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**Running Head:** ***Escherichia coli* O26 in feces of feedlot cattle**

# *Escherichia coli* O26 in feedlot cattle: Fecal prevalence, isolation, characterization and effects of an *E. coli* O157 vaccine and a direct-fed microbial

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Key Words: *E. coli* O26, Shiga toxin-producing *E. coli,* Cattle, Feces

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**Abstract**

*Escherichia coli* O26 is second only to O157 in causing foodborne, Shiga toxin-producing *E. coli* (STEC) infections. Our objectives were to determine fecal prevalence and characteristics of *E. coli* O26 in commercial feedlot cattle (17,148) that were enrolled in a study to evaluate an *E. coli* O157:H7 siderophore receptor and porin (SRP®) vaccine (VAC) and a direct-fed microbial (DFM; $10^6$ CFU/animal/day of *Lactobacillus acidophilus* and $10^9$ CFU/animal/day of *Propionibacterium freudenreichii*). Cattle were randomly allocated to 40 pens within 10 complete blocks; pens were randomly assigned to control, VAC, DFM, or VAC+DFM treatments. Vaccine was administered on days 0 and 21, and DFM was fed throughout the study. Pen floor fecal samples (30/pen) were collected weekly for the last four study weeks. Samples were enriched in *E. coli* broth and subjected to a multiplex PCR designed to detect O26-specific *wzx* gene and four major virulence genes (*stx*1, *stx*2, *eae*, and *ehx*A) and to a culture-based procedure that involved immunomagnetic separation and plating on MacConkey agar. Ten presumptive *E. coli* colonies were randomly picked, pooled and tested by the multiplex PCR. Pooled colonies positive for O26 serogroup were streaked on sorbose MacConkey agar and 10 randomly picked colonies per sample were tested individually by the multiplex PCR. The overall prevalence *E. coli* O26 was higher ($P < 0.001$) by the culture-based method compared to the PCR assay (22.7 vs.10.5%). The interventions (VAC and or DFM) had no impact on fecal shedding of O26. Serogroup O26 was recovered in pure culture from 23.9% (260 out of 1,089) of O26 PCR-positive pooled colonies. Only seven of the 260 isolates were positive for *stx* gene and 90.1% of the isolates possessed an *eaeβ* gene that codes for intimin subtype *β,* but not the *bfpA* gene, which codes for bundle-forming pilus. Therefore, the majority of the O26 recovered from feedlot cattle feces was atypical enteropathogenic *E. coli*, and not STEC.

## Introduction

Shiga toxin-producing *E. coli* (STEC) are major foodborne pathogens that cause illnesses in humans with symptoms ranging from diarrhea, with or without blood, to hemolytic uremic syndrome, and even death (Tarr et al., 2005). Among STEC, the serotype O157:H7 has caused a greater number of foodborne outbreaks than any other serotype (Rangel et al., 2005). Recent epidemiological data have shown that non-O157 STEC represent a larger portion of foodborne STEC infections than O157 (Scallan et al., 2011). Ruminants, particularly cattle, are considered to be major reservoirs of non-O157 STEC (Karmali et al., 2010). Of the non-O157 STEC, the serogroup O26 accounts for the largest proportion of infections (Brooks et al., 2005; Stigi et al., 2012; Gould et al., 2013). However, not much is known about fecal prevalence and factors affecting fecal shedding of O26 in cattle, mainly because a selective medium that can phenotypically distinguish O26 from other serogroups has not been optimized (Jenkins et al., 2008).

Preharvest intervention strategies that include the use of direct-fed microbials and vaccines have been shown to reduce the fecal shedding of *E. coli* O157:H7 in cattle. An *E. coli* O157:H7 siderophore receptor and porin protein-based (SRP®) vaccine was shown to reduce the overall fecal shedding of *E. coli* O157:H7 (Thomson et al., 2009; Thornton et al., 2009; Cull et al., 2012). Direct-fed microbials (DFM), particularly *Lactobacillus acidophilus*-based products, also have been shown to reduce fecal shedding of *E. coli* O157:H7 (Loneragan and Brashears, 2005; Jacob and Nagaraja, 2012). The effects of SRP vaccine or DFM on fecal shedding of non-O157 STEC in feedlot cattle have not been evaluated; but it is conceivable that the mechanisms responsible for *E. coli* O157:H7 reduction also may reduce other serogroups. Our objective was to determine fecal prevalence of serogroup O26 in cattle feces by culture- and PCR-based

methods, evaluate selective isolation procedures, characterize virulence genes of the isolates, and determine if vaccine (SRP®) and/or a *L. acidophilus*-based DFM product intended to reduce the prevalence of *E. coli* O157:H7 in feedlot cattle have effects on fecal shedding of *E. coli* O26.

