J. Appl. Zool. Res. (2017) **28**(1): 37-44
ISSN 0970-9304
NAAS Rating: 4.22

FLY MAGGOTS (DIPTERA: CALLIPHORIDAE, MUSCIDAE) AND ASSOCIATED MICROORGANISMS IN GABORONE

JOSEPH ALLOTEY, I. RANDOME, I. C. MOROBE, AND P. MONNAKOPO

Department of Biological Sciences, University of Botswana, Gaborone, Botswana.

Corresponding author e-mail: alloteyj@mopipi.ub.bw

Received: 28 March, 2017 **Accepted: 15 April, 2017**

ABSTRACT: Synathropic species of flies carry pathogenic organisms that result in diseases such as bacillary dysentery, salmonellosis, cholera and diarrhea. Microorganisms associated with fly maggots were isolated and identified in the present study in Gaborone. Fly maggots were collected randomly from four geographical areas of Gaborone i.e. Tlokweng, Ledumang, University of Botswana campus and Maruapula. The external and internal microbes of the fly maggots were isolated and examined microscopically. Determination of aerobic mesophilic and psychrotrophic plate counts were conducted. Tests for yeasts and molds were also done. Biochemical characterization, identification and confirmation of microbial isolates were done using Analytical Profile Index (API 20E) and Vitek II. Colony counts ranged from $1.4x10^3$ to $8.3x10^3$ colony forming units (CFU/g). *Salmonella* spp. was a common isolate in all the geographical areas while *Providencia stuartii, Pseudomonas aeruginosa, Pseudomonas fluorescens* and *Citrobacter braakii* were only identified in maggots from Tlokweng. The most common species of flies in Gaborone identified from the maggots were *Chrysomyia megacephala* and *Musca domestica,* and few *Syrphid* spp. The microorganism isolated from the maggots of these flies can be given as *Salmonella* spp., *Salmonella arizonae, Klebsiella oxytoca, Enterobacter cloacae, Providencia stuartii, Pseudomonas aeruginosa, Pseudomonas fluorescens* and *Citrobacter braakii* with *Salmonella* spp. as the dominant species

Key words: Flies, *Chrysomyia megacephala Musca domestica, Syrphid* spp. microorgaisms, *Salmonella* spp.

INTRODUCTION

Flies belong to the large and important order Diptera. The insect families in the Diptera most often represent and belong to the Muscidae (true flies such as the house fly) and Calliphoridae (blowflies) (SERVICE, 2008). *Musca domestica* and *Chrysomyia megacephala* are the main fly species found in Gaborone, Botswana (ALLOTEY *et al.*, 2015). Flies feed on substances such as sugar, milk, and almost all food of man, rotten vegetables and carcasses, excreta and vomit, and almost any organic material (SERVICE, 2008). They breed in a variety of human-controlled places including other organic substrates such as fecal and decaying matter and can cause problems in the food industry such as the food-processing plants, restaurants and slaughter houses (YEATES and WIEGMANN, 2005; MERSON *et al.*, 2012). Outbreaks of diarrheal diseases in urban and rural areas of developing countries are closely related to the seasonal abundance of flies and fly control is closely related to the decline in cases of enteric diseases (OLSEN, 1998, MERSON *et al.*, 2012). In urban environments, the flies that are closely related and associated with humans are encouraged by food and harborage provided by the dwellings and human activities (MERSON *et al.*, 2012; ALLOTEY *et al.*, 2015). Recently there had been cases where large numbers of maggots were found in the domestic dustbins around Gaborone because of late collection of garbage yet not much is known about the microorganisms associated with the maggots.

