

Isolation of *Campylobacter* and *Salmonella* from houseflies (*Musca domestica*) in a university campus and a poultry farm in Selangor, Malaysia

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Abstract. Insects, in particular house flies and cockroaches, have been shown to be associated with the spread of pathogens in livestock farms and in human disease outbreaks: among these pathogens are salmonellae and campylobacters. A total of 60 flies were caught in three locations: an animal teaching facility and a cafeteria in a university campus, and a poultry farm. Five percent (5%) and 13.3% of flies sampled were found to carry *Campylobacter* and *Salmonella*, respectively.

INTRODUCTION

Salmonella spp. is a serious foodborne threat to humans worldwide (Holt *et al.*, 2007). The organisms are ubiquitous in nature, can survive several weeks in a dry environment and several months in water (Wray & Davies, 2003). Animals, including aquatic vertebrates, birds and reptiles, are important carriers of *Salmonella* (Wray & Davies, 2003). *Campylobacter* spp. is the most common foodborne pathogen in industrialised countries (Hald *et al.*, 2004). Campylobacters are commonly associated with poultry and poultry products and frequently reported to be the common source of human campylobacteriosis (Humphrey *et al.*, 2007). Humans are frequently exposed to both of these organisms when they consume inadequately cooked or contaminated food; other possible sources include contaminated environment, water and in contact with infected animals (Kaneene & Potter, 2003; Wray & Davies, 2003).

Houseflies (*Musca domestica*) have been shown to be carriers of several species of bacteria; this is because of their

close association with decaying organic matters, garbage and faeces (Holt *et al.*, 2007). The hairy proboscis and feet with glandular hairs and pads that secrete sticky material enable the flies to pick up the pathogens onto their bodies. In addition, the regurgitation of vomitus and deposit of faecal droplets during feeding process contribute to the flies' ability to spread the pathogens (Rosef & Kapperud, 1983; Nazni *et al.*, 2005). A number of researchers have studied and isolated pathogens, such as *Vibrio cholera* (Fotedar, 2001), *Escherichia coli* O157:H7 (Sasaki *et al.*, 2000), *Salmonella* and *Campylobacter* (Olsen & Hammack, 2000; Nichols, 2005; Wales *et al.*, 2010) from houseflies and reported them as a potential source for transmission and spread of these pathogens. In a review by Wales *et al.* (2010) on carriage of zoonotic pathogens by arthropods it was reported that *Helicobacter pylori* was detected frequently by PCR examination of houseflies trapped around dairy, poultry and pig units.

The paucity of published works on the carriage of *Salmonella* and in particular *Campylobacter* by houseflies in Malaysia

led to this study with the aim of determining the presence of *Campylobacter* and *Salmonella* in and on houseflies caught in a university campus and in a poultry farm.

MATERIALS AND METHODS

Fly Sampling Sites

Samples of houseflies were collected from a large animal ward of an animal teaching facility and a cafeteria in a university campus and a poultry farm. The animal teaching facility was located in a university campus in Serdang; one of the cafeteria was located about 100 m from the facility and the other was 300 m away. The poultry farm was located in Jenderam Hilir, about 30 km from the campus. The flies were caught using an insect net and were immediately placed in sterile bottles. Three flies were placed in a bottle which was considered as one pooled sample (Rosef & Kapperud, 1983).

Sampling Bacterial Specimens

For each pooled fly sample, both the external body surface and the internal content of the flies were examined for the presence of *Campylobacter* and *Salmonella*. Two sterile cotton swabs were used to swab the external body surface of each dead fly in a sample, with one swab for *Campylobacter* isolation and the other placed in 10ml Buffered Peptone Water (Oxoid) for *Salmonella* isolation. The dead flies were then dipped in alcohol and dried. They were placed in a sterile plastic bag and crushed to release the internal contents. Another two sterile cotton swabs were used to collect the internal contents, and as above, one for *Campylobacter* and the other for *Salmonella* isolations.

