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Role of *Musca domestica* and S*tomoxys calcitrans* as a vector of *Escherichia coli* O157:H7 from different sites in Baghdad/ Iraq

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Abstract
This study was designed to approve the roles of house fly (HF) *Musca domestica* and Stable fly (SF) *Stomoxys calcitrans* as a vector for *Escherichia coli* O157: H7. For this purpose, (200) flies were collected from (4) different sites (3 locations near farmland and one residential area) in Baghdad/ Iraq to isolate and identify *Escherichia coli* and *Escherichia coli* O157:H7 from the external surface of the flies. Eosin Methylene Blue agar (EMB), Sorbitol MacConkey agar supplemented with Cefixime and Potassium tellurite (CT-SMAC) agar and Chrome agar, were used for isolation of *E. Coli*. The biochemical tests (IMVC) and (KCN) broth and serological test using Latex agglutination test (Oxoid) were used to identify the isolates. *E. coli* O157: H7 was identified in (62.5%) from houseflies and (40%) from stable flies with different isolation percentage between farmland location and residential area. In conclusion, the results of this study approved the isolation of *E. coli* O157: H7 from the external surface of *Musca domestica* and *Stomoxys calcitrans* for the first time in Iraq. The result was also determined the potential role of flies as carriers of pathogenic bacterial agents. The author recommends taking a high precautions procedures to reduce the risk of *Musca domestica* and *Stomoxys calcitrans* as sources of *Escherichia coli*, *Escherichia coli* O157: H7.

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Introduction

Flies are a cosmopolitan pest. It is distributed a worldwide and found throughout the country in close association with human activities. Pest has great medical and veterinary significance, and it is one of the most important mechanical vectors of human diseases (Lakmini *et al.*, 2013; Jones *et al.*, 2008). Houseflies (HF) are important nuisance pests of people and domestic animals. Flies also consider the most important vectors of foodborne and animal pathogens (Zurek and Gorham, 2010). Because of their random

movements, ability to fly long distances, attraction to both decaying organic materials, and placed where food is prepared and stored; the houseflies are significantly increased the risk of human exposure to foodborne pathogens (Magda *et al.,* 2014; Butler *et al.,* 2010). Houseflies can transport microbial pathogens from reservoirs (animals manure). Moreover, they present a minimal hazard for people to the food but pose a significant to carry and distribute the pathogenic bacteria in a hospital, houses, and restaurant (Lakmini *et al.,* 2013). Stable flies (SF) are blood-sucking insects. It is important pests for domestic animals and people and causes considerable economic losses in the livestock industry (Campbell *et al.,* 2001). Moreover, the ecology of various bacteria originating from animal manure can be affected by the presence of SF (Rochon *et al.,* 2005). Flies have a hairy proboscis and feet with glandular hairs and pads. These structures secrete sticky material enable the flies to pick up the pathogens on to their bodies. The flies able also to regurgitate the vomits and deposit of fecal droplets during the feeding process. All these processing enable the flies to spread the pathogens (Nazni *et al.,* 2005). Transmission of microorganisms takes place when the fly makes contact with people or their food. It has also approved that microorganisms may swarm over flies body and legs as many as (500000) bacteria (Todd *et al.,* 2009). Ruminant animals especially cattle are a major reservoir of pathogenic *E. coli* strain O157: H7 and approximately 30% of feedlot cattle shed *E. coli* O157: H7 (Hussein and Sakuma, 2005; Thirumalai *et al.,* 2008).
Enteric hemorrhagic Escherichia coli O157: H7 produces Shiga-like toxins (STEC). It is a well-known causative agent of hemorrhagic colitis (HC), hemolytic uremic Syndrome (HUS), Thrombotic Thrombocytopenic Purpura (TTP) and diffused intravascular coagulation (DIC) in humans (Tserenpuntsag *et al.,* 2005; Morgan *et al.,* 2005). The small infectious dose and high virulence of *E. coli* O157: H7 make this infection severe and life-threatening, especially for young children, the elderly, and those with weak immune systems. The main reservoir for *E. coli* O157: H7, is the intestinal tracts of healthy cattle (Kiranmayi et al., 2010). In 1999, Iwasa *et al*., studied the roles of flies in the transmission of E. coli O157: H7 in cattle, poultry, and pig farms (Iwasa *et al.,* 1999). The *E. coli* O157: H7-related hemorrhagic diarrhea found as the primary pathogenic strain that responsible on the outbreak in a Japanese nursery school. Moreover, the pathogenic strain responsibility was traced to the house flies that had originated from nearby cattle (Kurahashi *et al.,* 1999). Review of literature showed scarce studies regarding the isolation of *Escherichia coli* O157: H7 from the outer surface of different kinds of flies in Iraq. So, this study intended to isolate and identified *Escherichia coli, Escherichia coli* O157: H7 on the outer surface of flies collected from different locations in Baghdad governorate \ Iraq.

