NOTE

Detection of Pathogen DNA from Filth Flies (Diptera: Muscidae) Using Filter Paper Spot Cards

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ABSTRACT

Filth flies play a major role in the transmission of microbial organisms that cause disease in animals and humans. A procedure was developed using filter paper to collect filth fly fecal/regurgitation droplets at dairies and turkey production facilities that could be used to detect pathogen DNA carried by filth flies. Weekly fly fecal/regurgitation droplets were collected on 11 cm diameter filter paper that was either tacked to beams or stapled to 30 cm wooden stakes. Molecular diagnostics using polymerase chain reaction (PCR) procedures detected the presence of Escherichia coli H7, Campylobacter sp., and Cochlosoma anatis DNA in fecal/regurgitation droplets deposited by filth flies at two dairy and one turkey farm in Arkansas. This procedure provided a rapid and effective method to monitor pathogen presence in fly populations.

KEY WORDS

Filter paper, filth flies, fecal/regurgitation droplet, PCR

Campylobacter spp. (Doyle 1998), and Escherichia coli (Gyles 1993) have been reported to cause illnesses involving diarrhea, lethargy, severe abdominal pain, fever, vomiting, nausea, and kidney failure to occur in humans. Similar occurrences have been reported in livestock and poultry, and in various pathogen combinations may occur with more severity, resulting in economic losses. Rosef & Kapperud (1983) indicated that flies may contribute to the spread of Campylobacter by transmitting the bacteria from animals to human food. Alam & Zurek (2004) reported that house flies were a potential contributor to the transmission of E. coli O157:H7 in both farm and urban environments. Previous studies have shown that filth flies can disseminate viable pathogens such as Helicobacter pylori (Grubel et al. 1997), Salmonella (Greenburg 1965), and E. coli O157 (Kobayashi et al. 1999) to other substrates. Japan experienced outbreaks of E. coli O157:H7 during which students attending a nursery school located close to a farm became infected with the bacteria. House flies collected in the area were carrying the pathogen (Moriya et al. 1999), and fecal/regurgitation droplets on substrates within the school were suspected as a factor in the spread of the bacteria (Kobayashi et al. 1999). House flies have also been documented to carry the protozoan Cochlosoma anatis, which plays a role in turkey enteritis (McElroy et al. 2005). Filth flies such as the house fly, Musca domestica L., and black

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garbage fly, *Hydrotaea aenescens* (Wiedemann), often disperse away from the areas where eggs, larvae, and pupae are located. Hogsette & Jacobs (1999) found that *H. aenescens* were capable of dispersing 0.3 km away from release points. Lysyk & Axtell (1986) stated that *M. domestica* can travel up to 20 km from points of release and some move from dairies and poultry houses to other farms or urban environments. Often different types of fly movement in and among the various components of the environment occur and pathogens that cause disease to both humans and animals are dispersed throughout the Agro-Ecosystem. Spot cards are used to collect fecal/regurgitation droplets as a monitoring device to determine the density of filth fly populations (Lysyk & Axtell 1985), and filter paper has been shown to work well for preserving insect and pathogen DNA (Owens & Szalanski 2005). This study was conducted to determine if filter paper could be used to collect fly fecal/regurgitation droplets under field conditions at dairies and turkey production facilities to identify pathogen DNA in adult filth flies.

**Materials and Methods**

Two dairy farms and two turkey facilities located in Carroll County, Arkansas were used in this study: two dairy farms and one turkey farm were located near Green Forrest, Arkansas, and one turkey farm was located near Berryville, Arkansas. At both dairy farms, the most common filth flies were *Musca domestica* and *Hydrotaea aenescens* (McElroy 2005). Whatman 11 cm filter paper (Aloe Scientific, St. Louis, MO) was placed at all farms to collect filth fly fecal and regurgitation droplets during August to October, 2004. Filter paper was stapled to a 7.6 cm × 12.7 cm index card and hung inside turkey production facilities with thumbtacks on wooden support beams 1 m above the floor along the side-curtain opening of the houses where flies were observed entering and exiting the facilities. At dairies, cards were attached to wooden surfaces where cows and calves could not reach (Fig. 1). In addition, filter paper was stapled to 30 cm wooden stakes placed in the ground with the filter paper 20 cm above the surface around calf hutches and milking parlors where fly activity was noted. After 1 wk, cards were collected and replaced. In the field, the cards were placed in 568 ml zip-lock bags, transported to the laboratory, and stored at room temperature. Ten fly spots were chosen at random from each filter paper and 1.2 mm discs were punched from the filter paper using a Harris Micro Punch (Whatman). Each filter paper used in the field was tested separately by pooling 10 fly spots and extracting DNA using the Puregene DNA extraction kit (Gentra, Minneapolis, MN), which was resuspended in 50 ul Tris:EDTA pH 8.0 and frozen at −20°C. Contamination in the laboratory was avoided by performing all isolations in a Safety Class II hood cabinet. Extracted DNA was subjected to PCR in order to detect *E. coli* O157:H7, *Campylobacter* spp., and *Cochlosoma anatis*. PCR primers and PCR conditions for *E. coli* O157:H7, and *Campylobacter* sp. were from Szalanski et al. (2004), and the primers and PCR conditions for *Cochlosoma anatis* were from (McElroy et al. 2005). To visualize the presence of pathogens in all samples tested, 1% agarose gel electrophoresis was performed and recorded using a UVP biodoc-it system (UVP Inc., Upland, CA).
Results and Discussion

