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ABSTRACT:

This study was aimed at assessing the epidemiology of paragonimiasis in two coastal communities with definite interests in ascertaining the infection rates of *Sudanautes* with *Paragonimus uterobilateralis* metacercariae in those in their natural habitats and those purchased in local markets in the Cross River Basin. Crabs were trapped using the local methods used by the local population. In addition, crabs, supplied by local fishermen, were purchased from the market. The carapace of freshly collected or purchased crabs was broken and contents were examined under the dissecting microscope. The positive case infection was established by presence of metacercariae in the crab. In Akpabuyo and Calabar South, the highest monthly prevalence of *P. uterobilateralis* metacercariae in crabs in their natural habitats (6.6% and 8.3% respectively) and in those purchased in local markets (7.0% in both areas) was recorded in the month of September. The mean number of crabs collected from their natural habitats were significantly higher in the wet than dry season in both Akpabuyo ($p = 2.98$) and Calabar South ($p = 2.64$). In Akpabuyo, the overall prevalence of crab metacercariae infection among purchased crabs was higher than among those caught in their natural habitats, but this was not statistically significant ($p = 0.664$). In Calabar South, metacercariae infection among crabs caught from their natural habitats was not significantly higher than among those purchased in the local market ($p = 0.041$). The percentages of infected crabs were 5.3% and 5.8% in the wet season, and 5.9% and 4.4% respectively in the dry season. Overall, metacercariae infection rate of edible crabs (both those caught in their natural habitats and those purchased in markets pooled together) was 5.4% in Akpabuyo and 5.2% in Calabar South. There is a high metacercariae infection rate among crabs in South-eastern Nigeria, which necessitates an urgent need for innovative measures to discourage the local population from eating improperly cooked crabs.

Keywords: Metacercariae, edible crabs, paragonimiasis, seasonality

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INTRODUCTION:

Paragonimiasis is a neglected but re-emerging zoonotic parasite infection in Nigeria [1]. It is one of the most important food-borne parasitic zoonoses caused by one or more of the trematode species of the genus *Paragonimus* [2]. Known also as endemic haemoptysis or parasitical haemoptysis, it is a sub-acute to chronic inflammatory disease of the lung [3] affecting twenty million people [4], with 293 million at risk of the disease worldwide [5]. *Paragonimus* species infect more people globally than any other food-borne trematode, reportedly causing an estimated 196,710 disability adjusted life-years [6]. It might be more serious than reported, as these estimates do not account for infections in Africa. Paragonimiasis constitutes a major public health concern especially in the tropical and sub-tropical regions. The disease is endemic in many parts of Africa, Asia and South America [7]. In Africa, paragonimiasis is geographically clustered around the inter-tropical zone [8] as 80% of the 10 countries in the continent, where paragonimiasis has been reported, are located in this zone.

Edible crab species *Sudanautes* has been earlier confirmed as the intermediate host of *Paragonimus uterobilateralis* in south-eastern Nigeria [9]. Preliminary investigations into the crab-eating behaviour of an endemic population have been reported in six communities from two ethno-cultural clusters in South-eastern Nigeria and observations indicate that the risk of paragonimiasis is related to the frequency of eating of *Sudanautes* [1].

Most cases of paragonimiasis around the world have been associated with ingestion of improperly cooked crayfish, crabs, and prawns, while some are associated with raw crabs or crayfish used in traditional medications as obtainable in Korea, Japan, and some parts of Africa. These crabs, prawns, and crayfish are infected with infective metacercariae [8].

In Nigeria, endemic foci have been reported in the Cross River Basin with comparable prevalence rates in Yakurr Local Government Area as reported in different studies: 12 % [10], 9.6%; [11], and 8.6% [12]. Another endemic focus was reported in Akamkpa Local Government Area [13]. Furthermore, other geographic regions where high prevalence of paragonimiasis disease has been reported include Cameroon, an area contiguous to Akpabuyo and Calabar South; two areas included in our present study. The intermediate and definitive hosts of *P. africanus* are found throughout the contiguous forest of south-eastern Nigeria bordering Cameroon [14, 15], suggesting that *P. africanus* could be more widely distributed here than is currently appreciated.

Massive eating of crabs and crayfish has been reported to have continued in parts of south-eastern Nigeria decades after the second outbreak of paragonimiasis in the area [1]. This

has become a public health concern since paragonimiasis is acquired as a result of consumption of raw or improperly cooked or prickled freshwater crabs or crayfish harbouring infective metacercariae. Eating habits and closeness to water bodies is known to influence the transmission of parasitic infections since most of the fresh water products are infected with the infective metacercariae. It has become necessary to study the current status of paragonimiasis in coastal regions of Cross River State where this crab-eating practice is popular. This study provides empirical data to the stakeholders especially the health authorities that would help construct a more effective and robust paragonimiasis intervention plan in the area.

