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Investigation of crab-eating influence on Paragonimiasis infection in six communities of Abayong from Cross River State Nigeria.

Running Title: Investigation of Crab-eating influence on paragonimiasis.

7 Abstract

- Background: The investigation of paragonimiasis infection was carried out among
 inhabitants of Abayong communities in Cross River State, Nigeria.
- 10 Aim: The study was aimed at investigating Crab-eating influence on paragonimiasis.
- 11 **Material and Methods:** Crab-eating behaviour of the people was observed and sputum 12 samples examined for eggs/ova of *Paragonimus uterobilateralis*

Statistical Analysis: Infection between males and females was compared using Chi-square test, while Analysis of variance was used to compare infection between Age groups, occupation and location.

16 **Results:** Out of 830 sputum samples examined consisting 67 (19.2%) males and 56 (11.6%)

17 females, an overall paragonimiasis prevalence of 14.8% was recorded. There were more

- males infected than females with evidence of significant difference (p < 0.001) between
- 19 them. Higher frequency of crab-eating revealed more intensity of infection. The intensity of
- infection revealed that 56.96%, 33.3% and 1.0% persons showed low, moderate and high
 intensity respectively, of eggs/ova counts per 5ml of sputum.

22 Paragonimiasis infection was highest (24.9%) in Ijom Abayong and lowest (5.2%) in Abrijang.

23 Risk exposure of each occupation to paragonimiasis revealed that Food vendors had the

highest risk ratio of 1.025. Teachers, Farmers, Fishermen, Students and Artisans have 70%,

14%, 34%, 67% and 35% respectively of the risk of their non-exposed members to contract
 paragonimiasis.

Conclusion. This study revealed that paragonimiasis is a food-borne parasitic zoonosis ravaging the six communities of Abayong. Mass education of the inhabitants is advocated to

- create awareness of the consequences of eating improperly cooked crab meals, to reduce
- 30 infection and re-emergence of paragonimiasis.
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32 Key-words: Abayong Crab-eating influence on paragonimiasi

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Introduction. Paragonimiasis is a foodborne parasitic zoonosis caused by lung fluke species 34 of the genus Paragonimus.^[1] The disease is endemic in East and South East Asia, West 35 Africa, and south America.^[2, 3] In Africa, paragonimiasis is geographically clustered around 36 the intertropical zone,^[4] as 80% of the 10 countries in the continent where paragonimiasis 37 has been reported are located in this zone. Paragonimiasis is a sub-acute to chronic 38 inflammatory disease of the lungs.^[5] The disease is acquired as a result of consumption of 39 raw or improperly cooked or prickled fresh water crabs,^[1] or crayfish harbouring 40 metacercariae cysts.^[4, 6, 7] Paragonimus westermani is the most common human pathogen 41 in Asian countries.^[1] In West Africa, *P. uterobilateralis* and *P. africanus* have been 42 incriminated.^[4, 8, 9] About 200 million persons are at risk of being infected, while 20 million 43

44 are already infected, aggravating the public health and socioeconomic indices in the endemic areas.^[10] A wide range of fresh water snails and crabs as well as crayfish serve as 45 first and second intermediate hosts respectively of *Paragonimus* species.^[11,12] 46 Paragonimiasis is a neglected but re-emerging parasitic zoonotic infections in Nigeria, ^{[7, 13],} 47 and has not been a public health concern in Nigeria before 1964 when it was recorded.^[9] 48 But the civil war in Nigeria (1967-1970) which caused a collapse in socioeconomic indices 49 helped to make the disease a public health problem, with very many cases in Nigeria.^[9] The 50 severe shortage of traditional protein source and poor cooking facilities during the Nigerian 51 civil war led to the greatly increased consumption of inadequately cooked crabs.^[8] In effect, 52 the Paragonimus parasite sojourning naturally inside the common crabs were still verv 53 much alive when they gained ready entrance into the many beleaguered bodies.^[14] The 54 parasites infected the lungs, grew and matured naturally and the resulting cough was 55 coupled with copious bloody sputum or even frank bleeding.^[14] Following well planned 56 efficient and effective programme at the end of the war in Nigeria, the disease was reported 57 58 to be eradicated by 1980. No case of paragonimiasis was reported from 1980 to September 2007.^[9] Incidentally, re-emergence of the disease was reported in 2007 and until recently 59 only sporadic cases had been reported in Upper Igwun Basin.^[13, 15] Endemic foci had been 60 reported in Enugu and the areas around the Imo and Cross River, and their tributaries, [15] 61 Igwun River and Iduma including Abam, Arochukwu, Bende, and Ohafia towns among 62 others.^[13] Paragonimiasis infection has a gradual onset and is characterized by low grade 63 fever, excruciating chest pain, diarrhoea and blood stained sputum.^[16, 17] The prevalence of 64 Paragonimus uterobilateralis have been reported in Cross River Basin by several authors: 65 5.5%,^[18] 8.6%,^[19] 9.6%,^[20] and 12.27%.^[21] Uttah^[7] recorded 13.2% prevalence in South 66 Eastern Nigeria, and Udonsi^[7] had 16.8% in upper Igwun Basin, Nigeria. Confirmation of 67 patients with paragonimiasis is carried out in testing their sputa or faeces in the laboratory. 68 Observation of the characteristic golden yellow opercolated eggs of *Paragonimus* in the 69 samples identify positive sample.^[6] This study was aimed at determining the prevalence of 70 paragonimiasis among the inhabitants of Abayong in Biase Local Government Area of Cross 71 72 River State Nigeria.

