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Cytotaxonomic Identification and Diversity Indices of Cyclops in Fresh Water Ponds of Ebonyi State Implication for Re-Emergence

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ABSTRACT

Out of the 5529 Cyclops examined from 2004-2005 during this study using microscopic examination, 36 were found infected with Dracunculus larvae. The prevalence rate was 0.65%. Three species *Thermocyclops oblongatus nigerianus*, *Mesocyclops aquatorialis* and Tropocyclops confines were encountered and two out of these species, *Thermocyclops oblongatus nigerianus* and *Mesocyclops aquatorialis* were infected. Species diversity indices of these Cyclops showed that there was no significant difference (p>0.05) between Simpson's Dominance Index (C=0.4985) and Simpson's Index (D=0.4384) for cyclops species encountered during the study. Simpson's dominance index was weighted towards the abundance of the commonest species (*Thermocyclops oblongatus nigerianus* and *Mesocyclops aequatoriali*s). This indicates that the Cyclops are more abundance, now in the area than during the guinea worm eradication period when the first study was done. This may be because ponds were being treated with abate as a measure to control Cyclops but since after the eradication, ponds were no more being treated and Cyclops started growing more in abundance. This has an epidemiological implication and calls for serious attention by responsible authorities. The situation could be dangerous if dracunculus larvae are introduced into the communal water bodies and can result to outbreak of the guinea worm disease again.

Keywords: Cyclops, Cytotaxonomics, Dracunculus, Ponds, Re-emergence, Ebonyi State

INTRODUCTION

The transmission pattern by Cyclops depends on types of climate and amount of annual rainfall. There is little transmission in the rainy season when the ponds are flooded and becomes turbid and unsuitable for Cyclops; but in the dry season, the ponds assume their role as transmission sites as the volume of the ponds became low [3]. In areas where ponds are used, Cyclop tend to persist in large number and transmission period become longer. The ingested larvae develop to adult warm in humans. The first sign of dracunculiasis is blister formation which occurs when gravid female worm is about to emerge from the subcutaneous tissue.

[14] carried out a study on the ecology of Cyclops species in Nigeria and found out that Cyclopoid copepodsinfected with dracunculiasis larvae abound in water bodies in parts of Plateau State, where guinea worm is

also endemic. Five species of these Cyclops were encountered. However, only *Thermocyclops nigerianus and Mesocyclops aequatorialis* were found to be infected with dracunculiasis larvae. The other three species namely: *Microcyclops linjanticus. Tropocyclops confinis and Platycyclops phalevatus* were not infected. Of the infected Cyclopoid copepod, *Thermocyclops nigerianus* was the most dominant throughout the study. In this review, [7] stated that only one or two species of Cyclops are generally found naturally infected in each region where dracunculiasis is endemic even though there are usually other species present in the sore habitant which can easily be infected experimentally. The predominant species in a habitant is usually the most important host. When the larvae come in contact with the water it act as food for predatory Cyclops, man ingest the infected Cyclops in water and so the cycle continues. Low level of water in ponds during the dry season maximizes Cyclops densities giving peak ingestion by man. [6] noted that larvae which are released into the water remain infective to Cyclops for only about 4 days.

Very little is known about the numbers of infected copepod needed to maintain transmission of dracunculiasis [7]. In Nigeria, infection rates in Cyclopoid copepod in a community with a prevalence of dracunculiasis of 83% varied between 4.7% and 10.5% [12]. Near communities in Burkina Faso with prevalence greater than 10%, about 0.3%-19.3% of Cyclops were found infected [15]. The rapidity with which adult copepod stages reappear indicates that encysting takes places in an advanced copepods state. It has been reported that adult copepods have been found in a previously dry water source 2 hours after it is filled with rain, in this case, encystment could have occurred in the adult state. Adult copepods readily ingest free-swimming first stage larvae of *D. medinensis*. Larvae need approximately 5 days if not ingested by copepods, will run out of its food reserve. Once ingested, the 1st stage larvae penetrate into the copepod body cavity and moult 2 times. The 2 moults are completed in about 14 days, and the resultant third-stage larvae are infective for humans.

