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HUMAN PARAGONIMIASIS IN TWO COASTAL COMMUNITIES IN CALABAR

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Abstract

This study was aimed at investigating the epidemiology of paragonimiasis in two selected coastal communities in Calabar suburb, namely Akpabuyo and Calabar South. Seven sputum examinations per person were carried out for the presence of *Paragonimus uterobilateralis* eggs/ova. Structured questionnaire was administered for information regarding biodata, clinical manifestations, and crab-eating characteristics. Frequency of active coughing per 15 minutes by each candidate during the contact was recorded. The overall prevalence was 28.2% and 15.6% respectively. Prevalence was comparable in both sexes, increased with age, and highest among fishermen/women, farmers, and artisans. The Geometric Mean Intensity of *Paragonimus* eggs/ova was 87 and 63 per 5ml^{-1} of sputum respectively, and comparable between the sexes. In conclusion, paragonimiasis is endemic in the study areas. Urgent steps are needed to cur the scourge.

1. Introduction

Paragonimiasis is a neglected but re-emerging zoonotic parasite infection in Nigeria (Uttah *et al*., 2013). Endemic foci in eastern parts of the country have been identified to be around Enugu and the areas around the Imo and Cross River and their tributaries (Nwokolo, 1972), Cross River Basin (Arene *et al*., 1998), Igwun River and River Iduma basins including Abam, Arochukwu, Bende and Ohafia towns among others (Udonsi, 1987). Edible crab species Sudanautes has been earlier confirmed as the intermediate host of *Paragonimus uterobilateralis* in southeastern Nigeria (Udonsi, 1987). *Paragonimus* species are widely distributed in areas in which people habitually eat raw or undercooked freshwater crabs/crayfishes (Nakamura-Uchiyama *et al*., 2002). Paragonimiasis leads to pulmonary infection (Narain *et al*., 2005).

The diagnostic overlap of paragonimiasis with tuberculosis has been reported (Lane *et al*., 2009). Preliminary investigations into the crab-eating behaviour of an endemic population have been reported in six communities from two ethnocultural clusters in South-eastern Nigeria and observations indicate that the risk of paragonimiasis is affected by the frequency of eating of Sudanautes (Uttah *et al*., 2013).

Endemic foci have been reported in the Cross River Basin (Arene *et al*., 1998; Asor *et al*., 2003; Ibanga *et al*., 2003). High prevalence of the paragonimiasis disease have been found in areas contiguous to Akpabuyo and Calabar South, the two intended study areas (Kingdon, 2005; Abraham and Akpan, 2011).

Food shortages or unaffordability have led to increased consumption of inadequately cooked or raw crab, causing dramatic increase in cases of paragonimiasis (Nwokolo, 1972). In Nigeria patients have been seen in all age groups (Udonsi, 1987). Massive eating of crabs and crayfish has continued in parts of south-eastern Nigeria decades after second outbreak of paragonimiasis in the area (Uttah *et al*., 2013). Recent surveys reveal resurgence of paragonimiasis in south-eastern Nigeria (Aka *et al*., 2008; Blair, 2014).This study was aimed at determining the prevalence of human paragonimiasis in Akpabuyo and Calabar South communities.

2. Materials and methods

2.1 Description of the study area

The study was carried out in two riverine communities, namely Akpabuyo and Calabar South in Akpabuyo and Calabar South Local Government Areas respectively. These two communities are within the rain forest zone of Cross River State, Nigeria; but Akpabuyo is of rural setting while Calabar South is of semi-urban setting.

2.2 Akpabuyo

Akpabuyo is in Akpabuyo Local Government Area of Cross River State, which has an area of 28.5 km² and a population of 271,395 at the 2006 Census. Akpabuyo Local Government Area lies between latitude 4° 5' and 5° 40' and longitude 8° 25' and 8° 32 East. It lies within the vegetation belt of southern Nigeria and shares the Atlantic coastline with Bakassi to the East and the Republic of Cameroon to the West. The major languages spoken are Efik and English, while all the major ethnic groups share a common cultural and ancestral heritage. In these communities, crabs are caught by people in all categories of occupation for both subsistence and commercial purposes. The people of Akpabuyo are predominantly fishermen/ women, farmers and artisans. The area is rural in setting and is a monolithic population comprising mainly of the indigenous people. Akpabuyo is predominantly an agricultural area, and it is one of the Food Baskets of Cross River State. It produces cassava, cocoyam, kola nut, coconut, palm produce as well as sea foods. The land is rich in mineral deposits such as petroleum deposits, gold, limestone, sand and salt deposits to mention a few.

