

The blow fly, *Chrysomya megacephala*, and the house fly, *Musca domestica*, as mechanical vectors of pathogenic bacteria in Northeast Thailand

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Received 7 October 2013; received in revised form 8 January 2014; accepted 9 January 2014

Abstract. The Oriental latrine fly, *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) and the house fly, *Musca domestica* L., (Diptera: Muscidae) are synanthropic flies which are adapted to live in close association with human habitations, thereby making them likely mechanical vectors of several pathogens to humans. There were two main aims of this study. The first aim was to determine the prevalence of these two fly species from five types of human habitations including: fresh-food markets, garbage piles, restaurants, school cafeterias and paddy fields, in the Muang Ubon Ratchathani and Warinchamrap districts of Ubon Ratchathani province of Northeast Thailand. Flies collection were conducted monthly from September 2010–October 2011 using a reconstructable funnel trap, containing 1 day-tainted beef offal as bait. A total of 7 750 flies (6 401 *C. megacephala* and 1 349 *M. domestica*) were collected. The second aim was to examine the potential of these flies to carry pathogenic bacteria. Bacteria were isolated from 994 individual flies collected using a sweep net (555 *C. megacephala* and 439 *M. domestica*). A total of 15 bacterial genera were isolated from the external surfaces, comprising ten genera of gram-negative bacteria and five gram-positive bacteria. The most common bacteria isolated from both species were coagulase-negative staphylococci, followed by *Streptococcus* group D non-enterococci. Human pathogenic enteric bacteria isolated were *Salmonella* sp., *Shigella* sp., *Escherichia coli* O157:H7, *Salmonella typhi*, *Bacillus* sp., and *Enterococcus* sp., of which *S. typhi* is the first report of isolation from these fly species. Other human pathogens included *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Not only were the number of *C. megacephala* positive for bacteria significantly higher than for *M. domestica*, but they were also carrying ~11–12 times greater bacterial load than *M. domestica*. These data suggest that both fly species should be considered potential mechanical vectors of bacterial pathogens associated with human habitations year-round in this region of Northeast Thailand.

INTRODUCTION

The Oriental latrine fly, *Chrysomya megacephala* (Fabricius) and the house fly, *Musca domestica* L., are considered flies of medical importance worldwide. Their synanthropic behavior, adapting them to live in close association with human habitations combined with frequent visitation of filth

allows them to mechanically transmit several pathogens to humans through this close relationship (e.g., Greenberg, 1973; West & Peters, 1973). Numerous pathogens (e.g., bacteria, virus, parasitic eggs) have been transmitted by these two fly species. Pathogenic bacteria associated with *C. megacephala*, in Malaysia included: *Aeromonas hydrophila*, *Citrobacter*

freundii, *Enterobacter agglomerans*, *Klebsilla oxytoca*, *Burkholderia pseudomallei* (Sulaiman *et al.*, 2000), and from *C. megacephala* and *M. domestica* in Thailand included: *Escherichia coli*, *Klebsiella pneumonia*, *Morganella morganii*, *Enterobacter cloacae*, *Proteus mirabilis* (Sukontason *et al.*, 2007). Several parasitic eggs have also been recovered from both fly species, e.g., *Ascaris*, hookworm, *Trichuris trichiura*, *Taenia*, *Toxocara* (Monzon *et al.*, 1991; Graczyk *et al.*, 2005). In Thailand, investigations of fly abundance in the northern region using systematic methodology indicated that *C. megacephala* was the most common fly species collected (Ngoen-klan *et al.*, 2011), but data for other regions are more limited (Sucharit & Tumrasvin, 1981). The role of flies as mechanical vectors of pathogens is also scant (Echeverria *et al.*, 1983; Sukontason *et al.*, 2007).