Furthermore, familial clustering has been reported in some parasitic infections including schistosomiasis [16, 17] and trichuriasis [18]. Filarial disease has been reported to aggregate in families [19, 20], and microfilarial levels have also been reported to be attributable to genetic factors [21]. Susceptibility to filarial disease and its clinical spectrum have been said to be determined by a gene associated with the histocompatibility complex [22, 23, 24]. Indeed, host genetic factors play an important role in determining both the nature of the responses to some vector-borne infection and the variability observed in pathologic outcome as exemplified in filariasis [25, 26], and hydrocele [27]. However, in paragonimiasis, it seems that familial flocculation and spatial household clustering of infection in endemic area is more of a function of crab-eating frequency and behaviour [1, 28].

Furthermore, there is paucity of information on the seasonal abundance of *Sudanautes* and its seasonal rate of infection with *P. uterobilateralis* metacercariae. This is an important missing link in paragonimiasis research since seasonal variation in crab populations and the level of crab infection are important epidemiological factors in paragonimiasis transmission [29]. Thus seasonal investigation to obtain data on these parameters should make a significant addition to knowledge in the epidemiology of paragonimiasis. This study was therefore aimed at assessing the epidemiology of paragonimiasis in two coastal communities with definite interests in ascertaining the infection rates of *Sudanautes* with *P. uterobilateralis* metacercariae in those in their natural habitats and those sold in local markets in the Cross River Basin.

MATERIALS AND METHODS:

The study was conducted between the months of June and December, 2017.

Description of the study area:

The study was carried out in two coastal communities, 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.

Akpabuyo is a rural community with monolithic population in Akpabuyo Local Government Area (LGA) of Cross River State (CRS). It has a land area of 28.5 km² and a population of 271,395 in the 2006 national census [30]. Akpabuyo LGA lies between latitude 4° 5' and 5° 40' and longitude 8° 25' and 8° 32 East. It is 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 ethnic groups are the Efiks, Quas and Efuts. 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 comprised mainly of the indigenous people. Akpabuyo is predominantly an agricultural area, and is known as the Food Basket of CRS. 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 slat deposits to mention a few [31].

Calabar South is an urban community and cosmopolitan, it has a land area of 264 km² and a population of 191,630 from the 2006 national 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.

Collection of crabs:

Two groups of crabs were used in the present study. The first group consisted of crabs trapped using the local methods reported in an earlier study in an endemic area proximal to the study area [28]. This traditional trap method was adopted because that was the normal method used by the local population. The second group of crabs were those supplied by local fishermen, and also purchased from the markets.

Dissection of crabs:

Freshly collected or purchased crabs were dissected using standard procedures [15]. The carapace was broken using a hammer, and the contents were examined under dissecting microscope. The positive case infection was established by presence of metacercariae in the crab [15].

Ethical approval for the study was received from the Cross River University of Technology Ethical Committee.

Data analysis:

Epi Info version 6.0 was used in entering data, and SPSS for windows was used for data analysis. Statistical analyses were carried out on differences between prevalence of infection using chi-square tests. $P < 0.05$ was considered

statistically significant. ANOVA was used in comparing means of intensities.

RESULTS:

Infection rates *Paragonimus* metacercariae in edible crabs were assessed in two groups of crabs; those crabs caught in their natural habitats and those purchased in the markets in both Akpabuyo and Calabar South.

Monthly prevalence of *Paragonimus* metacercariae in edible crabs caught in their natural habitats and in those purchased in the markets in Akpabuyo is presented in Table 1. The overall prevalence of *Paragonimus* metacercariae among crabs purchased in the market was not significantly higher than among those caught in their natural habitats (p = 0.009). Prevalence of metacercariae infection among crabs caught in their natural habitat was higher in the months of July, October, and November while Crabs purchased in the markets had higher metacercariae infection than those caught in their natural habitat in the months of June, August, and September, but these were not statistically significant (p = 0.009).

Monthly prevalence of *Paragonimus* metacercariae in edible crabs caught in their natural habitats and in those purchased in the market in Calabar South is presented in Table 2. Metacercariae infection among crabs caught from their natural habitats was higher than among those sold in the local market both in the overall, and in the months of July, August, September, and November, but the differences were not statistically significant (p = 0.876).