73 Materials and methods. This study was carried out in six communities of Abayong in Biase 74 Local Government Area of Cross River State, Nigeria. The communities are Ijom Abayong, 75 Abapia, Abredang, Abrijang, Abamba and Abaribara, located in the north western part of the 76 Local government Area, sharing a common boundary with Abia State. These communities are rural in setting with various occupations such as teachers, farmers, Fishermen, food 77 vendors, students, health workers and artisans. A major geographical feature in the area is 78 79 Cross River and most of the inhabitants are engaged in crab hunting and fishing in all the 80 beaches along the river. The vegetation is tropical rain forest and thickly forested in areas 81 where man's activities are not very pronounced. Crab hunting by the school-age children 82 and preparation of delicacies with it for consumption is one of their food habits. Fish, crabs and other farm produce from this area are transported to other localities through the Cross 83 84 River and its tributaries.

85 Collection of samples The village heads of the six communities investigated were informed 86 before sample collection commenced. This enhanced proper education of their subjects on 87 the importance of the study. Individuals who consented to this study were recruited and 88 labelled specimen vials with biodata sex (male or female) and occupation given to them for 89 their morning sputum collection. Morning sputum samples are ideal for a direct wet smear 90 examination of the parasite ova. The rusty brown or blood-stained sputum usually contain numerous *Paragonimus* ova.^[22] These sputum vials were retrieved from them the following
 morning for examination.

Sample size A total of 830 sputum samples were collected and analyzed, comprising of 481
(58%) females and 349 (42%) males.

Sputum examination using concentration techniques All the retrieved sputum samples 95 were brought to the laboratory and examined in search of Paragonimus eggs/ova. Because 96 of the sensitivity concerns of sputum examination for analysis of eggs, seven sputum 97 examinations per person were carried out as recommended.^[10] Five millilitres (5ml) of 98 sputum sample was dropped into a centrifuge and 5ml of 10% caustic soda solution added 99 and allowed to stand for 10 minutes. Mucus in the sputum samples dissolved and the red 100 101 blood cells lysed by caustic soda solution, after centrifugation at 2000 rounds per minute, 102 for 10 minutes, leaving the sputum clear. The supernatant was discarded and the wet 103 preparation of the residue was viewed under 10X and 100X magnifications in search of the presence of Paragonimus eggs/ova. It should be noted that though the eggs of both species 104 are morphologically identical, those of *P. africanus* are significantly smaller.^[23] Observation 105 of the characteristic golden yellow operculate eggs of *Paragonimus* in the sample confirmed 106 107 positive sample.

108 Learning influence of crab consumption on paragonimiasis. A structured questionnaire was 109 distributed to consenting inhabitants for this study to provide answers on their regularity of 110 crab consumption; either yearly, every six month, monthly, weekly or occasionally.

111 **Intensity of Paragonimus infection.** To determine the intensity of paragonimiasis infection, 112 5ml of sputum was placed into sterile petri dish using a 5ml pipette. The petri dish and its 113 content were viewed on an illuminating microscope stage and the total egg count 114 determined per 5ml sputum.

Ethical clearance Ethical clearance was obtained from Cross River University of Technology
 Calabar, ministry of health in Biase Local Government Area, and individuals who consented
 to this study.

Data analysis was carried out on the differences of prevalence of infection between age groups and sex using chi-square test. Comparism of infection between age groups and intensity of infection and between occupation and location was made by the analysis of variance. P-value < 0.05 was considered statistically significant.

