Original article

Isolation of *Salmonella* from Flies in the Mekong Delta, Vietnam

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Summary

From July 2004 to April 2005, total of 494 flies, including 128 houseflies (*Musca domestica*) and 366 blowflies (*Genus Calliphora*), were captured at 6 pig farms, at a slaughterhouse and at 2 wet markets in the Mekong Delta, Vietnam to know the role of flies as a transmitter of *Salmonella*. *Salmonella* were isolated from 38 (7.7%) of the 494 flies samples. The isolation rates of *Salmonella* from flies captured at a slaughterhouse (15.5%) were significantly higher than that in pig farms (4.5%) and in wet markets (6.5%). The isolation rates of *Salmonella* from blowflies (9.8%) were higher than those houseflies (1.6%). From those 38 *Salmonella*-positive fly samples, 41 *Salmonella* strains were isolated and 14 serovars were identified. The predominant serovars were *S. Typhimurium*, *S. Panama*, *S. Newport*, *S. Derby*, *S. Bareilly*, *S. Lexington* and *S. Anatum*. Of the 41 *Salmonella* isolates examined, 38 (92.7%) showed resistance to at least one or following antibiotics ; SM, OTC, KM, NA, ABPC, SMX, CP, and GM. No isolates showed resistance to CEZ, CTRX and CPFX. Among the resistant isolates, 22 isolates were multi-drug resistance. Flies seem to play an important role, as an epidemiological link between humans and domestic animals in the Mekong Delta, Vietnam.

Keywords : housefly, blowfly, *Salmonella*, isolation, Vietnam

Introduction

Human *Salmonella* infection is endemic in developing tropical countries²⁴. *Salmonella* is well established as one of the most important causes of food borne illness worldwide and transmission is usually by the fecal-oral route². It is transmitted by food and water contaminated by animal excreta and human feces containing this organism. Flies are also recognized as contributing epidemiological factor in the spread of foodborne pathogens. They can transmit contagious diseases, such as *Salmonella*, to livestock and humans^{1,4,11,14,17,26}. The housefly is most frequently encountered around pig pens, and can be considered as critical disease conveyor¹⁰. The housefly is also categorized by Food and Drug Administration (FDA) as an important contributing factor in the dissemination of various infectious foodborne diseases such as cholera, shigellosis and salmonellosis¹⁵) and as

well as blowfly^{6,7}.

However, no report has been published regarding the role of houseflies and blowflies as a transmitter of *Salmonella* in the Mekong Delta, southern Vietnam. In the present study, we isolated *Salmonella* from flies in the Mekong Delta, Vietnam to know the role of fly as a carrier of *Salmonella*.

Materials and methods

Sample collection :

From July 2004 to April 2005, 494 fly samples were captured by sterilized hand net at 6 pig farms, 1 slaughterhouse and 2 wet markets in Can Tho city located in the Mekong Delta, Vietnam. The slaughterhouse is located near the center of Can Tho city, and the domestic animals slaughtered there were originated mainly from farms around the city. Flies captured were immediately frozen by dry ice and were immediately transported to Can Tho University for examination. Fly species were identified morphologically. The captured flies were identified as housefly (*Musca domestica*), and blowfly which belonging to the genus *Calliphora*.

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Isolation and identification of *Salmonella*

In the laboratory, each fly was put aseptically into a tube containing 9 ml of buffered peptone water (BPW, Oxoid, Hampshire, UK) for pre-enrichment and incubated at 37°C for 24 h. Then 1ml of the pre-enrichment broth in each tube was transferred to 9 ml of Hajna Tetrathionate broth (Eiken) and further incubated at 37°C for 24 h. One loopful of each enrichment tube was inoculated onto a plate of brilliant green agar (BGA, Oxoid, UK) supplemented with 20 µg novobiocin/ml and mannitol lysine crystal violet brilliant green agar (MLCB, Nissui). The plates were inoculated at 37°C for 24 h and three suspicious colonies morphologically similar to those of *Salmonella* from each plate were subcultured for biochemical examination. Biochemical characteristics were examined using triple-sugar iron agar (Eiken), VP medium (Eiken) and lysine indole motility medium (Eiken). When typical *Salmonella* reactions were seen, additional biochemical tests were performed as described by Farmer³⁾. Serotyping of *Salmonella* isolates was accomplished with commercial O and H antisera (Denka, Seiken, Japan) according to the method of Popoff and Le Minor¹⁸⁾.