Microorganisms are minute living, single-celled organisms such as bacteria, fungi and protozoa. They are found almost everywhere in the world and play a vital role in supporting and maintaining life and nature. There are beneficial and harmful microorganisms. They keep nature clean by removing toxins and some degrade dead plants and animals. However, some microorganisms are harmful as they cause many diseases. The fly usually acquires microorganisms by walking over materials containing them and feeding on them. Flies have been reported to carry pathogenic microorganisms and other pathogenic protozoans which include enteropathogenic *Escherichia coli,* enterotoxic *Escherichia coli*, *Klebsiella, Campylobacter, Staphylococcus aureus, Candida albicans, Mucor* and *Aspergillus* spp. The Calliphoridae, Sarcophagidae and Muscidae are the major fly vectors of pathogenic microorganisms. For example, *Musca domestica* is known to transfer pathogenic agents of medical and veterinary significance such as *Salmonella, Shigella* and *Entamoeba histolytica* (MERSON *et al.*, 2012).

Flies occupy many habitats due to their small size and the ability to fly. They are mostly found in areas where there is poor sanitation, especially around dustbins and in areas of increased air and water pollution. Flies are mostly found in homes especially in unprotected kitchens where there is plenty of food. They lay eggs in warm and moist places such as on waste and refuse materials and take nourishment in liquid form. They secrete saliva containing digestive enzymes on food. The digestive enzymes partially digest the food into semi-liquid. The fly vomitus contains bacteria that contaminate food, equipment, supplies and utensils. Flies have been reported to feed on refuse and later fly to human food where they deposit bacteria from the legs, wings, saliva and excrement (TRICKETT, 2001; SHIAO, 2008). Fly larvae cannot grow in substrate free of live microorganisms and this makes the maggots a potential source of pathogenic microorganisms. As mentioned earlier, there has not been previous study of microorganisms associated with maggots of flies (Diptera) in Gaborone. Thus the objective of the present study was to isolate and identify the microorganisms associated with fly maggots in Gaborone.

MATERIALS AND METHODS

Maggots of flies were randomly collected from two different dustbins per geographical area in Gaborone i.e. Tlokweng, Ledumang, University of Botswana campus and Maruapula. The maggots were collected physically using sterile forceps and carefully put into sterile collecting vials (7 x 8cm). The maggots were collected in the morning at 8 AM per area on specific days of the week Monday at Tlokweng, Tuesday at Ledumang, Wednesday at Maruapula and Thursday at the University of Botswana campus.

Isolation of microbes: Nutrient Agar (HIMEDIA M001, Mumbai, India) was prepared by putting 28g of the nutrient agar powder in 1liter bottle and adding 1liter of distilled water. The Plate count agar was made by putting 23g of plate count agar (BIOLAB Diagnostics 1006497, Midrand, Gauteng, South Africa) into 1litre bottle and adding 1litre of distilled water. The two bottles containing the agar suspension were boiled using Bunsen burner to completely mix the agar powder with the distilled water. After boiling the nutrient agar and the potato glucose agar (BCBN5257V, Sigma-Aldrich, Steinheim, Spain) bottles were autoclaved for 15minutes at 121^0C. After autoclaving the media were allowed to cool for some time; and before the media solidified in the bottles, it was poured onto petri dishes labeled nutrient agar and kept on top of benches to solidify before using it. Normal saline used for serial dilution contained 0.85% of sodium chloride. It was autoclaved for 15 minutes at 121^0C before using it.

The external microorganisms from each maggot were isolated by swabbing the surface of the maggot with a sterile clean swab before rubbing the swab in test tubes containing 6ml of peptone water. The test tubes with their contents were then incubated at 37^0C for 48 hours. After 48 hours, a sterile inoculating loop was dipped into each of the test tubes containing peptone water (Saarchem 49433 DN, Midrand, Gauteng, South Africa) with the external microorganisms, and used to streak into petri dishes containing nutrient agar and potato glucose agar. The petri dish was then placed in the incubator at 37^0C for 48 hours. The internal microorganisms were isolated from the maggots using surface sterilized by dipping each maggot into 3% sodium hypochlorite for 2 minutes then rinsing in distilled water. This procedure was repeated thrice for each maggot. The surface sterilized maggots were then placed in test tubes containing peptone water. Sterile rods were used to grind the maggots in the test tube containing the peptone water. The test tubes together with their contents were then incubated at 37^0C for 48 hours. After 48 hours a sterile inoculating loop was dipped into the test tubes containing peptone water with the internal microorganisms, and used to streak into duplicate petri dishes containing nutrient agar. Some plates were incubated anaerobically while others were incubated aerobically. The petri dishes were placed in the incubator at 37^0C for 48 hours.

