

Prevalence of *Paragonimus* Infection

¹Nworie Okoro, ²Reginald Azu Onyeagba, ³Chukwudi Anyim, ⁴Ogbuinya Elom Eda,
³Chukwudum Somadina Okoli, ⁵Ikechukwu Orji, ³Eucharia Chinyere Okonkwo,
³Uchechukwu Onyeukwu Ekuma and ³Maduka Victor Agah

¹Department of Biological Science, Faculty of Science and Technology, Federal University Ndufu Alike-Ikwo, Nigeria

²Department of Microbiology, Faculty of Biological and Physical Sciences, Abia State University, Uturu-Okigwe, Nigeria

³Department of Applied Microbiology, Faculty of Biological Science, Ebonyi State University, Abakaliki, Nigeria

⁴Hospital Management Board, Ministry of Health, Abakaliki, Nigeria

⁵Federal Teaching Hospital Abakaliki II (FETHA II), Abakaliki, Ebonyi, Nigeria

Received 2013-01-25, Revised 2013-02-19; Accepted 2013-05-20

ABSTRACT

Paragonimiasis (human infections with the lung fluke *Paragonimus westermani*) is an important public health problem in parts of Africa. This study was aimed at assessing the prevalence of *Paragonimus* infection in Ebonyi State. Deep sputum samples from 3600 individuals and stool samples from 900 individuals in nine Local Government Areas in Ebonyi State, Nigeria were examined for *Paragonimus* ova using concentration technique. The overall prevalence of pulmonary *Paragonimus* infection in the area was 16.30%. Six foci of the infection were identified in Ebonyi North and Ebonyi Central but none in Ebonyi South. The intensity of the infection was generally moderate. Of the 720 individuals examined, 16 (12.12%) had less than 40 ova of *Paragonimus* in 5 mL sputum and 114 (86.36%) had between 40 and 79 ova of *Paragonimus* in 5 mL sputum. While 2 individuals (1.52%) had over 79 ova of *Paragonimus* in 5 mL Sputum. Furthermore, there was higher prevalence of paragonimiasis in rainy season than in dry season. The results of this study indicated the growing public health threat posed by paragonimiasis in Ebonyi North and Ebonyi Central. A combination of chemotherapy, to bring relief to persons already infected by the disease and public health education related to paragonimiasis transmission to increase awareness of the infection in the areas is recommended.

Keywords: Paragonimiasis, Sputum, Prevalence, Chemotherapy, Intensity

1. INTRODUCTION

Paragonimiasis, also known as endemic haemoptysis, oriental lung fluke infection, pulmonary distomiasis, parasitical haemoptysis, parasitare haemopte, Gregarinosis pulmonum, is one of the most important food-borne parasitic zoonoses caused by one or more of the trematode species of the genus *Paragonimus*. The disease is endemic in many parts of Africa, Asia and South America (Procop, 2009). The natural definitive hosts of the parasite comprise large varieties of wild mammals of the canidae and felidae families and humans. A wide range of fresh water snails and crabs as well as crayfish served as first and second intermediate hosts, respectively (Miyazaki and Hirose,

1976). Infection in human occurs by ingestion of raw or undercooked freshwater crabs or crayfishes (Singh *et al.*, 2005). Paragonimiasis is an important cause of pulmonary infection in all races (Narain *et al.*, 2005). This disease is diagnosed in the laboratory by microscopic demonstration of *Paragonimus* ova in the sputum and other clinical specimens such as faces and pleural fluid or by specific *Paragonimus* serological tests (Shantikumar *et al.*, 2012).

The prevalence of many snail-borne and vector-borne parasitic diseases has been dramatically diminished in many areas where they were once common. However, the frequency for some food-borne parasitic diseases is increasing (Nawa, 1991; 2000). Just as the reemergence of tuberculosis has been associated

Corresponding Author: Nworie Okoro, Department of Biological Science, Faculty of Science and Technology, Federal University Ndufu Alike-Ikwo, Nigeria

with lapses in public health, so too paragonimiasis has also reemerged in areas of endemicity where public health measures of the past have also lapsed (Procop, 2009). *Paragonimus* infection has a gradual onset and is characterized by low grade fever, productive cough and occasionally, dry cough, night sweats, excruciating chest pain, diarrhea and blood stained, rust brown sputum (Heath and Marshall, 1997; Cheesbrough, 2005). This disease is frequently misdiagnosed as pulmonary tuberculosis. Indeed, pulmonary paragonimiasis has created overlapping public health problems in many countries where both diseases coexist (Asor *et al.*, 2003).