Antimicrobial susceptibility testing :

Salmonella isolates from fly samples were examined for susceptibility to 11 different antimicrobial agents by agar dilution method according to the National Committee for Clinical Laboratory Standards procedure M7-A5¹²⁾. The antimicrobial agents were ampicillin (ABPC), streptomycin (SM), kanamycin (KM), gentamycin (GM), oxytetracycline (OTC), chloramphenicol (CP), cefazolin (CEZ), ceftriaxone (CTRX), sulfamethoxazole (SMX), nalidixic acid (NA), and ciprofloxacin (CPFX). *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as a control strains according to NCCLS.

Statistical analysis :

Fisher's exact test with statistical significance set at the 95% confidence level ($P<0.05$) was used for statistical comparisons of prevalence of *Salmonella*.

Results

Salmonella was isolated from 38 (7.7%) of 494 flies captured in the present study. It was isolated from 9 of 199 (4.5%) flies at pig farms, 17 of 110 (15.5%) at a slaughterhouse, and 12 of 185

(6.5%) at Markets. The isolation rate of *Salmonella* from slaughterhouse was significantly higher than that from farms ($p<0.01$) and markets ($p<0.05$). It was isolated from 2 of 128 (1.6%) houseflies and 36 of 366 (9.8%) blowflies. The isolation rate of *Salmonella* from blowflies were significantly higher than that from houseflies ($p<0.01$) (Table 1). Of the 38 *Salmonella*-positive fly samples, 41 *Salmonella* strains were isolated and 14 serovars were identified. Of the 38 *Salmonella*-positive fly samples, each of 2 blowflies and 1 housefly harbored 2 *Salmonella* serovars. The predominant serovars from flies were *S. Typhimurium* (29.3%), *S. Panama* (9.8%), *S. Newport* (9.8%), *S. Derby* (7.3%), *S. Bareilly* (7.3%), *S. Lexington* (7.3%), and *S. Anatum* (7.3%). The other isolates were *S. London*, *S. Braenderup*, *S. Javiana*, *S. Senftenberg*, *S. Lagos*, *S. Irumu* and *S. Warnow*, but only one strain of each were isolated. Two strains were untypeable (Table 2). Of the 41 *Salmonella* isolates examined, 38 (92.7%) showed antimicrobial resistance against one or more antimicrobial agents. Thirty-five isolates (85.4%) were resistant to SM, 24 (58.5%) to OTC, 19 (46.3%) to KM, 19 (46.3%) to NA, 2 (4.9%) to ABPC, 2 (4.9%) to SMX, 1 (2.4%) to CP and 1 (2.4%) to GM. None of the isolates showed resistance to CEZ, CTRX, and CPFX (Table 3). Twenty-two (56.1%) of the 41 *Salmonella* isolates from flies showed multi-drug resistance (Table 4).

Discussion

In the present study, *Salmonella* was isolated from flies in the Mekong Delta, Vietnam at a high rate (7.7%). Also, it might be noted that among the predominant *Salmonella* serovars, that is, *S. Typhimurium* (29.3%), *S. Panama* (9.8%), *S. Newport* (9.8%), *S. Derby* (7.3%), *S. Bareilly* (7.3%), *S. Lexington* (7.3%) and *S. Anatum* (7.3%) isolated from the flies samples. Among those serovars, *S. Typhimurium*, *S. Derby*, *S. Lexington*, and *S. Anatum* was previous isolated from domestic animals, and were also the predominant serovars in foods including pork, beef, duck meat, chicken meat and shrimps in the Mekong Delta^{22, 23)}. Moreover, *S. Bareilly*, and *S. Typhimurium* was also the predominant serovars in human. Ogasawara, et al.¹³⁾ reported that *Salmonella* isolates originated from several sources such as domestic animals, and food in the Mekong Delta showed resistance to OTC, CP,

Table 1 Prevalence of *Salmonella* from flies by place in the Mekong Delta, Vietnam