Enumeration of microbes: Serial dilutions were made ranging from 10^{-1} to 10^{-3}. This was done by emulsifying maggot in 10ml of normal saline in a test tube using a sterile rod to make 10^{-1}. Then 1ml of the suspension was transferred using a sterile pipette into another test tube containing 9ml of normal saline to make 10^{-2} dilution then the suspension was mixed and 1ml of the 10^{-2} dilution was transferred to another 9ml test tube to make 10^{-3} dilution. This procedure was repeated for all the maggots. After the serial dilutions were made, 1ml of each dilution was transferred to duplicate petri dishes and cooled plate count agar was added to the petri dishes which were swirled to mix the agar with the bacterial suspension. The plates were allowed to solidify and then incubated for 24 hours 37^0C. After 24 hours enumeration of microorganism was done using the electric plate counter. Only plates with colonies from 30 to 300 were counted and the number of colonies was recorded. The colony forming units was then calculated by multiplying the average number of colonies by the dilution factor divided by the volume transferred.

Biochemical characterization, identification and confirmation of microbial isolates: After 24 hours the plates showing growth on the nutrient agar were selected and a gram stain was done for plates having pure colonies while those with mixed growth were sub cultured again for 24 hours 37^0C before gram stain was done on them. Gram stain was done by putting a drop of water on the slide then inoculating a pure colony on the drop of water and mixing the colony with water using inoculating loop. After mixing the colony and spreading it on the slide, it was air dried, after drying, crystal violet was flooded on it for 2 minutes, then rinsing under running water from the tap, solution was then flooded for 1 minute and rinsed again with water, the stain was then decolorized with 95% alcohol then rinsed again with water and lastly the slide was flooded with safranin for about 30 seconds then rinsed with tap water. The slide was blot dried and observed under the microscope to record the colony morphology and the color of the colonies.

For a confirmatory test, the Analytical Profile Index (API 20E) was then carried out to identify the bacterial isolates. Before performing the Analytical Profile Index all the plates with bacterial growth were sub cultured again for exactly 24 hours at 37^0C. After 24 hours one pure colony or two was emulsified into a test tube containing 3 ml of saline to create a homogenous bacterial suspension. A sterile Pasteur pipette was then used to

transfer the suspension into all the 20 micro tubes in Analytical Profile Index kit containing different biochemical tests. And with the same Pasteur pipette the citrate, Voges-Proskeur and gelatin micro tubes were filled up with the bacterial suspension. Tests of amino acid decarboxylase, lysine decarboxylase, hydrogen sulfide, urease and Ornithine decarboxylase were filled up with mineral oil. The strip was then covered and incubated for 24 hours 37^0C. After 24 hours 1 drop of TDA reagent was added into TDA test, 1 drop of JAMES reagent was added in the Indole test and 1 drop of VP1 and VP2 was added to VP tests. Observations were made to identify where reactions occurred. The pattern of the results was recorded on the results sheet which consisted of tests separated in 3 groups and a number 1, 2 and 4 is indicated for each. The numbers to the positive reactions within each group were added resulting in a 7 digit profile number. This number was used to identify the organism using the analytical profile index catalogue book. Vitek II instrument was also used to confirm the microbial isolates.

RESULTS AND DISCUSSION

Number and identification of maggots in different geographical areas around Gaborone: Maggots of flies were collected from different places in Gaborone in three weeks. Fig-1 shows that in three weeks, 75 maggots were collected from Tlokweng and UB, 50 from Maruapula and 45 from Ledumang. Table-1 shows that *Chrysomyia megacephala* is the predominant fly species in UB, Maruapula and Ledumang while *Musca domestica* is predominant in Tlokweng. *Syrphid* sp. was identified in Maruapula only.

