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Research Article Parasite and Microbial Load of Housefly Collected from Selected Houses in Amassoma Community, Bayelsa State, Nigeria

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Abstract

Background and Objective: Housefly infestation accounted for >90% of all flies in living houses in the rural communities. They are mechanical vector that transmit several disease pathogens, yet the awareness of their role in disease transmission is low. The study was undertaken to assess the parasite and microbial loads of housefly in living houses in Amassoma community. **Materials and Methods:** Sixty houseflies were collected from 6 houses in Amassoma community and analyzed for microbial and parasite surface contamination during March, 2019 -May, 2019. The procession of the housefly and the assessment of the microbial and parasite load in the housefly followed standard procedures. **Results:** All the houseflies caught were contaminated with at least one microbe's flora. The mean microbial load of the housefly across houses ranged from 0.9120×10^{-4} to 11.244×10^{-4} with a mean±standard of $3.50 \times 10 \pm 1.722 \times 10^{-4}$. The microbial flora isolated from the body surface of the housefly across the locations in order of abundance were the *Baccillus* spp., *Staphylococcus aureus, E. coli, Salmonella* spp., *Pseudomonas* spp., *Kiepsella* spp., *Erwinia* spp., *Micrococcus* spp., respectively (p<0.05). Ten (16.7%) of the housefly were contaminated with seven species of parasites fauna. The parasites species in order of abundance were *E. histolytica, Trichuris trichuira, Ascaris lumbricoides, Hook worm* and *Enteribius verminicularis*, respectively (p<0.05). **Conclusion:** It is evident from the results that houseflies are not only considered a nuisance but are also responsible for disease burden in humans. The high housefly density and parasite and microbial load surface contamination in the housefly is a call for public enlightenment campaign.

Key words: Synanthropic, housefly, contamination, Amassoma, Bayelsa State

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Housefly is a synanthropic animal that is widely distributed all over the world but is more adaptable in warm areas^{1,2}. They accounted for >90% of all flies in human habitation³. As house hold pests, housefly are responsible for most public health problems in the human environment⁴ where it accounts for >100 pathogens of wide range of diseases, such as cholera, typhoid fever, tuberculosis, aspergillosis, poliomyelitis, hepatitis, ascariasis, amoebic dysentery^{5,6}.

The increasing abundance of housefly's population in striving environment is worrisome A strong attraction to filth and human food is one of the major factors that incriminate housefly as mechanical carriers of disease agent⁷. Studies have also demonstrated that housefly are direct transmissive agent for several parasites such as cysts of Entamoeba histolytica, Entamoeba coli, Giardia lamblia and Oocysts of Toxoplasma gondii, isospora spp. and egg or larvge of Ascaris *lumbricoides, Trichuris trichiura* and Hook worm^{5,8,9}. The role of housefly in the transmission of disease pathogens has been given little attention in the rural communities of Bayelsa State. This study is therefore designed to assess the parasite and microbial load of body surfaces of Housefly (Musca domestica) in Amassoma community. This study shall provide the information to the individuals and the community about the role of housefly in community disease burden, call for proper hygiene life style and environmental sanitation at community level.

MATERIALS AND METHODS

Study area: The study was conducted in Amassoma community (6° 08'N and 4° 57'E) during March, 2019- May, 2019. It is an ancient community within the Wilberforce Island in the Southern Ijaw Local Government Area of Bayelsa State. The area is characterized by two seasons-wet and dry seasons. The dry season lasts between May and October with a peak rainfall in August. The daily mean temperature is $25^{\circ}C^{10}$. It has tropical rain forest, with most houses showing traditional architecture The major occupations of the people are fishing, farming and petty trading.

Study design: The study adopted a field survey study design to assess the parasites and microbial load of housefly from human living rooms.

Sample and sampling technique: The study population of the study comprises of all living houses in Amassoma community.

Six houses were randomly selected for the study. A total of 60 houseflies were collected using sweep net. Collected housefly samples were preserved in disinfected disposable Petri dishes and transported to the Microbiology Laboratory, Department of Biological Sciences, Niger Delta University for microbial and parasitological analysis.

