COMPARISON BETWEEN *MUSCA DOMESTICA* AND *CHRYSOMYA MEGACEPHALA* AS CARRIERS OF BACTERIA IN NORTHERN THAILAND

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Abstract. A comparison between the common house fly, *Musca domestica*, and the Oriental latrine fly, *Chrysomya megacephala*, was assessed for their potential as carriers of bacteria in urban areas of Chiang Mai Province, northern Thailand. *C. megacephala* was significantly more likely to carry bacterial species than *M. domestica*; however, no significant difference was found between the number of positive male and female flies within the same species. A total of 42 bacterial species were isolated. The most common bacterium isolated from *M. domestica* was coagulase-negative staphylococci (n=57) followed by *Escherichia coli* (n=10) and Viridans streptococci (n=10), while that of *C. megacephala* was non-fermentative gram-negative bacilli (n=59) followed by coagulase-negative staphylococci (n=54).

INTRODUCTION

The common house fly, *Musca domestica* L., and the Oriental latrine fly, *Chrysomya megacephala* (Fabricius), are medically-important insects worldwide. In addition to causing annoyance and myiasis (Zumpt, 1965), they are forensically-important fly species (Smith, 1986), with both being reported as mechanical carriers and/or reservoirs of several pathogens, *ie*, bacteria, viruses, protozoan cysts and helminth eggs (Greenberg, 1973; Echeverria *et al*, 1983; Levine and Levine, 1991; Monzon *et al*, 1991; Fotedar *et al*, 1992; Sulaiman *et al*, 2000).

In Thailand, the adult *M. domestica* was reported as being the most abundant of those flies collected, while *C. megacephala* was the second most abundant (Sucharit *et al*, 1976; Tumrasvin *et al*, 1978; Sucharit and Tumrasvin, 1981). When it came to mechanically carrying pathogens, *C. megacephala* played a more important role than *M. domestica* in the transmission of helminthic eggs (Monzon *et al*, 1991). Urban and Broce (1998) demonstrated the blow fly was twice as likely to be contaminated with enteric bacteria as any other fly. We studied the bacteria isolated from individual *M. domestica* and *C. megacephala* collected from domestic market places in Chiang Mai, northern Thailand. We also compared both fly species as mechanical carriers of the bacteria isolated.

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MATERIALS AND METHODS

Five fresh-food markets in urban areas of Chiang Mai Province (17-21ºN and 98-99ºE) were selected as collection sites where numerous adult *M. domestica* and *C. mega-*

cephala exist. These markets offer various kinds of foods, fresh (fruit, vegetables, meat, fish) and cooked food. One hundred thirty adult flies of each species were collected from these markets with insect sweep nets from April-June 1999. All fly specimens captured were separated individually into 20-ml sterile glass vials (2.5-cm diameter, 6-cm height), which were immediately covered with sterile caps. These vials were then transported to the laboratory of the Department of Parasitology, Faculty of Medicine, Chiang Mai University within one half hour. In the laboratory, all flies were killed while still in the vials by placing them in a freezer set at -20ºC for 30 minutes. The two species were then identified under a stereo microscope, while remaining segregated in their glass vials, by using the keys of Greenberg (1971) and Tumrasvin *et al* (1979).

After identification, 1 ml of sterile physiological saline solution was added to each vial, which was shaken vigorously for 1 minute with the fly remaining inside. The solutions were then examined immediately at the Microbiology Section, Central Laboratory, Faculty of Medicine, Chiang Mai University, for the presence of bacteria. The saline wash solution in each vial was inoculated onto the following plates: phenyl ethyl alcohol agar plate (for the isolation of *Staphylococcus* spp and *Enterococcus* spp), a MacConkey plate (for the isolation of gram-negative bacilli, *Shigella* spp and *Salmonella* spp), *Salmonella* and *Shigella* (SS) agar plates and Selenite - F broth (for the isolation of *Salmonella* spp and *Shigella* spp), thiosulfate citrate bile salt agar plate, and alkaline peptone water (for the isolation of *Vibrio* spp). The inoculated plates were subsequently incubated for 24-48 hours at 37ºC at normal atmosphere. The resulting isolates were characterized morphologically and further identifications were carried out following the methods of Koneman *et al* (1992).

A chi-square test was employed to determine if the number of positive male and female flies carrying bacteria differed significantly. A p-value of <0.05 was deemed significant. An analysis was carried out using the SPSS program version 7.5.1 for Windows.

RESULTS

From the total of 260 flies collected from 5 markets in urban areas of Chiang Mai, and examined for bacterial carriers based on bacteriological results, *C. megacephala* (87.7%; 114/130) was significantly more likely than *M. domestica* (66.2%; 86/130) to be carrying bacterial species [$\chi^2 = 15.79$; $p < 0.05$; Odds ratio = 0.27 (0.14-0.54)] (Table 1). However, no significant difference was found between the numbers of positive male and female flies within the same species [*M. domestica* ($\chi^2 = 0.11$; $p = 0.75$), *C. megacephala* ($\chi^2 = 0.02$; $p = 0.898$)]. Each positive fly harbored 1-8 isolated bacteria (Table 1).