Table-1: Identification of fly maggots from different geographical areas around Gaborone

Fly species	Morphology utilized	% incidence (Geographical area)
Chrysomyia megacephala	Posterior spiracles: three rod-like slits surrounded by peritreme; two per posterior end	70% Maruapula 70% UB
Musca domestica	Posterior spiracles: three serpentine slits surrounded by peritreme; two per posterior end	100% Tlokweng 30% UB
Syrphid sp.	Posterior spiracles with a long respiratory tube; one per posterior end.	30% Maruapula

Enumeration of microorganisms: There was a lot bacterial growth in many plates. Some of the colonies were too many to count. The Colony forming Units/ml for most of the maggots where the colonies were countable was very high. Colony forming Units/ml for different maggots in different dustbins from different geographical areas in Gaborone (Table-2) shows that maggots contain a lot of microorganisms; for example maggot replicate 2 in dustbin one in Tlokweng had 8.7×10^3 colony forming units/ml.

Identification of the microorganisms in fly maggots: The most prevalent microorganisms identified in maggots in all the places was *Salmonella* spp. followed *Salmonella arizonae*. *Salmonella* spp. was a common isolate from maggots in all the four geographical areas (Table-3). *Providencia stuartii, Pseudomonas aeruginosa, Citrobacter braakii* and *Pseudomonas fluorescens* were only isolated from maggots collected from Tlokweng. Table-4 shows that *Klebsiella oxytoca* was isolated from maggots collected from all the four geographical areas. *Salmonella* spp. was isolated from maggots collected from UB and Ledumang. *Enterobacter cloacae* were isolated from maggots

collected in Maruapula. Table-5 shows that *Salmonella* spp. and *Salmonella arizonae* were isolated from maggots collected from all the four geographical areas while *Proteus mirabilis* was isolated from maggots collected in Tlokweng and Ledumang.

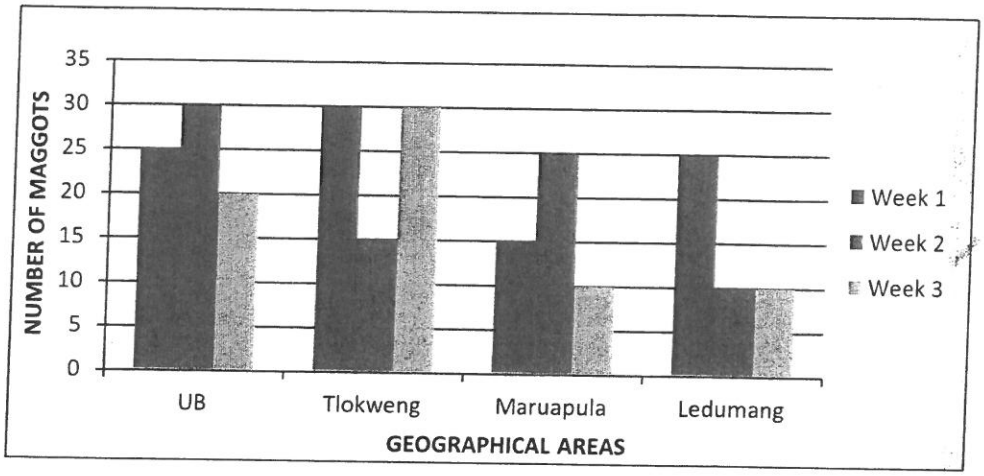

Fig-1: Number of maggots collected from different geographical areas in Gaborone

Table-2: Colony Forming Units/ml of microbes isolated from different geographical areas

