THE EYE FLY *SIPHUNCULINA FUNICOLA* (DIPTERA: CHLOROPIDAE) AS A CARRIER OF PATHOGENIC BACTERIA IN THAILAND

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Abstract. The oriental eye fly *Siphunculina funicola* (1.0-1.6 mm) is extremely annoying to humans and domestic animals, feeding on mucous membranes, secretions, wounds, eyes, and other moist surfaces of the host body. In many rural areas of Thailand heavy populations of this fly prevail where they aggregate on a variety of hanging substrates, such as strings, nest trailings, electrical lines, decorations, ropes, cob webs, clothes hangers, automobile radio antennae and other items in open shade close to their hosts. Both males and females feed voraciously on wounds and moist skin. With this type of persistent feeding, the eye flies are suspected to carry and transfer germs to their hosts. In the present study, bacteria were isolated from *S. funicola* captured from wounds, host seeking flies and from their resting sites. Some enriched and bacterial culture media were more suitable for isolation than others. A diverse group of bacteria (64 species), both gram-positive and gram-negative, most in risk category 2, were identified. Bacterial colony counts from Trypic soy broth ranged from 10 to $>3.0x10^3$ cfu/ml. The most common bacteria isolated were *Acinetobacter, Aeromonas, Bacillus, Corynebacterium, Enterobacter, Enterococcus, Escherichia, Kocuria, Pantoea, Pseudomonas, Staphylococcus* and others. These bacteria may cause disease conditions in humans and animals. This is the first time bacteria from *S. funicola* have been reported.

Key words: *Siphunculina funicola*, eye fly, bacterial isolation

INTRODUCTION

Eye flies (*Siphunculina*) are noxious pests for man and domestic animals and potential vectors of pathogenic organisms to man and domestic and wild animals. In the genus *Siphunculina*, the oriental eye fly (*Siphunculina funicola* de Meijere 1905) is the most widespread and important species in oriental and Southeast Asian regions. Graham-Smith (1930), Muirhead-Thomson (1954) and Syddiq (1938) reported this fly to be involved in the spread and transmission of the causal agent of catarrhal ophthalmia in India. The flies fre-

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quently attack humans and animals, feeding on wounds, scratches, mucous membranes, eyes, lips, moist skin, in-between toes, sweat, and other secretions of the body and are suspected of mechanically transmitting pathogenic organisms to their hosts. This eye fly species has a unique behavior of forming large and persistent aggregations very close to their hosts on resting sites in and around domestic and peri-domestic premises in rural areas. These aggregations were described for the first time and characterized by Mulla and Chansang (2007) and Chansang and Mulla (2008 a,b). Both males and females form aggregations and feed on their hosts voraciously through out daylight hours (Mulla and Chansang, 2007). With their persistent hovering and feeding behavior, especially on and in wounds, the eye flies are likely to be potential carriers of pathogenic organisms to human and animal hosts causing ophthalmic complications, and keeping wounds from healing. The transmission of pathogenic bacteria causing septicemia has been attributed to the feeding activity of eye flies (unpublished reports, 2008).

In nature, non-biting synanthropic and synzootic flies are potential carriers of pathogens and play a role in spreading and transmission pathogens such as bacteria, viruses, fungi, and parasites (Banjo *et al*, 2005). A number of species in the genus *Musca, Sarcophaga, Calliphora, Fannia, Lucilia* and *Stomoxys* have been shown to be carriers of multiple species of microorganisms (Greenberg, 1971, 1973; Förster *et al*, 2007). The house fly *Musca domestica* is considered as a mechanical vector of bacteria such as *Vibrio cholerae, Staphylococcus aureus* and *Salmonella* sp (Altekrues *et al*, 1997; Beutin, 1999; Garmendia *et al*, 2005). This fly has been found to carry strains of *Escherichia coli* (EHEC, EAEC, EPEC) (Iwasa *et al*, 1999; Kobayashi *et al*, 1999; Szalanski *et al*, 2004).