A total of 118 pools of 10 fly droplets each were tested (Table 1) with 15 (12.7%) of the samples testing positive for a pathogen. *E. coli* serotype H7 was detected in two samples from dairy farm 1 and five from dairy farm 2, totaling seven, or 5.9%, of the pooled samples. *Campylobacter* spp. was found in seven, or 5.9%, of the pooled samples, with three being from dairy farm 1, two from dairy farm 2, and two from turkey farm 1. *Cochlosoma anatis* was found in one, or 0.8% of the pooled samples at dairy farm 2. There was no detection of *E. coli* serotype O157 at

![Fig. 1. Filter paper card and stake used for collecting filth fly fecal/regurgitation droplets.](image)

<table>
<thead>
<tr>
<th>Farm</th>
<th><em>E. coli</em> H7</th>
<th>Campylobacter sp.</th>
<th>Cochlosoma anatis</th>
<th>Total fly pools</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy 1</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>Dairy 2</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>38</td>
</tr>
<tr>
<td>Turkey 1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Turkey 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>7</td>
<td>1</td>
<td>118</td>
</tr>
<tr>
<td>Percent positive</td>
<td>5.9</td>
<td>5.9</td>
<td>0.8</td>
<td>12.7</td>
</tr>
</tbody>
</table>

Table 1. Total number of filth fly fecal/regurgitation droplet pools positive for pathogens.
any farm. No pathogens were detected at turkey farm 2 which was located several km from other turkey production facilities and dairy farms.

Collecting filth fly fecal and regurgitation droplets on filter paper (Fig. 2) provided an effective way to monitor pathogen presence in filth fly populations at dairy and turkey production facilities. The filter paper placed at dairies and poultry facilities collected an abundance of fly fecal/regurgitation droplets. Filter paper attached to wooden stakes 20 cm above the surface collected greater numbers of droplets than filter paper hung by thumbtacks 1 m above the floor surface. This was likely due to the accessibility flies had to land on the stakes which were closer to manure on the ground. The method was also easier to place in areas where flies were congregating. Generally, more spots were deposited around the outer edges of the filter paper circles than towards the center area. There was a greater presence of pathogens in fly fecal/regurgitation spots collected on the filter paper from both dairies than from the turkey facilities. However, there was a greater abundance of spots on filter paper from dairies due to the easy access of placing the filter paper in areas where flies were congregating. Turkey farm 2 had no pathogens detected in fly spots, but also had no disease outbreaks occurring during times of collections, and this farm was

Fig. 2. Filth fly fecal/regurgitation droplets deposited on a filter paper card.
more isolated from other cattle or turkey production farms. In addition, the horse
and beef cattle production on this farm created no concentrated area of manure
necessary for filth fly breeding. In the case of an outbreak or epidemic it is vital
that the source of pathogen spread be identified and controlled. Flies have been
linked to the spread of many pathogens and currently, bio-terrorism has become
a concern.

Studies that have indicated the presence of anthrax and cholera bacteria in
flies (Fischer 1999) as well as the continuing discoveries in the vector competency
of filth flies leads to a need for improved methods in pathogen detection, and the
method of sample collection reported here offers quick, accurate results. This
method of field sampling combined with molecular diagnostics using PCR
procedures to detect pathogen DNA within 6 h after samples reach the laboratory
allows rapid application of filth fly management tactics to aid in controlling
disease outbreaks.

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