Maggot Replicates	Colony Forming Units/ml							
	Tlokweng D1	Tlokweng D2	Ledumang D1	Ledumang D2	Maruapula D1	Maruapula D2	UB D1	UB D2
1	4.8×10^{5}	2.04×10^{4}	3.7×10^{5}	2.4×10^{3}	3.4×10^{5}	8.1×10^{5}	2.7×10^{2}	8.1×10^{5}
2	8.7×10^{3}	6.4×10^{3}	2.4×10^{3}	2.1×10^{5}	6.4×10^{3}	7.7×10^{3}	6.4×10^{3}	n/a
3	6.4×10^{3}	5.4×10^{3}	2.1×10^{5}	n\a	2.1×10^{5}	5.4×10^{2}	2.2×10^{2}	8.1×10^{5}
4	n/a	n/a	$8.\times10^{5}1$	n/a	2.7×10^{2}	n/a	2.1×10^{3}	6.6×10^{5}
5	n/a	5.6×10^{5}	6.6×10^{5}	5.1×10^{5}	5.4×10^{5}	n/a	2.4×10^{5}	n\a
6	n/a	n\a	n/a	3.4×10^{3}	6.5×10^{5}	n\a	1.9×10^{5}	6.4×10^{3}
7	1.4×10^{3}	6.5×10^{3}	8.7×10^{3}	2.4×10^{4}	n/a	n\a	1.8×10^{5}	n/a
8	n/a	2.6×10^{5}	5.8×10^{5}	6.4×10^{3}	5.4×10^{5}	2.035×10^{4}	2.7×10^{2}	8.6×10^{5}
9	n/a	2.03×10^{4}	n/a	n/a	1.4×10^{5}	n/a	n/a	3.8×10^{3}
10	n/a	8.6×10^{5}	n/a	n/a	n/a	n/a	n/a	6.4×10^{5}

Key: n/a-colonies were not between 30-300; D = dustbin

In the present study the predominant species of flies identified was *C. megacephala* which was about 80% in UB, 65% in Maruapula and 100% in Ledumang. This species of flies is widely distributed and is found in New Zealand, Africa, Japan, South America, Central America, Australia and Palestric region. *C. megacephala* is a dipteran fly belonging to the family Calliphoridae, and is known as oriental latrine fly. The adult fly is metallic blue green in color and 10-12 mm (SHIAO, 2008). The larvae vary in size according to instar and are shaped more thickly towards the rear. The posterior spiracles

of the larvae identified in this study, had an incomplete peritreme enclosing 3 rod-like slits and the anterior spiracle had 8 marginal branches or spikes.

Table-3: External positive bacteria isolates from maggots in different geographical areas

Geographical areas	*Klebsiella oxytoca*	*Providencia stuartii*	*Pseudo-monas aeruginosa*	*Salmon-ella* spp.	*Salmonella arizonae*	*Citro-bacter braakii*	*Pseudo-monas fluorescens*
Tlokweng	35%	5%	5%	40%	0%	5%	5%
Ledumang	0%	0%	0%	60%	40%	0%	0%
Maruapula	0%	0%	0%	65%	35%	0%	0%
University of Botswana	20%	0%	0%	50%	30%	0%	0%

Table-4: Internal positive anaerobic bacterial isolates from fly maggots in different geographical areas in Gaborone.

Geographical areas	*Klebsiella oxytoca*	*Enterobacter cloacae*	*Salmonella* spp.	*Salmonella arizonae*	*Proteus mirabilis*
Tlokweng	5%	0%	0%	15%	60%
Ledumang	10%	0%	30%	30%	30%
Maruapula	55%	25%	0%	20%	0%
University of Botswana	70%	0%	30%	0%	0%

Table-5: Internal positive aerobic bacterial isolates from different geographical areas in Gaborone

Geographical areas	*Salmonella* spp.	*Salmonella arizonae*	*Proteus mirabilis*
Tlokweng	10%	45%	45%
Ledumang	45%	45%	10%
Maruapula	30%	70%	0%
University of Botswana	50%	50%	0%

The anterior spiracle of *C. megacephala* possesses 8-12 marginal branches, the posterior spiracle on the other hand has incomplete peritreme which is heavily dark structure encircling 3 relatively straight spiracular slits. Hundred per cent of flies from Tlokweng and 40% of flies in UB were identified to be *M. domestica*. These are the common houseflies. This species of flies is always found in association with humans or the activities of humans. The maggots which were identified to be *M. domestica* had a D-shaped posterior spiracle, with a complete peritreme enclosing three snake-like spiracular slits. The third species of flies identified was the *Sryphid* spp. This species belongs to the order Diptera under the family Syrphidae and is sometimes called the hover fly due to its behavior of hovering around. The Syrphid flies can be saprotrophs feeding on decaying plant and animal matter or insectivores and prey on aphids, thrips and other plant sucking insects. The Syrphid maggot identified was brown and legless with a long pipe at the anterior end which is used for respiration.