In Thailand, diarrheal disease is a major health problem and remains a significant source of mortality for young children. The Northeast region of the country is ranked second in the number of diarrheal disease cases reported in all of Thailand. Ubon Ratchathani province, reported diarrhea as the most common communicable disease during the period of 2003-2008 (Bureau of Epidemiology, Department of Disease Control). Investigations in the Chiang Mai province of northern Thailand, indicated that several bacterial species including those causing diarrheal disease could be isolated from both *C. megacephala* and *M. domestica* (Sukontason *et al.*, 2007). To date, there have been no studies on the mechanical vector potential of *C. megacephala* and *M. domestica* for the pathogenic bacteria associated with diarrhoeal disease in the community environments of Ubon Ratchathani Province of Northeast Thailand. Therefore, the objectives of this study were to investigate the prevalence of both filth fly species in two districts of Ubon Ratchathani Province where reported incidence could be used to classify areas of high and low diarrheal disease incidence. The second objective was to determine the types of bacteria carried by both fly species from

several types of locations where flies have potential access to both waste and food items in and around human habitations.

MATERIALS AND METHODS

Study area and fly collection

The study area consisted of two districts of Ubon Ratchathani province, northeast Thailand: Muang Ubon Ratchathani and Warinchamrap districts (Figure 1), the areas are representative of high and low diarrhea incidence in this province. Fly collections were performed monthly (30 ± 7 day-interval) for 12 months from September 2010 to October 2011. In each district, five collection sites were selected to observe flies with the opportunity to feed on both waste or unsanitary materials and human food items, including fresh-food markets, garbage piles, restaurants, school cafeterias and rice paddy fields. The geographic coordinates for each site are shown in Table 1. In each site, two reconstructable funnel traps containing 1 day-tainted beef offal as bait (Ngoen-klan *et al.*, 2011) were employed for adult fly collection, each trap set was located approximately 1 km apart. All flies captured were transported to the laboratory at College of Medicine and Public Health, Ubon Ratchathani University for identification.

Flies collected for bacterial isolation were collected via a sterilized sweep net from 10.00 AM-12.00 PM in the shaded areas. This collection was performed monthly in these two districts from September 2010 to October 2011 with 1 day-tainted beef offal used as bait.

Identification of pathogenic bacteria

After identification in the laboratory, individual *C. megacephala* and *M. domestica* captured from the five collection sites were transferred into separate 20 ml sterile glass vials using sterilized forceps. Bacteria from flies in each vial were isolated using standard bacterial isolation techniques. Two milliliters of sterile buffer peptone water were added to each vial and then shaken vigorously for two minutes to create a wash from each fly. Subsequently, 0.1 ml of the wash solution vial was inoculated onto selective culture media

