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Research Paper

Occurrence of *Escherichia coli* O157:H7 in Pest Flies Captured in Leafy Greens Plots Grown Near a Beef Cattle Feedlot

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ABSTRACT

Leafy greens are leading vehicles for *Escherichia coli* O157:H7 foodborne illness. Pest flies can harbor this pathogen and may disseminate it to produce. We determined the occurrence of *E. coli* O157:H7–positive flies in leafy greens planted up to 180 m from a cattle feedlot and assessed their relative risk to transmit this pathogen to leafy greens. The primary fly groups captured on sticky traps at the feedlot and leafy greens plots included house flies (*Musca domestica* L.), face flies (*Musca autumnalis* L.), stable flies (*Stomoxys calcitrans* L.), flesh flies (family Sarcophagidae), and blow flies (family Calliphoridae). *E. coli* O157:H7 carriage rates of house, face, flesh, and blow flies were similar ($P > 0.05$), ranging from 22.3 to 29.0 flies per 1,000 flies. In contrast, the carriage rate of stable flies was lower at 1.1 flies per 1,000 flies ($P < 0.05$). Differences in carriage rates are likely due to the uses of fresh bovine feces and manure by these different pest fly groups. *E. coli* O157:H7 carriage rates of total flies did not differ ($P > 0.05$) by distance (ranging from 0 to 180 m) from the feedlot. Most fly isolates were the same predominant pulsed-field gel electrophoresis types found in feedlot surface manure and leafy greens, suggesting a possible role for flies in transmitting *E. coli* O157:H7 to the leafy greens. However, further research is needed to clarify this role and to determine setback distances between cattle production facilities and produce crops that will reduce the risk for pathogen contamination by challenging mechanisms like flies.

HIGHLIGHTS

- *E. coli* O157:H7 was common in flies captured in leafy greens plots near a feedlot.
- *E. coli* O157:H7 carriage rates of house, face, flesh, and blow flies were similar.
- Stable flies had lower *E. coli* O157:H7 carriage than the other four fly groups.
- *E. coli* O157:H7 carriage of total flies was not affected by distance up to 180 m.
- Research is needed to determine risk for leafy green contamination by pest flies.

Key words: Cattle feedlot; *Escherichia coli* O157:H7; Leafy greens; Pest flies

Foodborne disease transmission associated with fresh produce has become a significant public health issue *(14, 15, 16, 31)*. Although several different produce items contaminated with any of a variety of pathogens have been implicated, leafy green vegetables contaminated with *Escherichia coli* O157:H7 have emerged as an important cause of foodborne illness outbreaks *(5, 15, 37)*. The combination of *E. coli* O157:H7 in leafy greens had the highest attribution risk ranking score among all other pathogen-produce item pairs in fresh produce–associated outbreaks in the United States *(5)*. Similarly, leafy greens were the produce item most frequently linked to outbreaks in Centers for Disease Control and Prevention (CDC, Atlanta, GA) produce-associated outbreak surveillance data in the period 2000 to 2009 *(15)*. Moreover, leafy vegetables were the second most commonly incriminated commodity, after beef, linked to U.S. foodborne disease outbreaks caused by Shiga toxin–producing *E. coli* (STEC) in the period 1998 to 2008 *(16)*.

Fresh produce products, such as leafy greens, tomatoes, melons, and berries typically are consumed raw, so preventing pathogen contamination during their production and processing is critical for the safety of these foods. Sources and routes of pathogen contamination in the preharvest production environment include contaminated soil or soil amendments (e.g., manures and composts), contaminated water (e.g., irrigation water, floodwaters, or runoff), bioaerosols from livestock facilities or the land application of manure, domestic livestock and wild animals,