This study showed that maggots of flies in different geographical areas in Gaborone contained a lot of microorganisms. The highest cfu/ml was 8.1×10^5 and the lowest cfu/ml was 1.4×10^3. Flies feed on feces and human waste food and therefore, carry with them the microorganisms carried on the waste. Flies were reported to carry many pathogenic

microorganisms and other pathogenic protozoans which include enteropathogenic *Escherichia coli,* enterotoxic *Escherichia coli*, *Klebsiella, Campylobacter, Staphylococcus aureus, Candida albicans, Mucor* and *Aspergillus* spp. (YEATES and WIEGMANN, 2005). *Salmonella* spp. was isolated in high numbers on the external body of maggots collected from all the geographical areas: Tlokweng (40%), Ledumang (60%), UB (50%) and Maruapula (65%); and lowest numbers internally as Tlokweng, (10%), Leduman (37.5%), UB (50%) and Maruapula (30%). *Salmonella* sp. is rod shaped, non spore forming, facultative anaerobes belonging to Enterobacteriaciae family. In a similar study, 11.8% of *Salmonella* sp. was isolated (FOTEDAR *et al.*, 1992).

Flies transmit the *Salmonella* sp. when they alight on human food and bacteria is carried on their legs and wings onto food and when they vomit on food. Consumption of food contaminated with the bacteria may cause salmonellosis. Symptoms of this infection include diarrhea, fever, vomiting and abdominal pains which last 12 to 72 hours after infection. In most cases, the illness lasts four to seven days, and most people recover without treatment. The diarrhea may be severe leading to dehydration and the patient will therefore, need to be hospitalized and be given intravenous fluids. Medications are given to relieve symptoms. *Salmonella* infection may spread from the intestines to the blood stream and other body sites resulting in Typhoid fever which is treated by antibiotics (WANG *et al.*, 2010). *Salmonella arizonae* which is a subspecies of *Salmonella* was isolated externally from maggots in the present study: UB (30%), Maruapula (35%) and Ledumang (40%); and isolated internally from maggots: UB (50%), Tlokweng (30%), Maruapula (25%) and Ledumang (37.5%). *S. arizonae* can cause salmonellosis if food contaminated by this bacterium is consumed. There have been several outbreaks in many countries due to eating food contaminated with *Salmonella. Salmonella* spp. isolated in maggots of flies could be transmitted to food and hence be causative agent for salmonellosis. *Salmonella* spp. was earlier isolated from *M. domestica* (OLSEN and HAMMACK, 2000).

Five per cent of *Providencia stuartii, Pseudomonas aeruginosa, Citrobacter braakii* and *Pseudomonas fluorescens* were isolated from the external body of maggots collected from Tlokweng only. All the fly maggots from Tlokweng were identified as *M. domestica. Pseudomonas* bacteria are known to cause food spoilage and diarrhoea from consumption of contaminated food. *Proteus mirabilis* was isolated and identified from fly maggots as follows: 60% was isolated in anaerobic conditions from Tlokweng; and in aerobic conditions, 30% from Ledumang, 45% and 10% in Tlokweng and Ledumang respectively. *Proteus mirabilis,* a Gram-negative, facultative anaerobe, rod-shaped bacterium causes food poisoning when consumed in contaminated food such as meat, vegetables, and seafood. *P. mirabilis* was isolated from fly maggots and could play a role in food poisoning from fly contaminated food. *Klebsiella oxytoca* was isolated from the external body of fly maggots: Tlokweng (35%), and UB (20%). It was isolated in anaerobic conditions: Tlokweng (5%), Ledumang (10%), Maruapula (55%) and UB (70%). *K. oxytoca*, a Gram negative and rod shaped bacterium that is the causative organism in at least some cases of antibiotic-associated hemorrhagic colitis (HÖGENAUER *et al.*, 2006).