The infective rate in Cyclops according to [13] varies from 4.7% to 10.92% with an average of over one larvae per liter of water and noted that no infected Cyclops were found during the rainy season in September. The entry of larvae into the Cyclops is by ingestion[6]. When the larvae come in contact with the water it acts as food for predatory Cyclops. In the gut of the Cyclops, the larvae undergo powerful bending movement and penetrate through the gut wall to the haemocele in 60-240 minutes depending on the temperature. The larvae penetrate through the head first, the process apparently being mechanical, caused by its flexion of the body against the lodged head and possibly helped by the action of the dorsal tooth. Larvae in water for more than 5 days are unable to develop 5 Cyclops [6], because they no longer have the energy to penetrate the gut wall.

There are species of the Cyclops in which the larvae will not develop after ingestion and the sluggish or integrating embryo can be found in the gut for some days after ingestion[5]. The inability of the larvae to penetrate the gut wall may be due to physiological resistance or because the feeding habit of the first stage larvae moult twice to become third stage larvae.

Water quality is the key to copepod density. Pipe borne water is copepods free and thereby completely safe from dracunculiasis. Draw wells have a few crustaceans (20 per 2 liters) but none is infective because "rope and bucket" access to water prevents leg immersion by *Dracucunliasis* carriers [10].

Within a pond, infected Cyclops tends to concentrate in the shallows, presumably because they require more oxygen. It has also been noted that Cyclops carrying third stage infected larvae, tend to sink deeper towards the bottom of a pond. From the disease transmission point of view, lower water levels in ponds at the end of the dry season, maximize copepod densities giving a peak in copepods ingestion by humans.

When the larvae come in contact with the water it act as food for predatory Cyclops, man ingest the infected Cyclops in water and so the cycle continues. Low level of water in ponds during the dry season maximizes Cyclops densities giving peak ingestion by man. [6] noted that larvae which are released into the water remain infective to Cyclops for only about 4 days.

MATERIALS AND METHODS

Collection of Cyclops: In this method, two strainers were used - a large pore tea strainer and a smaller pore plankton net. The tea strainer was used to strain off large materials collected with the Cyclops. The water sample was then centrifuged at 100 rpm for 5 minutes to settle out other dead materials collected along with the Cyclops and which pass through the tea strainer. The supernatant and measured samples with the Cyclops were then filtered through a sieve of 100 – mesh to an inch. This retained the entire Cyclops on the sieve. The Cyclops on the sieve was then washed into a Petri dish by inverting the sieve over the dish and pouring distilled water on it and were counted under the microscope.

Preservation of Cyclops: Since accounting the Cyclops alive is difficult, the Cyclops was fixed with 5% formalin before the counting was done. By filtering 10 liters of the pond water through monofilament nylon filter, the residue was collected in duplicate from each of the 60 ponds sampled. Each of the sample bottles contained 5% formalin. The preserved Cyclops was filtered and concentrated in 1ml of normal saline. Each drop of the concentrate was then observed and the number of Cyclops recorded and density per litre of the pond worked out and was expressed as the total number of Cyclops per a liter of water.

