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Research Article

Molecular detection of *Campylobacter* spp. and *E. coli* O157:H7 carried by filth flies (Diptera: Muscidae) associated with turkey production facilities

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Abstract: Filth flies (Diptera: Muscidae), such as those that breed in feces and other organic refuse, have been documented as being mechanical vectors of pathogenic bacteria including *E. coli* O157:H7, that cause hemorrhagic colitis in humans, and *Campylobacter* spp., which is the principal causative agent of human enteritis. Recently, a molecular diagnostic technique has been developed to identify filth flies that have been exposed to bacterial pathogens using PCR of insect DNA. We used this molecular diagnostic technique to screen over 5,000 adult filth flies for *Campylobacter* spp. and *E. coli* O157:H7 from two Arkansas turkey facilities during 2002 and 2003. An average of 20% of house flies, *Musca domestica* L., and 27.0% of black garbage flies, *Hydrotaea aenescens* (Weidemann), carried *Campylobacter* spp. Occurrence of *E. coli* O157:H7 was much lower, with 0.7% of the house flies and 1.0% of the black garbage flies testing positive. Flies were found carrying *Campylobacter* spp. during all 9 mo of the surveillance, with the highest proportion carrying *Campylobacter* spp. and *E. coli* O157:H7 during the summer months. We recommend that fly control be targeted towards flies found within poultry facilities and especially towards female flies, which carry a greater proportion of bacterial pathogens.

Keywords: *Campylobacter*, *E. coli*, house fly, *Hydrotaea aenescens*, DNA, molecular diagnostics

Introduction

Campylobacter spp. are important agents in acute gastroenteritis in humans causing 2.1 to 2.4 million cases of diarrhea (Tauxe 1992) and 50 to 150 deaths per year in the United States (Friedman et al. 2000). The main source of *Campylobacter* infections is the consumption of contaminated food, including raw milk (Patterson 2003), unwashed vegetables (Park and Sanders 1992), and contaminated meat products (Jones 2001, Zhao et al. 2001), with poultry being considered the most important reservoir (Deming et al. 1987, Bryan and Doyle 1995, Sahin et al. 2003). Escherichia coli O157:H7 (EHEC O157:H7) has emerged as the leading cause of enterohaemorrhagic colitis and is becoming one of the most important food-borne human pathogens of animal origin (Altekruse et al. 1997). An estimated 73,480 illnesses, 62,458 hospitalizations and 61 deaths occur each year in the United States from this pathogen (Mead et al. 1999). In poultry production facilities the occurrence of *Campylobacter* spp. (Montrose et al. 1985), and E. coli O157:H7 (Heuvelink et al. 1999) in fecal samples is well documented.

The house fly, *Musca domestica* L., and the black dump fly, *Hydrotaea aenescens* (Wiedemann), are filth flies that usually breed in substrates containing manure. *Musca domestica* has been shown to transmit *C. jejuni* in the laboratory (Shane et al. 1984, Skovgard et al. 2011), as well as *Campylobacter* spp. in Pakistan (Khalil et al. 1994), and in Norway (Rosef and Kapperud 1983). *Campylobacter* spp. positive filth fly samples have been collected from broiler houses in Georgia (Gregory et al. 1997) and Denmark (Hald et al. 2004). Seasonal increases in *Campylobacter* infections in humans have been hypothesized to be caused by direct or indirect contamination of people by small quantities of material carried by flies that have been in contact feces (Nichols 2004). House flies have been documented to carry *E. coli* O157:H7 from a livestock facility to a school lunchroom in Japan (Iwasa et al. 1999, Moriya et al. 1999). A study by De Jesus et al. (2004). found that house flies can cross contaminate *E. coli* with other surfaces with approximately 0.001% of the original *E. coli* numbers in the contaminated source. *Escherichia coli* O157:H7 has also been recovered from Diptera collected from dairy farms in Wisconsin (Shere et al. 1998), and house flies have been found to transit *E. coli* O157: H7 to cattle (Ahmad et al. 2007). Viable enteric bacteria has been demonstrated to survive on house fly corpses for up to five weeks (Cooke et al. 2003).

Polymerase Chain Reaction (PCR) has been successfully used to detect *Campylobacter* spp. and E. coli O157:H7 carried by filth flies in the poultry environment (Hald et al. 2004, Szalanski et al. 2004), and these studies have verified that some of the flies are carrying living pathogens and that the pathogens are present in the environment. Compared to the conventional culture methods, PCR can provide rapid detection, allowing intervention strategies to be quickly performed.