Aside from taxonomic descriptions of some 30 species of *Siphunculina* in the oriental region (de Meijere, 1905; Cherian, 1977; Kanmiya, 1982, 1989, 1994), no information is available regarding the isolation and detection of bacteria from this fly which can potentially cause infection in humans and animals. In this study, we focused our research on the presence and abundance of bacteria on the exterior of eye flies (*S. funicola*) captured aerially after leaving wounds and flying away, those aggregating on resting sites, and those hovering around human hosts before landing and feeding. To obtain this information, several visits were made to rural areas where eye flies were abundant and where large numbers were seen feeding on hosts, especially on wounds and scratches of the skin. To detect a broad-spectrum of bacteria, a variety of growth media were used; the results are reported here. This is the first time the presence and abundance of bacteria carried by eye flies (*S. funicola*) have been investigated.

MATERIALS AND METHODS

Study areas

This study was carried out in 2 villages, Ban Mab Charoen and Ban Kai Nao, of Bang Lamung District in the Chon Buri Province of central Thailand. Observations were made of eye flies seeking human or animal hosts, flies clustered on aggregation sites and in open agricultural fields adjacent to the study villages. Based on these observations we noted large populations of *S. funicola* in both villages.

Bacterial isolation

Eye flies were collected aerially (without touching the human host to prevent contamination, using sterile collecting

equipments) after they flew away from wounds. Aggregating and host-seeking flies were captured in the air close to aggregation sites or humans, using sterile equipment without touching the resting sites or hosts. A variety of enrichment media were used to isolate bacteria on the external surfaces of the eye flies. In the laboratory, all samples were transferred onto specific growth media followed by further culturing and identification of bacteria.

Media used

Several media were used to detect broad-spectrum bacteria present. These media were used for transport of isolates from the field to the laboratory and for culture and identification of the bacteria. The media used were: 1. Buffered Peptone Water (BPW) (Merck, Darmstadt, Germany) was used for isolation of bacteria found in the alimentary canal of vertebrate hosts; 2. Alkaline Peptone Water (APW) (Merck, Darmstadt, Germany) was used for the same purpose as the BPW medium; 3. Muller Broth (MU) (Difco, Detroit, Ml) was used for the isolation of aerobic and facultative bacteria; 4. Wilkins-Chalgren Broth (WCB) (Oxoid, Hampshire, England) was used for the isolation of anaerobic bacteria; 5.Trypic Soy Broth (TSB) (Difco, Detroit, Ml) was used for facilitating bacterial growth and bacterial colony counts (cfu/ml).

Eye fly collection in the field

Eye flies were found distributed throughout the infested areas. They were attracted to humans and animals, attempting to feed on moist surfaces of the body, especially wounds and scratches (Fig 1).

Eye flies feeding on wounds. These flies were collected by sterile Petri dish (diameter 9 cm) technique. This technique was developed during the course of this study,

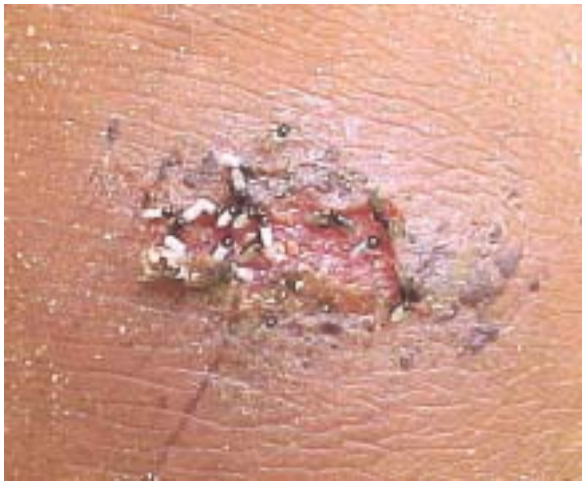

Fig 1–*Siphunculina funicola* on the wound of a human host.

capturing flies in the air after flying away from wounds. The two halves of the dish were held open when approaching the insects in the air, which were then captured by closing the two halves of the sterile dishes (without touching the host) (Fig 2A).

Eye flies from aggregation sites. The fly aggregation sites (Fig 3 A, B) were gently disturbed and the flies were captured using the same Petri dish technique as described above.

