



IN-VITRO STUDIES ON THE PUBLIC HEALTH IMPORTANCE OF *PERIPLANETA AMERICANA* IN THE TRANSMISSION OF HUMAN INTESTINAL PARASITES

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ABSTRACT

This study aims at determining the possible role of *Periplaneta americana* in the dissemination of ectoparasites and endoparasites of public health importance. The cockroaches used for this study were collected from two different sites, refuse dumps and residential areas 2km from the refuse dump. Parasites of medical importance were isolated from the external and internal surfaces of these cockroaches using the direct wet mount and molecular identification method and the data was analysed using the SPSS. A total of 200 cockroaches identified as *Periplaneta Americana* were used for this study, of this number, 170 were male adults and 30 were female adults. 150 cockroaches were collected from the refuse dump and 50 (4%) from residential areas carrying medically important parasites. Four parasites; *Entamoeba histolytica* (43.3%), *Entorobius vermicularis* (20%) *Asacaris lumbricoides* (19%) *Thelastoma bulhoesi* (28.6%) were isolated from these cockroaches. The refuse dump cockroach had at least one of these parasites either in their body surface or digestive tract, respectively while those from the residential areas had *Entamoeba histolytica* (4%) only at the external surface. In addition, yeast cells were also isolated only from the external and internal surfaces of the cockroaches from the residential areas. There was a significant difference ($p < 0.05$) in the number of cockroaches infected and that not infected. A significant ($p < 0.05$) difference in the external and internal parasites isolated from these cockroaches was also noted. The data from this study suggests that cockroaches, in refuse dumps can act as potential vectors of medical important parasites.

1.0 INTRODUCTION

Cockroaches are insects of the order DICTYOPTERA and of the suborder BLATTODEA. There are about 4,000 species of cockroach, of which 30 species are associated with human habitations and about four species are well known as pests.^[1,2] Cockroach is regarded as a primitive insect and fossil species from the carboniferous period (250 million years ago), are very like those known today. The majority of the cockroaches are tropical with only a few small species native to our wonderful climate. But those tropical ones which have found their way here, in the last 200 years are pretty much dependent upon our heated buildings, especially refuse dumps, hospitals, laundries, and kitchens where conditions are hot and steamy. Among the best known species are the American cockroach, *Periplaneta americana*, which is about 3 millimetres (1.2m) long, the cockroach, *Blatella germanica*, about 15 millimeters (0.59m) in length, and the oriental cockroach, *Blatta asahinai*, which is about 25 millimeters (0.98m) in length. Tropical cockroaches are often much bigger, and extinct related such as the

carboniferous *archimylaris* and the *Permian apthoroblathia* were several times as large as these.

Periplaneta americana is often found in close association with humans, living in homes, business and health facilities. The intimate association of these insects with humans has resulted in their relegation to pest status.^[3] *Periplaneta americana* is abundant in urban and rural areas where unsanitary conditions prevail, there are usually scarce where sanitary conditions are enforced. Because of their association with human environments, they may incidentally contact and acquire human pathogens. These pathogens may subsequently be mechanically transmitted to humans or susceptible surfaces which may then function as vehicles for indirect transmission.^[4] The American Cockroaches are potential vectors for bacteria (over 100 species of bacteria have been isolated or passed through cockroaches)^[5], fungi and parasites.^[6] Because the cockroaches feed on filth and feces, they may disseminate infections with the faecal oral route.^[7] This work is aimed at ensuring proper

collection of the cockroaches and isolation of parasites of medical importance from cockroaches collected from the refuse dump and residential area 2km from the dump.

2.0 METHODOLOGY

STUDY AREA

This research was carried out in Amakohia, Owerri, in Imo State. Owerri serves as Imo State Capital. The State accommodates Federal and State Government offices. It is accessible by road to all parts of the Eastern States; the location of an airport have improved private sector presence and attracted influx of people to the City. Owerri is located at approximately between latitude 5° 34' and 15°N and longitude 7° 30', having a population of about 693,039 and not too developed with rural areas surrounding it. It has a temperature range of between 20-35°C with a very high relative humidity of about 75% reaching 85% during the rainy season. Owerri is subdivided into Owerri Municipal, Owerri West and Owerri North Local Government Areas. Amakohia is a community in Owerri North Local Government Area with urban and rural dwellers. It shares borders with Owerri Municipal, North-East, and Owerri west Local Government in the west. There are many growing small scale industries, hotels and bars, government and private schools, hospitals, churches and a mini market, which serve the rural and urban dwellers. Source of drinking water is pipe borne water and borehole.