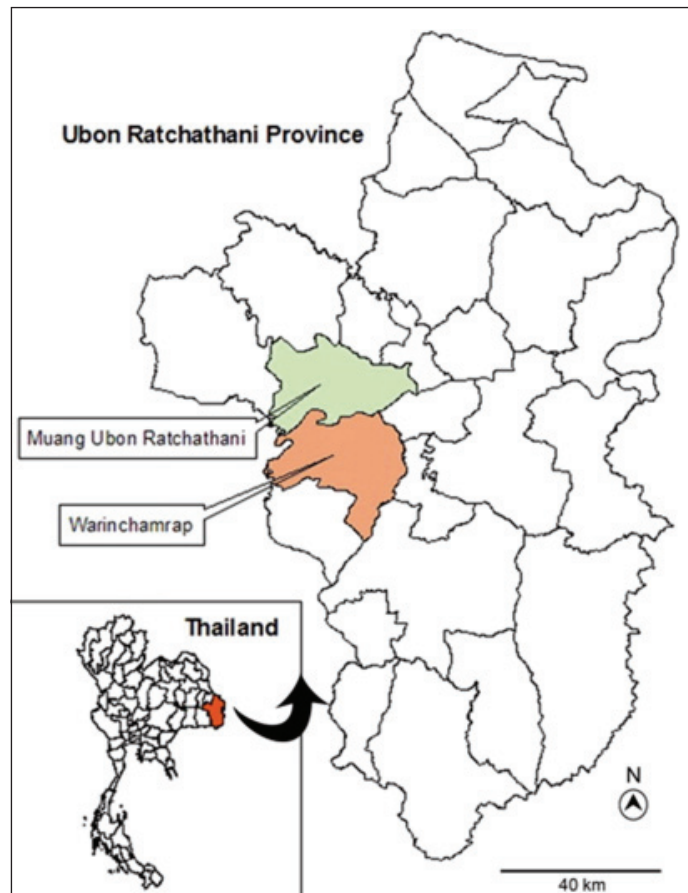


Figure 1. Location of the study area (Muang Ubon Ratchathani and Warinchamrap districts) of Ubon Ratchathani province, Northeast Thailand

Table 1. Geo-referenced locality of study sites for adult *C. megacephala* and *M. domestica* collection in Ubon Ratchathani province

Location site	Latitude	Longitude	Altitude (m)
Muang Ubon Ratchathani			
Fresh-food market	15°13'55.225"N	104°52'22.748"E	118
Garbage piles	15°15'35.620"N	104°50'55.601"E	121
Restaurant	15°16'29.668"N	104°49'27.471"E	132
School cafeteria	15°16'29.668"N	104°49'27.471"E	127
Paddy field	15°18'2.562"N	104°52'50.035"E	120
Warinchamrap district			
Fresh-food market	15°10'40.369"N	104°52'20.981"E	121
Garbage piles	15°11'52.340"N	104°52'10.045"E	133
Restaurant	15°7'43.603"N	104°54'0.579"E	145
School cafeteria	15°11'33.212"N	104°50'6.756"E	126
Paddy field	15°8'21.388"N	104°53'52.218"E	137

plates. Selective media were used to differentiate among closely related groups of bacteria and to isolate specific bacteria from mixed populations. The isolated bacteria were then identified by morphological and biochemical tests using the methods from Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Serological testing of *Escherichia*, *Salmonella*, *Shigella* and *Vibrio*

All samples identified as *Escherichia*, *Salmonella*, *Shigella* and *Vibrio* were serotyped by agglutination tests with specific antisera by Serosystem antisera (S & A Reagent Lab Limited, Thailand). *Escherichia* were serotyped by using *E. coli* O157 and H7 antisera; *Salmonella* were serotyped by using *Salmonella* O Polyvalent A-I antiserum and group D antisera; *Shigella* were serotyped by using *Shigella* Poly. D antiserum; and *Vibrio* were serotyped by using *Vibrio cholerae* O1 Polyvalent Antiserum and *Vibrio cholerae* Ogawa antiserum.

Statistical analysis

Descriptive statistics related to the bacterial species isolated from each fly collected from all site types were calculated in terms of number and percentage. The Chi-square test was used to compare the number of positive *C. megacephala* and *M. domestica* at each

site in each district, with p-value of < 0.05 used as the cut off for determination of significance. A one-way analysis of variance (ANOVA) was employed to test for statistical differences among bacterial carrying rates for *C. megacephala* and *M. domestica* at each site in each district. To analyze the bacterial carrying rates for flies over time an ANOVA was then conducted for each species. Significance for all tests was set at $p < 0.05$. All analysis were carried out using the SPSS program version 16.0 for Windows.

RESULTS

A total of 7 750 flies were collected from Muang Ubon Ratchathani and Warinchamrap districts, based on the 12 month collection period using a reconstructable funnel trap, having 1 day-tainted beef offal as bait. A significantly higher number of *C. megacephala* (6 401 in total: 4 036 in Muang Ubon Ratchathani, 2 365 in Warinchamrap) were collected than *M. domestica* (1 349 in total: 744 in Muang Ubon Ratchathani, 605 in Warinchamrap) ($\chi^2 = 974.22$; $p < 0.0001$) (Table 2). The total number of flies collected from Muang Ubon Ratchathani (4 780) was significant higher than from Warinchamrap district (2 970) ($\chi^2 = 29.42$; $p < 0.0001$). The highest numbers for both species were present at the restaurant site.

