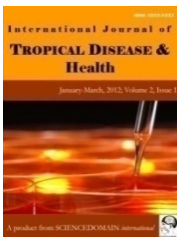

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A New Focus of Pleuro-Pulmonary Paragonimiasis in Manjo Health District, Littoral Region of Cameroon

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RMS, TN and CKA designed the study. Authors RMS, HBME, BEM and WKK performed the field study. Authors HBME and TN performed the statically analysis and drafted the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Background: Paragonimiasis is a food-borne parasitic disease caused by lung flukes of the genus Paragonimus. Cameroon has five known foci of paragonimiasis. Recently, 2 children from the Manjo health district were diagnosed of paragonimiasis at the near-by

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Ndoungué hospital. The aim of the present study was to determine whether Manjo district is endemic for paragonimiasis.
Materials and Methods: The study was conducted in Manjo health district, Littoral Region of Cameroon. A stool and a sputum samples obtained from each participant were examined in search of paragonimus eggs. Crabs from local streams were dissected in search of paragonimus metacercariae.
Results: Two hundred and fourteen participants were recruited (49.1% males and 50.1% females with an age range from 3-75 years and a mean of 15.73±10.72 years. 1.9% of subjects had eggs of paragonimus in their stools and/or sputum. Eight out of twenty crabs dissected (40%) were positive for metacercariae.
Conclusion: Manjo health district is endemic for paragonimiasis and should be considered as the 6th focus of the disease in Cameroon.

Keywords: Paragonimiasis; Manjo district; Cameroon.

1. INTRODUCTION

Paragonimiasis is common in Southeast Asia, South America and Africa with an estimated 22 million people infected and 293 million at risk of the disease worldwide [1].

Humans are infected through the ingestion of raw or undercooked crustaceans that harbour paragonimus metacercariae. These metacercariae when ingested in humans, lodge in the small intestine and pass through the intestinal wall, peritoneal cavity, diaphragm and pleural cavity to finally enter the lung parenchyma where they mature into adult flukes [2].

The acute phase of the infection (parasite invasion and migration) is clinically indicated by diarrhoea, abdominal pain, fever, urticaria, and hepatomegaly. The chronic phase (pulmonary infection) is characterised by pulmonary symptoms with radiological abnormalities that may persist for several years after effective treatment [3]. Extra pulmonary location of the adult fluke is rare. Cerebral paragonimias exists in severe forms. Ovarian paragonimiasis also exist, mimicking ovarian carcinoma [4].

The first case of paragonimiasis reported in Africa was in Cameroon [5]. Cameroon has five known foci of paragonimiasis. These foci are in the Kupe Mountain [6-8] and the Mundani foci [8] in the South West region, the Mbam and the Nyong foci in the Centre region and the Ntem in the South region [5].

Recently, two children from Manjo health district were diagnosed of paragonimiasis at the nearby Ndoungue Hospital. A pilot study conducted in this health district reported a positive case of paragonimiasis within pupils screened in different primary schools using parasitological methods.

The aim of the present study was to determine whether the Manjo health district was an endemic zone for paragonimiasis (presence of indigenous cases of the disease and fresh water crabs collected from local stream infected with paragonimus metacercariae).

2. MATERIALS AND METHODS

2.1 Study Site

The study was carried out in the Manjo health district, in the Littoral region of Cameroon. The study involved two localities (Manjo I and Manengoteng). The Manjo I locality is composed of a group of villages including Emeng, Manewa, Emal, Mikombe. Some subjects surveyed came from the Manengolle village (Fig. 1).

The Dibombé River is the major water body that crosses through the Manjo health district. This river is within the coastal river basin. It takes its source from the Kupe and Muanegouba Mountains where it flows south and passes through many villages of the health district and finally empties into the Wouri River.

The Manjo health district is found in a fertile rocky and volcanic valley; surrounded to the South West by Mount Kupe (2050m), the mountainous chains of Manengouba (2500m) in the North West and the Nlonako Mountain (1850m) in the North East. It has two seasons; a short dry season from November to March, followed by a long rainy season of 7 months with average temperatures varying between 20-31ºC. The total population of the district was estimated at 642 000 inhabitants following the census of the district health service in 2010. The autochthones indigenes are the Mbo. There are also many settlers from the North West and West regions attracted by the fertile soil for agricultural purposes. Agriculture is the main activity of the population. Plantains, coco-yams, vegetables, palm-nuts are crops produced for commercial and self-consumption.

2.2 Study Population

Inhabitants of the Manjo I and Manengoteng localities, irrespective of their age and sex were selected according to their eligibility. A total of six schools (three secondary and three primary schools) were also surveyed from these localities. With the aid of the president of the health committee, a door to door survey was undergone in houses. Consent forms were read, explained and signed by those willing to participate in the study. All children fulfilling the inclusion criteria and whose parents or legal guardian consented were recruited.

2.3 Clinical and Parasitological Investigations

The study was conducted from January to April 2011. Informed consent was obtained from subjects aged 15 years and above, or from the parents or legal guardians of children aged below 15 years, witnessed by the headmasters and the village chiefs. The study was reviewed and approved by the institutional review board of the Cameroon Institute of medical research and medicinal plants studies (authorization Nº13/CEI/IMPM11). Each participant provided a brief medical history and underwent a clinical examination for signs and symptoms of paragonimiasis (cough, haemoptysis and chest pain). Thereafter, they were given 2 small snap-cap plastic containers, 2 thin applicator sticks, and some toilet paper. They were instructed to transfer a small quantity of sputum into one container and a small quantity of stool into the other one, and return them to the research team. Each specimen was then mixed with a small quantity of sodium azide and transported to the base laboratory in Yaoundé for analysis. To each sputum sample was added twice its volume of 4% caustic soda followed by adequate mixing and centrifugation at 1500 rpm for 5 minutes. The resulting pellet was transferred unto a microscope slide and observed under a light

microscope at 10X and 40 X magnifications for the presence of paragonimus eggs. The stool samples were treated and analysed using a standard Rithie concentration technique to detect paragonimus eggs and the presence of other co-infecting parasites. All the paragonimiasis patients were treated with praziquantel at a dose of 3×25mg/Kg of body weight per day for 3 consecutive days.