To reduce the risk of campylobacteriosis and enterohaemorrhagic colitis, studies are needed to determine the role of filth flies in the ecology of *Campylobacter* and E. coli O157:H7 at poultry production facilities. Therefore, the purpose of this study was to detect *Campylobacter* spp. and E. coli O157:H7 from filth flies sampled from two turkey facilities over a two year period using molecular diagnostics techniques.

Methods

Filth fly Samples. House flies and black garbage flies were collected inside and outside of turkey finishing facilities at two turkey farms located in northwest Arkansas during 2002 and 2003. Description of the farms and methods used to sample flies is described in Szalanski et al. (2004). In the laboratory, flies were sexed,identified to species, and stored at -80ºC. Voucher specimens, preserved in 95% ethanol, are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR, U.S.A.

DNA extraction and PCR Amplification. Total genomic DNA was extracted from individual filth flies using the Puregene DNA extraction kit (Gentra, Minneapolis, MN) per Szalanski et al. (2004). Genomic DNA for *Campylobacter jejuni* subsp. *jejuni* (Jones et al.) Veron and Chatelain (ATTC number 33560D), was obtained from the American Type Culture Collection (Manassas, VA). *E. coli* serotype O157:H7 genomic DNA was obtained from the USDA ARS National Animal Disease Center (Ames, IA). The MD16S1 and MD16S2 PCR primers amplified a 857 bp region of the mtDNA 16S gene from *Campylobacter* spp. (Denis et al. 2001), RfbF and RfbR yield a 292 bp amplicon for *E. coli* serotype O157 (Hu et al. 1999), and $FLIC_{h7}$-F and $FLIC_{h7}$-R amplify a 625 bp portion of the *E. coli* H7 flic gene (Gannon et al. 1997). PCR reactions were conducted using the three PCR primer sets using conditions described by Szalanski et al. (2004). The PCR profile consisted of a denaturation step of 94ºC for 2 min, followed by 35 cycles of 94ºC for 45 s, 48ºC for 45 s, and 72ºC for 1 min, with a final extension step of 72ºC for 5 min. PCR products were visualized using one percent agarose gel electrophoresis and diagnostic PCR amplicons were visualized and documented using a UVP BioDoc-It Imaging System (UVP Inc. Upland, CA).

Results and Discussion

A total of 6,300 house flies and black garbage flies were collected from the two turkey facilities from June to November 2002 and May to August 2003. From the sampled flies, 5,517 were subjected to DNA

analysis for *Campylobacter* and 3,987 for *E. coli* O157:H7 (Tables 1 and 2). The PCR products from the *Campylobacter* and *E. coli* H7 and O157 primer sets were previously identified as *C. jejuni*, *E. coli* H7, and *E. coli* O157, respectively, by subjecting the PCR product from three separate filth fly amplifications to DNA sequencing (Szalanski et al. 2004). That study also confirmed that a subset of the flies used in this study were carrying viable *Campylobacter jejuni* and *E. coli* O157:H7, and also confirmed that *Campylobacter jejuni* was present at the two farms by testing turkey fecal samples. *Campylobacter* spp. was found from flies from every field collection (Tables 1 and 2) and was detected from 20.0% of the house flies, and 27.1% of the black garbage flies (Table 3). The *E. coli* H7 antigen was detected from 3.3% of the house flies, and 4.0% of the black garbage flies. The Rfb primer set which produces a PCR product for the O157 antigen, was positive for 0.8% of the house flies and 0.6% of the black garbage flies. *E. coli* O157:H7 was detected from 0.7% of the house flies and 1.0% of the black garbage flies collected during 2002 (Table 3). None of the flies collected during 2003 were positive for *E. coli* O157:H7. The flies positive for H7 but not for O157 were probably carrying another H7 serotype, which could include O22:H7, O159:H7, or O76:H7 (Hu et al. 1999).

Presence of cattle in the area adjacent to the turkey facilities was shown to provide a source of *Campylobacter* and *E. coli* O157:H7 in fecal droppings (Szalanski et al. 2004) that could be a source of bacterial contamination by filth fly activity. Previous research has shown that house flies carry viable *E. coli* O157:H7 at cattle farms in Japan (Iwasa et al. 1999), and that the flies were capable of carrying and mechanically transmitting the pathogen from cattle farms to humans (Moriya et al. 1999) and to food (Sasaki et al. 2000). Hald et al. (2004) found 8.2 % of the filth flies collected outside of a broiler house in Denmark carried viable *C. jejuni*.