Eye flies from hosts. Eye flies hovering close to humans were captured in the air using the same Petri dish technique described above without touching the host (Fig 2B). All the flies were removed from the Petri dish using sterile forceps after immobilizing them by placing the dishes in the freezer. Each insect was dipped in the various media for one second before being removed and discarded.

Bacterial culture and identification of species

All isolated bacteria were subsampled and cultured on group specific media,

(A) Capturing of flies after flying from the wound.

(B) Capturing of flies hovering close to a human host.

Fig 2–The capturing of flies in the air without touching the host by using a sterile Petri dish (9 cm), (A) after flying from the wound; (B) when hovering close to humans.

(A) Rusty wire

(B) Thatch roof

Fig 3–Examples of resting sites where eye flies (*S. funicola*) were collected for bacterial isolation; (A) eye flies on rusty wire, (B) eye flies on a sheath of a thatched roof over a deck.

such as blood agar (Merck, Darmstadt, Germany) and chocolate agar (GC Agar base, Difco, Detroit, Ml; Hemoglobin, Difco, Detroit, Ml; Isovitalex, Difco, Detroit, Ml), depending on the bacteria. After subculturing on these media the bacteria were Gram stained. The gram-positive cocci were subjected to catalase, oxidase, and other biochemical tests for species identification. The gram-positive bacilli were evaluated for morphologic features and categorized as large spore or small non-sporeforming. The sporeforming group was subjected to biochemical tests and the non-sporeforming group was subjected to catalase and biochemical tests for species identification. Automatic identification of species was carried out using the VITEK 2 Compact Instrument (Biomerieux, France) instead of using biochemical tests.

Table 1
Positivity of samples for bacteria from *Siphunculina funicola* flies from wounds, resting sites and host seeking flies. All isolations were made in Ban Mab Charoen Village of Bang Lamung District, Chon Buri Province in 2007 and 2008.

Medium	Flies from wounds		Flies from resting sites		Host seeking flies			
	No.	Positive	No.	Positive	No.	Positive	Total	Positive
BPW	6	0	9	0	8	0	23	0
MU	10	5	21	16	23	7	54	28
APW	6	0	11	4	8	0	25	4
WCB	6	0	23	0	7	0	36	0[a]
TSB	48	23	62	36	62	29	172	88
				Grand total			310	120

[a]The WCB host seeking insect samples were kept in a refrigerator and inoculated in the medium after 24 hours.

Table 2
Isolation of bacteria (risk category 2) from external surface of *Siphunculina funicola* captured after feeding and flying away from wounds of a human host in two enrichment media in Central Thailand, 2007/2008. [a]

Muller broth (10 samples) (5 positive)[b]	Trypic soy broth (48 samples) (23 positive)[c]	
Corynebacterium minutissimum	*Acinetobacter lwoffii*	*Sphingomonas paucimobilis*
Enterococcus durans	*Acinetobacter haemolyticus*	*Staphylococcus hominis*
Enterococcus faecalis	*Aeromonas salmonicida*	*Staphylococcus aureus*
Enterobacter agglomerans	*Burkholderia cepacia*	*Staphylococcus intermedius*
Esherichia coli	*Enterococcus faecalis*	*Streptococcus agalactiae*
Pantoea sp	*Pantoea* sp	*Streptococcus pyogenes*
Ralstonia mannitolilytica	*Serratia marcescens*	*Wauteria paucula*
Streptococcus parasanguinis	*Pseudomonas luteola*	
Streptococcus sp		

[a]From 3 collections and isolations. Bacterial species in risk category level 2 on a scale of 1 to 4 as classified by the ABSA (1999) and WHO (2004).
[b]1 non-pathogenic bacterium omitted.
[c]7 non-pathogenic bacteria omitted, 5 unidentifiable

RESULTS

Detection of bacteria

Files collected from different sites and cultured with various types of media had different positivity rates for bacteria. In these isolations, MU, TSB and APW media gave good rates of positivity, while BPW and WCB detected no bacteria. BPW is specific for bacteria causing diarrheal diseases and WCB is used for the detection of anaerobes. The positivity rates for bacteria isolated on various media from eye flies from wounds, resting sites and

Table 3
Isolation of bacteria (risk category 2) from external surface of host seeking *Siphunculina funicola* captured in the vicinity of human hosts in two enrichment media in Central Thailand, (2007/2008)[a].