Identification of Cyclops: The Cyclops in the unfixed sample was processed for identification of species. The identification was carried out as follows: Each Cyclops was pipette from the preservative with a dropper and placed on a glass slide with a drop of glycerin on it. The slide was then placed in a desiccators set at a temperature of 45°c to evaporate water from the glycerin. The specimen was again transferred one by one with a dissecting pin to another slide containing pure lactic acid. The lactic acid cleared the muscle and because of its viscosity, provided a convenient medium for dissection. The treated sample was then examined under a dissecting microscope at a magnification of X40 which allowed the detection of dracunculus larvae. During observation a moving dracunculus larvae was detected in the haemocele part of the Cyclops. Only one larva was seen in each infected Cyclops. The Cyclops species was identified using the identification key of [3]. The Cyclops dissected and found infected with larvae of dracunculus was further investigated to identify the particular specie of the Cyclops using the identification key described by [3]. The cyclops observed and identified have large and posteriorly curved lateral wing of seminal receptacle, a characteristic feature of *Thermesocyclops oblongatus nigerianus* described by Boxshell and Braide (1991). The numbers with these peculiar characteristics were recorded as *T. oblongatus nigerianus*. Also observed was another species of Cyclops which has maxillary palp without row of spiracles and dorsal seta on caudal ram shorter than other apical seta as was also described in the identification key of [3] for *Mesocyclops acquatorialis*. This particular specie seen was recorded as *M. acquatorialis*. During the observation care was taken not to confuse larvae of dracuncunlus nematode with that of camallanidae. The camallanidae larvae is shorter and broader than that of Dracunculus larvae which has a larger buccal capsule and trifid tail. The Cyclops found infected during dissection was separated from the uninfected ones. The identification of these species were based on identification key of [3] but not confirmed by higher authority

Identification of guinea worm-infected Cyclops: This was estimated by straining 10 litres of water collected through the monofilament nylon filter. The Cyclops was sedimented with a few drops of normal saline and transferred to Petri dishes. Infected Cyclops were detected by placing the Petri dishes in a refrigerator for 10 hours after which they were removed and allowed to stand at room temperature (28^{o}c) for 15 minutes. This inactivated the Cyclops and observation of *D. medinensis* larvae was carried out under the microscope. 0.05ml of HCL was added to enhance the observation as this destroyed the exoskeleton of the Cyclops and activated the larvae in the infected ones. The infected Cyclops were easily seen by the active movement of the larvae of *D. medinensis* in the Cyclops under a state of coma. Infection of the Cyclops was based on mature Cyclops only.

RESULTS

Table 1: Geocyclops survey and guinea worm-infection status in Ebonyi State

S/N	LGA	Pond		Cyclops		
		Examined	Cyclops present	Infestation	Infected with larvae of *Dracunculus medinensis*	
		No.	No.	No. per liter pond water	No.	Prevalence (%)
1.	Izzi	10	1	902	1	0.11
2.	Ikwo	10	3	911	6	0.66
3.	Ezza North	10	5	993	9	0.91
4.	Ishielu	10	5	947	10	1.06
5.	Ebonyi	10	3	772	6	0.78
6.	Ohaukwu	10	2	1001	4	0.40
Mean ± SD		**10**	**3.17±1.46**	**921±76.29**	**6±3**	**0.65±0.32**