Seasonality of Mean abundance and infection rate of edible crabs:

Seasonality of relative abundance of crabs caught, and of percentages of total infected crabs caught in Akpabuyo and Calabar South is presented in Figure 1. The mean number of crabs caught in the wet season was 294 and was significantly higher than that (135) for the dry season (p = 2.98). The percentage of infected crabs caught in the wet season (5.3%) was lower than that (5.9%) in the dry season, but the difference was not statistically significant (p = 0.664).

Seasonality of relative abundance of crabs caught, and of percentages of total infected crabs caught in Calabar South is also presented in Figure 1. The mean number of crabs caught in the wet season was 264 and was significantly higher than that (124) for the dry season (p = 2.64). The percentage of infected crabs caught in the wet season (5.8%) was not significantly higher than that (4.5%) in the dry season (p = 0.041).

The monthly infection rate of edible crabs with *Paragonimus* metacercariae in Akpabuyo and Calabar South, both those caught in their natural habitats and those purchased in markets were pooled together and presented in Table 3. The total number of edible crabs examined in Akpabuyo was 2,046. The monthly number of edible crabs examined was highest (448) in August and lowest (188) in November.

The overall infection rate was 5.4%. The monthly infection rate was highest (6.7%) in September and lowest (3.8%) in July. In Calabar South, total number of edible crabs examined was 1,903. The monthly number of edible crabs examined was highest (433) in August and lowest (179) in November. The overall infection rate was 5.2%. The monthly infection rate was highest (8.0%) in September and lowest (3.7%) in October.

Table 1: Comparison of Monthly prevalence of Paragonimus metacercariae between edible crabs caught in their natural habitats and those sold in the market in Akpabuyo

Month	Crabs caught from natural habitats		Crabs purchased in market		Chi-square (p=0.05)
	Number examined	Positive crabs N (%)	Number examined	Positive crabs N (%)	
June	244	14 (5.7)	100	7 (7.0)	0.197
July	299	12 (4.0)	100	3 (3.0)	0.213
August	348	17 (4.9)	100	5 (5.0)	0.002
September	286	19 (6.6)	100	7 (7.0)	0.150
October	181	11 (6.1)	100	6 (6.0)	0.000
November	88	5 (5.7)	100	5 (5.0)	0.043
Total	1446	78 (5.4)	600	33 (5.5)	0.009

Table 2: Comparison of Monthly prevalence of Paragonimus metacercariae between edible crabs caught in their natural habitats and those sold in the market in Calabar south

Month	Crabs caught from natural habitats		Crabs purchased in market		(Chi-square) (p=0.05)
	Number examined	Positive crabs N (%)	Number examined	Positive crabs N (%)	
June	185	8 (4.3)	100	5 (5.0)	310.42 $p < 0.05$
July	261	11 (4.2)	100	3 (3.0)	72.04 $p < 0.05$
August	333	19 (5.7)	100	3 (3.0)	126.2; $p < 0.05$
September	277	23 (8.3)	100	7 (7.0)	83.64; $p < 0.05$
October	168	6 (3.5)	100	4 (4.0)	17.28; $p < 0.05$
November	79	5 (6.3)	100	5 (5.0)	2.60;$p > 0.05$
Total	1303	72 (5.5)	600	27 (4.5)	260.46; $p < 0.05$

Figure 1: Seasonality of relative abundance of crabs caught and of percentages of total infected crabs in Akpabuyo and Calabar South.