Table 2. Number of *C. megacephala* and *M. domestica* collected using reconstructable funnel traps at various human habitations in Ubon Ratchathani province from September 2010 to October 2011

Collection site	Muang			Warinchamrap		
	CM	MD	Total	CM	MD	Total
Restaurants	2479	391	2870	688	69	757
School cafeterias	683	102	785	531	146	677
Paddy fields	427	25	452	621	36	657
Fresh-food markets	208	132	340	121	296	417
Garbage piles	239	94	333	404	58	462
Total ^a	4036	744	4780*	2365	605	2970*

CM = *Chrysomya megacephala*, MD = *Musca domestica*

*Total flies collected from Muang district was significant higher than Warinchamrap district ($\chi^2 = 29.42$; $P < 0.0001$)

^aTotal number of *C. megacephala* was significant higher than *M. domestica* ($\chi^2 = 974.22$; $P < 0.0001$)

The presence of bacteria from different sample sites revealed a high number of positives in both *C. megacephala* (96.4–100%; n = 42-60) and *M. domestica* (80.7–100%; n = 20-60) (Table 3). For *C. megacephala*, no significant difference was observed in the positive rates among all five sites (fresh-food market, garbage pile, restaurant, school cafeteria and paddy field) from both the Muang Ubon Ratchathani ($\chi^2 = 4.716$; $p = 0.166$) and Warinchamrap ($\chi^2 = 0.090$; $p = 1.000$). Similarly, no significant difference was observed in the positive rate of *M. domestica* from all five sites in Warinchamrap ($\chi^2 = 7.523$; $p = 0.089$), but statistical differences were observed among sites in Muang Ubon Ratchathani ($\chi^2 = 13.989$; $p < 0.008$).

The abundance of positive flies collected for each month along with the number of reported diarrhea cases, from Ubon Ratchathani Health Provincial Office, at that same time are displayed in Figure 2. In Muang Ubon Ratchathani - the area of high diarrhea incidence – four dramatic peaks of positive *C. megacephala* occurred in October, December, February and June; while four peaks of positive *M. domestica* occurred in September, December, March and June. The number of diarrhea cases did not appear

to be correlated with the observed positive flies. In the Warinchamrap district - the area of low diarrhea incidence – both positive fly species displayed a single peak, observing in June. The number of diarrhea cases in this district seemed likely correlated with these positive flies captured.

Table 4 displays the number of bacterial species observed for each fly examined for each month, as well as average climatic data (temperature and humidity). Up to eleven bacterial species were isolated from flies collected in September and March. The details of each fly species and the number of bacterial species they harbored are displayed in Table 5. Each positive *C. megacephala* and *M. domestica* harbored anywhere from 1-11 isolated bacterial species, with the most common number of simultaneous species being 5 or 6. *C. megacephala* was significantly more likely than *M. domestica* to be carrying bacteria ($\chi^2 = 137.574$; $p < 0.0001$).

Identification of pathogenic bacteria from the collected flies indicated a high diversity of species of human pathogens. A total of fifteen bacterial genera were isolated, comprising ten gram-negative bacterial genera and five gram-positive bacterial genera (Table 6). The most common bacterium isolated from both fly species was

Table 3. Presence of bacteria from flies collected from the different sample sites taken from Ubon Ratchathani province from September 2010 to October 2011

Collection site	Muang district		Warinchamrap district	
	% positive CM ^a	% positive MD ^c	% positive CM ^b	% positive MD ^d
Fresh-food market	100 (60/60)	80.7 (46/57)	100 (43/43)	95.9 (47/49)
Garbage pile	96.7 (58/60)	85.0 (51/60)	100 (56/56)	97.5 (39/40)
Restaurant	100 (60/60)	96.7 (58/60)	100 (60/60)	84.0 (42/51)
School cafeteria	100 (59/59)	100 (38/38)	100 (42/42)	92.7 (38/41)
Paddy field	96.4 (53/55)	85.0	100 (60/60)	91.3