2.3 Calabar South

This study was conducted in Henshaw Town in Calabar South and is known for the Henshaw Town Beach Market (or Marina Market, or Beach Market). The town has an area of 264 km² and a population of 191,630 from the 2006 Census. The area hosts a great influx of non-indigenous people from other regions of Nigeria. The area is semi-urban in setting and is comprised of people of various occupations including fishermen, artisans, traders, students, and civil servants. In this community, crabs are regarded as traditional delicacy and are caught, sold in markets in the local or neighbouring communities.

2.4 Public enlightenment and mobilization for the study

The village heads and leaders of thought were consulted and fully intimated about the research objectives and other details. This was to enable wider sensitization of the entire population on the objectives of the study and the methods to be employed in the study.

2.5 Enlistment into the study

The study population was randomly selected from the two communities. Every third consenting person was selected and enlisted into the study. For minors, the consent of their parents or guardians weresought before they were enlisted. For Akpabuyo, a total of 1,780 persons were selected into the study, out of which 593 persons were enlisted into the study. For Calabar South, a total of 2,774 persons were selected into the study, out of which 924 persons were enlisted.

2.6 Administration of structured questionnaire

A well-structured questionnaire were administered on all consenting persons, but for minors, their parents or guardians provided answers on their behalf. The structured questionnaire consisted of questions such as sex, age, occupation, crab-eating status, crab-eating frequency, active cough, haemoptysis, chest pain, and other questions.

3. Collection of Samples

All consenting persons were provided with vials and asked to use them to provide their sputum. All the vials were retrieved the following morning, and brought to the laboratory. The method of Cheesbrough (2005) was employed in the sputum collection.

3.1 Examination for *Paragonimus* spp: Sputum samples were examined for the presence of *Paragonimus uterobilateralis* eggs/ova to provide a diagnosis of paragonimiasis. To make up for the sensitivity concerns of sputum examination for detection of eggs (Blair *et al*., 2007), seven sputum examinations per person were carried out (Toscano *et al*., 1995). All individuals found to have eggs in their sputum were treated with praziquantel at a dose of 3×25 mg/kg of body weight per day for 3 consecutive days (Nkouawa *et al*., 2009).

3.2 Direct Microscopic examination of sputum: Direct smears of sputum samples especially those with haemoptysis were made for culturing. Using a plastic bulb pipette, a drop of the desired portion of the sputum were transferred to a clean glass slide and covered with a cover slide. The preparations were carefully observed under 10X and 40X objective lenses using low light intensity.

3.3 Sputum Examination using Concentration Technique: Concentration technique was employed too. Five millilitres (5 mL) of the thoroughly mixed sputum sample were put into a centrifuge and 5 mL 10% sodium hydroxide (NaOH)solution was added and allowed to stand for 10 minutes, then centrifuged at 2,000 Revolution Per Minute (RPM) for 10 minutes, and discarded the supernatant. A wet preparation of the sediment was viewed under the low power (10X) and high (40X) objective lens of the microscope.

3.4 Examination of Sputum by Saline Preparation for Paragonimus: For coloured and stingy sputum samples, small quantity of sputum were transferred on to a slide. Then a drop of physiological saline was added, mixed and covered with a cover glass. The preparation was examined for *Paragonimus* egg using the 10X objective with closed condenser iris diaphragm.

3.5 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 was observed and reported. The intensity was calculated by taking the average epg of triplicate samples for each individual. Egg counts of 1–50 eggs (or ova) per 5 mL−1 sputum, 51–100 eggs (or ova) per5 mL−1 sputum, and above 100 eggs (or ova) per 5 mL−1 was regarded as low, moderate, and high intensities, respectively after Uttah (2013b).