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and fowl and other birds *(4, 8, 15, 39)*. Cattle are a common reservoir of *E. coli* O157:H7, and pest flies carrying this pathogen and other non-O157 STEC can be found near cattle *(3, 19, 24, 32, 38)*. Several fly species are ubiquitous in cattle production environments, in which cattle feces and manure are important feeding, breeding, and/or landing sites, depending upon the fly species *(17)*. Flies can subsequently contaminate other surfaces with pathogens from manure by regurgitation, defecation, or mechanical transfer *(17)*. House flies have been demonstrated to transmit *E. coli* O157:H7 to cattle *(2)* and have been implicated in the transmission of *E. coli* O157:H7 from cattle to humans via food contamination *(28)*. The ability of these insects to transmit disease agents to both animals and humans is well understood, and more recently, they have attracted attention as potential vectors of bacterial pathogens to preharvest produce. Laboratory studies have shown that house flies can transmit *E. coli* O157:H7 from manure to spinach leaves *(40, 44)*. In addition, Talley et al. *(40)* detected genes associated with STEC in filth flies (families Muscidae and Calliphoridae) that were captured in a field of lettuces located near rangeland being grazed by cattle. However, there is limited information regarding the carriage of viable *E. coli* O157:H7 by different fly species that may be found in leafy green produce grown near cattle production. Additional work is warranted to further investigate a potential role for flies in the preharvest contamination of fresh produce with *E. coli* O157:H7.

This study was conducted at the same time as our previously reported research *(8),* which examined the effects of environmental conditions and proximity to cattle production on the potential airborne transmission and contamination of leafy greens with *E. coli* O157:H7. Leafy green contamination was associated with dry, dusty feedlot pen surfaces and cattle activity that produced airborne dust. The objectives of the current work were to (i) determine the occurrence of *E. coli* O157:H7–positive pest flies in leafy greens planted near a cattle feedlot, and (ii) compare pulsed-field gel electrophoresis (PFGE) types and subtypes of *E. coli* O157:H7 isolated from pest flies, feedlot surface manure (FSM), and leafy greens.

MATERIALS AND METHODS

Study site and experimental design. The field site and experimental design have been described in detail for our previous 2-year study *(8)*. Briefly, leafy greens (spinach, mustard greens, and/or turnip greens) were planted every 2 to 3 weeks in plots that were 60, 120, and 180 m (three plots at each distance) from the northernmost edge of the 6,000-head-capacity feedlot at the U.S. Meat Animal Research Center (Clay Center, NE). Leafy greens, FSM, and air samples were collected at several intervals throughout the June to September sampling season in each of 2011 and 2012. All three sample types were analyzed for *E. coli* O157:H7, and all confirmed *E. coli* O157:H7 isolates were subtyped by PFGE *(34)*.

For the current study, cattle pest flies were collected during the same June to September sampling season, on eight occasions in 2011 and four occasions in 2012. At each sampling occasion, two yellow sticky traps (Starbar EZ Trap, Wellmark International, Schaumburg, IL) were placed at each of the nine leafy greens plots and at three locations next to the feedlot pens (12 sites and 24 traps per sampling occasion). The traps were hung 1.2 m from the ground on the plot fences or on fence posts near the edge of the feedlot pens. In 2011, the traps were baited with fly attractant (Starbar Terminator fly attractant, Wellmark). To ensure that pest flies were not being attracted to the leafy greens plots by the bait, in 2012 we used the same yellow sticky traps without bait. The traps were deployed for 24 to 48 h and then taken to the laboratory for immediate analysis.

Analyses of pest flies and *E. coli* O157:H7. The fly species or fly families on each trap were identified and counted separately. On each sampling occasion, the presence of *E. coli* O157:H7 was determined in up to 10 fly pools from the two traps at each of the 12 sampling sites (target: 120 fly pools). The flies were pooled by species or family and up to 10 flies were added to each pool. The number of flies and the species or family were recorded for each fly pool. When there were fewer than 100 flies and/or fewer than 10 flies of one species or family, there were fewer flies per pool; however, the overall approach was to sample and analyze all available species or families at each site and to include as many flies as possible.