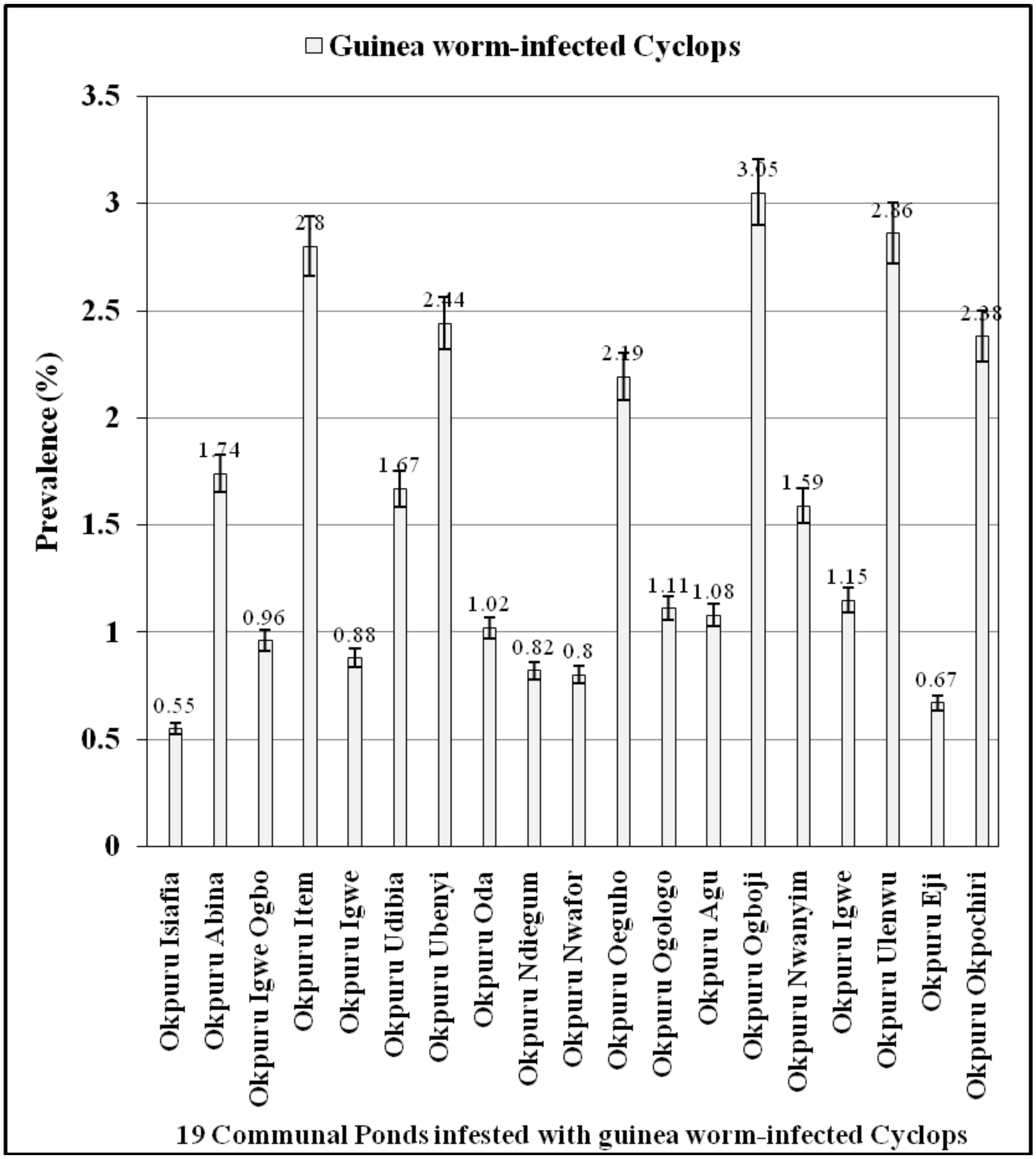
Guinea worm-infected Cyclops
Prevalence (%)
3.5
3
2.5
2
1.5
1
0.5
0
0.55
1.74
0.96
2.8
0.88
1.67
2.44
1.02
0.82
0.8
2.19
1.11
1.08
3.05
1.59
1.15
2.86
0.67
2.38
Okpuru Isiafia
Okpuru Abina
Okpuru Igwe Ogbo
Okpuru Item
Okpuru Igwe
Okpuru Udibia
Okpuru Ubenyi
Okpuru Oda
Okpuru Ndiegum
Okpuru Nwafor
Okpuru Oeguho
Okpuru Ogologo
Okpuru Agu
Okpuru Ogboji
Okpuru Nwanyim
Okpuru Igwe
Okpuru Ulenwu
Okpuru Eji
Okpuru Okpochiri
19 Communal Ponds infested with guinea worm-infected Cyclops

Table 2: Species diversity indices of Cyclops encountered during the study (2004-2005)