Fly species	No. of <i>Salmonella</i> positive samples/ No. of samples examined (%)			
	Farm	Slaughterhouse	Market	Total
Housefly	0/ 38 (0.0)	2/ 56 (3.6)	0/ 34 (0.0)	2/128 (1.6)
Blowfly	9/161 (5.6)	15/ 54 (27.8)	12/151 (7.9)	36/366 (9.8)
Total	9/199 (4.5)	17/110 (15.5) ^{a,b}	12/185 (6.5)	38/494 (7.7)

^a Slaughterhouse > Farm ($p<0.01$)

^b Slaughterhouse > Market ($p<0.05$)

NA, SM, KM and ABPC. In the present study, *Salmonella* isolates from flies also showed resistance to OTC, CP, NA, SM,

KM and ABPC.. These results indicate that *Salmonella* isolates from flies seem to be originated from the same sources of *Salmonella* strains isolated from domestic animals, foods and human in the Mekong Delta. Therefore, flies seem to play an important role as a transmitter of *Salmonella* between human and environment in the Mekong Delta.

In the present study, *Salmonella* isolation rate from blowflies (9.8%) was higher than that from houseflies (1.6%). Furthermore, the blowflies at slaughterhouse (27.8%) harbored *Salmonella* at a high rate rather than those in the farms (5.6%) and in markets (7.9%). Greenberg, et al.⁶⁾ reported that the most obvious source of *Salmonella* for flies was carrion derived from livestock, and the blowflies which were found exclusively on carrion, had high infection rates and the greatest diversity of *Salmonella* types at slaughterhouse in Mexico. Therefore, the reason that blowflies of this study harbored *Salmonella* at a high rate seems to be due to a behavior of blowflies which thrive in higher density in carrion.

In the present study, 92.7% of *Salmonella* isolates showed antimicrobial resistance against one or more antimicrobial agents, and 23 (56.1%) of the 41 *Salmonella* isolates showed multi-drug resistance (Table 4). *S. Typhimurium* DT 104, which showed resistance to up to 9 antimicrobial agents, has emerged as a global health problem in human and animal medicine during the last dec

Table 2 Serovars of *Salmonella* from flies in the Mekong Delta, Vietnam

Serovar	No. of isolate (%)
<i>S. Typhimurium</i>	12 (29.3)
<i>S. Panama</i>	4 (9.8)
<i>S. Newport</i>	4 (9.8)
<i>S. Derby</i>	3 (7.3)
<i>S. Bareilly</i>	3 (7.3)
<i>S. Lexington</i>	3 (7.3)
<i>S. Anatum</i>	3 (7.3)
<i>S. London</i>	1 (2.4)
<i>S. Braenderup</i>	1 (2.4)
<i>S. Javiana</i>	1 (2.4)
<i>S. Senftenberg</i>	1 (2.4)
<i>S. Lagos</i>	1 (2.4)
<i>S. Irumu</i>	1 (2.4)
<i>S. Warnow</i>	1 (2.4)
Untypeable	2 (2.9)
Total	41 (100)

Table 3 Antimicrobial resistance of *Salmonella* from flies in the Mekong Delta, Vietnam

Serovars	No. of isolates examined	No. of resistant isolates ^a (%)											No of resistant ^b (%)
		ABPC	OTC	CP	NA	SM	KM	GM	CEZ	CTRX	CPFX	SMX	
<i>S. Typhimurium</i>	12		8		7	11	7						12 (100.0)
<i>S. Panama</i>	4	1	3		3	4	3	1				1	4 (100.0)
<i>S. Newport</i>	4	1	4	1	2	3	2						4 (100.0)
<i>S. Derby</i>	3					3							3 (100.0)
<i>S. Bareilly</i>	3					2							2 (66.7)
<i>S. Lexington</i>	3					2							2 (66.7)
<i>S. Anatum</i>	3		3		2	3	1					1	3 (100.0)
<i>S. London</i>	1		1		1	1	1						1 (100.0)
<i>S. Braenderup</i>	1		1		1	1	1						1 (100.0)
<i>S. Javiana</i>	1					1							1 (100.0)
<i>S. Senftenberg</i>	1		1		1		1						1 (100.0)
<i>S. Lagos</i>	1		1		1	1	1						1 (100.0)
<i>S. Irumu</i>	1					1							1 (100.0)
<i>S. Warnow</i>	1		1		1	1	1						1 (100.0)
Untypeable	2		1			1	1						1 (50.0)
Total	41	2 (4.9)	24 (58.5)	1 (2.4)	19 (46.3)	35 (85.4)	19 (46.3)	1 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.9)	38 (92.7)