A total of 42 bacteria species were isolated (Table 2). The most common bacterium isolated from *M. domestica* was coagulase-negative staphylococci (n=57) followed by *Escherichia coli* (n=10) and Viridans streptococci (n=10), while that of *C. megacephala* was non-fermentative gram-negative bacilli (n=59) followed by coagulase-negative staphylococci (n=54).

DISCUSSION

The routes of pathogen dissemination by flies include the external surface, regurgitation of food via vomit drops (in saliva) and defecation (Kettle, 1995; Grübel *et al*, 1997; Kobayashi *et al*, 1999). Which of these routes is the most important is still in question. Adeyemi and Dipeolu (1984) indicated that the external organs of *M. domestica* (legs, wings and mouthparts) constituted a source of 76.9% of bacteria they isolated, while 23.2% of those isolated came from the midgut or crop. In contrast, McGuire and Durant (1957) found approximately 20 times more internal

Table 1
Bacterial carrying rates for *M. domestica* and *C. megacephala* collected from an urban area of Chiang Mai Province, northern Thailand.

No. of bacterial species isolated from each fly	No. of *M. domestica*			No. of *C. megacephala*		
	Male	Female	Total	Male	Female	Total
1	29	27	56	11	13	24
2	6	8	14	10	7	17
3	0	6	6	12	7	19
4	0	3	3	10	14	24
5	2	4	6	4	9	13
6	0	1	1	4	9	13
7	0	0	0	1	2	3
8	0	0	0	0	1	1
Total positive flies[a,b]	37	49	86	52	62	114
Total negative flies	21	23	44	7	9	16
Total flies examined	58	72	130	59	71	130

[a] No significant difference was found between the numbers of positive male and female flies within the same species; *M. domestica* (χ^2 = 0.11; df = 1; p = 0.75) and *C. megacephala* (χ^2 = 0.02; df = 1; p = 0.898).
[b] Significant difference was found between the total numbers of positive flies of *M. domestica* and *C. megacephala* [χ^2 = 15.79; df = 1; p = 0.00; Odds ratio = 0.27 (0.14-0.54)].

bacteria in *M. domestica* than they did external bacteria. Contrary to these investigations, Fotedar *et al* (1992) noted that no significant difference was observed regarding the external surface and internal organs of *M. domestica* carrying various microorganisms including bacteria. This controversy is possibly due to the variability of microbial species and their ability to survive on the integument of house flies (Prívora *et al*, 1969). In this study, individual adult *M. domestica* and *C. megacephala* were placed in sterile vials and then shaken vigorously after adding sterile physiological saline solution, which indicates possible contamination via external surfaces. However, during the identification of fly specimens under the stereo microscope, defecated substance from the flies in the vials was also frequently seen as drops of brown color, which indicate possible transmission through fly defecation. Saline solution mixed with this defecated substance before bacterial isolation was performed would suggest at least two routes of bacterial dissemination by the fly specimens in this study, or three routes if vomitus were present.

The results of this study indicated that *C. megacephala* plays a greater role as a mechanical carrier of bacteria than *M. domestica,* which is similar to that reported by Sulaiman *et al* (2000). This may be explained by the research of Maldonado and Centeno (2003). These authors used the Mihályi's danger-index to quantify eight fly species as to the potential capability of transporting and passing infective pathogen. Several parameters were used to calculate this index with the viewpoint of potential pathogen transmission, including visiting human feces, feeding on feces, feeding on infectious secretions, the synanthropic status, and the body size of each fly. *C. megacephala* had the highest transmission, potential 5 times greater than *M. domestica*.

Generally, the species of bacteria isolated from adult flies in Thailand were similar to those isolated from *C. megacephala, Chrysomya*

Table 2
Bacteria isolated from *M. domestica* and *C. megacephala* collected from an urban area of Chiang Mai, northern Thailand.