S/N	Species of Cyclops	*ni*	$pi = \frac{ni}{N}$	$pi^2 = (\frac{ni}{N})^2$	$\frac{ni(ni-1)}{N(N-1)}$
1.	*Thermocyclops oblongatus nigerianus*	2979	0.5391	0.290615	0.230557
2.	*Mesocyclops_aequatorialis*	2520	0.4560	0.207959	0.207877
3.	*Tropocyclops_confines*	27	0.0049	0.000023	0.000023
Σ		***N*= 5526**	**1.0000**	**0.498597**	**0.438457**

ni = number of individuals in the *ith* species, *N* = Number of individual (the total abundance), *pi* = proportion of individuals found in the *ith* species ($pi = \frac{ni}{N}$), *In* = $\log_{e}$, *S* is number of species encountered

Table 3:Distribution of Cyclops according to type of communal water source

	Communal water source		Cyclops		
S/N	Type	Number sampled	Number per litre of water	Relative %	Number infected
1	Large natural pond	10	636	44.08	Nil
2	Small natural pond	8	318	22.04	
3	Small man-made pond	7	210	14.55	
4	Large man-made pond	4	180	12.47	
5	Periodic stream	3	96	6.65	
6	Draw well	4	3	0.21	
Total		**36**	**1443**	**100**	

DISCUSSION

Some species of Cyclops were encountered during the first stage of study of abundance, distribution and infectivity of Cyclops (2004 to 2005). Two out of the three species of Cyclops identified *Thermocyclops and Mesocyclops* were implicated as the intermediate host for dracunculiasis Prevalent in the study area. The same species were implicated as the major intermediate host for dracunculiasis in Nigeria (Boxshall and Braide 1991. A similar report of these species has been documented in Ebonyi LGA Ebonyi State by [2] and [9] at Azara Awa LGA Plateau Sate Nigeria and [2] and [14] who reported 4.9% infection rate. [12] in Western Nigeria reported 5.1% of *Thermocyclops Oblongatus Nigerianus* infection. Our observation showed that only two of the species found – *Thermocyclops oblongatus nigerianus and Mesocyclops acquatorialis*_harboured infective larvae of *D. medinesis.* Whenever these species occurred, some population of the area could be infected with larvae of dracunculus. Specie distribution of the cyclops in the study area gave reliable information of the water sources that can harbour cyclops infected with *D. medinensis* larvae. This type of information worldwide is useful in planning Guinea Worm eradication programme since it provides an indication of dracunculiasis endemic foci. The result of the study showed 0.65% infection rate of Cyclops in the area. The low infection rate is due to massive and integrated cyclops control programme embarked upon by Global 2000 and [8] as part of activities for dracunculiasis eradication in the state. The objective of the eradication programme is the extermination of the parasite known as *Dracunculus medinensis* and not merely the Cyclopoid intermediate hosts. The evidence of infection of cyclops in the studied area presents a classical sign of erstwhile Guinea Worm free villages returning endemic should there be continued inadequate supply of portable water and the people continue to use pond as the their main water source. Seasonal variation exist in the abundance and infectivity of cyclops which were usually higher during the peak of the dry season (February – April) when the people source water from ponds and other source of water bodies. This is in agreement with observations of [11]. It has also been observed that the transmission potential of the cyclops depend solely on the abundance and levels of infectivity especially in stagnant ponds [16, 17].