CM = *Chrysomya megacephala*, MD = *Musca domestica*

^aNo significant difference was found within same column among numbers of positive *C. megacephala* for bacteria isolation from each site of Muang Ubon Ratchathani district ($\chi^2 = 4.716$; $P = 0.166$)

^bNo significant difference was found within same column among numbers of total positive *C. megacephala* for bacteria isolation from each site of Warinchamrap district ($\chi^2 = 0.090$; $P = 1.000$)

^cSignificant difference within same column among numbers of total positive *M. domestica* for bacteria isolation from each site of Muang Ubon Ratchathani district ($\chi^2 = 13.989$; $P < 0.008$)

^dNo significant difference within the same column among number of positive *M. domestica* for bacterial isolation from each site of Warinchamrap district ($\chi^2 = 7.523$; $P = 0.089$)

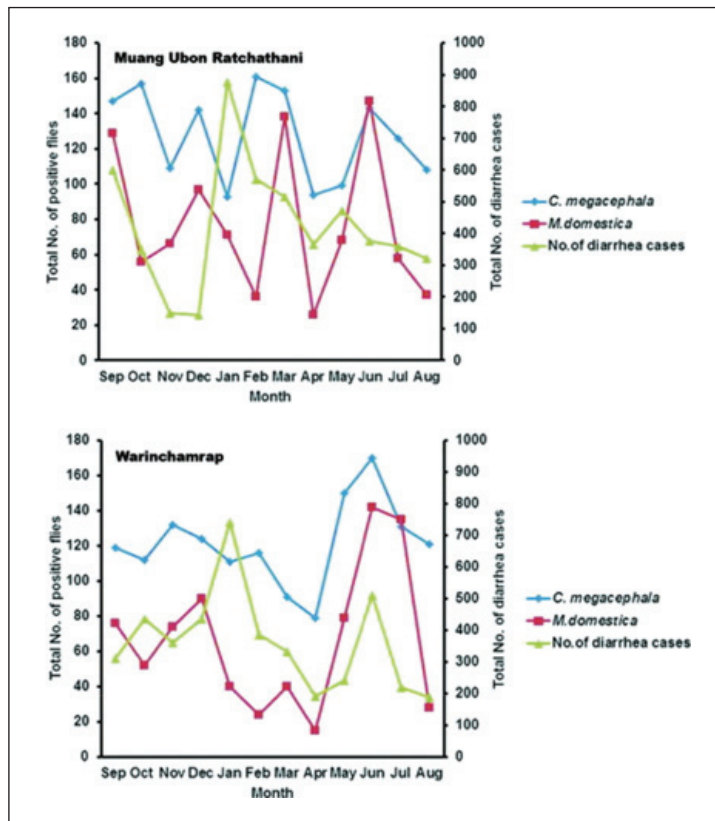


Figure 2. Total number of positive *C. megacephala* and *M. domestica* carrying pathogenic bacteria and total number of diarrhea cases in two districts of Ubon Ratchathani province, Northeast Thailand, from September 2010 to October 2011

Table 4. Comparison of bacterial carrying rates of *C. megacephala* and *M. domestica* in Ubon Ratchathani province from September 2010 to October 2011

Month	Average Temperature (°C)*	Average Humidity (%)*	No. flies collected ^{1**}	No. bacterial species isolated from each fly
September, 2010	25.7/28.1	88/62	83	2–11
October, 2010	27.8/28.2	82/NA	84	0–9
November, 2010	22.7/23.8	63/NA	90	0–10
December, 2010	25.7/25.1	71/NA	86	0–8
January, 2011	24.0/23.2	63/NA	80	0–8
February, 2011	27.6/26.6	60/NA	72	0–9
March, 2011	27.3/27.1	55/NA	78	0–11
April, 2011	30.2/28.3	56/NA	58	0–7
May, 2011	30.3/29.4	70/NA	100	0–9
June, 2011	27.7/28.1	82/NA	100	3–9
July, 2011	27.1/30.2	85/NA	92	0–8
August, 2011	28.0/29.0	79/NA	71	0–9