Paragonimiasis is a neglected but reemerging zoonotic parasite infection in Nigeria. It is now two and a half decades since endemic paragonimiasis was last reported in Southeastern Nigeria (Udonsi, 1987). Endemic foci has been reported in Enugu and the areas around the Imo and Cross Rive and their tributaries (Nwokolo, 1972), Igwun Igwun River and River Iduma including Abam, Arochukwu, Bende and Ohafia towns among others (Udonsi, 1987). Our personal observation in some new foci in Eastern Nigeria have revealed massive eating of the crab species *Sudanautes*, which has been earlier confirmed as the intermediate host of *Paragonimus uterobilateralis* in Eastern Nigeria (Udonsi, 1987). This study was therefore aimed to ascertain the prevalence of paragonimiasis among six Local Government Areas in Ebonyi State.

2. MATERIALS AND METHODS

2.1. Study Area

The study was carried out in Ebonyi State, South East Nigeria. The State is made up of thirteen (13) Local Government Areas viz: Abakaliki, Afikpo North, Ebonyi, Afikpo South, Ezza North, Ezza South, Ikwo, Ishielu, Ivo, Izzi, Ohaozara, Ohaukwu and Onicha.

The State has a population of about 1.8 million and a total land area of about 5,935 square kilometer (km²), which gives a population density of over 300 persons per square kilometer. The state is located between latitude 7° 30' East and 8° 30' west and longitude 5° 40' North and 6° 45' South within the rain forest zone of Nigeria. The annual rain is over 10 mm. the mean daily maximum and minimum temperature are 32°C and 25°C, respectively.

The fertile and rich soils of Ebonyi State encourage large-scale agriculture. Available statistics indicate that agriculture provides productive employment to over 85% of Ebonyians. Thus, agriculture is the mainstay of the

State's economy. Nevertheless, some Ebonyians engage in fishing (crayfish) and hunting of animals including crabs.

Also, some of the samples used in this study were collected in the Microbiology Laboratory of the following major hospitals: Federal Teaching Hospital Abakaliki II (FETHA II), Mile Four Hospital Ishieke, Presbyterian Joint Hospital Uburu and Mater Misericordiae Hospital Afikpo. Other samples were collected from Health Centres located at Ikwo, Effium, Ngbo, Isu, Ishielu and Ugwulangwu.

2.2. Nature of Sample

Two types of samples were collected viz: Sputum and stool.

2.3. Sample Size

A total of three thousand six hundred (3,600) sputum samples and nine hundred (900) stool samples, were collected and analyzed.

2.4. Collection of Samples

Heads of selected villages in the study area were informed before the sampling dates. This was to enable them inform and educate their wards on the importance of the study. The technique employed in sputum collection was the method of Cheesbrough (2005).

2.5. Examination of Specimen

Direct Microscopic examination of sputum from which direct smears were made were those highly suspected to be positive for paragonimiasis especially those showing haemotypsis. This was done as to culture the samples immediately. Using a plastic bulb pipette, a drop of the desired portion of the sputum was transferred to a clean glass slide and covered with a cover slide. The cover slide was gently pressed to make a thin even spread preparation and to exclude any air bubbles. Then, the preparation was carefully observed under 10X and 40X objective lenses using low light intensity.

2.6. Sputum Examination using Concentration Technique

Five milliliter (5 mL) of the thorough mixed sputum sample was put into a centrifuge and 5 mL 10% sodium hydroxide (NaOH) solution was added and allowed to stand for 10 min. The NaOH dissolved the mucus in the sputum sample and lysed the Red Blood Cells (RBCs), leaving the sputum clear, after centrifuging the mixture at 2,000 Revolution Per Minute (RPM) for 10 min, the supernatant was discarded; with a graduated pipette, a wet preparation

of the sediment was viewed under the low power (10X) and high (40X) objective lens of the microscope.

Observation of the characteristics golden yellow operculate eggs of paragonimiasis in the sputum sample identified positive sample.

