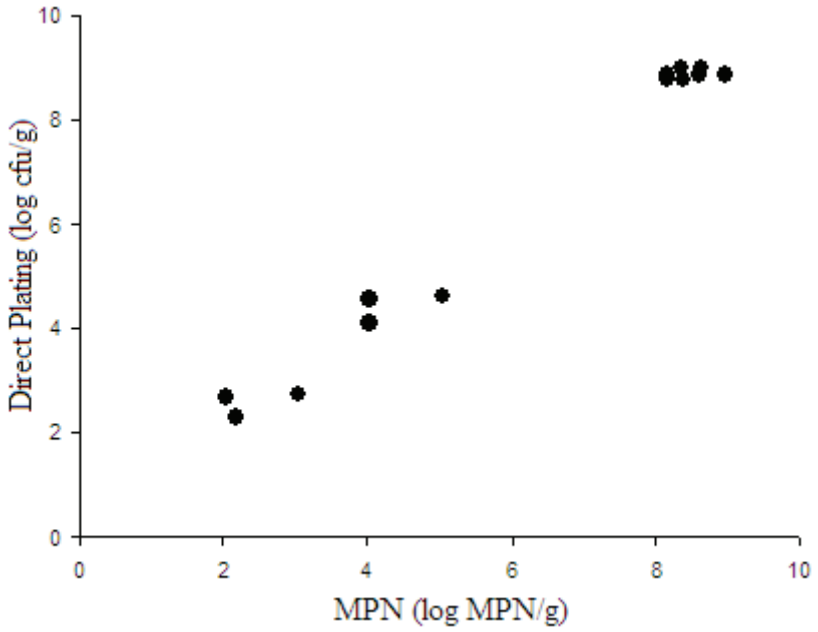

Fig. 3. MPN (log MPN/g) verse direct plating (log cfu/g) for the data points in all of the studies (5 studies x 3 replications per study = 15 data points).