Sterile forceps were used to remove the flies from the traps, and each pool was placed into a separate sterile, filtered sample bag (Nasco, Ft. Atkinson, WI). Up to 5 mL of 1.5× strength brilliant green bile broth (BD, Sparks, MD) *(24)* per 10 flies were added to each bag, and the flies were crushed by running the bag between the rollers of a manual pasta machine (setting 2, model 150P, Weston Supply, Strongsville, OH). The bags containing the flies and enrichment broth were incubated for 6 h at 37°C and then held at 4°C until the following day. The presence of *E. coli* O157:H7 was determined by immunomagnetic separation using anti-O157 Dynabeads (Invitrogen Corp., Carlsbad, CA) and plating of the beads onto CHROMagar O157 (DRG International, Inc., Mountainside, NJ) containing 5 mg L^{-1} novobiocin and 2.5 mg L^{-1} potassium tellurite (ntCHROMO157). For immunomagnetic separation, 500 μL of the fly enrichments were mixed with 500 μL of phosphate-buffered saline with Tween (Sigma, St. Louis, MO) and 20 μL of anti-O157 Dynabeads in individual wells of 96-well, deep-well blocks. The ntCHROMO157 plates were incubated at 37°C overnight and suspect mauve-colored colonies were tested with *E. coli* O157 latex agglutination reagents (Oxoid Ltd., Basingstoke, UK). The identities of agglutination-positive colonies were confirmed by multiplex PCR for genes for O157, H7 flagellin, intimin, and Shiga toxins 1 and 2, as previously described *(8)*.

Confirmed *E. coli* O157:H7 isolates were subjected to PFGE subtyping using the CDC PulseNet protocol and the restriction endonuclease *Xba*I *(34)*. PFGE banding patterns of *E. coli* O157:H7 isolated from pest flies were analyzed and compared with those isolated from FSM and leafy greens *(8),* using Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium). Isolates were categorized as subtypes (100% Dice similarity) and types (>95% Dice similarity), with position tolerance settings of 1.5% optimization and 1.5% band tolerance.

Prevalence computations and statistical analyses. Prevalence of *E. coli* O157:H7 in fly populations was estimated as *E. coli* O157:H7 carriage rates per 1,000 individuals using Pooled-InfRate which computes the bias-corrected, maximum likelihood estimate with skewness-corrected 95% confidence intervals *(9)*. Differences in carriage rates were assessed using the two-tailed Fisher exact test *(41)*. Differences were considered significant at $P < 0.05$ and were considered tendencies at $0.05 < P < 0.10$.