Processing of house fly sample: The samples were pre-rinsed according to standard techniques¹¹. The pre rinsed samples were added to sterile plastic bags and gently shaken for 5 min. Bacteria in the rinsed water were collected onto 0.2 mm filters (Corning, Inc., Tewksbury, MA, USA) by vacuum filtration. Swabs and filters were stored at -20°C. One milliliter of each sample was put into well labelled sterile test tubes containing 10 mL of 0.85% normal saline. The tubes were vigorously shaken to discard the microbes associated with the surfaces of the samples into the saline solution. After which, test tubes containing 9 mL of normal saline were set up in test tube racks and labelled. Tenfold serial dilution was done and 1 mL of the inoculums from the original bacteria stock (10 mL normal saline tube) was collected aseptically and transferred into the first dilution tube (10⁻¹). The procedures for serial dilution followed standard techniques. The samples were diluted three times in order to obtain an acceptable colony count. The tubes were covered swiftly with cotton wool to prevent contamination of the samples. Plating was done in triplicates with the third dilution tube (10^{-3}) using pour plate method. 1 mL of the inoculums was aseptically collected with a syringe and was poured into the Petri dishes. 20 mL of the molten agar was poured into the Petri dishes and were swirled gently to spread the inoculums evenly in the medium. The plates were allowed to set (solidify) and were inverted and thereafter incubated at 37°C for 24 h. After the incubation time, the plates were observed for the number of colonies. The number of colonies (Total colony forming units in grams) were recorded and expressed in T CFU g⁻¹. The colonies were randomly selected and were picked off with sterile wire loop. The colonies were sub-cultured on fresh nutrient agar plates by streaking colonies on the agar surface. The sub cultured plates were inverted and incubated at 37°C for 24 h to obtain pure isolates.

The pure isolates obtained were subjected to a series of biochemical tests, gram stain and motility test. During the biochemical tests, aseptic techniques and good laboratory practice was strictly adhered to so as to obtain the best and accurate results. Also, standardization of the quantity of reagents and media was done as per standard procedures in Cheesbrough¹¹. **Isolation of microorganisms:** Each colony was isolated in a pure form by subculturing. Distinctive morphological properties of each pure culture such as colony form, elevation of colony and colony margin were observed. Further microbial identification was based on the methods of Akogun and Badaki⁹. Colonies were counted at the expiration of incubation period using a colony counter (Stuart Scientific, UK) after 24 h incubation at 37°C. Counts were expressed as colony forming U mL⁻¹ of sample homogenates.

Gram staining technique: Colonies from different pure culture plates were emulsified into a drop of distilled water on a slide and a thin preparation was made. The smear was allowed to air dry, covered with crystal violet stain for 60 sec and was rapidly washed off with clean water. Lugol's iodine was added for 60 sec and was washed off. The smear was decolorized with acetone alcohol and washed off rapidly. The smear was counter stained with safranin for 60 sec and washed off. Finally, the smear was examined under the microscope at \times 100 objective lens. The confirmation of each of the microorganism isolated was carried out using different standard laboratory test procedures. These tests include, Indole test, Kligler iron agar slant test, citrate utilization test, catalase test and coagulase test.

Parasitological analysis of the housefly: The experimental procedures for parasitic analysis followed standard technique in Arora, 2010. The second part of the elution of eggs and cysts of parasites from the housefly was done

using a concentration method¹². Each preparation was dispensed into clean centrifuge tubes and centrifuged at 1500 rpm for 5 min. The supernatant was discarded into disinfectant jar and the sediment was mixed with a few of lugol iodine. A drop was applied on the center of a clean grease-free slide and covered with slip. The slide was examined under the microscope for parasites using ×10 and ×40 objectives. Identification of parasite followed pictorial key in Arora and Brij¹³.

Statistical analysis: The proportion of houseflies collected from each houses and the frequency of the bacteria and parasite isolate from the houseflies were analyzed using descriptive statistics such as mean, standard deviation and percentages. The significant differences between bacteria and parasite isolate were confirmed using t-test at 0.05% level of confidence.

RESULTS AND ANALYSIS

Microbial load of the body surface of housefly: Sixty houseflies were collected from 6 houses in Amassoma community and analyzed for microbial and parasite surface contamination. Hundred percent of the houseflies collected from the study were contaminated with different bacteria species. The mean \pm standard deviation of the microbial load of the houseflies does not vary significantly across location (t = 15.722, p>0.05) as shown in Table 1 and 2.

Table 1: Analysis of significant differences of microbial load of housefly across locations

Variables	Test value = 0						
		df	Significant (2-tailed)	Mean difference	95% confidence interval of the difference		
	t-value				Lower	Upper	
One sample test							
Population	15.742	59	0.000	3.50000	3.0551	3.9449	
Triplicate	8.177	59	0.000	1.39950	1.0570	1.7420	

Table 2: Mean difference of the microbial load of the body surface of housefly across location

Location	Mean±standard deviation	Standard deviation error	t-value
A	$1.003 \times 10^{-4} \pm 0.288 \times 10^{-4}$	0.0910×10 ⁻⁴	10.995ª
В	$11.244 \times 10^{-4} \pm 3.189 \times 10^{-4}$	0.1008×10 ⁻⁴	1.115 ^b
С	$0.912 \times 10^{-4} \pm 0.340 \times 10^{-4}$	0.1122×10 ⁻⁴	8.137ª
D	$1.079 \times 10^{-4} \pm 0.410 \times 10^{-4}$	0.1298×10 ⁻⁴	8.314ª
E	$1.138 \times 10^{-4} \pm 0.526 \times 10^{-4}$	0.1664×10 ⁻⁴	6.837ª
F	$1.138 \times 10^{-4} \pm 0.526 \times 10^{-4}$	0.1254×10 ⁻⁴	9.781ª