Muller broth (23 samples)[b] (6 positive)	Trypic Soy broth (54 samples)[c] (29 positive)	
Alloiococcus otitidis	*Acinetobacter junii*	*Pseudomonas oryzihabitans*
	Acinetobacter lwoffii	*Serratia marcescens*
	Bordetella bronchiseptica	*Sphingomonas paucimobilis*
	Burkholderia cepacia	*Staphylococcus aureus*
	Escherichia coli	*Staphylococcus epidermidis*
	Pantoea sp	*Stenotrophomonas maltophilia*
	Pseudomonas luteola	*Streptococcus parasanguinis*

[a] From 3 collections and isolations. Bacteria species in risk category level 2 on a scale of 1 to 4 as categorized by the ABSA (1999) and WHO (2004).
[b] 3 nonpathogenic bacteria omitted, 1 unidentifiable.
[c] 9 nonpathogenic bacteria omitted, 3 unidentifiable.

host seeking flies are shown in Table 1.

Eye flies from wounds

After preliminary studies using the 5 media, external bacteria on eye flies collected aerially after flying from wounds were isolated on 3 dates in MU and TSB. In total, 28 out of 58 samples were positive for bacteria. Positive samples were identified as shown in Table 2. Thirty-one bacterial species identified were from risk category level 2. Five isolates were not identifiable.

Eye flies seeking hosts

MU and TSB, used for isolating bacteria from eye flies captured from wounds, were also used on 3 different dates. Of the specimens cultured using these two media, 36 out of 85 were positive for bacteria (Table 3), 27 were identifiable and 4 unidentifiable. Most bacteria were in the risk category level 2. Some were isolated from eye flies captured flying from wounds.

Eye flies from resting sites

Eye flies captured from aggregation sites on 4 dates were inoculated on MU, APW and TSB. Fifty-six of 94 specimens were positive for bacteria (Table 4). Thirty-five bacterial species identified and 3 were unidentifiable. Most of the bacteria identified were in risk category level 2, the remaining species posed no threat to human. Some bacteria isolated here were from wound and host seeking flies.

Bacterial density

The bacteria isolated with TSB medium from eye flies from wounds, resting sites and host seeking flies, were cultured on group specific media, then subjected to identification and quantitation. Isolates from flies from wounds ranged in density from 10 to > 3.0 x 10^3 cfu/ml (Table 5). The bacterial species at densities of ≥ 2,000 cfu/ml were *Acinetobacter lwoffii*, *Aeromonas salmonicida, Enterococcus faecalis, Sphingomonas paucimobilis* and *Staphylococcus aureus*. The densities of bacteria isolated from flies captured from resting sites were relatively low, ranging from 10 to 1,740 cfu/ml (Table 5). *Klebsiella ozaenae* and *Providencia alcalifaciens* (1,175 cfu/ml), *Bacillus thuringiensis* (1,630 cfu/ml), *Staphy-*

Table 4
Isolation of bacteria (risk category 2) from external surface of *Siphunculina funicola* captured from their resting sites in 3 enrichment media in Central Thailand (2007/2008)[a].

Muller broth (21 samples)[b] (16 positive)	Alkaline peptone water (11 samples) (4 positive)	Trypic soy broth (62 samples)[c] (36 positive)
Pantoea sp	*Bacillus cereus*	*Acinetobacter lwoffii*
Pseudomonas aeruginosa	*Proteus penneri*	*Bacillus cereus*
Pseudomonas luteola	*Staphylococcus aureus*	*Burkholderia cepacia*
Serratia marcescens	*Staphylococcus intermedius*	*Enterococcus faecalis*
Staphylococcus aureus		*Klebsiella ozaenae*
Staphylococcus intermedius		*Pantoea* sp
Staphylococcus lugdunensis		*Providencia alcalifaciens*
Streptococcus pyogenes		*Rhodococcus* sp
		Serratia marcescens
		Staphylococcus aureus
		Staphylococcus hominis
		Staphylococcus intermedius
		Staphylococcus lugdunensis
		Stenotrophomonas maltophilia
		Streptococcus salivarius
		Wauteria paucula