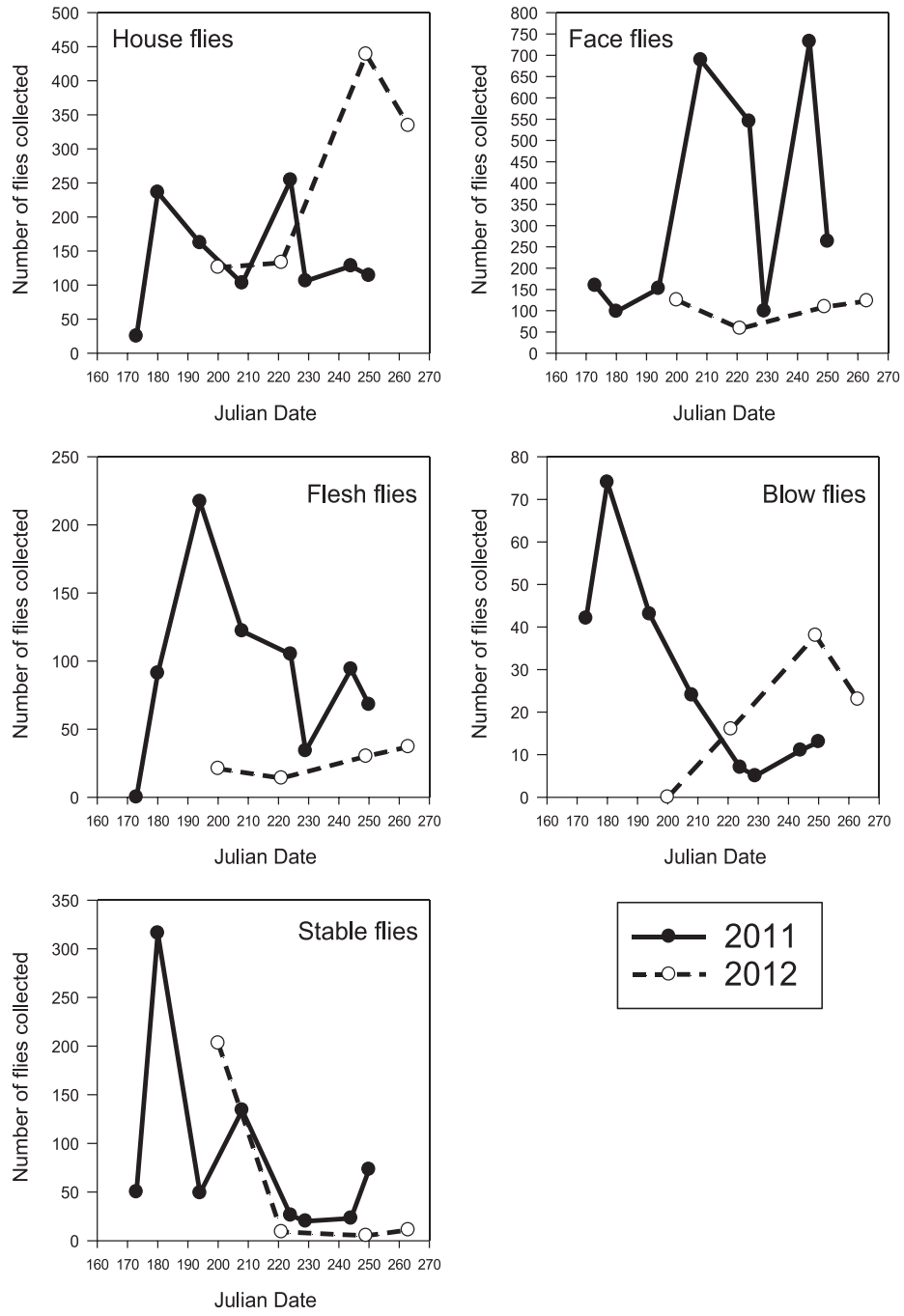


FIGURE 1. *The numbers of flies collected for each pest fly population on each sample date in 2011 and 2012. Fly attractant was used on the traps in 2011. Note that the* y *axis scales differ for the different fly populations so that their changes in abundance on different sample dates can be visualized.*

RESULTS AND DISCUSSION

Initial sampling identified house flies (*Musca domestica* L.), face flies (*Musca autumnalis* L.), stable flies (*Stomoxys calcitrans* L.), flesh flies (family Sarcophagidae), and blow flies (family Calliphoridae) as the most common fly species or families that were collected. Among 7,618 individual flies collected on the traps during the study, 7,360 (96.6%) of the flies belonged to one of these five groups. Horn flies (*Haematobia irritans* L.) and members of the family Syrphidae were among the fly groups that were less commonly observed. Analyses for carriage of *E. coli* O157:H7 were focused on the five common fly groups, and 6,228 individual flies in 1,055 pools were screened for the pathogen over the two sampling seasons. Figure 1 shows the numbers of flies collected for each of the five populations on each sample date, which suggests that there is considerable temporal variation in fly numbers. Variation between the 2 years may be due, in part, to the use of fly attractant on the traps in 2011, which resulted in an average of 721 total flies collected per sample date, compared with an average of 464 flies collected per sample date in 2012 when no attractant was used. Variation both between and within the 2 years may also be due to a number of other

TABLE 1. *Numbers of flies collected and analyzed, and* E. coli *O157:H7 carriage rates of pest fly species and families, 2011 and 2012*[a]

Species or family	No. of flies collected	No. of flies analyzed	No. of positive pools/no. tested	Carriage rate (95% CI)[b] No. of flies/1,000 flies
House flies	2,160	2,092	56/328	29.0 (22.2–37.1) A
Face flies	3,152	2,167	49/312	24.4 (18.4–31.9) A
Stable flies	919	876	1/159	1.1 (0.07–5.48) B
Flesh flies	833	809	17/168	22.3 (13.5–34.8) A
Blow flies	296	284	7/88	25.0 (11.2–48.4) A

