CALLIPHORIDAE (DIPTERA) IN MANAUS: IV. BACTERIA ISOLATED FROM BLOWFLIES COLLECTED IN STREET MARKETS.[1]

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ABSTRACT — *Staphylococcus aureus*, *Escherichia coli*, *Proteus* sp., *Providencia* sp., *Citrobacter* sp. and *Klebsiella* sp. were isolated from calliphorid flies collected in eight street markets in the city of Manaus, Amazonas State, Brazil. The presence of *E. coli* in the samples suggests that faecal contamination is occurring and that these flies are potential vehicles of enteropathogenic bacteria to exposed foods.

Key words: Bacterial contamination, Calliphoridae, Manaus, street markets.

Calliphoridae (Diptera) em Manaus: IV. Bactérias Isoladas de Moscas Coletadas em Feiras Livres.

RESUMO — *Staphylococcus aureus*, *Escherichia coli*, *Proteus* sp., *Providencia* sp., *Citrobacter* sp. e *Klebsiella* sp. foram isoladas de moscas califorídeas coletadas em oito feiras livres na cidade de Manaus, estado do Amazonas, Brasil. A presença de *E. coli* nas amostras sugere que está ocorrendo contaminação fecal e que estas moscas são veículos potenciais de bactérias enteropatogênicas para os alimentos expostos.

Palavras-chave: Contaminação bacteriana, Calliphoridae, Manaus, feiras livres.

INTRODUCTION

The potential of flies to act as mechanical vectors of gastro- intestinal pathogens has been well established (GIUGLIANO *et al.*, 1986; GREENBERG, 1988). In Brazil, association between enteropathogenic bacteria and synanthropic flies has been demonstrated in slaughterhouses and open markets (FURLANETTO *et al.*,1984; IMBIRIBA, 1979).

The arrive in Brazil of calliphorid flies of the genus Chrysomya Robineau-Desvoidy, probably from Africa and the Far East (IMBIRIBA *et al.*, 1977; GUIMARÃES *et al.*, 1978; AZEREDO-ESPIN & PAVAN, 1983) has caused concern among health workers and entomologists because of the great capacity for geographical expansion shown by these insects (PRADO & GUIMARÃES, 1982).

In the city of Manaus, flies proliferate in the widespread street markets, and the incidence of diarrhoea, particularly in children, is very high. There is, however, little information on the possible role of local synanthropic flies in the spread of the aetiological agents (GIUGLIANO *etal.*, 1986) of diarrhoea.

The purpose of the present work is to identify bacteria associated with

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calliphorid flies in street markets of Manaus, and to assess the importance of these flies in the spread of these bacterial pathogens to humans.

MATERIALS AND METHODS

Blowflies were collected in August 1990 (period of low rainfall) and March 1991 (high rainfall) in eight street markets in the districts of Coroado, Raiz, Alvorada, Compensa, São José Operário, Cidade Nova, Flores and Prata dos Remédios, in the city of Manaus, Northern Brazil.

Each sample of 9 to 30 flies (August) or 19 to 46 flies (March) was taken in a clean plastic bag which was subsequently discarded.

In the laboratory, the flies were lightly anaesthesized with ether and washed by agitating each batch in 5 ml of 0,85 % sterile saline for one minute, after which they were separated for identification. The saline was centrifuged at 3,000 r.p.m. for three minutes and the sediment inoculated on Salmonella-Shigella Agar (SS), Mac Conkey Agar (MC) and Baird Parker Agar (BP) in Petri dishes which were then incubated at 37°C for 24 hours. Two dishes of each medium were used for each sample. Colonies growing on SS and MC media were inoculated in lysine-iron agar (LIA) and triple sugar-iron agar (TSI), incubated for a further 24 hours at 37°C, and identified according to EDWARDS & EWING (1972). Black colonies with haloes growing on BP medium were confirmed as *S. aureus* by Gram, catalase, coagulase, fermentation and oxidation of glucose and thermonuclease tests (VASCONCELOS, 1987). Only the presence or absence of the respective bacterium was considered for each sample.

RESULTS AND DISCUSSION

Among the 440 flies examined (Tab.1), *C. megacephala* (Fabricius) was the most numerous, followed by *C. putoria* (Wiedemann), *Phaenicia eximia* (Wiedemann) and *C. albiceps* (Wiedemann).

The results of bacteriological analyses are shown in Tables 2 and 3.

Staphylococcus aureus was the specie most frequently isolated (87.5 %) and was absent only from two dryseason sample. *Proteus* sp. was equally prevalent in August and March and occured in 75 % of the samples. *Escherichia coli* and *Providencia* sp were most abundant in the dry season. *Citrobacter* sp and

Table 1. Percent relative and total numbers ( ) of calliphorid sppecies in samples from street markets in Manaus. Pooled samples, August 1990 + March 1991.

Species	Districts								Total
	A	B	C	D	E	F	G	H	
Chrysomya megacephala	50,0(29)	84,4(27)	36,5(23)	41,5(27)	90,9(50)	3,1(2)	86,7(52	71,4(30)	(240)
C. putoria	48,3(28)	9,4(3)	50,8(32)	58,5(38)	7,3(4)	96,9(63)	10,0(6)	16,(77)	(181)
C. albiceps	1,7(1)	0,0	4,8(3)	0,0	0,0	0,0	0,0	0,0	(4)
Phaenicia eximia	0,0	6,2(2)	7,9(5)	0,0	1,8(1)	0,0	3,3(2)	11,9(5)	(15)
Total	(58)	(32)	(63)	(65)	(55)	(65)	(60)	(42)	(440)

A= Coroado B= Raiz C= Alvorada D= Compensa E= Praça dos remédios F= S. José Operário G= Cidade Nova H= Flores

Table 2. Occourrency of bacterial isolation from blowflies in eight street markets of Manaus, according to location and season. 1= August 1990; 2 = March 1991.