Materials and Methods

Collection of flies

Flies collected from 4 sites. Three sites were farmland and including the following locations: Al-dahib Al-abiahd, Veterinary college farm, Fadhallia and one was residential site / Baghdad Algadeda Animal market area. The specimens included (200) flies (160) house flies (40) from each site and (40) *Stomoxys calcitrans* (10) from each location. Flies captured and placed in a sterile glass tube with sand. Later on, all samples

transferred in a cooling box to the laboratory until further processing. The flies that captured from different locations were classified according to (Furman and Catts, 1982) before bacterial isolation.

Bacterial Isolation and Identification

The bacterial isolates from each fly placed in test tubes containing enriched brain-heart infusion (BHI) broth and incubated at 37 °C for 24 hours. Sub culturing done for each isolate in Petri dishes containing Eosin Methylene Blue (EMB), MacConkey, Sorbitol MacConkey agar that supplemented with Cefixime and Potassium tellurite (CTSMAC) agar and Chrome agar culture media. Colonies selected according to their morph physiological features and submitted to preliminary bacterial identification assays as follow: Gram staining and Biochemical tests (IMVC) and (KCN) broth (Aseel *et al.*, 2013).

Serotyping of *E. coli* O157 by Latex Agglutination Kit

The nonsorbitol fermenting colonies on SMA and the violate colonies on Chrome agar suspected as *Escherichia coli* O157. These colonies subjected to slide test agglutination test with the *E. coli* O157 Latex kit (Oxoid) (Aseel, 2014).

Results

The isolation procedure done only from the external surface of the collected flies in this study. The flies were expected to act as mechanical vectors, and being able to transfer the microorganism with the aid of their leg or/and mouth parts. Totally, the isolation rates for *E. coli* and *E. coli* O157: H7 were 131 (81.8%) and 100 (62.5%) respectively out of 160 in *Musca domestica*. Moreover, the isolation rate of *E. coli* according to the site of the collection were 33/40 (82.5%), 34/40 (85%), 36/40 (90%) and 28/40 (70%) from Al-dahib Al-abiahd, Veterinary college farm, Fadhallia and Baghdad Algadeda animal market respectively. While, the isolation rate of *E. coli* O157:H7 according to site of collection were 25/40 (62.5%), 28/40 (70%), 27/40 (67.5%) and 20/40 (50%) from Al-dahib Al-abiahd, Veterinary college farm, Fadhallia and Baghdad Algadeda animal market respectively (Table.1). The result of isolation rates from *Stomoxys calcitrans* is presented in (Table. 2). Totally, *E. coli* and *E. coli* O157: H7 were isolated from 28 (70%) and 16 (40%) respectively out of 40 flies. According to the site of collection, *E.coli* was isolated from 7/10 (70%), 7/10 (70%), 8/10 (80%) and 5/10 (50%) from Al-dahib Al-abiahd, Veterinary college farm, Fadhallia and Baghdad Algadeda animal market respectively. Moreover, the isolation rate of *E. coli* O157: H7 according to site of collection were 4/10 (40%), 5/10 (50%), 5/10 (50 %) and 2/10 (20%) from Al-dahib Al-abiahd, Veterinary college farm, Fadhallia and Baghdad Algadeda animal market respectively (Table.2).