[a] Within-column values followed by the same letter are not significantly different ($P > 0.05$).
[b] CI, confidence interval.

factors, including the availability of food sources and breeding sites and how those factors are influenced by seasons and weather conditions. Weather data were collected during the project periods in both years and were previously reported *(8)*. There was substantially less rainfall and more days with temperatures $\geq$32.2°C in 2012 than there was in 2011 *(8),* and those hotter, drier conditions may also have affected the differences in fly numbers between the 2 years.

PooledInfRate software was used to compute the *E. coli* O157:H7 carriage rates of the captured flies *(9)*. This program is available from the CDC for estimation of the prevalence of arbovirus infection in mosquitoes and is useful for situations in which infection rates or carriage rates are low and pooling of individual insects is needed for more-efficient detection. Furthermore, the software can account for differences in the numbers of individuals in pools. *E. coli* O157:H7 carriage rates of the five pest fly species or families are shown in Table 1. Carriage rates of house, face, flesh, and blow flies ranged from 22.3 to 29.0 flies per 1,000 flies and were not significantly different ($P > 0.05$). In contrast, the *E. coli* O157:H7 carriage rate of stable flies was 1.1 flies per 1,000 flies, which was significantly lower than the other four fly populations ($P < 0.05$). These results are consistent with the findings of Puri-Giri et al. *(33)* who found only 1.1% of stable flies collected in a commercial beef feedlot were positive for STEC. Numerous studies have reported the carriage of *E. coli* O157:H7 and other non-O157 STEC by house flies found in or near feedlots, dairy farms, and agricultural fairs *(3, 24, 28, 32)*. *E. coli* O157:H7 prevalence of individual house flies collected near cattle ranging from 1.8 to 5.6% typically are reported *(3, 22, 28, 35)*. Although studies are limited, *E. coli* O157:H7 and non-O157 STEC have also been isolated from blow flies *(1, 24)* and flesh flies *(1)*. The carriage rates of the combined five fly species or families in 2011 and 2012 were 24.5 and 17.0 flies per 1,000 flies, respectively (data not shown) and were not significantly different ($P = 0.14$). This indicates that the use of the fly attractant did not affect the overall carriage rate of the flies collected on the traps.

The differences in *E. coli* O157:H7 carriage rates of house, face, blow, and flesh flies, compared with the carriage rate of stable flies, is most likely due to how these different pest fly groups make use of fresh bovine feces and manure. When flies use feces or manure as food sources or as part of the life cycle (oviposition and development), they can ingest *E. coli* O157:H7 that is present in the excrement or be directly contaminated with the pathogen on their external surfaces. In livestock environments, house flies will feed on feces, manure, and feeds, and oviposition and larval development typically occur in the same substrates *(20, 27)*. Female face flies oviposit in freshly deposited feces, and both male and female face flies will feed on liquids from fresh manure *(18, 20)*. Blow flies and flesh flies are members of the families Calliphoridae and Sarcophagidae, respectively, and many species in these families cause myiasis *(36)*. However, the blow flies collected in our study were blue-bottle and green-bottle flies, which commonly use carrion and dead tissue (e.g., placental carrion) for oviposition and feces for feeding *(11, 36)*. Likewise, the flesh flies that we collected were primarily red-tailed flesh flies (*Sarcophaga haemorrhoidalis*), which typically feed on feces *(36)*. Unlike these previous four fly groups, stable flies are biting flies that feed on blood. Although commonly found in or on cattle, they typically oviposit in decayed vegetation, with or without aged manure *(20, 27)*. These differences in breeding and feeding substrates reduce the exposure of stable flies to fresh feces or manure, which typically contain more *E. coli* O157:H7.