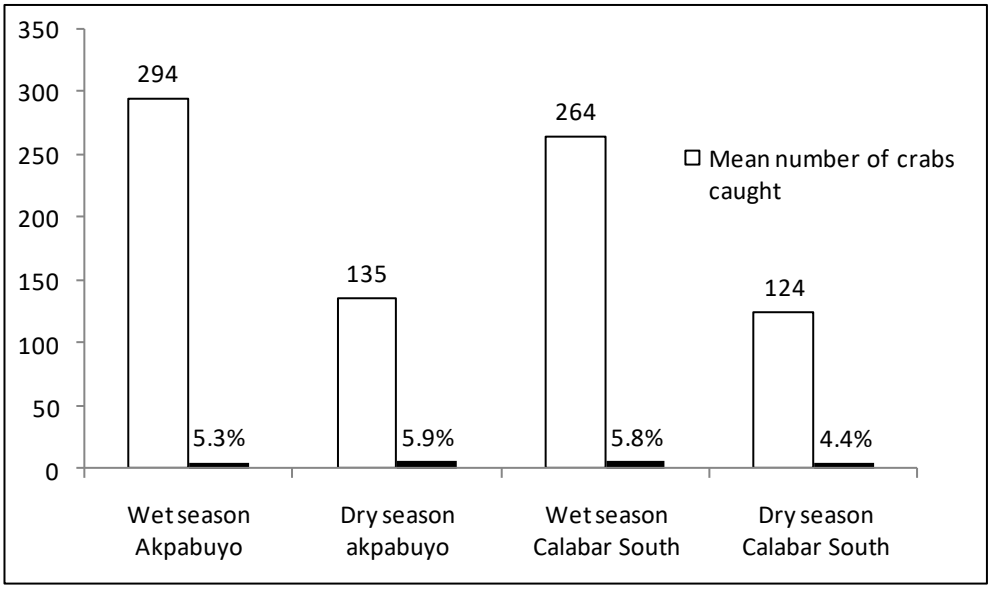

Table 3: Overall monthly infection rate of edible crabs (both those caught in their natural habitats and those purchased in markets pooled together) with Paragonimus metacercariae in Akpabuyo and Calabar South

Months	Akpabuyo		Calabar South	
	Number of crabs	Number of crabs found positive (%)	Number of crabs	Number of crabs found positive (%)
June	344	21 (6.1)	285	13 (4.6)
July	399	15 (3.8)	361	14 (3.9)
August	448	22 (4.9)	433	22 (5.1)
September	386	26 (6.7)	377	30 (8.0)
October	281	17 (6.0)	268	10 (3.7)
November	188	10 (5.3)	179	10 (5.9)
Total	2046	111 (5.4)	1903	99 (5.2)

DISCUSSION:

Although transmission of paragonimiasis is not seasonal in South-eastern Nigeria [28], metacercariae infection rate in edible crabs seems to be at its pick in the month of September as observed in this study and also

reported in an earlier study [1]. The epidemiological parameters recorded from crabs caught in their natural habitats showed high infection rate. Massive consumption of crabs in the area is driven mostly by the level of education and therefore could be attributed to extent of awareness of the health implications of such dietary behaviour [29]. Better awareness of the population regarding the implications to their health of consuming infected crab would lead to better and proper preparation of crab meals before consumption. On the other hand, better level of education most times could translate into better economic wellbeing, which means being in a better position to afford meat rather than relying on cheaper alternatives such as crab meals [1]. Behavioural change must be a prominent part of any successful control strategies in South-eastern Nigeria. However, provision of alternative sustainable and inexpensive solutions to cause behavioural change has also been a restraint for many control programs [32]. Human behaviour plays a fundamental role in the epidemiology of parasitic infections such as paragonimiasis, both its emergence and spread. Human behaviour is further complicated by the impacts of cultural, religious, ethnic, age and gender related variables. To have a desirable outcome in paragonimiasis control in the area, behavioural change must be directed to either curbing the frequency of crab-eating or to properly cook them before eating. Secondly, there must be an alternative plan to encourage massive breeding and provision of infection-free crabs on a commercial scale for committed eaters. The crab species *S. africanus* is reported to have high fecundity, producing many eggs per individual, which is an indication that they are viable for farm production, and can be bred in captivity [33]. Selective breeding for rapid growth can be engineered to obtain more attractive and delicious species that will compete favourably with their naturally occurring counterparts.

The monthly prevalence was significantly different between the two groups of crabs in all the months in both study areas except for the month of November in Calabar South where it was comparable. Metacercariae infection was not consistently higher in any particular group of crabs in this study. However, the overall metacercariae infection rate of edible crabs in this study was relatively low compared to that reported from some endemic countries outside Africa. In the Amazon, *P. mexicanus* metacercariae were found in 96.1% of the crab *Moreirocarcinus emarginatus* (formerly referred to as *Zilchiopsis ecuadoriensis*) individuals examined, whereas none of the *Trichodactylus faxoni* (formerly referred to as *Trichodactylus maytai*) crabs captured were parasitized [34].

CONCLUSION:

There was a high metacercariae infection rate among crabs in South-eastern Nigeria, which necessitates an urgent need for innovative

measures to discourage the local population from eating improperly cooked crabs. Mass education and awareness campaign to provoke changes in customs and food preparation practices of crabs should be emphasized in South-eastern Nigeria. The epidemiology and economic importance of paragonimiasis should be explained in such a way that majority of the people would be adequately informed about the risks of their food choices and food preparation preferences.

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