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Results The study was carried out to determine the prevalence of paragonimiasis among inhabitants of six endemic communities in Abayong of Biase Local Government Area from Cross River State. Out of the 830 sputum samples examined consisting of 67 (19.2%) males and 56 (11.6%) females, an overall paragonimiasis prevalence of 14.8% was recorded (Table 1). There were more males infected than females. There was evidence of significance difference (P = 001) in the infection rate between males and females (Table 1).

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Location	Male	9	Fen	nale	Total	Number
	Number	Number	Number	Number	Number	Positive (%)
	Examined	positive (%)	examined	positive (%)	examined	
ljom	72	22 (30.6)	121	26 (21.5)	193	48 (24.9)
Abapia	54	9 (16.7)	81	8 (9.9)	135	17 (12.6)
Abredang	80	15 (22.5)	90	12 (13.3)	170	27 (15.9)
Abrijang	25	2 (8.0)	52	2 (3.8)	77	4 (5.2)
Abamba	58	7 (12.1)	65	3 (4.6)	123	10 (8.2)
Abaribara	60	12 (20.0)	72	5 (6.9)	132	17 (12.9)
Total	349	67 (19.2)	481	56 (11.6)	830	123 (14.8)

Table 1. Prevalence of *Paragonimus uterobilateralis* infection in relation to sex and locationin Abayong, Biase Local Government Area Nigeria.

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Table 2 revealed the intensity of Paragonimus uterobilateralis eggs/ova counts per 5ml of 141 sputum samples in relation to age in Abayong. The intensity of infection ranged from the 142 143 highest 46 (25.6%) in children less than 10 years to the lowest 4 (2.9%) in adults more than 144 50 years of age. There was progressive decline in the intensity of infection from children to 145 adults. The intensity of egg counts between 1–50 per 5ml of sputum was considered to be 146 low, 51-100 counts per 5ml of sputum viewed as moderate, and above 150 egg counts was 147 regarded as being high. In view of this, a total of 70 (56.96%) persons showed low intensity of egg counts, 4 (33.3%) persons were observed to have moderate intensity of egg counts, 148 149 while 9 (1.0%) had high intensity of egg counts (Table 2).

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152 Table 2. Intensity of *Paragonimus* eggs/ova counts in relation to age in Abayong, Biase Local

153 Government Area Nig

Government Area Nigeria						
Age	Number	Number	Intensity of infection per 5 ml sputum			
(Years)	examined	positive (%)				
			1 – 50	51 – 100	101 – 150	>150
< 10	180	46 (25.6)	25 (13.9)	12 (6.7)	6 (3.3)	3 (1.7)
11 – 20	120	30 (25.0)	20 (16.7)	5 (4.2)	3 (2.5)	3 (2.5)

21 – 30	160	23 (14.4)	14 (8.8)	4 (2.5)	3 (1.9)	2 (1.25)
31 – 40	130	12 (9.2)	6 (4.6)	3 (2.3)	2 (1.5)	1 (0.77)
41 – 50	100	8 (8.0)	3 (3.0)	3 (3.0)	2 (2.0)	0 (0.0)
> 50	140	4 (2.9)	2 (2.9)	2 (2.9)	0 (0.0)	0 (0.0)
Total	830	123 (14.8)	70 (8.4)	29 (3.5)	16 (1.9)	9 (1.0)

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156 Tabe 3 showed the evaluation of the risk of exposure of each occupation to paragonimiasis compared to any other occupation in Abayong communities. An Odds ratio (OR) is a 157 158 measure of association between an exposure and an outcome. The Odds ratio and Risk ratio 159 of Food vendors in this study were 1.027 and 0.023 respectively, greater than 1 (one) and 160 therefore the association was positively related to the disease paragonimiasis. The Odds 161 ratios of Teachers, Farmers, Fishermen, Students and Artisans are 0.32, 0.83, 0.57, 0.29, and 0.58 respectively, while the Risk ratios of Teachers, Farmers, Fishermen, Students and 162 Artisans are 0.30, 0.86, 0.66, 0.33 and 0.65 respectively. These mean that the exposed 163 164 Teachers, Farmers, Fishermen, Students and Artisans have 70%, 14%, 34%, 67% and 35% 165 respectively of the risk of their non-exposed members to contract paragonimiasis.