We are unaware of previous reports of *E. coli* O157:H7 carriage by face flies. Face flies are important pests for cattle in pasture and are not typically associated with feedlot cattle. However, in this experiment, both the feedlot and field containing the leafy greens plots were surrounded by pastures. House flies and face flies are similar in appearance. Personnel evaluating the traps were instructed by, and worked alongside of, professional entomologists with extensive experience in pest fly identification. For this reason, we have confidence in our assessments of the captured house and face flies. However, because of the potential for misidentification, we acknowledge this possibility and also note that these two species were the most abundant of the five fly species or groups that were collected and that the carriage rates of the house and face flies were not different (Table 1). Hence, risk for transmission of *E. coli* O157:H7 to leafy greens from these two fly populations would be similar whether considered separately or in combination. Interestingly, face flies, primarily the females, also feed on secretions from eyes, mouth, and nostrils of cattle *(18, 20)*. Previous studies have shown that *E. coli* O157:H7 can be isolated from the bovine oral cavity *(6, 23)*.

TABLE 2. *Numbers of flies collected and analyzed, and* E. coli *O157:H7 carriage rates of pest flies collected at different distances from the feedlot, 2011 and 2012*[a]

Distance from feedlot (m)	No. of flies collected	No. of flies analyzed	No. of positive pools/no. tested	Carriage rate (95% CI)[b] No. of flies/1,000 flies
0	3,068	2,079	53/284	28.0 (21.3–36.2) A
60	1,788	1,651	30/282	19.0 (13.1–26.6) A
120	1,295	1,293	23/253	18.6 (12.1–27.3) A
180	1,209	1,205	24/236	21.0 (13.8–30.6) A

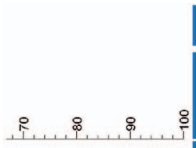
[a] Values are for all flies of the five common pest fly populations (house, face, stable, flesh, and blow flies). Within-column values followed by the same letter are not significantly different ($P > 0.05$).
[b] CI, confidence interval.

In combination with our findings of the carriage of this pathogen by face flies, these data suggest a previously unexplored route for the acquisition and dissemination of *E. coli* O157:H7 by flies.

When this study was conducted in 2011 and 2012, food safety guidelines provided to California and Arizona leafy greens growers by the Leafy Green Products Marketing Agreements (LGMA) in each state suggested a guidance distance of 400 ft (120 m) between the edge of the crop and concentrated animal feeding operations *(12)*. In the previous companion report *(8),* we examined leafy green contamination at 60, 120, and 180 m from the edge of the feedlot and concluded that a set-back distance of 120 m may not be sufficient to protect the crop from *E. coli* O157:H7 contamination via airborne dust. Flies are quite mobile and capable of dispersal distances of several kilometers *(18)*. *E. coli* O157:H7 carriage rates did not differ ($P > 0.05$) for the combined total of all five pest fly populations that were collected at distances ranging from 0 up to 180 m from the feedlot (Table 2). When stable flies were removed from the analysis because of their low carriage rate, the combined carriage rates of the remaining four fly populations were 35.3 and 20.7 flies per 1,000 flies at 0 and 60 m, respectively, and tended to differ ($P = 0.08$). These data suggest that much greater distances between leafy greens and cattle feedlots would be needed to limit the risk of *E. coli* O157:H7–positive flies. The recently revised LGMA food safety guidelines extend the guidance distances to 1,200 ft (366 m) from the edge of concentrated animal feeding operations with >1,000 head and 1 mi (1.6 km) from the edge of concentrated animal feeding operations with >80,000 head *(13)*.

A clear role for pest flies as vectors of *E. coli* O157:H7 and other human foodborne bacteria to leafy greens and other produce in the preharvest production environment has not yet been determined; however, the attraction of many fly species to vegetation is well understood. Many fly species perch in vegetation (e.g., trees, weeds, and grass) during rest *(18)*. In addition, numerous species will feed on pollen and nectar of flowering plants, including stable, face, and blow flies *(18, 20)*. Male face flies appear to spend much of their time in trees and tall weeds or on fences and gates, and nectar from flowering plants may be their principal food source *(18, 20)*. Talley et al. *(40)* captured house flies and blow flies in a lettuce field located near cattle rangeland and hypothesized that those flies may have been attracted to honeydew secreted by aphids that were on the lettuce. Subsequent work showed that house flies were attracted to honeydew produced by several honeydew-producing insects on different produce plants *(21)*. Regardless of the specific attraction or whether its presence is coincidental, if a fly has acquired *E. coli* O157:H7 and subsequently rests on produce, there is a risk that the fly may transfer the pathogen by regurgitation, defecation, or simple mechanical transfer.