4. Data analysis

The Epi Info version 6.0 was used in entering data, and SPSS for windows used for data analysis. The geometric mean intensity (GMI) of eggs/ova was calculated as antilog (∑log(x +1), with x being the egg/ova counts per 5 mL of sputum in infected individuals and the number of infected individuals examined. Proportions such as prevalence and percentages were tested using Chi-square or Fisher's exact test, while the relative risks of infection was assessed using the Odds ratio (OR). Differences in intensity was tested using Students't-test or ANOVA. P-values < 0.05 will be considered statistically significant.

5. Results

Prevalence of *Paragonimus* infection in relation to age and sex

The prevalence of *Paragonimus* infection in relation to age and sex in Akpabuyo is presented in Table 1. The overall prevalence was 28.2%. The prevalence between males (27.7%) and females (28.6%) were comparable ($\varkappa^2$-test; p > 0.05). Prevalence increased with age.

The prevalence of *Paragonimus* infection in relation to age and sex in Calabar south is presented in Table 2. The overall prevalence was 15.6%. The prevalence was comparable (p<0.05) between males (15.1%) and females (16.1%). 0.05). Prevalence increased significantly with age from 6.0% in the youngest age group to 36.7% in the oldest age group ($\varkappa^2$-test; p < 0.05 for all tests).

5.1 Intensity of *Paragonimus* infection

The intensity of *Paragonimus* infection in relation to age and sex in Akpabuyo is presented in Table 3. The overall Geometric Mean Intensity (GMI) of *Paragonimus* eggs/ova in Akpabuyo was 87 per $5ml^{-1}$ of sputum. The GMI of females (89 per $5ml^{-1}$ of sputum) was higher than that of males (85 per $5ml^{-1}$ of sputum), but the difference was not statistically significant (t-test; p > 0.05).

The intensity of *Paragonimus* infection in relation to age and sex in Calabar South is presented in Table 4. The overall Geometric Mean Intensity (GMI) of *Paragonimus* eggs/ova in Calabar South was 63 per $5ml^{-1}$ of sputum. The GMI between the sexes was comparable (t-test; p > 0.05).

Table 1. Prevalence of *Paragonimus* infection in relation to age and sex in Akpabuyo

Age group (years)	Males		Females		Total	
	Examined	No. positive (%)	Examined	No. positive (%)	Examined	No. positive (%)
1-9	62	6 (9.7)	65	5 (7.7)	127	11 (8.7)
10-19	72	13 (18.1)	69	11 (15.9)	141	24 (17.0)
20-39	68	17 (25.0)	78	26 (33.3)	146	43 (29.5)
40-59	64	30 (46.9)	68	32 (47.1)	132	62 (47.0)
60+	23	14 (60.9)	24	13 (54.2)	47	27 (57.4)
Total	289	80 (27.7)	304	87 (28.6)	593	167 (28.2)

Table 2. Prevalence of *Paragonimus* infection in relation to age and sex in Calabar south

Age group (years)	Males		Females		Total	
	Examined	No. positive (%)	Examined	No. positive (%)	Examined	No. positive (%)
1-9	90	6 (6.7)	92	5 (5.4)	182	11 (6.0)
10-19	105	9 (8.6)	124	14 (11.3)	229	23 (10.0)
20-39	132	22 (16.7)	137	23 (16.8)	269	45 (16.7)
40-59	80	16 (20.0)	85	20 (23.5)	165	36 (21.8)
60+	38	14 (36.8)	41	15 (36.6)	79	29 (36.7)
Total	445	67 (15.1)	479	77 (16.1)	924	144 (15.6)

Table 3. Geometric Mean Intensity of *Paragonimus* eggs/ova in relation to age and sex in Akpabuyo

Age group (years)	Males per 5 ml^{-1}	Females per 5 ml^{-1}	Total per 5 ml^{-1}
1-9	18	20	19
10-19	25	23	24
20-39	42	46	44
40-59	87	101	94
60+	122	134	128
Total	85	89	87

Table 4. Geometric Mean Intensity of *Paragonimus* eggs/ova in relation to age and sex in Calabar South

Age group (years)	Males	Females	Total
1-9	9	9	9
10-19	11	10	11
20-39	22	28	25
40-59	66	72	69
60+	88	78	83
Total	62	65	63

5.2 Prevalence of *Paragonimus* infection in relation to occupation

The prevalence of *Paragonimus* infection in relation to occupation in Akpabuyo is presented in Table 5. Prevalence was highest (62.9%) among those in fishing and least among the preschool children (0.0%) and civil servants (6.8%). The prevalence among fishermen/ women was significantly higher than prevalence in any other occupation in Akpabuyo (χ^2-test; $p < 0.05$ for all tests). The second highest prevalence was observed among artisans for males and among traders for females.