166 The association between frequency of crab eating and paragonimiasis infection is shown in

167

168 Table 3. Evaluation of the risk of exposure of each occupation to paragonimiasis compared 169 to any other occupation in Abayong, Biase Local Government Area Nigeria.

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Occupation	Odds ratio	Risk ratio
Teachers	0.32	0.30
Farmers	0.83	0.86
Fisher men	0.57	0.66
Food vendors	1.027	1.025
Students	0.29	0.33
Artisans	0.58	0.65

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173 Table 4. The risk of infection of inhabitants who ate crabs weekly was 15 times more than

those who ate yearly, 5 times more than those who ate every six months, 3 times more than

those who ate monthly and 7 times more than those who ate occasionally. The higher the frequency of crab consumption was the more the risk of paragonimiasis infection.

Thus, it was observed that children were more among inhabitants who ate crabs weekly andso more infected than adults.

- 179
- 180 Table 4. Comparism between the frequency of crab consumption and paragonimiasis
- 181 infection in Abayong, Biase Local Government Area, Nigeria.
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Frequency	Crab eating individuals	Number infected	Prevalence (%)
Yearly	25	1	4
Every six months	220	28	12.7
Monthly	200	37	18.5
Weekly	78	46	59
Occasionally	127	11	8.7
Total	650	123	18.9

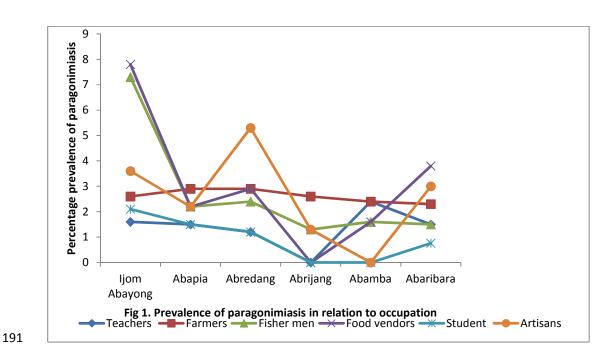
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Figure 1 represent distribution of paragonimiasis in Abayong in relation to occupation *Paragonimus uterobilateralis* infection was highest (7.8%) among Food vendors, followed by Fishermen (7.3%), Artisans (5.2%), Farmers (2.9), Teachers (2.4%) and finally Students (Fig. 1).

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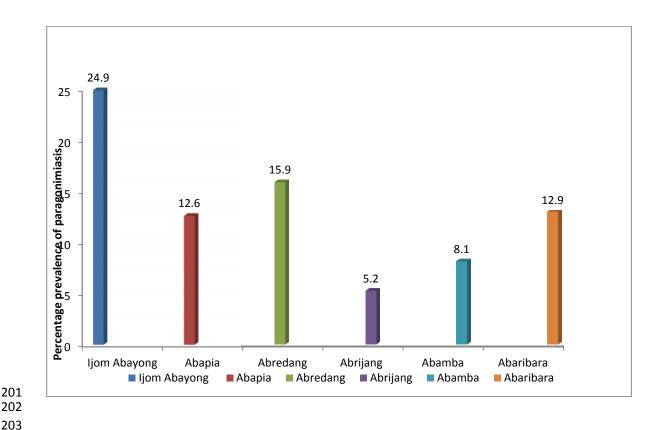
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The prevalence of paragonimiasis among communities ranged from 24.9% at Ijom Abayong to 5.2% in Abrijang (Fig 2). There was evidence of significant difference (P = 0.01) in prevalence of paragonimiasis between communities as exemplified by the various levels of

197 prevalence of paragonimiasis between communities as exemplified by the various levels of 198 infection (Fig 2.). The order of infection recorded was Ijom Abayong 24.9%, Abredang 15.9%,

Abapia and Abaribara 12.9% each, Abamba 8.1% and finally Abrijang 5.2% (Fig. 2).

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204 Fig 2. Prevalence of paragonimiasis among communities.