Isolation and identification of *Campylobacter*

Each swab was streaked directly onto Campylobacter Blood Free Selective Agar (Oxoid) plate supplemented with CCDA Campylobacter Selective Supplement (Oxoid). All the plates were incubated at 42°C for 48 h, under microaerophilic

condition which was generated by using an anaerobic jar containing a gas generating pack (GasPak EZ Campy, BD). The plates were examined for colonies typical of *Campylobacter* namely, round translucent colonies, raised, convex and glistening, with an entire edge and a tendency to spread along streaking lines. The suspected colonies were then examined for oxidase positive, gram negative, slender, spiral curved rods which also appeared as s-shape and gull-winged shape, with typical corkscrew, twirling and darting movements under hanging drop examination. Two to three colonies were then selected and transferred onto a Columbia Blood Agar (Oxoid) plates with 5% defibrinated horse blood added, incubated at 37°C for 24 h under aerobic condition.

Biochemical tests was performed on colonies isolated from the blood agar plates which consisted of hippurate hydrolysis, indoxyl acetate hydrolysis and urease tests using MAST ID™ CAMP Identification System (Mast Diagnostics). These tests were able to differentiate *Campylobacter* isolates into four species, namely *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari* and *Campylobacter upsaliensis*.

Isolation and identification of *Salmonella*

Each swab was placed in a bottle containing Buffered Peptone Water (Oxoid) and all bottles were incubated aerobically at 37°C for 24 h for pre enrichment. A drop of each pre-enriched sample was then transferred into 10ml of Rappaport Vassiliadis (RV) enrichment broth (Oxoid) incubated aerobically at 42°C for 48 h. A loopful of each enriched sample was then streaked onto Xylose-Lysine-Tergitol-4 (XLT4) agar (Merck) plate and all plates were incubated aerobically at 37°C for 24 to 48 h.

The suspected colonies, pink to red colonies with black centers, were picked and examined for oxidase positive, gram negative rods. Biochemical tests were performed on presumptive *Salmonella* colonies which include in Triple Sugar Iron

(TSI) agar (Oxoid) and Lysine Iron Agar (LIA) (Oxoid). The colonies that showed typical characteristics of *Salmonella* were then subjected to slide agglutination test (SAT) using *Salmonella* agglutinating antiserum Poly A-S (Serotest). Those colonies that gave positive reaction to SAT were cultured on nutrient agar (Oxoid) slant and sent to Veterinary Research Institute (VRI) in Ipoh for further confirmation and serotyping.

A total of eight sample pools were found positive for *Salmonella* with seven from a large animal ward, three from a cafeteria and two from a poultry farm. *Salmonella* were isolated from external body surfaces (2 samples) and from internal contents (6 samples) (Table 1). Serotyping of *Salmonella* isolates identified *Salmonella* Hadar (3 isolates), *S. Muenster* and *S. Indiana* (2 isolates each) and *S. Newington* (1 isolate).

RESULTS

Twenty pools of housefly samples were collected from each of the three locations; thus a total of 60 fly specimens were examined. Isolation results are as presented in Table 1.

Three sample pools, all from a poultry farm, were positive for *Campylobacter*. *Campylobacter* were isolated from two external body surfaces and one from the internal contents. All fly samples from a large animal ward and a cafeteria were negative for *Campylobacter*. Two isolates were identified as *C. coli* and one as *C. jejuni*. *Campylobacter lari* was not isolated.