CONCLUSION: It is important to keep good sanitation around homes especially kitchens. Proper, and regular disposal of wastes from dust bins are very crucial in keeping out flies. From this study, fly maggots were found to be a source of pathogenic microorganisms. It is therefore recommended that food should always be kept covered and kitchen utensils be kept clean and preferably in closed cupboards where they cannot be reached by flies. The use of fly nets is recommended to keep out flies. *C.*

megacephala, *M. domestica* and a few *Syrphid* spp. were identified from maggots collected in dustbins from different areas in Gaborone. The microorganism isolated from the fly maggots include *Salmonella* spp., *Salmonella arizonae, Klebsiella oxytoca* and *Enterobacter cloacae, Providencia stuartii, Pseudomonas aeruginosa, Citrobacter braakii* and *Pseudomonas fluorescens,* with *Salmonella* spp. predominating.

ACKNOWLEDGEMENT: We thank the University of Botswana for providing facilities for this study and for technical assistance from the Department of Biological Sciences, University of Botswana.

REFERENCES

ALLOTEY, J., PATELELLO, S. O., MAKATE, N. and MPHO, M. 2015. Study of Muscid and Calliphorid flies in Gaborone (Insecta: Diptera) *Uttar Pradesh J. Zool.* **35**:185-194.

FOTEDAR, R., BANERJEE, U., SINGH, S. SHRINIWA, S. and VERMA, A. K. 1992. The housefly (*Musca domestica)* as a carrier of pathogenic microorganisms in a hospital environment. *J. Hosp. Infect.* **20**: 209-15.

HÖGENAUER, C., LANGNER, C. BEUBLER, E., LIPPE, I. T., SCHICHO, R., GORKIEWICZ, G., KRAUSE, R., GERSTGRASSER, N., KREJS, G. J. and HINTERLEITNER, T. A. 2006. *Klebsiella oxytoca* as a causative organism of antibiotic-associated hemorrhagic colitis. *N. Engl. J. Med.* **355**: 2418-2426.

MERSON, H. M, BLACK, R. E. and MILLS, A. J. 2012. Global Health: disease programs, systems and policies, *3rd edition, Jones and Bartlett Publishers, Canada.* 976 pp.

OLSEN, A. R.1998. "Regulatory Action Criteria for Filth and Other Extraneous Materials*1 III. Review of Flies and Foodborne Enteric Disease". *Reg. Toxicol. Pharmacol.* **28**: 199-211.

OLSEN, A. R, and HAMMACK, T. S. 2000. Isolation of *Salmonella* spp. From the Housefly, *Musca domestica* L., and the Dump Fly, *Hydrotaea aenescens* (Wiedemann) (Diptera: Muscidae), at Caged-Layer Houses. *J. Food Prot.***63**: 958-960.

SERVICE, M. 2008. Medical Entomology for Students, *4th Edition, Cambridge University Press, USA.* 226 pp.

SHIAO, S. 2008. Larval Competition of *Chrysomya megacephala* and *Chrysomya rufifacies* (Diptera: Calliphoridae): Behavior and Ecological Studies of Two Blow Fly Species of Forensic Significance. *J. Med. Entomol.* **45**: 785-799.

TRICKETT, J. 2001. The Prevention of Food Poisoning, *4th Edition, Nelson Thomas Publishers, United Kingdom.* 176 pp.

WANG, Y., ZHANG, S. Yu, J. ZHANG, H. YUAN, Z. SUN, Y. ZHANG, L. ZHU, Y. and SONG, H. 2010. An outbreak of *Proteus mirabilis* food poisoning associated with eating stewed pork balls in brown sauce, Beijing. *Food Control.* **21**: 302-305.

YEATES, D. K, and WIEGMANN. B. M. 2005. The Evolutionary Biology of Flies, *1st Edition, Columbia University Press, New York.* 430 pp.