COLLECTION OF COCKROACHES

The specimens were collected into suitable sized, clean, dry, leak proof plastic containers with cork screw by handpicking and sticky trap method according to Poulin.^[8] The cockroaches collected were not stored in the same container; they were stored in separate containers for those cockroaches from the refuse and those from the residential areas.

EXAMINATION OF SAMPLES

Several methods were adopted for the examination and identification of parasites from the external and internal surfaces of the cockroaches collected. Such methods include macroscopic examination using the direct wet method and molecular identification.

Macroscopic Examination and Identification

The cockroaches were examined macroscopically to note the colors, type of wings, the sex, the legs, antenna, eyes, mouthparts and abdomen. The cockroaches collected were all identified macroscopically as adults measuring about 1-1/2 inch long and uniformly reddish-brown over the entire body except for the area behind the head and in front of the wings which is reddish-brown with a tan margin.

Microscopic Examination and Identification

After the macroscopic examination, the samples were anaesthetized at zero degree for 5min^[9], examined under a dissecting microscope (40x) and identified using standard taxonomic keys for Blattidae.^[10]

Identification of Parasites from External Surfaces

After morphological identification, the cockroaches were each transferred from the container into test tubes and closed. 2ml of sterile Normal saline (0.09%) was added to each of the test tubes and cockroaches were thoroughly shaken for 2min. A fixed volume (1ml each) of the washing was centrifuged at 2000 revolution for 5min, decanted and the deposit examined after staining with 1% Lugol's iodine under light microscope using first a 10x magnification to focus and later a 40x magnification to identify the parasites according to Ukaga^[11] and Chessbrough.^[12]

Identification of Parasites from Internal Surfaces

After external washings, cockroaches were placed in tubes rinsed with 70% alcohol for 5min (to decontaminate external surfaces, transferred to sterilized tubes, and allowed to dry at room temperature under sterile conditions. Cockroaches were then washed with sterile normal saline for 2-3 min to remove traces of alcohol. After being immobilized they were set for dissection,

DISSECTION

The head was first severed out and the next are the legs with the help of the fine pointed forceps and scissors. Next the body was pinned to a small dissecting board with thin rigid pins. The ligaments on the right hand side of the abdominal sternites were cut beginning at the rear end and ventral plate to release towards the left side, clearing its adhesions to the internal organs with sharp needles. The thin and sharp teasing needles were used to remove the fat that surrounds the abdominal organs. The alimentary canal was isolated and set free from its ties. The digestive tracts were liberated with needles, and cut at the level of the cloaca. The intestinal caecae was identified and the intestine carefully macerates with a blunt forceps and transferred in a centrifuge tube containing 2ml normal saline. The resulting macerate was then centrifuged at 2000 revolution for 5min.

Molecular Identification

After the identification of the endo and ecto parasites of the cockroaches it was further subjected to purification and DNA isolation, sequencing and identification of the parasites.

Purification of Samples and Backup PCR

The PCR samples were purified as done previously using GENECLEAN® Turbo Kit, EtOH, and NH₄OAc. A repeat PCR reaction was also performed using the leftover unspooled chromosomal DNA from the extraction as well as a 1/5000 dilution of the spooled chromosomal DNA. A new PCR reaction was performed and gels run with these products. The band was cut out and gel purified. Another PCR reaction was then run and the Eukaryotic band extracted (gel purified).

Analysis of Sequences

Sequences were copied from the BioEdit program (Ibis Therapeutics) into a Microsoft Word document. The FB sequences were taken for all samples (Eub and Euk) and the first 500-900 base pairs were recopied. The unnecessary N's from the sequences were also deleted. The sequences were then placed into the BLAST website (NCBI), their relationships were observed, and phylogenetic trees were constructed from the relationships with other parasites. The edited sequences from these organisms were compared with the sample sequences in ClustalW (EMBL-EBI).

3.0 RESULT

Of the 220 cockroach samples collected, 20 were lost in transit; only 200 cockroaches in good condition were examined. The findings of the study revealed that all the cockroaches collected from the two sites are the

American cockroach (*Periplaneta Americana*). 147(73.5%) out of 200 cockroaches had parasites isolated from both the external and internal surfaces. In 145 (96.7%) of the 150 cockroaches from the refuse bin were isolated parasites from the external and internal surfaces, while 2(4%) out of 50 residential cockroaches had parasites only at the external surface.