Discussion The wide spread of paragonimiasis in most communities of the world is due to 208 change in food habit, cultural influences and extremely successful parasites which are 209 widely geographically distributed.^[3,9,24] During the Nigerian Civil War of 1967-1970, the 210 Federal Government blockade of traditional protein sources to the secessionist Eastern 211 region, resulted in a desperate shift to the consumption of Crabs as substitute.^[8] 212 Consequently, the conservative cultural habits of preparing crab meals and eating 213 214 improperly cooked crabs precipitated the re-emergence of paragonimiasis during and after the war.^[9] Paragonimus species are extremely successful parasites and are widely 215 geographically distributed, being endemic in Asia, the Americas and Africa.^[3] It has been 216 estimated recently that 293 million persons are at risk of infection of Paragonimus 217 uterobilateralis^[25], while over 22 million persons were infected yearly.^[24] 218

In this study, the reported 14.8% prevalence of paragonimiasis is greater than 2.56% and 219 5.2% observed by,^[18,23] among school children in Cameroon and South Eastern Nigeria 220 respectively. The high prevalence of *P. uterobilateralis* in the study area is also confirmed by 221 the infection rates of 9.36% and 12.27% earlier recorded in Cross River State. [19, 21] 222 However, this finding is comparable with the 14.4% and 16.8% reported in Okigwe, ^[20] and 223 in Igwun River Basin.^[13] It was observed that more males (19.11%) were infected than 224 females (11.6%) with evidence of significant difference (P = 001) in infection between them. 225 This finding is in consonance with several reports, ^[13, 23, 24] but in sharp disagreement with 226 some other work, ^{[7, 20, 27],} who observed more infection in females than males. That more 227 males are infected than females buttressed the fact that males hunt for crabs and carry out 228

229 fishing activities, thus more exposed to improperly cooked crab meals than females. This 230 study reported the highest intensity of P. uterobilateralis infection 25.6% among children 231 below 10 years of age, compared with the lowest (2.9%) in adults. This finding is certainly an 232 underestimate of the actual situation in the study area, considering the suboptimal 233 sensitivities of the use of eggs/ova in sputum, compared to the stringency of standard diagnostic method such as ELISA used to identify typical operculated ova in sputum. This 234 observation is in line with the reports of some researchers. [23, 28, 29] Such tools were 235 unfortunately not employed in this study due to their cost. However, the repeated sputum 236 examination per person adopted in this study is known to improve the sensitivity of the 237 parasitological Method.^[10] There was evidence of significant difference (P = 05) between P. 238 uterobilateralis infection and age. Furthermore, prevalence of paragonimiasis among 239 240 communities showed that Ijom Abayong recorded the highest infection rate (24.9%) and the 241 least (5.2%) by Abrijang. There was evidence of significant difference (P = 001) in infection 242 rate between communities. However, prevalence among occupation indicated that Food 243 vendors have the highest (7.8%) infection rate, followed by Fishermen (7.3%). There was no 244 evidence of significant difference (p > 0.05) in infection rate among occupations. The risk of 245 paragonimiasis posed by each of the occupation compared to other occupations in this 246 study showed that there was strong association between Food vendors and paragonimiasis as against Fishing and river-food processing earlier reported. ^[26] The risk ratio of food 247 vendors in this study is greater than 1 (one) and therefore the association is positively 248 249 related to the disease paragonimiasis. The risk ratio of Teachers, Farmers, Fishermen, 250 Students and Artisans are 0.30, 0.86, 0.66, 0.33 and 0.65 respectively. These mean that the 251 exposed Teacher, Farmers, Fishermen, Students and Artisans have 70%, 14%, 34%, 67% and 252 35% respectively of the risk of their non-exposed members to contract paragonimiasis. This 253 study revealed that there was association between the frequency of crab eating and 254 paragonimiasis infection among inhabitants of Abayong. It wass observed that the higher 255 the frequency of crab consumption, the more was the risk of paragonimiasis infection. 256 Weekly crab eaters had the highest risk of paragogonimiasis infection. This finding is in consonance with previous report. ^[7] 257

258 It should be borne in mind that the high prevalence of paragonimiasis in the study area, which is contiguous to the endemic Eastern region of former Biafra, is its overlap with 259 tuberculosis and its consequent diagonistic confusion. [30] Proper identification of 260 Paragonimus eggs/ova requires skill technicians and standard diagnostic methods which are 261 262 lacking in most rural communities of the state. Furthermore, due to the high cost of wide-263 scale screenings for this disease, training of technicians in anti-tuberculosis centres would 264 be the most realistic attitude to detect mycobacteria and or Paragonimus eggs/ova during the same sputum examination. [4] 265

In conclusion, this study has revealed that paragonimiasis is a food borne parasitic infection, vectored by *Sudanautes africanus* which ravages the six communities of Abayong in Biase Local Government Area of Cross River State. There is therefore the need for proper education of the inhabitants, to create awareness on the consequences of eating improperly cooked crab meals in the study area, to reduce infection and re-emergence of paragonimiasis.

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