^a ABPC : Ampicillin, SM : Streptomycin, KM : Kanamycin, GM : Gentamycin, OTC : Oxytetracycline, CP : Chloramphenicol, CEZ : Cefazolin, CTRX : Ceftriaxone, NA : Nalidixic acid, CPFX : Ciprofloxacin, SMX : Sulfamethoxazole

^b Isolates resistant to at least one antimicrobial agent

Table 4 Antimicrobial resistance patterns of *Salmonella* isolates from flies in the Mekong Delta, Vietnam

No. of antimicrobial agents	Resistance pattern ^a	No. of resistant isolates	Serovars (No. of isolates)
1	SM	14	Lexington (2), Bareilly (2), Irumu (1), Typhimurium (4), Derby (3), Panama (1), Javiana (1)
	OTC	2	Typhimurium (1), Newport (1)
2	OTC + SM	1	Anatum (1)
3	OTC + SM + NA	1	Anatum (1)
	OTC + KM + NA	2	Warnow (1), Senftenberg (1)
4	OTC + NA + SM + KM	15	Braenderup (1), Panama (2), Typhimurium (7), Anatum (1), London (1), Newport (2), Lagos (1),
	ABPC + OTC + CP + SM	1	Newport (1)
5	OTC + NA + SM + KM + SMX	1	Anatum (1)
7	ABPC + OTC + NA + SM + KM + GM + SMX	1	Panama (1)
Total		38	

^a ABPC : Ampicillin, OTC : Oxytetracycline, SM : Streptomycin, KM : Kanamycin, GM : Gentamycin, NA : Nalidixic acid, , SMX : Sulfamethoxazole, CP : Chloramphenicol,

ade^{5, 8, 19, 20, 21}). However, *S. Typhimurium* DT 104 strain was not isolated from any fly samples in the present study. Ogasawara et al.¹³) also reported that *S. Typhimurium* DT 104 was not isolated from animals, meats and foods in the Mekong Delta. However, Vo, et al.²⁴) isolated *S. Typhimurium* DT 104 from pig and human in Vietnam at a low rate although it was still unclear whether these isolates were multi-drug resistant strains or not. Since *S. Typhimurium* DT 104 have been isolated from neighbor countries such as Thailand¹⁶) and Taiwan²⁷), further continuous survey should be needed to clarify the presence of *S. Typhimurium* DT 104 in Vietnam.

It can be concluded that the flies are carrying viable *Salmonella* associated with domestic animals, foods and environments. It is possible that flies may function as an epidemiological link between humans and domestic animals in the Mekong Delta, Vietnam. Therefore, flies control should be pay more attention to reduce the risk from pathogenic bacteria such as *Salmonella* in the Mekong Delta.

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原 著

ベトナム・メコンデルタのハエからのサルモネラの分離

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要旨

2004年7月から2005年4月までの間に、ハエのサルモネラの媒介者としての役割を知るために、ベトナム・メコンデルタの養豚場6ヶ所、と畜場1ヶ所および市場2ヶ所でイエバエ128匹とクロバエ366匹の計494匹のハエを捕獲した。サルモネラは494匹中38匹(7.7%)から分離された。と畜場(15.5%)で捕獲したハエからのサルモネラの分離率は、養豚場(4.5%)や市場(6.5%)のものに比べ有意に高かった。また、クロバエ(9.8%)からの分離率はイエバエ(1.6%)に比べ有意に高かった。38匹のハ

エから分離されたサルモネラ41菌株は、14血清型に型別され、Typhimurium, Panama, Newport, Derby, Bareilly, Lexington および Anatum の次に多かった。分離されたサルモネラ41株中38株(92.7%)がSM, OTC, KM, NA, ABPC, SMX, CP および GM のいずれか1つかそれ以上に耐性を示したが、CEZ, CTRX および CPFX に耐性を示した株は認められなかった。ハエはベトナム・メコンデルタにおいて、人と家畜との間で疫学的に重要な役割を果たしているものと思われる。

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