2.7. Examination of Stool Sample Using Concentration Technique

About 1 g of faecal material was emulsified in 10% formol saline (15 mL). This was mixed thoroughly and sieved. Exactly 1 mL filtrate and 3 mL ether were mixed together, then corked with stopper and shaken 3 times. This was spun at 2,500 rpm for 10 min. The supernatant decanted.

2.8. Intensity of *Paragonimus* Infection

Exactly 5 mL of sputum was emptied into a sterile Petri dish using a sterile 5 mL pipette. The content of the Petri dish was placed on the stage of illuminating microscope and examined for the presence of *Paragonimus* eggs. The total egg count per 5 mL were observed and reported.

2.9. Examination of Sputum by Saline Preparation for *Paragonimus*

A small quantity of sputum especially that which was coloured and stingy was transferred on to a slide. A drop of physiological saline was added, mixed and covered with a cover glass. Using the 10X objective with the condenser iris diaphragm closed sufficiently to give good contrast, the preparation was examined for *Paragonimus* egg. Positive preparations revealed golden yellow operculate eggs.

2.10. Ethical Clearance

Ethical approval for the study was obtained from Ministry of Health in the nine respective Local Government Areas in Ebonyi State, which are Abakaliki, Ebonyi, Ohaukwu, Ikwo, Ezza North, Ishielu, Afikpo North, Onicha and Ohaozara.

3. RESULTS AND DISCUSSION

This study investigated the prevalence of paragonimiasis infections in Ebonyi State. A total prevalence of 16.3% (587 out of 36000) was observed in this study (**Table 1**). This result is close to the 12.27% infection rate observed by Arene *et al.* (1998) in Cross River State; the 14.4% noticed by Asor *et al.* (2003) in Okigwe. Also, the 9.36% noticed by Ibenga *et al.* (1997) all in Cross River State and the 19.7% recorded by Uttah *et al.* (2013) all in Cross River State.

Table 1. Relationship between *Paragonimus* infection and location in nine Local Government Areas (LGAs) in Ebonyi State

Location	Number examined	Number positive	Prevalence of infection (%)
Abakaliki LGA	400	206	51.50
Ebonyi LGA	400	90	22.50
Ohaukwu LGA	400	70	17.50
Ikwo LGA	400	78	19.50
Ezza North LGA	400	80	20.50
Ishielu LGA	400	41	10.25
Afikpo North LGA	400	10	2.50
Onicha LGA	400	07	1.75
Ohaozara LGA	400	05	1.25
Total	3600	587	16.30

This result also ranks Ebonyi State as one of the major foci of paragonimiasis in Nigeria.

Also, age had some significant influence of the distribution and prevalence of *Paragonimus* infection in the studied areas ($p \leq 0.05$). Children of 11-15 years were more infected than other age brackets with percentage prevalence of 11.50% (414/3600) followed by those with 16-20 years of age (2.56%). Children in the studied area frequently collected crabs from nearby streams especially at 11-15 years of age (**Table 2**); while doing their collection, the children occasionally consume raw, broken-off parts of the crabs' tissues and hence may contribute to the higher prevalence of the infection recorded in children. However, before 10 years, most children are not courageous enough to go hunting for crabs in the area while above 20 years might leave the villages for urban areas or for higher schools. The result is in consonance with the results of (Ibenga *et al.*, 1997; Arene *et al.*, 1998; Uttah *et al.*, 2013) who independently reported highest prevalence rate in 11-16 years of age. Nevertheless, this result disagrees with the work of Udonsi (1987) in Cross River who reported that individuals within the age range of 10-12 years were more infected than others.

More so, the present study revealed that males (11.11%) were significantly more infected than females (5.19%) ($p \leq 0.05$) (**Table 3**). This too agrees with the reports made by (Singh *et al.*, 1986; Udonsi 1987; Uttah *et al.*, 2013) but disagrees with the results of (Ibenga *et al.*, 1997; Asor *et al.*, 2003; Uttah, 2013) who reported that more females were infected than males. Many other researchers have reported no sex-related difference in prevalence (Uchiyama *et al.*, 1999; Ashitani *et al.*, 2000). It is pertinent to note that, although both sexes can acquire the infection through food or by wrong sense of remedy to certain ailments, the males encounter crabs more than females.