*Data from Muang Ubon Ratchathani district/Warinchamrap district, respectively. Data from the Meteorological Center, Ubon Ratchathani province. NA: not available

**Flies were collected at the same time of a day (10.00 AM-12.00 PM)

Table 5. Bacterial species loads for each fly species collected and district from Ubon Ratchathani province from September 2010 to October 2011

No. of bacterial species isolated from each fly	Fly species			Districts		
	CM	MD	Total	Muang	Warinchamrap	Total
1	21	52	73	42	31	73
2	22	59	81	44	37	81
3	42	55	97	56	41	97
4	95	41	136	75	61	136
5	104	56	160	87	73	160
6	103	62	165	74	91	165
7	90	38	128	64	64	128
8	45	21	66	34	32	66
9	24	7	31	16	15	31
10	4	3	7	6	1	7
11	0	2	2	2	0	2
Total positive flies ^{a,b}	550	396	946	500	446	946
Total negative flies	5	43	48	29	19	48
Total flies examined	555	439	994	529	465	994

CM = *Chrysomya megacephala*, MD = *Musca domestica*

^aSignificant difference of bacterial carrying rates in the same row between *C. megacephala* and *M. domestica* ($\chi^2 = 137.574$; $P < 0.0001$)

^bNon significant difference of bacterial carrying rates in the same row between Muang and Warinchamrap district ($\chi^2 = 12.679$; $P = 0.315$)

Table 6. Bacteria isolated from *C. megacephala* and *M. domestica* collected from different habitat sample sites taken from Ubon Ratchathani province from September 2010 to October 2011

	No. of positive flies for bacteria isolated (%)	
	<i>C. megacephala</i>	<i>M. domestica</i>
<i>Bacillus</i> sp.	391 (70.45)	222 (50.57)
<i>Citrobacter</i> sp.	179 (32.25)	96 (21.87)
Coagulase-negative staphylococci	463 (83.42)	305 (69.48)
<i>Enterobacter</i> sp.	34 (6.13)	15 (3.42)
<i>Enterococcus</i> sp.	99 (17.84)	100 (22.78)
<i>Escherichia coli</i>	178 (32.07)	86 (19.59)
<i>Escherichia coli</i> O157:H7(EHEC)	16 (2.88)	8 (1.82)
<i>Klebsiella</i> sp.	288 (51.89)	145 (33.03)
<i>Morganella</i> sp.	90 (16.22)	30 (6.83)
<i>Proteus</i> sp.	352 (63.42)	159 (36.22)
<i>Providencia</i> sp.	74 (13.33)	30 (6.83)
<i>Pseudomonas aeruginosa</i>	146 (26.31)	97 (22.10)
<i>Salmonella</i> sp.	116 (20.90)	76 (17.31)
<i>Salmonella typhi</i>	66 (11.89)	62 (14.12)
<i>Shigella</i> sp.	53 (9.55)	25 (5.69)
<i>Staphylococcus aureus</i>	45 (8.11)	13 (2.96)
<i>Streptococcus</i> group D non-enterococci	398 (71.71)	255 (58.09)
Total positive flies ^a	551 (99.28)	397 (90.43)
Total negative flies	4 (0.72)	42 (9.57)
Total flies examined	555 (100.00)	439 (100.00)

^aSignificant difference between total positive flies between *C. megacephala* and *M. domestica* ($\chi^2 = 22.140$; $P < 0.0001$)

coagulase-negative staphylococci, followed by *Streptococcus* group D non-enterococci and *Bacillus* sp. Human pathogenic enteric bacteria isolated included *Salmonella* sp., *Shigella* sp., *Escherichia coli* O157:H7, *Salmonella typhi*, *Enterococcus* sp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