Confirmed *E. coli* O157:H7 isolated from the flies (131 strains) was subjected to PFGE fingerprinting to reveal linkages with leafy green and FSM isolates (75 and 1,096 strains, respectively). Over both years, 93 unique PFGE subtypes (100% Dice similarity) and 36 PFGE types (95% Dice similarity) were identified, and their distribution across years and sample sources are shown in Figure 2. LeJeune et al. *(25)* reported 56 unique PFGE subtypes among 230 *E. coli* O157:H7 isolates from feces and water samples collected in eight pens of feedlot cattle over a 4-month period. Similarly, Sanderson et al. *(35)* found 34 unique PFGE subtypes and 11 similar PFGE types among 466 *E. coli* O157:H7 isolates from a variety of environmental samples (cattle and bird feces, flies, pen floor surfaces, water, and feed) collected weekly for 13 weeks from 12 feedlot pens. We previously described the occurrence of long-term predominant PFGE subtypes among the *E. coli* O157:H7 from FSM *(8),* a phenomenon that has been documented in other studies of this pathogen on feedlots and dairy farms *(25, 35, 38)*. As observed for *E. coli* O157:H7 found in leafy greens (74 of 75), most fly isolates (119 of 131) were among the predominant PFGE types 8, 9, 20, and 29 (Fig. 2). PFGE type 28 was found only in a single isolate from a leafy green sample. In contrast, three PFGE types were found only in isolates from flies, and an additional seven less-abundant PFGE types were seen in fly isolates. Sanderson et al. *(35)* also reported unique *E. coli* O157:H7 genotypes in houseflies captured during a longitudinal feedlot study. This likely is a reflection of the ability of flies to move about in agricultural environments and potentially acquire and disseminate new strains of *E. coli* O157:H7 from different sources. Although the isolation of the same predominant PFGE types in feedlot surface manure and leafy greens demonstrates a direct link to cattle production as a source of this pathogen, the finding of these same PFGE types in the flies does not provide direct evidence that the flies were responsible for transmitting the



			Study year		Sample type			Pest fly species or family				
PFGE type	No. PFGE subtypes	No. *E. coli* O157:H7 isolates	2011	2012	Feedlot surface manure	Leafy greens	Total pest flies	House flies	Face flies	Flesh flies	Blow flies	Stable flies
1	1	1		1	1							
2	1	1		1	1							
3	1	3		3	3							
4	1	1	1		1							
5	1	1		1	1							
6	2	31	31		29		2	2				
7	3	4	3	1	3		1	1				
8	24	460	299	161	395	19	46	16	21	7	2	
9	7	47	17	30	38	5	4	2	2			
10	2	2		2	2							
11	3	38	1	37	38							
12	1	2	2		2							
13	2	2	2		1		1	1				
14	2	4	4		3		1		1			
15	1	1	1		1							
16	1	1	1		1							
17	1	1	1				1		1			
18	1	1	1		1							
19	4	61	61		59		2	1		1		
20	1	343		343	284	38	21	13	3	1	3	1
21	1	3		3	3							
22	1	1		1	1							
23	1	1		1	1							
24	1	1		1	1							
25	1	4		4	4							
26	1	3		3	2		1		1			
27	1	1		1			1	1				
28	1	1		1		1						
29	17	264	258	6	204	12	48	18	19	9	2	
30	1	4	4		3		1	1				
31	1	1		1			1		1			
32	1	1		1	1							
33	1	1		1	1							
34	1	3	2	1	3							
35	2	3	3		3							
36	1	5		5	5							
Totals :	93	1302	692	610	1096	75	131	56	49	18	7	1

FIGURE 2. *Comparison and distribution of* E. coli *O157:H7 PFGE types and subtypes among study years and sample types. The dendrogram was generated from PFGE banding patterns of* Xba*I-digested genomic DNA from representative isolates from each of the 36 PFGE types.*

pathogen. However, finding the same PFGE types in flies that were collected in the leafy green plots does suggest that these insects likely are one potential means of dissemination of *E. coli* O157:H7 to the leafy greens. Other potential means of dissemination of *E. coli* O157:H7 in the field site include windborne dust and birds *(8)*.