## Materials and Methods

### *Animals and study design*

The animals, study location and study design have been previously described by Cull *et al*., (2012). The study involving 17,148 cattle housed in 40 pens was a randomized complete bloc design with a 2 x 2 factorial treatment structure of vaccine (VAC), DFM, and VAC plus DFM and control (CON; neither DFM nor VAC). The DFM was Bovamine® (Nutrition Physiology Corp., Guymon, OK) was mixed into the cattle diets to provide $10^6$ CFU/animal/day of *L. acidophilus* and $10^9$ CFU/animal/day of *Propionibacterium freudenreichii* throughout the study for the DFM and VAC + DFM groups. Cattle in the VAC and VAC + DFM groups received a 2 ml subcutaneous dose of the vaccine (*E. coli* SRP® vaccine, Zoetis Animal Health, New York, NY) on the day cattle were allocated to the study and 21 days later. During the 4 weeks prior to the end of the study date for each respective block, fresh pen floor fecal samples (n=30/pen/week) were collected. Samples were assigned sequential numbers, thus blinding laboratory personal to treatment assignments.

### *Culture-based detection of* E. coli *O26*

Fecal samples were processed within 24 h after collection. Samples were enriched in *E. coli* broth (EC; Difco™, Becton Dickinson Co., Sparks, MD; Paddock et al., 2012) and 1.0 ml of the enriched fecal suspension was subjected to immunomagnetic separation (IMS) with Dynabeads®

VTEC/STEC O26 (Invitrogen, Carlsbad, CA) and the rest of the sample was stored at -80° C for subsequent PCR assay. Bead suspensions (20 µl) were pipetted onto MacConkey agar (BD Biosciences, Franklin Lakes, NJ) and streaked with an inoculating loop to obtain isolated colonies. Plates were incubated at 37° C for 24 h and up to ten lactose-fermenting colonies were randomly picked and suspended in 1 ml of peptone broth. A 100 µl aliquot of the pooled colon mixture was boiled for 10 min and subjected to an 11-plex PCR (mPCR; Bai et al., 2012) to detect the seven STEC serogroups (O26, O45, O103, O111, O121, O145, O157) and four major virulence genes (*stx*1, *stx*2, *eae* and *ehx*A). The remaining colony mixture was preserved with glycerol (15% final concentration) and stored at -80° C. Pooled colony mixtures that were PCR-positive for serogroup O26 were then used to isolate *E. coli* O26 in pure culture.

*PCR detection of* E. coli *O26*

The frozen enriched fecal suspension was thawed and a 1.0 ml sample was boiled for 10 min and centrifuged (9,300 x *g* for 5 min). DNA was extracted and purified with a GeneClean Turbo kit (MP Biomedicals, Solon, OH) and subjected to the 11-plex PCR (Bai et al., 2012).

*Evaluation of culture media and isolation of* E. coli *O26 from PCR-positive pooled colony mixtures*

A lactose-free MacConkey agar base (BD Biosciences, Sparks, MD) with sorbose (10 g/l; SorboseMAC) as the sole sugar source (Possé et al., 2007; Hiramatsu et al., 2002) was evaluated for the isolation of serogroup O26. The sorboseMAC medium was evaluated with supplementation of no additives, novobiocin (8.0 mg/l; Sigma-Aldrich, St. Louis, MO), potassium tellurite (2.5 mg/l; Sigma-Aldrich), or novobiocin and potassium tellurite. The

evaluation of the selectivity and ability to differentiate O26 serogroup from others were done with pure cultures of O26 (n = 36) and non-O26 (n = 116) STEC. The strains were streaked onto each medium to determine growth and sorbose fermentation after 24 h incubation at 37° C. Based on the initial evaluation of culture media, sorboseMAC agar was chosen to isolate *E. coli* O26 from PCR-positive pooled colony mixtures. The frozen pooled colony mixtures were thawed and 10 µl of the mixture was streaked, with a calibrated loop, onto each medium. Following 24 h incubation at 37° C, sorbose-fermenting (pink colored) colonies (up to 10) were selected and individually tested by the mPCR. Isolates that were confirmed as O26 were stored on CryoCare beads at -80° C.