Based on our study, our results indicate that house flies and black garbage flies are carriers of *E. coli* O157:H7, and *Campylobacter* spp. within the turkey, cattle and human components of the agro-ecosystem. Recent studies have revealed that the use of fly screens can reduce *Campylobacter* spp. prevalence among broiler chicken flocks from 41.4% to 10.3% (Bahrndorff et al. 2013). Also, control of filth flies using insecticides, waste management or biological methods should target female filth flies and flies located within poultry facilities. It is interesting to note that that *E. coli* O157:H7 was found on house flies collected within and outside of the poultry facilities but was only recovered from black garbage flies from within the facility. This suggests that house flies may be moving *E. coli* O157:H7 from cattle feces to within poultry facilities, since at each of the studied farms cattle were maintained immediately adjacent to the poultry structures. Filth flies have a high potential for the distribution of many pathogens into the human population living in close proximity to animal production facilities that harbor fly populations. Future studies should be implemented to determine the dispersal of filth flies among the animal production and human components of the agro-ecosystem.

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Table 1. Percentage occurrence of *Campylobacter* spp., *E. coli* H7, and *E. coli* O157 from *Musca.domestica* (MD) and *Hydrotaea aenescens* (HA) collected from turkey production farms 1 and 2 from July to November 2002

			Percent					
Farm	**Month**	**Species**	**Campylobacter (n)**	***E. coli* O157 (n)**	**E. coli H7 (n)**	***E. coli* O157:H7 (n)**	**n***	**n****
1	Jul	MD	13.8 (53)	3.6 (8)	3.6 (8)	1.4 (3)	385	222
		HA	25.7 (28)	0.0 (0)	5.8 (5)	0.0 (0)	109	86
1	Aug	MD	16.4 (29)	0.0 (0)	11.1 (10)	0.0 (0)	177	111
		HA	23.8 (38)	0.9 (1)	3.7 (4)	0.0 (0)	160	107
1	Sep	MD	14.6 (42)	1.4 (4)	6.9 (20)	0.7 (2)	288	288
		HA	24.4 (40)	5.5 (9)	5.5 (9)	1.2 (2)	164	164
1	Oct	MD	7.8 (30)	0.5 (2)	5.2 (20)	0.0 (0)	383	383
		HA	4.5 (3)	0.0 (0)	1.5 (1)	0.0 (0)	66	66
1	Nov	MD	20.9 (24)	0.0 (0)	2.6 (3)	0.0 (0)	115	115
		HA	100.0 (3)	0.0 (0)	0.0 (0)	0.0 (0)	3	3
2	Jul	MD	20.9 (39)	4.6 (9)	5.6 (11)	3.1 (6)	187	196
		HA	30.0 (15)	27.3 (3)	54.5 (6)	27.3 (3)	50	11
2	Aug	MD	13.8 (27)	1.0 (1)	1.9 (2)	0.0 (0)	195	103
		HA	15.8 (9)	0.0 (0)	0.0 (0)	0.0 (0)	57	33
2	Sep	MD	9.8 (22)	1.8 (4)	9.8 (22)	0.9 (2)	225	225
		HA	23.5 (31)	0.8 (1)	4.5 (6)	0.8 (1)	132	132
2	Oct	MD	7.1 (23)	0.0 (0)	0.6 (2)	0.0 (0)	326	326
		HA	13.3 (2)	0.0 (0)	0.0 (0)	0.0 (0)	15	15
2	Nov	MD	22.6 (21)	1.1 (1)	1.1 (1)	0.0 (0)	93	93
		HA	100.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)	1	1

*number of flies examined *Campylobacter*, ** *E. coli*

Table 2. Percentage occurrence of *Campylobacter* spp., *E. coli* H7, and *E. coli* O157 from *Musca domestica* (MD) and *Hydrotaea aenescens* (HA) collected from turkey production farms 1 and 2 from May to August 2003