DISCUSSION

The results of this study revealed several important observations regarding fly abundance at common human habitations and the bacteria present on flies in these areas. More *C. megacephala* (n = 6 401) were captured than *M. domestica* (n = 1 349) at all five of the human habitations sampled (fresh-food markets, garbage piles, restaurants, school cafeterias and rice paddy fields), in the Muang Ubon Ratchathani and Warinchamrap districts of Ubon Ratchathani province of Northeast Thailand. This finding is similar to that observed in previous study in 2011 of Chiang Mai province of Northern Thailand (Ngoen-klan *et al.*, 2011). However, these observations are in contrast with those of Sucharit *et al.* (1976), who found that *M. domestica* collected in Bangkok and neighboring provinces of the central region in 1976 were much higher than *C. megacephala*, and the work of Echeverria *et al.* (1983) in Nakorn Rajsima province of Northeast Thailand in 1983. The reason for current increase in the *C. megacephala* population over *M. domestica* in Thailand is unknown. It might be as a result of different kinds of bait used to attract flies. Putrid fish was used as bait by Sucharit *et al.* (1976). The areas of collection of *M. domestica* included animal pens, yard, local toilet or kitchen in the study conducted by Echeverria *et al.* (1983). The differences may also possibly be related to land use changes in the development of the Northeast region of Thailand that *C. megacephala* is capable of adapting to, thus resulting in higher population density over *M. domestica* since the 1983 study (Echeverria *et al.*, 1983).

Investigation of the possible role of bacterial carriage by filth flies dwelling in

association with humans is important, in determining the dissemination of pathogenic bacteria into surrounding areas. Previous publications indicated that *C. megacephala* was more efficient at pathogen carriage than *M. domestica* (Sulaiman *et al.*, 1988; 2000; Monzon *et al.*, 1991; Sukontason *et al.*, 2007). Not only the more positive rate of pathogen carriage by *C. megacephala* than *M. domestica*, but also more species of pathogens carriage by each fly, either bacteria, helminthic eggs or protozoan cysts. Result obtained from this study adds up such phenomenon.

When comparing the potential *C. megacephala* and *M. domestica* in Thailand, to act as mechanical vectors of bacteria a higher percentage of *C. megacephala* were positive for bacteria (96.4–100%) and 85.0–100% of *M. domestica* were positive (see Table 3). These percentages are higher than the 87.7% of *C. megacephala* and 66.2% of *M. domestica* positive flies observed in the Chiang Mai province of Northern Thailand (Sukontason *et al.*, 2007). Moreover, the number of bacterial species carried by individual flies in the present study was higher (up to eleven species), than the eight species previously reported in Chiang Mai (Sukontason *et al.*, 2007). This might reflect the fact that the collection sites for filth flies used in this study (fresh-food markets, garbage piles, restaurants, school cafeterias and paddy fields) in Ubon Ratchathani were more diverse than one site type sampled (fresh-food markets) in Chiang Mai.

The routes of pathogen transmission by flies involve the external surfaces, regurgitation of food via vomit droplets (in saliva) and defecation (Grübel *et al.*, 1997; Kobayashi *et al.*, 1999). Several human pathogenic enteric bacteria were isolated from the external surface of both *C. megacephala* and *M. domestica*, e.g., *Salmonella*, *Shigella*, *Escherichia coli* O157:H7, *Salmonella typhi*, *Bacillus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Studies in Malaysia have revealed that these bacteria (*Bacillus*, *Enterobacter* and *Proteus*) can be isolated from both the external surface and gut contents of *M. domestica* (Nazni *et al.*,