*Characterization of* E. coli *O26 isolates*

Isolates were further characterized with individual PCR assays for additional genes; *bfp*A (bundle forming pilus; Gunzburg et al., 1995), *eae*β1 (intimin; Blanco et al., 2003), *esp*P (secreted effector protein; Cookson et al., 2006), *fliC*$_{H11}$ (flagellar gene for H11; Durso et al., 2005), and *tir* (translocated intimin receptor; Bardiau et al., 2011). Two strains, TW01597, a STEC O26 (Thomas Whittam *E. coli* Reference Laboratory at Michigan State University) and EDL933 (ATCC 700927; American Type Culture Collection, Manassas, VA), a STEC O157:H7 were used as positive controls. Strains and isolates were grown on blood agar, DNA was extracted from 1 or 2 colonies by mixing with 1.0 ml of dd$H_20$, boiling for 10 min and centrifugation at 9,300 x *g* for 5 min.

*Statistical analysis*

Data on whether samples were positive or negative for each serogroup or virulence gene, were analyzed as pen-level proportions in generalized linear mixed models (GLMM; Proc Glimmix; SAS Version 9.2, SAS Institute Inc., Cary, NC) assuming a binomial distribution and utilizing a logit link function as described by Cull et al. (2012). Treatment, sampling week and treatment × sampling week interaction were evaluated as predictors. Block and sampling pens over time with blocks were included as random effects to account for the study design and lack of independence between the four sample periods within each pen. Model-adjusted prevalence means, and corresponding standard errors and confidence intervals, were generated. For all analyses, $P$ values < 0.05 were considered statistically significant. To determine if an individual fecal sample was more likely to be O26 positive by direct PCR feces compared to culture method (IMS separation, plating on MacConkey and testing of pooled colonies), data were analyzed by GLMM where the outcome was considered binary for each sample for each detection method. Block was included as a random effect. As before, pen over time was included as a repeated effect in all models accounting the lack of independence between the four samples from each pen.

**Results**

Based on the culture method (IMS followed by plating on MacConkey agar), the apparent prevalence of serogroup O26 in cattle feces collected weekly ranged from 2.5 to 36.7% of the samples (Fig 1A). The prevalence of serogroup O26 by week, based on the mPCR assay, ranged from 0.4 to 41.5% (Fig 1B). Overall, 10.5% (502/4,800) of the fecal samples were positive for the O26 serogroup, based on the mPCR assay, and the prevalence of the four virulence genes, *stx*1, *stx*2, *eae,* and *ehxA,* were 12.4% (597/4,800), 24.0% (1,151/4,800), 45.4% (2,179/4,800),

and 84.7% (4,066/4,800), respectively (Table 1). Vaccine, DFM, or both had no significant effects on the fecal prevalence of the serogroup O26 or the four virulence genes in cattle feces (Table 1).

When a mixture of 10 randomly picked colonies, obtained from plating O26 IMS beads on MacConkey agar, were tested by the mPCR, the O26 serogroup was detected in 22.7 % (1,089 out of 4,800) of the fecal samples (Table 2). Although O26 IMS beads were intended to retrieve O26 from the enriched sample, the pooled colonies also contained one or more of the six other STEC serogroups that were part of the mPCR assay. The other serogroups, in the order of predominance, were 9.8 % O103 (470/4,800), 5.7 % O111 (273/4,800), 0.8 % O145 (37/4,800), 0.8% O157 (38/4,800), 0.4 % O121 (21/4,800) and 0.4 % O45 (18/4,800). The prevalence of virulence genes in the pooled colonies were 2.4% of *stx*1 (117/4,800), 10.8% of *stx*2 (519/4,800), 7.9% of *eae* (378/4,800) and 16.2% of *ehx*A (776/4,800). Of the 1,089 samples that were O26 positive based on testing of the pooled colonies, 561 (51.5%) were negative for all four virulence genes and the remaining (528/1,089; 48.5%) contained at least one of the four virulence genes (Table 2). Among the O26-postive pooled colonies, 45 (4.1%), 216 (19.8%), 179 (16.4%) and 311 (28.6%) were positive for *stx*1, *stx*2, *eae* and *ehx*A, respectively (Table 2). Only a small proportion (10.4%) of O26 positive pooled colonies contained a combination of *stx*1 or *stx*2 and *eae*. Among the other six serogroups detected in the pooled colonies, only O103 and O111 were in high numbers (Table 2). The proportions of pooled colonies positive for O103 and O111 that contained *stx*1 or *stx*2 and *eae* were 6.6 and 10.0%, respectively. Of the 1,089 O26-positive pooled colonies, only a few (4 to 13) contained O45, O121, O145 or O157 (Table 2). The prevalence estimates of O26 based on IMS, plating on MacConkey agar and testing 10 pooled

colonies picked randomly were not significantly associated with treatment group and no treatment by sampling week interaction was observed.