A-F: Designation of the houses used for sample collection), ^{a,b}Significant difference

Microbial load







Fig. 2: Percent occurrence of parasite isolate in the housefly

Microorganism composition of the housefly in the study location: Eight bacteria species were isolated from the housefly. The bacteria in the order of their abundance are *Baccillus* spp., *Staphylcoccus*, *E. coli, Salmonella*, *Pseudomonas*, kiepsiella spp., *Erwinia* and *Micrococcus* as shown in Fig. 1. The differences of the bacteria isolate from the housefly vary significantly across the study locations (t = 7.016, p<0.05).

Parasite load in the body surface of housefly across location: Thirty houseflies were analyzed for body surface parasite infestation. Out of the thirty housefly, ten (10) houseflies representing 33.3% were infected with four species of parasites. The parasites in the order of their abundance were, *Entoemoeba histolytica, Trichuris trichuira, Ascaris lumbricoides, Hookworm* spp and *Enterobius verminicularis.* The differences of the parasite species infestation were significant (t = 4.247, df = 4 p<0.05) as shown in Fig. 2.

DISCUSSION

Houseflies are associated with unhealthy, dirty and insanitary environments^{14,15}. They are more attracted to environment where garbage, feces and carcasses predominant. In such environment, the tendency for houseflies to migrate inside living houses becomes high. The habitation of houseflies in the living room and kitchen in this present study is an indication that the surrounding and houses in the study location were filthy^{3,16}. Houseflies had been considered a mechanical vector of several disease pathogens. Their ability to carry pathogens from place to place is due to their structural hairy body and sticky jointed appendages pad. In this study, hundred percent of the housefly were contaminated with bacteria, parasitic protozoan and helminths ova. The surface body contamination of the housefly is an indication that the housefly may have interacted with dumpsites, garbage and fecal materials around the surroundings. This observation is consistent with earlier report by Oghale et al.¹⁵ and Mawak and Olukose¹⁷.

Bacteria are normal flora of most dirty and unhealthy environment. The isolation of eight bacteria species from the body surface of housefly in this present study highlighted that the housefly may have inhabited and fed from environment that supports the growth of the bacteria species¹⁸. The high bacteria count in housefly have been reported elsewhere in Nigeria^{3,7,19}. The bacteria species isolate of the housefly in this present study was comparable with the report of Sulaiman *et al.*²⁰, who isolated seven bacteria species from housefly in Malaysia.

The high prevalence of *Baccillus* and *Staphaloccocus* species agrees with other report by Vazirianzadeh *et al.*²¹ and Adeleke *et al.*²². All reported that the two bacteria species are normal flora in all living tissues. However, they can become pathogenic where possible. The presence of *Salmonella* spp. and *Klebsiella* spp., is an indication that the housefly may have contracted the pathogens from other contaminated objects within the environment. The isolation of *E. coli* from the housefly highlighted their association with fecal contaminant during feeding. *Salmonella* spp. in any medium is considered as bio-indicator of fecal contamination.

The recovery of the five parasites *(Entoemoeba histolytica cyst,* ova of *Ascaris lumbricoides, Hookworm, Trichuris trichuira* and *Enterobius verminicularis* from housefly collected in human kitchen have also been reported elsewhere in Nigeria^{8,19}. These are gastrointestinal parasites, which show either direct or indirect transmission pattern. The cyst or the ova are usually seeded to the environment through human excreta. Housefly is synanthropic animal that always

interact with fecal contaminants. Their hairy body may have aided their ability to transport the parasite. It is thus recommended that measures must be taken to control the fly population in Amassoma and Bayelsa State at large. Government should set hygiene standards for places like markets, slaughterhouses (abattoirs), hospitals, public toilets, eateries (restaurants) and packaging industries. Public awareness should be created to enlighten the masses on the essence of maintaining a fly-free environment by avoiding such activities as open refuse, dumping, open defecation and the benefits of screening houses restaurants and other human habitations from flies using nets.

CONCLUSION

It is concluded from this study that adult houseflies are not only a nuisance to animals, they carry many pathogens which may be transmitted to man and animals resulting in outbreaks of diseases of both parasitic and bacteria origin causing a reduction in animal production, human work h and financial losses through the treatment and loss of infected animals by death.

SIGNIFICANCE STATEMENT

This study has discovered salient information that can be beneficial for the control of pathogenic diseases in the rural communities. Most of the infectious diseases in the rural environment were not traced to a known source. This study has uncovered the critical role of housefly in the spread of gastrointestinal parasites as well as pathogenic bacteria. Their link between the dirty environment and human habitation is the source of dissemination of pathogens among human. Reducing the housefly- human contact can go along way to reducing the menace of housefly related diseases.

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