2005b). For *E. coli* O157:H7, this bacterium persisted in the crop of adult house fly for at least 4 days (Sasaki *et al.*, 2000), it was also detectable for up to 13 days on the body surface after exposure (Wasala *et al.*, 2013). Another important aspect is that *E. coli* O157:H7 can proliferate in the microhabitat of the pseudotrachea of the labella of adult house fly, suggesting not only simply mechanical transmission, but “bioenhanced transmission” (Kobayashi *et al.*, 1999). The proliferation of *Enterococcus faecalis* OG1RF:pmV158 in the house fly digestive tract under laboratory conditions also supports this hypothesis (Doud & Zurek, 2012). Such phenomenon agree with previous authors, indicating higher bacterial transmission via gut content than the external surface. McGuire & Durant (1957) reported that internal bacteria in *M. domestica* were ~20 times higher than on the external surfaces. Similar findings were recently published by Pava-ripoli *et al.* (2012) showing that the prevalence of food-borne pathogens was three times greater in the guts of the flies than on their body surfaces. Gupta *et al.* (2012) also showed that the house fly gut is an environmental reservoir for a vast number of bacterial species, which may have impacts on pathogen transmission. The flight range of the house fly is up to 7 km, based on mark release recapture data, which may allow for the widespread dispersal of these pathogens by flies (Nazni *et al.*, 2005a). However, it is noteworthy that the recovery of pathogens from the flies does not necessary implicate them in the transmission of disease. There are other routes of infection, and the role of the flies transmission can be variable.

Bacterial species detected from this present study were very similar to those isolated in both fly species captured in Chiang Mai, but also included *Shigella* sp., *Salmonella* spp. and *Salmonella typhi*. Based on the limited investigations, mechanical carriage of *S. typhi* by both fly species is initially documented by the results of this study. The isolation of several bacterial species in Muang Ubon Ratchathani and Warinchamrap districts is also reflected in the existence of these pathogens associated with human disease in this particular area. In the time

analysis data, *Shigella* sp. was isolated from 53 *C. megacephala* and 25 *M. domestica* in both districts (see Table 6). Approximately 5.5 times more *Shigella*-positive *C. megacephala* and 4.4 times *M. domestica* were detected from Muang Ubon Ratchathani than Warinchamrap districts (data not shown). Although no apparent evidence association between these flies and *Shigellosis*-cases, the higher diarrhea cases in Muang Ubon Ratchathani than Warinchamrap districts is still challenged.

The link between diarrheal disease incidence and fly populations is indirect, such that reductions in fly populations lead to reductions in specific disease incidence. A few studies have documented reductions in *Shigella* caused diarrheal disease attributed to large-scale insecticide use. One of the first of these studies was conducted by Watt and Lindsay (1948) in Hidalgo County, Texas, where DDT was used to knock down fly populations. The authors found that there was a reduction in *Shigella* but not *Salmonella* caused diarrheal disease incidence. Lindsay *et al.* (1953) followed this study up in an area where the mortality caused by the high diarrheal disease incidence was higher and found similar results. In both of these studies, the flies’ ability to rapidly develop insecticide resistance led to increased fly populations and increased diarrheal disease incidence. These findings as well as others (reviewed in Esrey 1991) suggest that reduction of fly populations can reduce diarrheal disease incidence, of at least certain bacterial species and that an integrated approach to fly control must be implemented for long term reductions in diarrheal disease.

In conclusion, *C. megacephala* and *M. domestica* captured in different types of human habitations in Muang Ubon Ratchathani and Warinchamrap districts of Ubon Ratchathani province are potential mechanical vectors of multiple pathogenic bacteria. Several of the human pathogenic enteric bacteria isolated included: *Salmonella* sp., *Shigella* sp., *E. coli* O157:H7, *S. typhi*, *Enterococcus* sp., *S. aureus* and *P. aeruginosa*. Importantly, characteristics of the fly population should be monitored to investigate the impact of control on fly

abundance in human habitations which may be a reasonable approach to decreasing diarrheal disease in the Ubon Ratchathani province. Further work should be completed to document diarrheal disease before and after active intervention to reduce fly populations to determine the importance of flies in disseminating pathogenic bacteria in this region of Thailand.

Acknowledgements. This study was financially supported by the Thailand Research Fund and Office of the Higher Education Commission and Ubon Ratchathani University (MRG5380283 to TC). We thank the College of Medicine and Public Health, Ubon Ratchathani University for facilities and publication cost.

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