The prevalence of *Paragonimus* infection in relation to occupation in Calabar South is presented in Table 6. Prevalence was highest (59.5%) among those in fishing and least among the preschool children (0.0%) and civil servants (0.6%). The prevalence among fishermen/ women was significantly higher than prevalence in any other occupation in Calabar South ($\varkappa^2$-test; $p < 0.05$ for all tests). Among male's prevalence was comparable between traders and artisans ($\varkappa^2$-test; $p > 0.05$), while among females prevalence was significantly higher among artisans than among traders ($\varkappa^2$-test; $p < 0.05$).

Table 5. Prevalence of *Paragonimus* infection in relation to sex and occupation in Akpabuyo

Occupation	Males		Females		Total	
	Examined	No. positive (%)	Examined	No. positive (%)	Examined	No. positive (%)
Preschool	22	0 (0.0)	26	0 (0.0)	48	0 (0.0)
Student	99	12 (12.1)	101	14 (13.9)	200	26 (13.0)
Fishing	53	32 (60.4)	44	29 (65.9)	97	61 (62.9)
Farming	36	12 (33.3)	43	16 (37.2)	79	28 (35.4)
Trading	27	10 (37.0)	51	21 (41.2)	78	31 (39.7)
Artisan	28	12 (42.9)	19	6 (31.6)	47	18 (38.3)
Civil servant	24	2 (8.3)	20	1 (5.0)	44	3 (6.8)
Total	289	80 (27.7)	304	87 (28.6)	593	167 (28.2)

Table 6. Prevalence of *Paragonimus* infection in relation to sex and occupation in Calabar South

Age group (years)	Males		Females		Total	
	Examined	No. positive (%)	Examined	No. positive (%)	Examined	No. positive (%)
Preschool	32	0 (0.0)	37	0 (0.0)	69	0 (0.0)
Student	104	11 (10.6)	118	10 (8.5)	222	21 (9.5)
Fishing	63	33 (52.4)	58	39 (67.2)	121	72 (59.5)
Farming	8	1 (12.5)	11	3 (27.3)	19	4 (21.5)
Trading	89	12 (13.5)	136	17 (12.5)	225	29 (12.9)
Artisan	76	10 (13.2)	21	7 (33.3)	97	17 (17.5)
Civil servant	73	0 (0.0)	98	1 (1.0)	171	1 (0.6)
Total	445	67	479	77	924	144

6. Discussion

Paragonimiasis is endemic in the study communities, especially in the rural Akpabuyo with an indigenous monolithic population with an age-long tradition of crab-eating. The prevalence of 28.2% was significantly higher than 15.6% reported at proximal but semi-urban Calabar South that hosts a lot of settlers from other regions of Nigeria. Prevalence of paragonimiasis varies from locality to locality. Prevalence reported to be 13.2% in other communities in south-eastern Nigeria (Uttah, 2013a). Geographic regions that have or have had high prevalence of paragonimiasis include Cameroon, which is contiguous to the study areas in Calabar suburbs in the eastern flank of southern Nigeria. Prevalence is equally high in other regions of the world, and ranged from 7 to 15% in the general population in India (Singh *et al*., 2012), but reportedly higher among tuberculosis patients than in the general population (Singh *et al*., 2009). Most times, paragonimiasis is highly underdiagnosed (Roy*et al*., 2016).

Prevalence of infection could be higher than reported in this study but for the suboptimal sensitivities of the method adopted in this study, that is, the use of eggs/ova in sputum (Vidamaly *et al*., 2009), compared to the good sensitivity and specificity of the ELISA method, especially ELISA using partially purified cysteine proteinases method (Ikeda *et al*., 1996; Nkouawa *et al*., 2009). However, the repeated sputum examinations per person adopted in this study are known to improve the sensitivity of the parasitological method (Toscano, 1995). Another limitation imposed on this study by the parasitological method adopted is that the egg/ova detection in sputum cannot detect those in the pre-patent period of infection or extrapulmonar paragonimiasis (Mahajan, 2005).