Bacteria isolated	No. (%) of bacterial species isolated	
	M. domestica	*C. megacephala*
Coagulase-negative staphylococci	57 (43.8)	54 (41.5)
Non-fermentative gram-negative bacilli	7 (5.4)	59 (45.4)
Streptococcus group D non-enterococci	7 (5.4)	35 (26.9)
Escherichia coli	10 (7.7)	28 (21.5)
Klebsiella pneunoniae	9 (6.9)	22 (16.9)
Viridans streptococci	10 (7.7)	20 (15.4)
Morganella morganii	3 (2.3)	20 (15.4)
Enterobacter cloacae	7 (5.4)	11 (8.5)
Providencia stuartii	5 (3.8)	13 (10.0)
Enterococcus spp	6 (4.6)	10 (7.7)
Providencia alcalifaciens	3 (2.3)	12 (9.2)
Providencia rettgeri	1 (0.8)	14 (10.8)
Citrobacter freundii	1 (0.8)	11 (8.5)
Enterobacter agglomerans	5 (3.8)	6 (4.6)
Bacillus spp	2 (1.5)	8 (6.2)
Proteus mirabilis	4 (3.1)	6 (4.6)
Mixed gram-negative bacilli	3 (2.3)	6 (4.6)
Proteus vulgaris	0 (0)	9 (6.9)
Aeromonas sobria	0 (0)	4 (3.1)
Citrobacter amalonaticus	2 (1.5)	2 (1.5)
Enterococcus faecalis	1 (0.8)	3 (2.3)
Klebsiella oxytoca	0 (0)	4 (3.1)
Aeromonas hydrophila	0 (0)	3 (2.3)
Enterobacter aerogenes	1 (0.8)	2 (1.5)
Pseudomonas aeruginosa	0 (0)	3 (2.3)
Talumella ptyseos	0 (0)	3 (2.3)
Enterococcus fergusonii	0 (0)	2 (1.5)
Proteus penneri	1 (0.8)	1 (0.8)
Pseudomonas spp	1 (0.8)	1 (0.8)
Acinetobacter baumannii	0 (0)	1 (0.8)
Citrobacter diversus	0 (0)	1 (0.8)
Diphtheroid bacilli	0 (0)	1 (0.8)
Escherichia hermanii	0 (0)	1 (0.8)
Enterococcus faecium	0 (0)	1 (0.8)
Edwardsiella tarda	0 (0)	1 (0.8)
Hafnia alvei	0 (0)	1 (0.8)
Klebsiella ozaenae	0 (0)	1 (0.8)
Micrococcus spp	2 (1.5)	0 (0)
Vibrio cholerae non-01	0 (0)	2 (1.5)
Providencia rustigianii	0 (0)	1 (0.8)
Staphylococci spp	1 (0.8)	0 (0)
Staphylococcus aureus	1 (0.8)	0 (0)

rufifacies (Macquart), *M. domestica* and *Musca sorbens* Wiedemann, which were collected in food stalls and wet markets in downtown areas of Kuala Lumpur, Malaysia (Sulaiman *et al*, 2000). In this study, most of the bacteria isolated were medically important, including those causing diarrhea in humans, such as, *Aeromonas hydrophila* (Echeverria *et al*, 1984), *Edwardsiella tarda* (Marsh and Gorbach, 1982; Vandepitte *et al*, 1983; Janda and Abbott, 1993; Strauss *et al*, 1997), and *Pseudomonas aeruginosa* (Adlard *et al*, 1998). The pathogenic effect of these bacterial enteric pathogens results from the enterotoxin produced by certain strains. Although the enterotoxin was not investigated in this study, there was evidence of toxin produced by *E. coli* (ETEC) and enterotoxin produced by *V. cholerae* non-01. These were isolated from *M. domestica* collected in rural areas of northeastern Thailand (Echeverria *et al*, 1983). Interestingly, no enteropathogenic bacteria, such as *Shigella* or *Salmonella,* were isolated. This is possibly because the adult flies collected in this study had not been exposed to excreta, as indicated by Bidawid *et al* (1978). In the urban areas of Chiang Mai Province, human excrement would not be exposed to adult flies, since latrines are used regularly. *Burkholderia pseudomallei* was not found in this study, corresponding with only 4.4% prevalence surveyed in northern Thailand (Vuddhakul *et al*, 1999). Although it was the dominant bacteria isolated from *C. megacephala* in Malaysia, Sulaiman *et al* (2000) remarked that it was unusual, and may be a first report of its isolation.

Besides those causing diarrhea, many bacterial species isolated in this study have been previously reported as the causative agents of several diseases elsewhere, such as infective endocarditis caused by coagulase-negative staphylococci, streptococcus group D non-enterococci, *Viridans* streptococci (Mansur *et al*, 1992; Pearlman *et al*, 1998); endophthalmitis caused by *Enterobacter cloacae* (Okhravi *et al*, 1998); spinal epidural abscesses or impetigo-like vegetating nasal lesions caused by *Klebsiella pneumoniae* (Fragoulis *et al*, 2005; Kangwanprasert and Young, 2005); ecthyma gangrenosum-like eruptions and opportunistic pathogenic infections caused by *Morganella morganii* (Del Pozo *et al*, 1998; Gebhart-Mueller *et al*, 1998); empyema thoracis and lung abscesses caused by *Viridans* streptococci (Jerng *et al*, 1997); and chronic otitis media caused by *S. aureus, P. aeruginosa* and *Proteus mirabilis* (Obi *et al*, 1995).

Greenberg (1971) indicated four conditions involving the mechanical transmission of house flies: eusynanthropy, the consumption of both contaminated and non-contaminated food, great flight activity and dispersal, and the constant alternation between feces and humans. Based on the results of this study, both *C. megacephala* and *M. domestica* may play a role as mechanical carriers of bacteria in the urban areas of Chiang Mai. Fly control strategies as well as hygiene promotion programs should be promoted, as previously pinpointed out by Curtis *et al* (2000).

ACKNOWLEDGEMENTS

This work received support from Dr Natth Bhamarapravati's Fund for Science Research at Chiang Mai University. We thank the Faculty of Medicine, Chiang Mai University, for funding the cost of this publication.

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