Table 2. Relationship between paragonimiasis and age in nine Local Government Areas (LGAs) in Ebonyi State

Age range (yrs) location	6-10			11-15		16-20		21-25	
	NE	NI	PR (%)	NI	PR (%)	NI	PR (%)	NI	PR (%)
Abakaliki	400	18	4.50	108	27.00	62	15.50	18	4.50
Ebonyi	400	07	1.75	68	17.00	12	3.00	03	0.75
Ohaukwu	400	06	1.50	61	15.25	03	0.75	00	0.00
Ikwo	400	10	2.50	65	16.25	02	0.50	01	0.25
Ezza North	400	04	1.00	60	15.00	06	1.50	10	2.50
Ishielu	400	01	0.25	30	7.50	07	1.75	03	0.75
Afikpo North	400	00	0.00	10	2.50	00	0.00	00	0.00
Onicha	400	00	0.00	07	1.75	00	0.00	00	0.00
Ohaozara	400	00	0.00	05	1.25	00	0.00	00	0.00
Total	3600	46	1.28	414	11.50	92	2.56	35	0.97

Key: NE = Number Examined, NI = Number Infected and PR (%) = Percentage Prevalence Rate

Table 3. Relationship between *Paragonimus* infection and sex in nine Local Government Areas (LGAs) in Ebonyi State

Location	Male			Female	
	NE	NI	PR (%)	NI	PR (%)
Abakaliki	400	132	33.00	74	18.50
Ebonyi	400	77	19.25	13	3.25
Ohaukwu	400	62	15.50	08	2.00
Ikwo	400	30	7.50	48	12.00
Ezza North	400	57	14.25	23	5.75
Ishielu	400	21	5.25	20	5.00
Afikpo North	400	10	2.50	00	0.00
Onicha	400	06	1.50	01	0.25
Ohaozara	400	05	1.25	0	0.00
Total	3600	400	11.11	187	5.19

Table 4. Relationship between *Paragonimus* infection and occupation of individuals in nine LGAs in Ebonyi State

Location	Cl			Fa			Fi			St			Tr			Total		
	NE	NI	%Inf	NE	NI	%Inf	NE	NI	%Inf	NE	NI	%Inf	NE	NI	%Inf	NE	NI	%Inf
Abakaliki	80	36	45.00	80	38	47.50	80	32	40.00	80	66	82.50	80	34	42.50	400	206	51.50
Ebonyi	80	4	5.00	80	20	25.00	80	16	20.00	80	40	50.00	80	10	1.30	400	90	22.50
Ohaukwu	80	7	8.80	80	13	16.30	80	14	17.50	80	30	37.50	80	6	7.50	400	70	17.50
Ikwo	80	8	10.00	80	28	35.00	80	10	12.50	80	40	50.00	80	2	2.50	400	78	19.50
Ezza north	80	7	8.80	80	25	31.30	80	8	10.00	80	27	33.80	80	13	16.30	400	80	20.00
Ishielu	80	2	2.50	80	6	7.50	80	2	2.50	80	27	33.80	80	4	5.00	400	80	20.00
Afikpo north	80	0	0.00	80	0	0.00	80	0	0.00	80	10	12.50	80	0	0.00	400	10	2.50
Onicha	80	0	0.00	80	0	0.00	80	0	0.00	80	7	8.78	80	0	0.00	400	7	1.75
Ohaozara	80	0	0.00	80	0	0.00	80	0	0.00	80	5	6.25	80	0	0.00	400	5	1.25
Total	720	64	8.89	720	130	18.06	720	82	11.39	720	252	35.00	720	69	9.58	720	626	86.94

Key: NE = Number Examined, NI = Number Infected, %Inf = Percentage Infected, Cl = Civil Servants, Fa = Farmers, Fi = Fishermen, St = Students and Tr = Traders

The males are involved in crab hunting, fishing and similar activities which bring them close to crabs during which crab limbs are often chewed raw thereby becoming infected by this means.

Furthermore, the present study revealed that there was a significant ($p \leq 0.05$) variation between *Paragonimus* infection and location as various levels of infection in the nine LGAs studied in Ebonyi State

were recorded. The result revealed that Abakaliki LGA had the highest prevalence rate of 51.50% followed by Ebonyi LGA (22.50%), Ezza North LGA (20.0%), Ishielu LGA (20.00%) and then Ikwo LGA, Ohaukwu, Afikpo North and Onicha LGAs recorded 19.50, 17.5, 2.5 and 1.75% respectively while the least prevalence rate, 1.25% was observed in Ohaozara LGA (Table 4).