Table 1: Shows the prevalence of *E. coli* and *E. coli O157:H7* in *Musca domestica*

Site of sample collection	No. of sample	No. positive of *E coli*	Percentages (%)	No. positive of *E coli O157:H7*	Percentages (%)
Al-dahib Al-abiahd	40	33	82.5%	25	62.5%
Veterinary college farm	40	34	85%	28	70%
Fadhallia	40	36	90%	27	67.5%
Baghdad Algadeda animal market	40	28	70%	20	50%
Total	160	131	81.8%	100	62.5%

Table 2: Shows the prevalence of *E. coli* and *E. coli O157:H7* in *Stomoxys calcitrans*

Site of sample collection	No. of sample	No. positive of *E coli*	Percentage (%)	No. positive of *E coli O157 : H7*	Percentages (%)
Al-dahib Al-abiahd	10	7	70%	4	40%
Veterinary college farm	10	7	70%	5	50%
Fadhallia	10	8	80%	5	50%
Baghdad Algadeda animal market	10	5	50%	2	20%
Total	40	28	70%	16	40%

Discussion

Musca domestica is a worldwide-distributed pest and the dominant synanthropic fly species in animal production, homes, restaurants, and hospitals. The major problem for most livestock farming is the control of the housefly population. *Musca domestica* has abilities to breed and feed on animal manure, trash, human excrement, and other decaying material (Forster *et al.,* 2009). In this study different, flies were collected from various locations to approve its ability to carry and transfer the pathogens. The three sites of samples collection, were near the "farmland", while the fourth site was a residential area. The files usually prefer to accumulate in the farmland locations because the availability of decaying materials, animals, and manure. Moreover, all kind of flies is prone to pick up and transport the pathogens that present in the feeding material. Moreover, they have the ability to travel to distance places such as private houses, restaurants and hospitals (Zurek and Gorham 2010; Cláudia *et al.,* 2013). Previous studies approved the role of houseflies in the dissemination of *E. coli O157: H7* between animals as well as to the surrounding environment (Alam and Zurek, 2004; Castro *et al., 2013*), thus becoming major risk vectors (Caprioli *et al.,* 2005). The results of the current study approved the isolation of pathogenic *E. coli* from both *Musca domestica* and *Stomoxys calcitrans. E.coli* and *E.coli* O157: H7 isolated from *Musca domestica* that collected from the first three farmland sites Al-dahib Al-abiahd, Veterinary college farm and Fadhallia, as well as from the residential area Baghdad Algadeda animal market. The higher and lower percentage of isolation were 90% and 70% in Fadhallia and Baghdad Algadeda animal market respectively. These results showed that the flies that collected from farmland have a higher ability to carry the pathogens in compare to a residential area. So far as aware, no previous study has been reported the isolation of *E.coli and E.coli* O157: H7 in Iraq. However, a screening study was done by Ahmad *et al., (2007)* and they approved the presence of a fecal coliform (95.4%) from 350 house fly, meanwhile, the percentage of *E. coli* O157: H7 was 2.9%. This study approved that houseflies have the ability for transmitting *E. coli O157: H7* to the cattle and play a role in the ecology of this human foodborne pathogen in the cattle production environment (Chakrabarti *et al.,* 2010).

The results of the current study also showed the ability of stable flies to carry and transfer the Shiga toxin *E.coli* O157: H7. The higher percentage of isolation was 50 % in Fadhallia and Veterinary college farm. However, the lower rate was 20 % and also from Baghdad Algadeda animal market. The results of the current study showed higher isolation percentage than the previous survey in Brazil (Rochon *et al.,* 2005). *Rochon et al.,* (2005) approved the isolation of *E. coli* in 19.5% of the stable flies in Brazil; while Shiga toxin genes detected in 13% of the isolated *E. coli.* There were variations between the isolation percentages that occurred in different studies. The degree of the contamination of the place was an important factor. Besides, the period of shedding of bacteria in animal manure which pollute the surrounding area, building and plant, revealed also significant variations (Chakrabarti *et al.,* 2010; Dahiru and Enabulele, 2013; Haus-Cheymo *et al., 2006;* Matthews *et al.,* 2006). In conclusion, this study approved the ability of both house and stable flies to carry and transport the pathogenic *E.coli*. Moreover, Shiga toxin *E.coli* O157: H7 isolated in the high percentages in farmland site and a low rate in a residential area. The author recommends taking a strong precautions procedures to reduce the risk of *Musca domestica* and *Stomoxys calcitrans* as sources of *Escherichia coli, Escherichia coli* O157: H7. A Higher level of cleaning, food inspection programs and avoiding the direct contact of food and water with the house and stable flies are the most important precautions that should be taken.

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