			Percent					
Facility	**Month**	**Species**	**Campylobacter (n)**	***E. coli* O157 (n)**	**E. coli H7 (n)**	***E. coli* O157:H7 (n)**	**n***	**n****
1	May	MD	25.0 (55)	0.0 (0)	0.9 (2)	0.0 (0)	220	220
		HA	47.4 (36)	0.0 (0)	3.9 (3)	0.0 (0)	76	76
1	Jun	MD	22.0 (60)	0.0 (0)	10.6 (29)	0.0 (0)	273	273
		HA	45.5 (41)	0.0 (0)	8.8 (8)	0.0 (0)	90	90
1	Jul	MD	26.2 (100)	0.0 (0)	3.6 (5)	0.0 (0)	382	137
		HA	12.1 (7)	0.0 (0)	8.3 (1)	0.0 (0)	58	12
1	Aug	MD	39.1 (52)	0.0 (0)	0.0 (0)	0.0 (0)	133	0
		HA	20.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)	5	0
2	May	MD	55.0 (122)	0.0 (0)	0.9 (2)	0.0 (0)	222	222
		HA	52.4 (11)	0.0 (0)	0.0 (0)	0.0 (0)	21	21
2	Jun	MD	24.7 (64)	2.3 (6)	2.3 (6)	0.0 (0)	259	259
		HA	35.0 (14)	0.0 (0)	0.0 (0)	0.0 (0)	40	40
2	Jul	MD	19.7 (82)	0.0 (0)	0.0 (0)	0.0 (0)	416	51
		HA	42.3 (11)	0.0 (0)	0.0 (0)	0.0 (0)	26	6
2	Aug	MD	20.5 (41)	0.0 (0)	5.5 (2)	0.0 (0)	200	36
		HA	16.7 (1)	0.0 (0)	0.0 (0)	0.0 (0)	6	0

*number of flies examined *Campylobacter*, ** *E. coli*

Table 3. Percentages of house flies and black garbage flies carrying *Campylobacter* spp. and *E. coli* O157:H7 inside and outside each turkey facility during 2002 and 2003

Campyloblobacter		House flies (*n**)			Black garbage flies (*n*)			Total filth flies (*n*)
Facility	Year	In	Out	Total	In	Out	Total	
1	2002	15.9 (710)	10.2 (638)	14.1 (1348)	23.1 (334)	20.2 (168)	22.1 (502)	15.6 (1850)
2	2002	15.1 (578)	10.0 (448)	12.9 (1026)	23.0 (174)	22.2 (81)	22.7 (255)	14.8 (1281)
1	2003	30.5 (639)	19.5 (369)	26.5 (1008)	39.2 (176)	30.2 (53)	37.1 (229)	28.5 (1237)
2	2003	33.6 (670)	21.8 (386)	29.3 (1056)	42.0 (69)	37.5 (24)	40.9 (93)	30.2 (1149)
Total		23.9 (2597)	14.4 (1841)	20.0 (4438)	28.6 (753)	23.6 (326)	27.1 (1079)	21.4 (5517)
E. coli								
1	2002	0.2 (666)	0.9 (453)	0.4 (1119)	0.7 (296)	0 (130)	0.5 (426)	0.6 (1545)
2	2002	0.8 (487)	1.1 (356)	0.9 (843)	2.9 (136)	0 (56)	2.1 (192)	1.2 (1035)
Total		0.4 (1153)	1.0 (809)	0.7 (1962)	1.4 (432)	0 (186)	1.0 (618)	0.8 (2580)

*number of flies examined

Table 4. Percentages of male and female house flies and black garbage flies carrying *Campylobacter* spp. and *E. coli* O157:H7 at each facility during 2002 and 2003

Campylobacter		House flies (*n**)		Black garbage flies (*n*)	
Facility	Year	Male	Female	Male	Female
1	2002	9.4 (715)	17.5 (633)	12.3 (236)	30.1 (266)
2	2002	8.8 (532)	17.2 (494)	19.4 (170)	29.4 (85)
1	2003	23.1 (527)	30.1 (481)	29.1 (79)	41.3 (150)
2	2003	28.4 (584)	30.3 (472)	37.5 (32)	42.6 (61)
Total		17.0 (2358)	23.3 (2080)	18.8 (517)	34.7 (562)
E. coli					
1	2002	0.4 (557)	0.5 (562)	1.1 (185)	0 (384)
2	2002	0.2 (440)	1.7 (403)	0 (136)	7.1 (56)
Total		0.3 (997)	1.1 (965)	0.6 (321)	0.9 (440)

*number of flies examined