DISCUSSION

The present study found houseflies contaminated with *Campylobacter* (5%) and *Salmonella* (13.3%). In a separate study on eight broiler farms where 10 flies were collected from each farm, flies from seven farms were found positive for *Campylobacter* (unpublished data). Holt *et al.* (2007) reinforced the findings that flies residing in environments contaminated with human pathogens become contaminated themselves. In a report by Newell & Fernley (2003), insects which include flies, darkling beetle and cockroaches found in and around chicken farms carried campylobacters and the bacteria can

Table 1. Carriage of *Campylobacter* and *Salmonella* by houseflies

Locations	No. of samples	No. positive for <i>Campylobacter</i>		<i>Campylobacter</i> species identified
		External body surface	Internal contents	
Large animal ward	20	0	0	
Cafeteria	20	0	0	
Poultry farm	20	2	1	<i>C. jejuni</i> (1 isolate) <i>C. coli</i> (2 isolates)
		No. positive (%) for <i>Salmonella</i>		<i>Salmonella</i> serovar identified
		External body surface	Internal contents	
Large animal ward	20	1	3	<i>S. Hadar</i> (3 isolates)
Cafeteria	20	0	2	<i>S. Muenster</i> and <i>S. Indiana</i> (2 isolates each)
Poultry farm	20	0	2	<i>S. Newington</i> (1 isolate).

survive on or within these insects for a few days. Rosef & Kapperud (1983) showed the *Campylobacter* carrier rate of houseflies captured on a chicken farm was 51% while Hald *et al.* (2004) reported 8.2% carriage rate. Chicken populations in many countries worldwide have been reported to be colonized with campylobacters (Humphrey *et al.*, 2007) and in Malaysia, the prevalence rates have been shown up to 100% in a number of farms (Saleha, 2003). Campylobacters are shed in faecal materials and as such, flies could have picked up the organisms from the chicken litter. Free-flying birds are also colonized with campylobacters (Newell & Fernley, 2003; Saleha, 2003) and were observed flying around the chicken farm; their faeces could be the source of campylobacters (and possibly salmonellae) in chickens, the environment and for the flies. *Campylobacter* was not recovered from the other two locations; it is probably that the organisms were not present in these environments.

Salmonella were isolated from flies caught from all the three locations. The chickens and the environment of the farm could have been colonized with salmonellae. Moreover, monitor lizards were reported by the farm workers to frequent the farm and could have been a source of salmonellae. According to Corrente *et al.* (2004), reptiles are common reservoirs of salmonellae with high faecal carriage rate. The environment around the ward could have been contaminated with salmonellae as it housed sick animals such as cattle, goats, sheep and horses. The flies captured around a cafeteria could possibly be contaminated with salmonellae from the surrounding environment, cats' faeces, garbage bins or elsewhere. *Salmonella* Hadar was the most common serotype identified. Pereira *et al.* (2007) reported that in the last two decades, *Salmonella* Hadar has become the second commonest

serotype isolated from foodborne disease in European countries and often the outbreaks caused by this serovar were associated with food of animal origin as meat and poultry products.

The studies on carriage of bacteria by houseflies have shown that the bacteria were carried on the external body surface as well as inside the body and that flies may act either as mechanical or biological vectors (Humphrey *et al.*, 2007). Holt *et al.* (2007) indicated that initially *Salmonella* serovar Enteritidis were more readily isolated from the interior of the fly compared to the exterior; however upon rinsing with detergent, their finding showed the recovery of the *Salmonella* serovar Enteritidis from both fly interior and exterior were similar. It is interesting to note the study by Holt *et al.* (2007) which showed that contaminated flies administered to hens initiate an infection and that *Salmonella* serovar Enteritidis residing on or in a fly does not eliminate its infectivity.

Hence, houseflies need to be regarded as important mechanical vectors of gastrointestinal diseases such as campylobacteriosis and salmonellosis. Campylobacteriosis requires a low infectious dose of a few hundred cells of the bacteria (Humphrey *et al.*, 2007) which lead to a suggestion that fly transmission of campylobacters may be the most important source of infection in the kitchen scenario and in establishments that sell ready-to-eat foods may be sources of campylobacters, if there is no effective fly control (Nicholls, 2005) and possibly for *Salmonella* too. Thus, there should be increased public awareness on health hazards brought about by flies and to carry out more effective fly control measures such as keeping the environment clean by proper disposal of wastes to reduce breeding places for flies.

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