The prevalence was comparable between males and females in this study. This is in tandem with the findings in neighbouring southwest area of Cameroon, where prevalence was comparable in both sexes (Moyou-Somo and Tagni-Zukam, 2003). Furthermore, published reports elsewhere indicate that prevalence of paragonimiasis is without sex-related differences (Uchiyama *et al*., 1999; Ashitani *et al*., 2000; Mukae *et al*., 2001; Nakamura-Uchiyama *et al*., 2001). This could be explained by the fact that crab-eating behaviour was also comparable between the sexes in this study. However, a study in proximal south-eastern Nigeria reported a significantly higher prevalence among females than among males (Uttah, 2013). In contrast Singh and co-workers reported a higher prevalence among male patients in Manipur, India (Singh *et al*., 1986). However, all the published reports agree that paragonimiasis is found in children, adults, and all categories of persons (Ashitani *et al*., 2000). Furthermore, prevalence pattern in populations could be determined by the crab-eating pattern by that population (Uttah, 2013).

Age-related prevalence of paragonimiasis varies from locality to locality. Prevalence in the two populations in this study increased with age. This corroborates earlier findings in Nigeria that patients have been seen in all age groups (Udonsi, 1987), and is also comparable to that of another study in neighbouring south-eastern Cameroun (Nkouawa *et al*., 2009). However some studies in India reported of higher prevalence among younger age groups than among the older ones. One of such studies reported sputum egg positivity of 20.9% and 4.1% in children less than 15 years of age and adults (those more than 15 years of age) respectively (Jyothimol and Ravindran, 2015). Another study had it that antibody positivity against excretory-secretory antigen of the adult worm in children and adults was 51.7% and 18.7%, respectively (Devi *et al*., 2007b). Furthermore, when a nation-wide survey for paragonimiasis was conducted in Japan in the 1950s and 1960s, the majority of patients were children (Uchiyama *et al*., 1999). Recently, however, patients found in Japan are mostly middle-aged men because they have a conservative affinity for traditional dishes (Uchiyama *et al*., 1999; Mukae *et al*., 2001). In the present study, prevalence was significantly higher among those 40 years of age or older than among their younger counterparts. This could be a reflection of the crab-eating pattern as crab-eating was significantly higher among those 40 years of age or older than among those younger. The mean age of crab-eaters in this study was 42 years and 43 years in Akpabuyo and Calabar South populations respectively, while the mean age for non-eaters were 20 and 23 years respectively. This scenario agrees with findings in an earlier study in south-eastern Nigeria, where the mean age of crab eaters was significantly older (43 years) than that of non-crab eaters (26 years), indicating that crab eating is not as popular among the younger persons in Eastern Nigeria as among the older ones (Uttah, 2013a). This relatively diminishing desire for crab-eating by the younger persons in communities in south-eastern Nigeria is encouraging as it would lower the risk of paragonimiasis infection over time.

The overall Geometric Mean Intensity (GMI) of *Paragonimus* eggs/ova in the two study communities ranged from 63 to 87 per $5ml^{-1}$ of sputum. The GMI for 6 different ethno-cultural rural communities in south-eastern Nigeria was 83, which is comparable to the GMI of rural Akpabuyo in this study (Uttah, 2013a). The present study also agrees with Uttah (2013a) in the comparability of the GMI between the sexes.

Prevalence of *Paragonimus* infection in relation to occupation in the two study populations revealed highest prevalence among fishermen and women while artisans, farmers and traders recorded high prevalence rates also. This indicates that there is high clustering of paragonimiasis around occupation in the study area. Fishermen and women have greater access to fresh crabs than others, being most involved in trapping and catching freshwater edible crabs than other occupational groupings. Since occupation in the study areas is a function of one's level of education, and whereas those in fishing occupation in the study populations are those with little or no education, it can be inferred that level of education is an important factor in the epidemiology of paragonimiasis in the rural and semi-urban suburbs of Calabar. This inference is further strengthened by the fact that prevalence was lowest among the civil servants who were the most educated occupational group in the study populations. However, further studies on the impact of level of education on the epidemiology of paragonimiasis are needed to make further conclusions. Indeed, socio-economic status and cultural practices influence the perpetuation of paragonimiasis in endemic areas (Belizario *et al.*, 2014).

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