Bacteria	Districts															
	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	F1	F2	G1	G2	H1	H2
Staphylococcus aureus	x	x	x	x	x	x	x	x		x		x	x	x	x	x
Proteus sp	x	x			x		x	x	x	x	x	x	x	x		x
Escherichia coli			x				x		x		x	x	x			
Providencia sp			x	x	x		x			x	x	x			x	
Klebsiella sp				x								x				
Citrobacter sp							x			x						

A= Coroado B= Raiz C= Alvorada D= Compensa E= Praça dos Remédios F= S. José Operário G= Cidade Nova H= Flores

Table 3. Frequency of bacterial isolation from flies in eight street markets of Manaus, according to season.

Bacteria	Frequency (%)		
	August-90	March-91	mean
Staphylococcus aureus	75.0	100.0	87.5
Proteus sp	75.0	75.0	75.0
Escheriachia coli	62.5	12.5	37.5
Providencia sp	62.5	37.5	50.0
Klebsiella sp	0.0	25.0	12.5
Citrobacter sp	12.5	12.5	12.5

Klebsiella sp were represented in only two samples each.

LINDSAY & SCUDDER (1956) state that the presence and numbers of flies at a site can be used to indicate the magnitude of a problem concerning to solid organic waste disposal. The high density of introduced blowflies, associated with precarious sanitary conditions, observed in the street markets of Manaus, is cause for public health concern. Although in our samples we did not detect Salmonella or Shigella, important agents of gastroenterites, these bacteria have been isolated from *Chrysomya* blowflies on several occasions (BRYGOO *et al.*, 1962; FURLANETTO *et al.*, 1984). GIUGLIANO *et al.* (1986) who found flies contaminated with *E. coli* in the vicinity of Manaus, and remarked that, although diarrhoea is a major cause of infant mortality in this city, very little is known about the dynamics of the disease or sources of infection.

We conclude that foods exposed for sale in the street markets of Manaus are subject to human faecal contamination by means of flies carrying *E. coli* and probably also enteropathogenicbacteria. Although we did not estimate the population density of synanthropic flies in these markets quantitativaly, it is extremely high. According to BIDAWID *et al.* (1978), were public health statistics are lacking, bacteriological studies with flies can provide essencial epidemiological information.

AZEREDO-ESPIN, A.M.L.; PAVAN, C. 1983. Karyotypes and possible regions of origin of three species of Calliphoridae (Diptera) recently introduced in Brazil. *Rev. Brasil. Genet.*, 6(4): 619-638.

BIDAWID, S.P.; EDESON,J.F.K.; IBRAHIM, J.; MATOSIAN, R. M. 1978. The role of non-biting flies in the transmission of enteric pathogens (*Salmonella* species and *Shigella* species) in Beirut, Libanon. *Ann. Trop. Med. Parasitol.*, 72: 117-122.

BRYGOO, E.R.; SUREAU, P.; LeNOC, P. 1962. Virus et germes fecaux des mouches de l'agglomeration urbaine de Tananarive. *Bull. Soc. Path. Exot.*, 55: 866-881.

EDWARDS, P. R.; EWING, W. H. 1972. *Identification of enterobacteriaceae.* Burgess Publishing.

FURLANETTO, S.P.M.; CAMPOS, M.L.C.; HARSI, C. M.; BURALLI, G. M.; ISHIATA, K. 1984. Microorganismos enteropatogênicos em moscas africanas pertencentes ao gênero *Chrysomya* (Diptera: Calliphoridae) no Brasil. *Rev. Microbiol.*, 15: 170-174.

GIUGLIANO, L.G.; BERNARDI, M.G.P.; VASCONCELOS, J.C.;COSTA, C. A.; GIUGLIANO, R. 1986. Longitudenal study of diarrhoeal disease in a peri-urban community in Manaus (Amazon-Brazil). *Ann. Trop. Med. Parasitol.*, 80: 445-450.

GREENBERG, B. 1988. *Chrysomya megacephala* (F.) (Diptera: Calliphoridae) collected in North America and notes on *Chrysomya* species present in the New World. *J. Med. Entomol.*, 25:199-200.

GUIMARÃES, J.H.; PRADO, A.P.; LINHARES, A.X. 1978. Three newly introduced blowfly species in southern Brazil (Diptera: Calliphoridae). *Revta. bras. Ent.*, 22:53-60.

IMBIRIBA, A.S. 1979. Incidência de enterobactérias encontradas em lotes de moscas em abatedouros de Curitiba-PR e arredores. *Arq. Biol. Tecnol.*, 22: 197-206.

IMBIRIBA, A.S.; IZUTANI, D.T.; MILHORETTO, I.T.; LUZ, E. 1977. Introdução de *Chrysomya chloropyga* (Wiedemann, 1818) na Região Neotropical (Diptera: Calliphoridae). *Arq. Biol. Tecnol.*, 20:35-39.

LINDSAY, D.R.; SCUDDER, H.I. 1956. Nonbitting flies and disease. *Annu. Rev. Entomol.*, 1:323-346.

PRADO, A.P.; GUIMARÃES, J.H. 1982. Estado atual da distribuição e dispersão das espécies do gênero *Chrysomya* R-D na região Neotropical (Diptera: Calliphoridae). *Revta. bras. Ent.*, 26:225-231.

VASCONCELOS, J.C. 1987. *Microorganismos indicadores, Staphylococcus aureus enterotoxigênico, Bacillus cereus, Clostridium perfringens A e Salmonella sp em linguiça fresca comercializada em feiras livres no município de São Paulo, SP. São Paulo.* Doctoral Thesis.

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