In order to obtain pure culture of O26 from pooled colonies from samples that were PCR positive for O26 (n=1,089), sorbose sugar in lactose-free MacConkey agar was chosen because the sugar is fermented by all strains of *E. coli* O26 and only a limited number of other *E. coli* serogroups (Posse et al., 2007). Initially, growth and fermentation of sorbose by pure cultures of O26 strains (n=36) and non-O26 STEC strains (O45, O103, O111, O121, O145, and O157; n=116) on media without or with potassium tellurite (2.5 µg/ml) and or novobiocin (8 µg/ml) were tested. All O26 strains fermented sorbose (pink-colored colonies), while sorbose fermentation was highly variable among the other six serogroups (Table 3). Only few strains of O45, O103, O111, O121, and O145 and none of the O157 strains (n=20) fermented sorbose. Inclusion of potassium tellurite (2.5 µg/ml) inhibited the growth of 4 of 36 strains of O26, 2 of 2 O45, 9 of 40 O103, 0 of 29 O111, 6 of 9 O121, 2 of 16 O145, and none of the 20 O157 strains. Novobiocin (8 µg/ml) inclusion in the medium had no effect on the growth of O26 or other STEC strains, except one strain of O111 was inhibited (Table 3). Based on this data, sorbose MacConkey agar without potassium tellurite or novobiocin was used to isolate O26 from all the PCR positive pooled colonies. Of the1,089 samples of pooled colonies, O26 was obtained in pure cultures from 260 samples, representing a recovery rate of 23.9%.

Of the 260 isolates of O26 obtained, only seven isolates carried *stx* genes, with six carrying *stx*1 and one carrying *stx*2 (Table 4). Of the seven O26 STEC, six different virulence gene profiles were observed. All seven O26 STEC were positive for the *eae*β1 and *tir*. Six of the 7 O26 STEC were O26:H11. Only two isolates were *ehx*A positive and three isolates were *esp*P positive. Interestingly, 59 additional isolates were recovered that contained one or both *stx* genes

but did not belong to the seven serogroups detectable by the mPCR assay. Majority of the isolates (n=253) that were negative for *stx* carried the *eae*β1gene (n=236) and the H11 flagellar gene (n=197), suggesting they may be enteropathogenic *E. coli.* None of the isolates was positive for the *bfp*A, a gene characteristic of the typical enteropathogenic *E. coli* (EPEC), suggesting the strains belong to atypical EPEC (aEPEC). The majority of O26 aEPEC (234 out of 236) was positive for *tir,* which is commonly found on the locus of enterocyte effacement (LEE) with *eae*β1, and the presence of *fli*$C_{H11}$ identifies that these isolates were O26:H11 aEPEC. Fewer of the aEPEC isolates (37 out of 236) carried the *tir* and *eae*β1 but not the *fli*$C_{H11}$. The O26 aEPEC possessed a variety of virulence gene profiles (Table 4). A small number of isolates of aEPEC O26 (7 out of 236) was positive for *tir*, *fli*$C_{H11}$ and *ehx*A. Only three O26 aEPEC isolates were positive for *tir* and *fli*$C_{H11}$, as well as *esp*P which are commonly observed in STEC strains. Seventeen of the 260 O26 isolates carried none of the five additional genes, *eae*β1, *bfp*A, *tir*, *fli*$C_{H11}$ and *esp*P. One of the 17 isolates was positive for the H11 gene.

**Discussion**

The study focused on the serogroup O26 because, according to the CDC, it is the most frequent serogroup involved in human non-O157 STEC infections in the US (Brooks et al., 2005; Gould et al., 2013). The cattle fecal samples used in the present study to detect O26 were part of a study that was designed to evaluate the effects of a *E. coli* O157 vaccine and a *L. acidophilus*-based DFM on fecal shedding of *E. coli* O157:H7 (Cull et al., 2012). That study demonstrated that a two-dose vaccination significantly reduced fecal shedding of *E. coli* O157:H7 and DFM had no effect on fecal shedding of *E. coli* O157:H7 (Cull et al., 2012). The vaccine is based on siderophore receptor and porin proteins of *E. coli* O157:H7 and the efficacy in reducing fecal

shedding is attributed to induced immunity that blocks bacterial uptake of iron (Thornton et al., 2009). Because *E. coli* O26 may have surface proteins that are antigenically similar to that of O157, it was of interest to test whether the vaccine has an effect on fecal shedding of O26. The effects of *E. coli* O157:H7 vaccine and the DFM on fecal shedding of O26 or any other non-O157 STEC have not been reported. Neither vaccine nor DFM had any effect on fecal O26 strains estimated by either culture-based method or mPCR assay. The lack of vaccine effect may be because the surface proteins of O26 strains that are involved in iron uptake are antigenically different from those of *E. coli* O157:H7. The lack of DFM effect on O26 was not unexpected because the product also had no effect on fecal shedding of *E. coli* O157:H7 (Cull et al., 2012).