From table 1 below, a total number of 147(73.5%) of the cockroach samples were infected while 53 (26.5%) were not infected by parasites. 45(96.7%) of the infected samples came from the refuse dump while 2 (4%) was collected from residential areas. 5 (3.3%). Refuse dump cockroaches had no parasite isolated from them while 48(96%) of the residential cockroaches had no parasite isolated from it. A significant high ($p < 0.05$) number of refuse dump cockroaches were infected.

Table 1: Number of Infected and Non-Infected Cockroach Samples.

| Sites | No. Examined | No. Infected (%) | No. Not infected (%) |
|-------|--------------|------------------|----------------------|
| R. D | 150 | 145(96.7%) | 5(3.3%) |
| R,A | 50 | 2(4%) | 48(96%) |
| Total | 200 | 147(73.5%) | 53(26.5%) |

Keys:

R.D: Refuse dump

R.A: Residential areas

From table 2 below, 96.7% of the groups of cockroaches from refuse dump infected by parasites, 72.4% had Endo Parasites isolated from their internal surface while 27.6% (40) had Ecto-parasites on their external surface. For the other group of cockroaches from residential

areas, 4% had external parasites only, none had internal parasite isolated from its internal surface. There is thus significant difference ($p < 0.05$) in the external and internal parasites isolated from these groups of cockroaches.

Table 2: Rate of recovery of parasites from cockroaches at 2 different sites.

| Sites | No Examined | No. of Endo-parasite isolated (%) | No. Of Ecto-parasite isolated (%) |
|-------|-------------|-----------------------------------|-----------------------------------|
| R. D | 150 | 40(27.6%) | 105(72.4%) |
| R,A | 50 | 2(4%) | - |
| Total | 200 | 42(21%) | 105(52.5%) |

From table 3 below, out of the 150 cockroaches examined from the refuse dump; 25 (62.5%) of the parasites were *Entamoeba histolytica* while for the residential cockroaches had the least. *ntamoeba*

histolytica parasite on their surfaces. A low carriage rate that is statistically not significant at ($p < 0.05$) parasitic cysts ova were observed among the groups of cockroaches.

Table 3: Rate of parasites Recovery from cockroach surface of cockroach.

| External Parasite Isolated | R.D cockroach (%) | R.A cockroach (%) |
|--------------------------------|-------------------|-------------------|
| <i>Entamoeba histolytica</i> | 25 (62.5%) | 2(100) |
| <i>Entorobius vermicularis</i> | 15 (37.5%) | - |
| Total | 40 | 2 (100) |

From table 4 below, highest number of endoparasites were isolated from cockroaches collected from the refuse dump while none was isolated from cockroaches collected from the residential areas. From the refuse dump cockroaches, *Entamoeba histolytica* 40 (38.1%), *Enterobius vermicularis* 15(14.3%), *Ascaris lumbricoides* 20(19%), *Thelastoma bulhoesi* 30(28.6%) were were predominant among the refuse dump cockroaches.

Table 4: Rate of recovery of parasites from internal surface of cockroaches.

| Internal Parasite Isolated | R.D cockroach (%) | R.A cockroach (%) |
|--------------------------------|-------------------|-------------------|
| <i>Entamoeba histolytica</i> | 40 (38,1) | - |
| <i>Enterobius vermicularis</i> | 15 (37.5) | - |
| <i>Ascaris lumbricoides</i> | 20 (19.0) | - |
| <i>Thelastoma bulhoesi</i> | 30 (28.6) | - |
| Total | 105 (100) | |

The seasonal change in the collection of these samples showed (Table 5) that the greatest collection of the samples was during the rainy season from both the refuse dump and the residential areas. 43.3% of the refuse dump cockroaches were collected during the dry season as at 56.7% collected during the rainy season while 40% of

the residential cockroaches were collected during the dry season and 60% during the rainy season (Table 5). This showed no significant difference ($p < 0.05$) in rate of collection from both groups of cockroaches with respect to seasonal changes.