[a]From 4 collections and isolations. Bacteria in risk category level 2 on a scale of 1 to 4 as categorized by the ABSA (1999) and WHO (2004).
[b] 7 non-pathogenic bacteria omitted.
[c]12 non-pathogenic bacteria omitted, 3 unidentifiable.

lococcus intermedius and *Streptococcus salivarius* (1,740 cfu/ml) exhibited the greatest densities. Flies collected from around human hosts had low to high densities of bacteria ranging from 10 to > 3.0 x 10^3 cfu/ml (Table 5). Those in high abundance were: *Pantoea* sp, *Pseudomonas oryzihabitans*, *Streptococcus parasanguinis* (> 3.0 x 10^3 cfu/ml) and *Kocuria kristinae* (1,770 cfu/ml).

Bacterial species

In total 64 species of bacteria were isolated and identified from positive samples taken from eye flies. Twelve species could not be identified with the protocol used. The numbers of species for each category of bacteria were: gram-positive bacilli (8 species) which included *Bacillus* spp, *Corynebacteriun* spp and *Rhodococcus* sp; gram-positive cocci (26 spp) which includes *Enterococus*, *Staphylococcus* spp and *Streptococcus* spp; gram-negative non-fermenting bacilli (17 spp) which included *Acinetobacter* spp, *Pseudomonas* spp and *Ralstonia* spp; and gram-negative fermenting bacilli (13 spp) which included *Klebsiella* spp, and *Serratia* species. We did not detect gram-negative cocci in this study.

DISCUSSION

Many studies have evaluated the association between pathogenic bacteria and filth-breeding synanthropic and synzootic flies. These non-biting flies have been

Table 5
Densities of bacteria isolated from *Siphunculina funicola* collected from wounds resting sites and host questing flies, cultured on Trypic soy medium, Chon Buri Province Thailand (2007/2008)[a].

	Densities of bacteria on eye flies		
cfu/ml	After flying from wound	Flying from resting sites	Host questing
10-300	*Acinetobacter haemolyticus*	*Bacillus cereus*	*Acinetobacter junii*
	Pseudomonas luteola	*Serratia marcescens*	*Acinetobacter lwoffii*
	Serratia marcescens	*Staphylococcus aureus*	*Bordetella bronchiseptica*
	Staphylococcus aureus	*Staphylococcus hominis*	*Staphylococcus aureus*
	Staphylococcus hominis	*Staphylococcus lugdunensis*	*Staphylococcus epidermidis*
	Staphylococcus intermedius	*Streptococcus agalactiae*	-
	Streptococcus pyogenes	-	-
	Streptococcus agalactiae	-	-
301-800	-	*Bacillus cereus*	*Staphylococcus aureus*
	-	*Enterococcus faecalis*	*Escherichia coli*
	-	*Rhodococcus* sp	*Bordetella bronchiseptica*
1,201-2,000	-	*Klebsiella ozaenae*	*Kocuria kristinae*
	-	*Providencia alcalifaciens*	-
	-	*Staphylococcus intermedius*	-
	-	*Streptococcus salivarius*	-
>2,001-3,000	*Acinetobacter lwoffii*	-	*Pantoea* sp
	Aeromonas salmonicida	-	*Pseudomonas oryzihabitans*
	Enterococcus faecalis	-	*Streptococcus parasanguinis*
	Sphingomonas paucimobilis	-	-
	Staphylococcus aureus	-	-

[a]From 4 collections and isolations (2007/2008).

found to carry a variety of pathogenic bacteria with the potential for transmitting them to humans and animals (Greenberg, 1971, 1973). Non-biting flies have been shown to carry and be involved in the transmission of enteric pathogens, such as *Salmonella* and *Shigella* species in Beirut, Lebanon (Bidawid *et al*, 1978) and in rural areas of Thailand (Echeverria *et al*, 1983). Förster *et al*, (2007) studied 12 species of non-biting Muscidae and Calliphoridae around animal quarters and isolated microorganisms associated with these flies. Pathogenic and nonpathogenic bacteria and a few strains of *Escherichia coli* were isolated and identified from these flies. They isolated several bacterial species which we also found in the present study associated with eye flies (*S. funicola*) in Thailand. Sukontason *et al* (2007) studied bacteria densities on two non-biting fly species, the house fly and the Oriental latrine fly, *Chrysomya megacephal*a, both more than 100 times larger than the eye fly, in northern Thailand. They isolated 42 bacterial species; the Oriental latrine fly, as expected, carried a greater number of bacteria species than the house fly. The most common bacteria isolated from the house fly were coagulase-negative staphylococci and *Escherichia coli*. The dominant bacteria from the Oriental latrine fly were non-fer-