Based on PCR assay targeting the O26 serogroup-specific gene ($wzx_{O26}$), fecal prevalence estimates of 6.5 % (Lynch et al., 2012), 23.0% (Hofer et al., 2012), 80.0 % (Renter et al., 2004) and 82.5 % (Paddock et al., 2012) have been reported. However, the subsequent recovery rate of *E. coli* O26 isolates from positive samples has been low (4.3 to 35.7% of PCR-positive fecal samples) presumably because of lack of selective isolation procedures (Hofer et al., 2012; Lynch et al., 2012; Paddock et al., 2012). In the present study, two methods (PCR- and culture-based) were used to obtain fecal prevalence estimates of the serogroup O26. Regardless of the treatment group, fecal prevalence estimates differed between the two methods of detection. The overall prevalence was higher by the culture-based detection method compared to the detection by mPCR assay (22.7 vs. 10.5%), in spite of plating the O26 specific-IMS beads on MacConkey agar, a relatively nondifferential medium, for detection and isolation. Jenkins et al. (2003) have reported that IMS beads were 2.5 times more sensitive than a procedure that used PCR to detect *stx* and then plating the positive samples on MacConkey to detect by colony hybridization with *stx* probes. The difference in sensitivity may be because of the volume of sample used in the

detection procedure. In the PCR assay, the volume of sample was 1 µl, which requires the sample to contain a minimum of 1,000 cells per ml to assure that the sample volume in the PCR reaction contains DNA from at least one cell. The detection limits for enrichment, IMS, and plating for O157 or non-O157 serogroups have been reported to be 20-90 CFU/25 g of feces (Verstraete et al., 2010). Although commercial IMS beads were coated with O26-specific antibodies, and therefore, intended to selectively retrieve O26 from cattle feces, the use of relatively non-differential MacConkey agar and mPCR assay allowed us to identify the other six serogroups contained in the pooled colonies. Of the six serogroups, O103 and O111 were identified more frequently than the other four serogroups (O45, O121, O145, and O157). The higher frequency of detection of O103 and O111 suggests nonspecific binding or perhaps some cross reactivity of the O26 antibodies with O-antigens of O103 and O111.

Only a few studies have been reported on the detection and or prevalence of O26 serogroup in cattle or sheep feces (Barlow and Mellor, 2010; Brandal et al., 2012; Cobbold et al., 2004; Hall et al., 2006; Jenkins et al., 2003; Monaghan et al., 2011; Pearce et al., 2004; Pradel et al., 2000; Renter et al., 2005, 2007; Sasaki et al., 2011; Wells et al., 1991). The majority of cattle or sheep shedding O26 are healthy; however, the serogroup has been isolated from calves and lambs with diarrhea (Blanko et al., 2003; Caprioli et al., 2005; Cid et al., 2001; De et al., 2002). The limitation of culture-based methodology to detect or isolate O26 serogroup is lack of a selective medium, similar to sorbitol MacConkey agar for O157, for presumptive identification based on phenotypic characteristic (colony color). Possé et al. (2008) have described a selective (enteric Gram negative bacteria) and differential MacConkey agar base medium supplemented with a mixture of sugars (sucrose, sorbose, rhamnose) with inhibitory components (potassium tellurite and novobiocin) and a chromogenic compound to signal β-galactosidase activity that

allowed color-based identification of O26 and other non-O157 *E. coli* (O103, O111, and O145). Based on sugar fermentation characteristics of non-O157 STEC described by Possé et al. (2008), sorbose fermentation was used for phenotypic identification of O26 cultured on a lactose-free MacConkey agar. The use of sorbose MacConkey agar allowed isolation of O26 in pure culture from 23.9% (260/1,089) samples of pooled colonies that were PCR positive for the serogroup O26. The poor recovery may be reflective of the lack of selectivity of the medium and the logistical limitation of finding a positive O26 from a mixture of 10 colonies that were pooled together. Fukushima and Seki, (2004) enriched and performed IMS on 605 fecal samples and streaked the resulting beads onto Chromocult agar, a commercial chromogenic medium. A colony sweep from Chromocult agar was screened with a PCR to detect *stx* genes and subsequently recovered *stx*-positive pure cultures from only 50% of the *stx* positive colony sweeps.