Table 5. Relationship between *Paragonimus* infection and location using stool specimens

Location	Number examined	Number infected	Prevalence of infection (%)
Abakaliki	100	4	4.00
Ebonyi	100	1	1.00
Ohaukwu	100	1	1.00
Ikwo	100	1	1.00
Ezza north	100	0	0.00
Ishielu	100	0	0.00
Afikpo north	100	0	0.00
Onicha	100	0	0.00
Ohaozara	100	0	0.00
Total	900	7	0.78

Table 6. Distribution of number of persons infected with *Paragonimus* in nine LGAs in Ebonyi State

Location	NE	NI	<20	20-39	40-59	60-79	80-99	100
Abakaliki	80	28	0	2	4	20	2	0
Ebonyi	80	22	0	1	3	18	0	0
Ohaukwu	80	20	0	2	18	0	0	0
Ikwo	80	17	0	2	10	5	0	0
Ezza north	80	19	0	1	11	7	0	0
Ishielu	80	18	0	0	11	7	0	0
Afikpo north	80	5	2	3	0	0	0	0
Onicha	80	6	3	3	0	0	0	0
Ohaozara	80	2	1	1	0	0	0	0
Total	720	137	6	15	57	57	2	0

This result is in agreement with the result obtained by Asor *et al.* (2003) who suggested that the prevalence of human paragonimiasis was higher in Upper District part of Cross River Basin than in Lower District. The highest prevalence of *Paragonimus* infection recorded in Abakaliki LGA could be attributed to poor knowledge of the biology of the parasite and transmission mechanism of the disease, which permitted unrestricted consumption of raw or partially cooked crabs. Also, the abundance of the intermediate hosts (crab and snail) in the most affected areas cannot be over emphasized; the crabs and snails were commonly hawked along Abakaliki-Enugu expressway, Ishieke Junction as well as Abakaliki-Afikpo road by Akpoha bride. The low prevalence observed in Ohaozara could have resulted from the scarcity of the intermediate hosts in the area. The few cases recorded might have been acquired as a result of migrations to areas where paragonimiasis is endemic in Ebonyi State.

In terms of occupation, this study revealed that *Paragonimus* infection was most prevalent among students with 35.0% prevalence rate followed by farmers (18.06%), fishermen (11.39%), traders (9.58%) and lastly civil servants (8.89%) (Table 4). This was probably because those people living in the various areas

examined especially the highly infected areas fell within the age group (11-15 years) that usually engaged themselves in hunting for crabs and snails and other water related activities after school periods. The possibility of these students eating the crabs raw or undercooked cannot be over emphasized. The prevalence rates recorded in farmers and fishermen can be attributed to the nature of their works, which bring them closer to the intermediate host, thereby promoting *Paragonimus* infection through the consumption of partially cooked crabs and snails. However, the civil servants recorded the least in prevalence owing to the fact that they could have been enlightened on the transmission mechanisms of food borne infections, especially paragonimiasis. Therefore, the low prevalence rate (8.89%) recorded among civil servants who were obstinate and never believed they could contract the disease.

The study revealed a very low prevalence of *Paragonimus* infections with stool samples. A total prevalence rate of only 0.78% (7 out of 900) was obtained, out of 900 stool samples examined for *Paragonimus* eggs, only 7 were positive for the parasites eggs. Abakaliki has the highest prevalence rate of 4 (4.0%) followed by Ebonyi, Ohaukwu and Ikwo Local Government Areas (LGAs) with 1(1.0%) prevalence rates of infection each. However, no egg(s) were detected in the stool samples examined in Ezza North, Ishielu, Afikpo North, Onicha and Ohaozara LGAs (Table 5). The low prevalence recorded compared to that obtained in sputum sample could be attributed to the fact that paragonimiasis is a lung infection and eggs are expected to occur highly in sputum and not in stool samples. It is few cases of swallowing of sputum that probably gave rise to the occurrence of the few eggs observed in the stool samples examined.