It was observed during the study that the supporting Agencies (Global 2000, NIGEP, UNICEF and Yakubu Gowon Centre(YGC) as well as Ebonyi State Rural Water and Sanitation Agency(EBRUWASA) contributed towards eradication of dracunculiasis in the studied area through provision of safe water to the endemic communities. The state Government on its part made concise efforts to supply boreholes and hand dug wells to all communities in collaboration with UNICEF and NIGEP, but these have not been fully implemented as some communities are without boreholes or hand dug well. Global 2000 and NIGEP encouraged the communities to dig community wells. Consequently, many communities that the government has not supplied boreholes with were beginning to sink their own boreholes and hand-dug-wells for safe water supply. The state government ensures the provision of pipe borne water in some endemic areas. In addition, some seasonal streams in some of the endemic communities have been developed by NIGEP field Officers for all year round provision of clean water to all the endemic communities, and the communities are encouraged to maintain the streams by weeding the edges and surroundings. Monthly abate treatment of the village ponds with Abate for the control of the Cyclops are also the responsibility of the Global 2000. Global 2000 and NIGEP ensured that the filters are distributed to families in the endemic area to prevent ingestion of the Cyclops by the people. They train one village based health workers in each village except in some large villages where there could be two or more (VBHWs) to carry out these intervention strategies. There is also state, local government and village taskforce who see at each level that the activities are effectively carried out in the area. The State Taskforce (STF) on guinea worm eradication which functions within the Ministry of Health in Ebonyi State coordinates the activities of the eradication programme. Health Education was being carried out in all the communities and villages where the disease is endemic by NIGEP and Global 2000. [4] demonstrated the effectiveness of the combined use of filters and health education in dracunculiasis eradication and observed that inadequacy of filters may hinder the easy realization of the eradication. Another intervention strategy applied was the use of Abate to kill the cyclop intermediate hosts in the water bodies. It was observed that the water sources were treated with this chemical monthly by the Global/NIGEP field Officers on regular basis in the villages studied. There was no shortage of Abate and the villagers seemed to have accepted this intervention strategy. The Abate treatment of water in a synergistic manner with health education had reduced the abundance and infectivity rate of cyclops in the area. Some of the problems observed in this intervention strategy included under dosing or overdosing of the chemical during the water treatment, as determination of actual volume of any water sources takes a very tedious process due to shortage of trained personnel, inadequate number of NIGEP trained personnel and cultural belief of the people as earlier reported for some villages in Ishielu LGA where treatment of the pond is never accepted and transmission was high in the area. Other intervention strategies observed included case-containment strategy (CCS) and enforced legislation. However, it is established that due to peculiar epidemiology of the disease, people living in the contagious area with local water sources are still at risk of dracunculus infection. The result of the first stage study which showed 5529 abundance of Cyclops recorded in 2004/ 2005, when compared to the third stage result which recorded 1443 within May/June, it indicates that the Cyclops is more abundant in the area than during the guinea worm eradication period. This is because, during the period when guinea worm eradication activities were in progress, the community ponds were being treated with Abate as a measure for control of Cyclops which transmit the guinea worm disease but since after the eradication, those ponds were no being treated with Abate and Cyclops have started growing more in abundance. This has epidemiological implication. The situation could be dangerous if an infected guinea worm patient happen to introduce dracunculus larvae into the communal pond. The implication is that there could be re-emergence of dracunculus infected Cyclops and possible re-occurrence of guinea worm infection in the area already certified free of guinea by [18]. Therefore to ensure that there is no re-occurance of the disease in the area, the following co-ordinated and collaborative measures should be taken: Intensive surveillance of the guinea disease especially at the communities and village level by NIGEP with a view to ensuring early dictation of outbreak of the disease. Most of the community members have accepted health education aimed at avoiding contamination of water sources by infected persons, and prevention of drinking of unfiltered pond water. NIGEP and Global 2000 responsible for distribution of filters to the people should make more effort to supply filter to all households to ensure 100% filter coverage. Low coverage of the study area with safe water was observed in the course of this study. Most of the endemic villages visited for the study have only one hand dug well (HDW) or borehole (BH) or none at all and these people depend solely on pond water which is the main source of dracunculiasis. The safe water sources required for the communities could be boreholes, pipe borne water, deep hand dug well fitted with pump or properly protected spring water. More researches should be carried out on Cyclops and Dracunculus and Sampling of all drinking water sources to assess the effect of Abate treatment on the Cyclops density should be a matter of routine measure.

In conclusion having proclaimed the state along with other states in Nigeria free of guinea worm disease by WHO and by the findings of this study in which none of the Cyclops encountered were found infected with guinea worm larvae, it is therefore confirmed that guinea worm disease has been eradicated in Ebonyi State.

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