*Escherichia coli* O26 serogroup is broadly classified into STEC and enteropathogenic (EPEC) based on virulence factors, although both are capable of causing attaching and effacing lesions (Kaper et al., 2004). The genetic determinants for the production of A/E lesions are located on the LEE, a pathogenicity island that contains genes encoding the intimin, a type III protein secretion system, a number of secreted (Esp) proteins and the translocated intimin receptor (Tir) (McDaniel et al., 1995). Intimin is the outer membrane protein responsible for the intimate attachment between *E. coli* and enterocytes and the 280-amino acid residues at the C-terminus has antigenic variation that allows classification of distinct subtypes among STEC and EPEC strains (Adu-Bobie et al., 1998). Strains of EPEC produce a characteristic adherence, called local adherence, in which bacterial cells form microcolonies or clusters. This type of adherence is associated with the presence of a plasmid, called EAF (EPEC adherence factor)

plasmid, which also has a cluster of genes that encode bundle-forming pili (BFP; Nataro and Kaper, 1998). Strains of EPEC carrying *bfpA* gene are called typical EPEC. In contrast to typical EPEC, certain strains that carry *eae* gene, but do not have the EAF plasmid encoding *bfpA,* are called atypical EPEC (aEPEC; Chen and Frankel, 2005). A total of 260 strains of O26 were obtained in the study and seven were STEC, with 6 possessing *stx*1and one strain had *stx*2. Generally, STEC strains of bovine origin, including O26, are more likely to carry *stx*1 than *stx*2 (Pearce et al., 2004, 2006; Aktan et al., 2007). The majority of the O26 isolates (236/260; 90.8%) obtained in the study contained *eae*β1, but none was positive for *bfpA,* suggesting they belonged to atypical EPEC. Sekse et al., (2011) and Kobayashi et al., (2001) screened O26 isolated from ruminant feces (n = 142 and 9, respectively) and also reported that none of them carried the *bfpA*.

In conclusion, serogroup O26 was detected in in 10.5% (502/4,800) and 22.7% (1,089/4,800) of fecal samples collected from feedlot cattle based on PCR- and culture-based methods, respectively. The interventions (vaccine and or DFM) had no impact on fecal shedding of O26. Only 260 of the 1,089 culture positive samples yielded pure cultures of O26. Only seven of the 260 strains of O26 were STEC and majority of the strains was aEPEC.