Table 5: Rate of collection of cockroaches seasonally.

| Seasons | No. Of refuse dump cockroaches (%) | No, of Residential roaches (%) |
|---------------------------------|------------------------------------|--------------------------------|
| Dry season Oct-Mar) | 65(43.3%) | 20(40%) |
| Rainy season (April) | 85(56.7%) | 30(60%) |
| Total | 150(100.0%) | 50(100%) |

4.0 DISCUSSION

This study Demonstrated the presence of parasite on the internal (52.5%) and external (21%) surfaces of an American cockroach *Periplanata americana*. The high prevalence of the Endo-parasites especially from cockroaches from the refuse dump may be attributed to their feeding habits. The finding in this study showed that, the 200 cockroach samples collected from the refuse dump and residential areas were all identified as belonging to the American cockroach, *Periplanata americana*. In the analysis, 4 species of parasites were isolated, 3 species were parasitic nematodes that are located at the hindgut of the cockroach and the last and Protozoa. It has been reported that an impressive array of parasites has also been identified from cockroaches from residential area^[13] and from sewage.^[14] The four parasite species identified in this study have earlier been reported^[15] as the commonest nematodes and protozoa that inhabit the internal surface of cockroach causing diarrhea, cholera, typhoid fever etc, when this arthropod contaminate foods with their feces. This finding suggests the cockroaches in the refuse dump carry medically important parasites.

The rate of recovery of these parasites from cockroaches in both the refuse dump and residential areas (Table 2). Out of 200 cockroaches analysed, 147(73.5%) were infected, 42(21%) had external parasited isolated from them while 105(52.5%) had the internal parasites. Regarding the importance of cockroaches as carriers of parasitic worm, cysts or eggs, there are some reports of the presence of parasitic forms on or in cockroaches^[16]^[17] in their study on the role of insects as possible mechanical vectors of helminthes in domiciliary and predomiciliary environment, 54 Blattodea, 275 Diptera and 371 Hymenoptera were used. *Periplanata americana*

was the Blattodea specie used and these parasites were isolated from it; Oxyuridae eggs 36.5% Ascaridae 28.4%, Nematode larva 4.8% Cestoda egg 3.5% Toxocaridae 0.08% other nematode 0.08%

From this group of cockroach, Out of 150 refuse dump cockroaches, 62.5% had *Entamoeba histolytica* as external parasites while 37.5% had *Enterpbius vermicularis* as external parasites. For the residential groups of cockroaches, 2 had Entamoeba as external parasite while none had *Enterobius vermicularis*. This study showed that *Entamoeba histolytica* has a high prevalence rate, despite the low carriage rate it was statistically insignificant ($p < 0.05$) for the important parasite among the two groups of cockroaches. Hsui Hua Pai, et al^[18] study isolated *Entamoeba* cyst on cuticle and/or digestive tract of *Periplanata*. Their study showed that cockroaches may harbor cysts and play a role as potential disseminators.

Adamson and Noble^[19] in Canada, showed that *Thelastoma bulhoesi* nematode species had a highest prevalence (75% and 81%), but in this study is was low as 28,6% as is the case in another study carried out by Jose^[14] on *Periplanata americana* parasitic fauna of Linnacus in a district of Lima, one hundred specimens were collected from sewage of district of Pueblo Libre, 5 parasites were identified with prevalence and intensity of infection as follows: *Cephalobellus tipulae* (38%,3,5), *Leidynema appendiculatum* (Nematode) (35% and 2.85). *Thelastoma bulhoesi* (Nematode) (1% and 7) *Moniliform moniliform* (Acanthocephala) (2% and 1.5) and *pimeliaphilus cunlikkei* (Axarina) (62% and 5.03)

Table 5 five shows the rate of collection of cockroaches seasonally, during the rainy season a huge number

(56.7%) of cockroach was collected from the refuse dump, though a food number was also collected from residential area, this may be compatible with Walter^[15] study were he found that torrential rains of the season (June-July) force the cockroaches to leave their hiding places even in daylight, terrifying housewives that take care of their house hygiene. The difference observed in the seasonal ($p < 0.05$) showed that collection of cockroach does not have any association with seasonal change, this may be so because in the rainy season the samples were only collected in April unlike in the word of Wolda and Fisk^[20] which concluded that in both climatically seasonal and non seasonal localities in Panama, cockroach adults were most common between April & July corresponding to the beginning of the rainy season, but does not show absence of inactivity of adults.

CONCLUSION AND RECOMMENDATION

In an attempt to eradicate the menace of cockroaches in most homes, good recommendations of methods employed in the destruction of these cockroach that has formed a potential vector for the dissemination of medical important parasites and micro organisms should not be over emphasizes. These methods will include proper sanitation both at home and around the residential environs, good water drainage system, proper sewage disposal and siting of refuse dump far away from residential areas and the use of insecticides.

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