The prevalence of *E. coli* O157:H7 shedding by cattle typically is highest in the summer months *(7, 42)*. Rather than evaluate the prevalence of shedding, we determined the prevalence of the pathogen on the surfaces of the 10 feedlot pens that were nearest the field that contained the leafy greens plots *(8)*. *E. coli* O157:H7 prevalence peaked in August in each of 2011 and 2012 (94.0 and 97.0%, respectively) *(8)*. Interestingly, the highest *E. coli* O157:H7 carriage rates of the combined five fly populations were also observed in August 2011 and 2012. On 17 August 2011, total fly carriage rate was 114.5 flies per 1,000 flies, which was significantly higher ($P = 0.001$) than the total fly carriage rate of 72.6 flies per 1,000 flies on 8 August 2012 (data not shown). These carriage rates on these two dates

were significantly higher ($P < 0.05$) than total fly carriage rates on any other sample date in either year, which ranged from 4.86 flies to 34.6 flies per 1,000 flies.

As indicated by the carriage rate units of "number of flies per 1,000 flies," as fly populations increase, so increases the risk for *E. coli* O157:H7 transmission by the flies. Although carriage rates of the four fly species or families were similar, house and face flies occurred in greater numbers than blow or flesh flies, indicating the potentially greater risk for transmission by these more-abundant species. The coinciding seasonal increases of *E. coli* O157:H7 shedding by cattle, house fly density *(10, 29),* and human foodborne illness *(43)* have been noted and previously discussed by Puri-Giri et al. *(32),* leading to speculation of the involvement for house flies as important links in the transmission of this pathogen both within and outside of cattle production. If pest flies do indeed transmit *E. coli* O157:H7 to fresh produce, a similar scenario of greater risk might occur for produce grown near cattle production when both *E. coli* O157:H7 prevalence and fly populations are high. Although further work is needed to clarify the significance of flies in this role, the coincident occurrences do suggest that focusing fly control resources and efforts on reducing the most abundant species may have the most impact on reducing *E. coli* O157:H7 transmission risk. However, factors other than simple abundance can affect fly transmission risk. Maldonado and Centeno *(26)* compared danger-index values for several blow fly species and the house fly (*Musca domestica* L.), which were calculated from variables representing body size and a number of synanthropic parameters. Their analysis suggests that some blow fly species pose a greater risk for transmitting pathogens in comparison to the house fly. Pace et al. *(30)* recently showed that black blow flies and house flies can differ in both their acquisition of pathogenic bacteria from bovine manure and their deposition of pathogens onto lettuce, and that blow flies may be more efficient at transmission. Furthermore, they also found that acquisition and/or deposition by the flies could differ between *E. coli* O157:H7 and *Salmonella enterica*. Hence, differences in the biology of flies, bacteria, and their interactions can further affect the risk for pathogen transmission.

In conclusion, *E. coli* O157:H7–positive pest flies of several species were common in leafy greens planted within 180 m of a beef cattle feedlot. The isolation of the same PFGE types from the FSM, flies, and leafy greens suggests that flies can disseminate this pathogen from cattle production to nearby produce crop fields. However, a definitive role for pest flies to transmit pathogens to preharvest leafy greens has not yet been demonstrated, so additional research is needed to confirm these occurrences and to guide the development of management strategies to protect fresh produce from contamination in the preharvest production environment, including the determination of appropriate setback distances from cattle production that will reduce risk from hard-to-exclude pathogen sources, such as airborne dust or flies.

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