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**Disclosure Statement**

No competing financial interests exist

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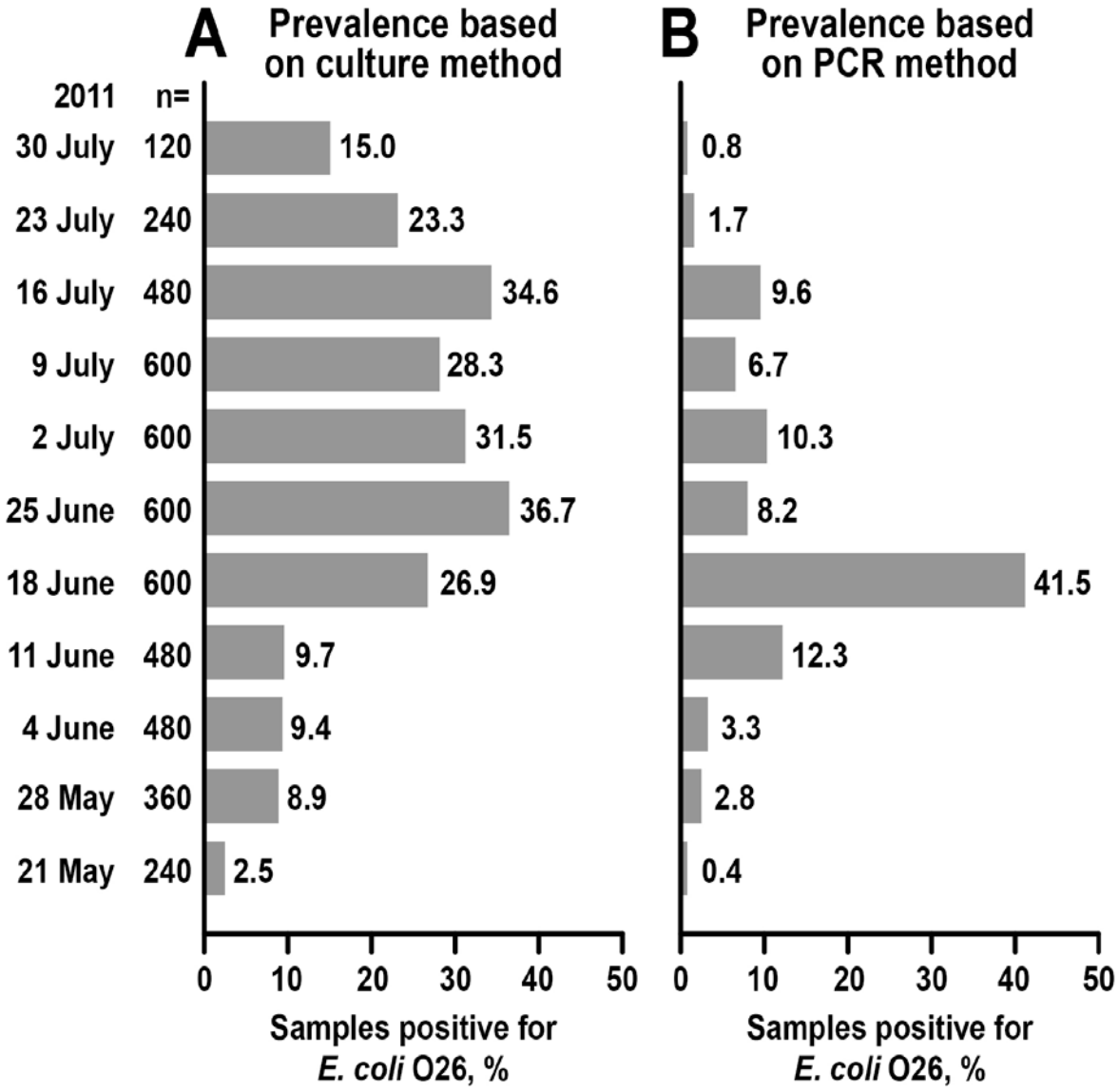


Figure 1. Prevalence of *Escherichia coli* serogroup O26, based on culture method (A; immunomagnetic separation and plating on MacConkey agar) or multiplex PCR assay (B), in feces of feedlot cattle (n=4,800). The day of sampling and the number of samples collected are indicated in the Y axis.

TABLE 1. PREVALENCE OF *ESCHERICHIA COLI* O26 SEROGROUP AND MAJOR VIRULENCE GENES, BASED ON MULTIPLEX PCR, IN *ESCHERICHIA COLI* BROTH-ENRICHED FECAL SAMPLES OF FEEDLOT CATTLE THAT RECEIVED NO TREATMENT (CONTROL), VACCINE (SIDEROPHORE RECEPTOR AND PROTEINS-BASED), DIRECT-FED MICROBIALS (*LACTOBACILLUS ACIDOPHILUS* AND *PROPIONIBACTERIUM FREUDENREICHII*) OR BOTH.

| *Serogroup and virulence genes* | *Total (n=4,800)* | *Number of samples (n=1,200) positive (%)* | | | |
|---|---|---|---|---|---|
| | | *Control* | *Vaccine* | *Direct-fed microbials* | *Vaccine + Direct-fed microbials* |
| O26 | 502 (10.5) | 110 (9.2) | 87 (7.3) | 179 (14.9) | 126 (10.5) |
| *stx*1 | 597 (12.4) | 118 (9.8) | 130 (10.8) | 226 (18.8) | 123 (10.3) |
| *stx*2 | 1,151 (24.0) | 263 (21.9) | 251 (20.9) | 341 (28.4) | 296 (24.7) |
| *eae* | 2,179 (45.4) | 537 (44.8) | 546 (45.5) | 629 (52.4) | 467 (38.9) |
| *ehx*A | 4,066 (84.7) | 1,047 (87.3) | 967 (80.6) | 1,068 (89.0) | 984 (82.0) |

There were no significant differences among treatment groups ($P$ values > 0.05).