mentative gram-negative bacilli and coagulase-negative staphylococci. Szalanski *et al* (2004) isolated and identified pathogenic bacteria, such as *Escherichia coli* 0157:H7 and *Campylobacter* spp from filth breeding flies from Turkey farms. Both these groups of bacteria can cause enteric diseases in humans.

In all the above studies, the non-biting flies were associated with filth (both human and animal waste) or carrion. It is likely these flies picked up enteric bacteria. Flies frequenting animal and human waste are prone to pick up and carry enteric bacteria, such as *E. coli, Shigella,* and *Salmonella.* The eye fly (*S. funicola*), the subject of the current study, has not been associated with animal breeding activities or frequenting human or animal waste, therefore, we did not isolate enteric or anaerobic bacteria from eye flies, despite the fact we used culture media specific for isolating and detecting these enteric organisms (Table 1, 4 Wilkin-Chalgren broth and all saline peptone water). Eye flies (in the oriental region), relatives of eye gnats (in the western hemisphere) (Mulla, 1962) are believed to breed in the soil of agricultural fields (Mulla and Chansang, 2007). Both male and female adults are attracted to humans and animals for feeding. Some of the bacteria isolated from *S. funicola* in the present study were of soil or plant origin or other environmental sources.

The present study is the first of its kind determining bacteria (potentially pathogenic and non-pathogenic) isolated from the synanthropic and synzootic non-biting eye flies (*S. funicola*) in the family Chloropidae. Eye flies were found to carry a large number of bacteria (gram-positive and gram-negative), most of the bacteria are in risk category 2 (WHO, 2004). These bacteria are unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment under most situations. However, they could cause infections in immune suppressed individuals or under certain environmental conditions.

In our present study it was noted that the eye flies collected from different sources carried some of the same bacteria. These bacteria were: *Pantoea* sp, *Sphingomonas paucimobilis, Staphylococcus aureus* and others. Twenty-seven bacterial species were isolated from host seeking eye flies and 31 species were isolated from flies that had fed on wounds. Thirty-five bacterial species were isolated from flies from resting sites. A number of bacterial species were common among the three sources of flies. It is likely the eye flies pick up bacteria from the environment and during feeding on the skin, wounds and moist surfaces of their host. It is also likely they carry and transfer those bacteria to their hosts.

To assess the densities of bacteria isolated, they were grown on specific culture media, identified and then counted. The bacterial densities ranged from 10 to $>3x10^3$ cfu/ml. The bacteria with the highest densities in host seeking flies were *Pantoea* sp, *Pseudomonas oryzihabitans* and *Streptococcus parasanguinis* ($>3.0x10^3$), from eye flies from resting sites were *Staphyloccocus intermedius* (1,740) and *Bacillus thuringiensis* (1,630), and from flies captured flying away from wounds were *Aeromonas salmonicida, Sphingomonas paucimobilis, Acinetobacter lwofii, Enterococcus faecalis* and *Staphylococcus aureus* ($>3x10^3$).

In this study, we isolated a large variety of bacteria from the eye fly *S. funicola.* gram-positive bacilli, gram-positive cocci, and gram-negative bacilli (non-fermenters) were isolated and identified. No gram-negative cocci were detected. Normally gram-negative cocci are fastidious and

need special media for isolation which we did not do in our study. Sixty-four species of bacteria were identified and 12 specimens were not identifiable with our study protocol. Eye flies carry a variety of bacteria and have the potential to transmit these bacteria to humans and animals due to their target-specific feeding patterns, which include humans, equines, bovines, canines and others.

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