In terms of distribution of number of persons infected with *Paragonimus*, Abakaliki recorded the highest (28), followed by Ebonyi (22) and Ohaukwu (20). While Ohaozara recorded the least (2) (Table 6). The result revealed that Abakaliki LGA had the highest prevalence rate followed by Ebonyi and Ohaukwu, while Ohaozara had the least prevalence rate. The current study also showed that the intensity of *Paragonimus* infection (number of eggs in 5 mL sputum) declined with age (from 20 years of age onwards). In all the LGAs examined, the highest intensity of *Paragonimus* infections was recorded within 9-11 years. This compared favourably with the result obtained by Arene *et al.* (1998) who demonstrated that the highest intensity of infection was observed in subjects within the age of 10-21 years old.

Table 7. Relationship between age and intensity (eggs/ mL of sputum) of *Paragonimus* infection in three LGAs in Ebonyi State

Location	Age	NE	1-20	21-40	Above 40	Total
Ikwo	6-8	6	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
	9-11	16	7(43.75%)	6(37.50%)	1(6.25%)	14(87.50%)
	12-14	16	4(25.00%)	10(62.50%)	0(0.00%)	14(87.50%)
	15-17	16	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
	18-20	16	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
	Total	70	11(15.71%)	16(22.86%)	1(1.43%)	28(40.00%)
Afikpo north	6-8	6	0(0.00%)	3(50.00%)	0(0.00%)	3(50.00%)
	9-11	16	1(6.25%)	8(50.00%)	0(0.00%)	9(56.25%)
	12-14	16	0(0.00%)	3(18.75%)	1(6.25%)	4(25.00%)
	15-17	16	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
	18-20	16	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
	Total	70	1(1.43%)	14(20.00%)	1(1.43%)	16(22.86%)
Ikwo	6-8	6	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
	9-11	16	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
	12-14	16	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
	15-17	16	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
	18-20	16	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
	Total	70	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)

Table 8. Relationship between intensity (eggs/ mL of sputum) of *Paragonimus* infection and occupation of individuals in three LGAs in Ebonyi State

Location	Eggs/5 mL of sputum	Civil servant	Farmers	Fisher men	Students	Traders	Total
Abakaliki	1-20	3(13.04%)	7(30.43%)	2(8.70%)	7(30.43%)	4(17.39%)	23(82.14%)
	21-40	0(0.00%)	0(0.00%)	0(0.00%)	2(50.00%)	2(50.00%)	4(14.29%)
	Above	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	1(100.00%)	1(3.57%)
	Total	3(10.71%)	7(25.00%)	2(7.14%)	9(32.14%)	7(25.00%)	28(100.00%)
Ikwo	1-20	0(0.00%)	0(0.00%)	0(0.00%)	8(100.00%)	0(0.00%)	8(50.00%)
	21-40	0(0.00%)	2(28.57%)	0(0.00%)	5(71.43%)	0(0.00%)	7(43.75%)
	Above	0(0.00%)	1(100.00%)	0(0.00%)	0(0.00%)	0(0.00%)	1(6.25%)
	Total	0(0.00%)	3(18.75%)	0(0.00%)	13(81.25%)	0(0.00%)	16(100.00%)
Afikpo north	1-20	0(0.00%)	0(0.00%)	0(0.00%)	3(100.00%)	0(0.00%)	3(60.00%)
	21-40	0(0.00%)	2(100.00%)	0(0.00%)	0(0.00%)	0(0.00%)	2(40.00%)
	Above	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
	Total	0(0.00%)	2(40.00%)	0(0.00%)	3(60.00%)	0(0.00%)	5(100.00%)

This result still agrees with the fact that highest infection occurred with 11-15 years (**Table 7**). In terms of occupation, this study revealed that the highest intensity of *Paragonimus* infection (number of eggs in 5 mL sputum) was observed in students followed by farmers (**Table 8**). This underscores the possibility of these students eating raw or inadequately cooked crabs or snails. The intensity observed in farmers could be attributed to the nature of their occupation, which brings them closer to the intermediate host, hence promoting *Paragonimus* infection.

4. CONCLUSION

The result of this study notifies the need to increase public health awareness campaign in the epidemiology, clinical manifestations and control of paragonimiasis in

Ebonyi State. Subsequently, mass education and awareness campaign to incite changes in food preparation practices and customs should be emphasized in Ebonyi State. Sputum examination for *Paragonimus* eggs is essential before concluding a case as paragonimiasis negative. A combination of chemotherapy, to bring relief to persons already infected by the disease is recommended.

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