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TABLE 2. PREVALENCE OF THE SIX SEROGROUPS OF *ESCHERICHIA COLI* AND FOUR MAJOR VIRULENCE GENES IN POOLED COLONIES (N=10) FROM MACCONKEY AGAR PLATED WITH FECAL SAMPLES (N=4,800) THAT WERE ENRICHED IN *ESCHERICHIA COLI* BROTH AND SUBJECTED TO O26 IMMUNOMAGNETIC BEADS SEPARATION AND IDENTIFIED AS PCR-POSITIVE (N=1,089) FOR THE SEROGROUP O26

| *Serogroups* | *PCR positive* | *Number of samples positive for virulence genes (%)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *None* | *stx*1 | *stx*2 | *eae* | *ehxA* | *stx*1 + *eae* | *stx*2 + *eae* | *stx*1 and/or *stx*2 + *eae* |
| O26 | 1,089 | 561 (51.5) | 45 (4.1) | 216 (19.8) | 179 (16.4) | 311 (28.6) | 7 (0.64) | 52 (4.8) | 55 (5.1) |
| O45 | 4 | 2 | 1 | 2 | 0 | 1 | | | |
| O103 | 168 | 75 (44.6) | 12 (7.1) | 44 (26.2) | 29 (17.3) | 54 (32.1) | 1 (0.6) | 8 (4.8) | 11 (6.6) |
| O111 | 259 | 112 (43.2) | 6 (2.3) | 50 (19.3) | 104 (40.2) | 57 (22.0) | 1 (0.4) | 23 (8.9) | 26 (10.0) |
| O121 | 13 | 4 | 0 | 4 | 6 | 3 | | | |
| O145 | 12 | 2 | 1 | 2 | 6 | 7 | | | |
| O157 | 10 | 10 | 2 | 5 | 7 | 7 | | | |

TABLE 3. GROWTH AND FERMENTATION OF SORBOSE IN LACTOSE-FREE MACCONKEY AGAR WITH OR WITHOUT POTASSIUM TELLURITE AND OR NOVOBIOCIN OF PURE CULTURES OF THE SEVEN SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI*

| | *Number of strains fermenting sorbose/Number of strains that grew on the medium* | | | | | | |
|---|---|---|---|---|---|---|---|
| *Culture medium* | *O26 (n=36)* | *O45 (n=2)* | *O103 (n=40)* | *O111 (n=29)* | *O121 (n=9)* | *O145 (n=16)* | *O157 (n=20)* |
| Lactose-free MacConkey agar base with: | | | | | | | |
| Sorbose (10 g/l) | 36/36 | 1/2 | 14/40 | 2/29 | 3/9 | 4/16 | 0/20 |
| Sorbose (10 g/l) and potassium tellurite (2.5 mg/l) | 32/32 | 0/0 | 13/31 | 2/29 | 3/3 | 3/14 | 0/20 |
| Sorbose (10 g/l) and novobiocin (8 mg/l) | 36/36 | 0/0 | 14/40 | 2/28 | 3/9 | 3/16 | 0/20 |
| Sorbose (10 g/l) + potassium tellurite (2.5 mg/l) + novobiocin (8 mg/l) | 32/32 | 0/0 | 13/31 | 2/28 | 3/3 | 3/14 | 0/20 |

TABLE 4. PATHOTYPES AND VIRULENCE GENE PROFILES OF *ESCHERICHIA COLI* O26 (N=260) ISOLATED FROM CATTLE FECES.

| *Pathogroup* | *No. of isolates* | *Virulence genes* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *stx1* | *stx2* | *eaeβ1* | *ehxA* | *bfpA* | *tir* | *fliC$_{H11}$* | *espP* |
| Shiga toxin-Producing *E. coli* O26 | 7 | 6 | 1 | 7 | 3 | 0 | 7 | 6 | 3 |
| Profile 1 | 2 | + | - | + | + | - | + | + | + |
| Profile 2 | 1 | + | - | + | - | - | + | + | + |
| Profile 3 | 1 | + | - | + | - | - | + | + | - |
| Profile 4 | 1 | + | - | + | + | - | + | + | - |
| Profile 5 | 1 | + | - | + | - | - | + | - | - |
| Profile 6 | 1 | - | + | + | - | - | + | + | - |
| Atypical enteropathogenic[1] *E. coli* O26 | 236 | 0 | 0 | 236 | 7 | 0 | 234 | 197 | 3 |
| Profile 1 | 187 | - | - | + | - | - | + | + | - |
| Profile 2 | 37 | - | - | + | - | - | + | - | - |
| Profile 3 | 7 | - | - | + | + | - | + | + | - |
| Profile 4 | 3 | - | - | + | - | - | + | + | + |
| Profile 5 | 2 | - | - | + | - | - | - | - | - |
| Avirulent *E. coli* O26 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Profile 1 | 16 | - | - | - | - | - | - | - | - |
| Profile 2 | 1 | - | - | - | - | - | - | + | - |

[1]Atypical enteropathogenic classification is based on the presence of *eae* and absence of *bfp*A and *stx* genes.