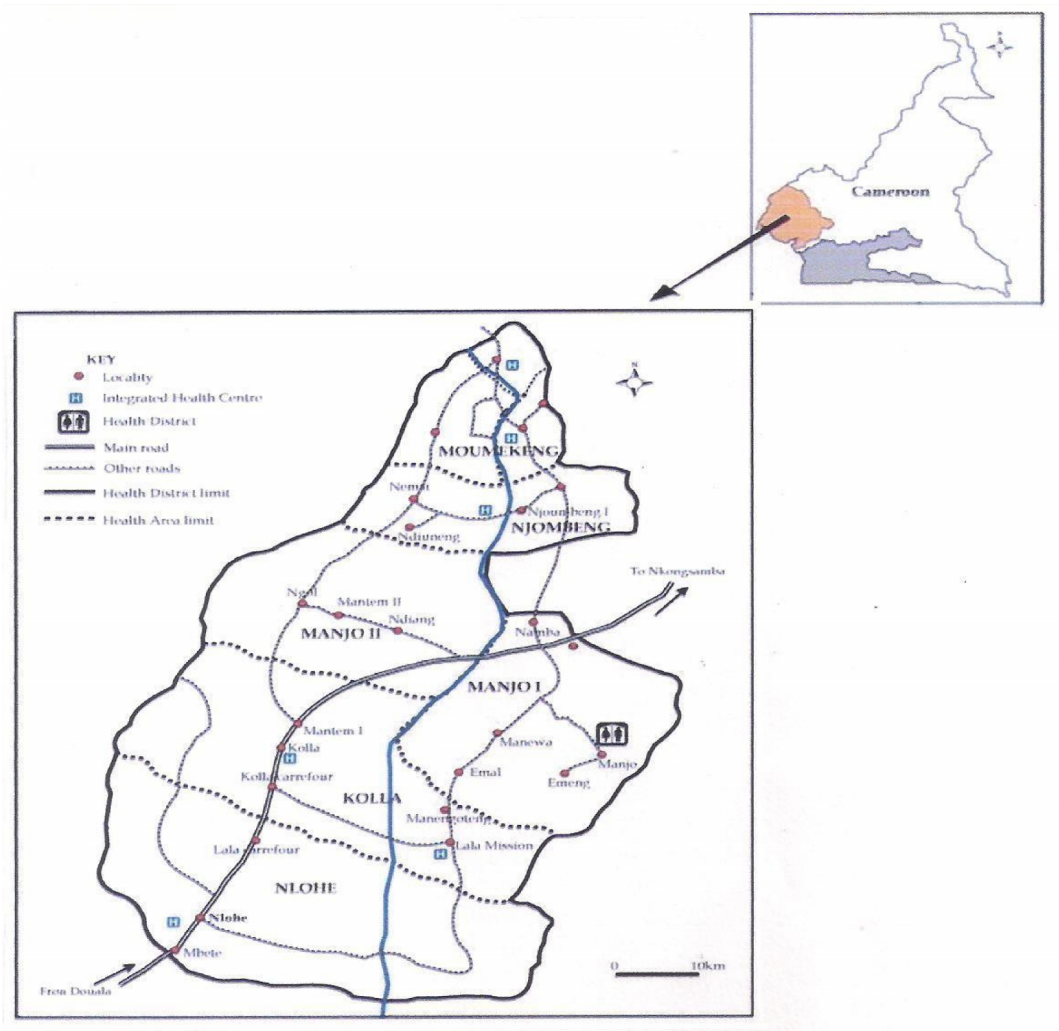

Fig. 1. Map of the Manjo health district showing the surveyed villages

2.4 Collection and Dissection of Crabs

With the assistance of the pupils and indigenes, crabs were collected from the neighboring streams in each of the villages using fishing baskets tufted with palm nuts and cassava. The baskets were placed at various points in the streams overnight and only checked the next day for the presence of crabs. Some crabs were equally dug out of small borrows along the stream beds or picked under some stones in the streams. The collected crabs were transported in perforated plastic buckets each with a lid to the laboratory where they were fed with palm nuts and slices of cassava until dissected. The hard shell of each crustacean

was removed using a small metal hammer and the muscle tissues beneath extracted using a small metallic spatula, into a petri dish. The obtained sample was then examined under a dissecting microscope at a magnification of 10X and 25X.

2.5 Data Analysis

Collected data was recorded on a pre-formed questionnaire which was computed and validated using EPI INFO software, version 3.4.3, 20. All entries were further checked on paper, item by item to ensure accuracy of the data entered. The data were exported to the Microsoft Excel 2003 and the Statistical Package for Social Sciences software, SPSS (version 12.0) compatible file for further analysis.

Data analysis was done using SPSS software version 12 for windows, Graph pad Prism version 5 and Microsoft Excel 2003. Frequencies were compared using the chi-squared test. Values of p less than or equal to 0.05 were considered statistically significant.

3. RESULTS

3.1 Socio-Demographic Data

Two hundred and fourteen (214) participants were recruited, including 105 (49.06%) males and 109 (50.93%) females giving a male to female ratio of 0.99. Their age ranged from 3 to 75 years with a mean of 15.73±10.72 years. The most represented age group was the 11-20 years (Fig. 2).

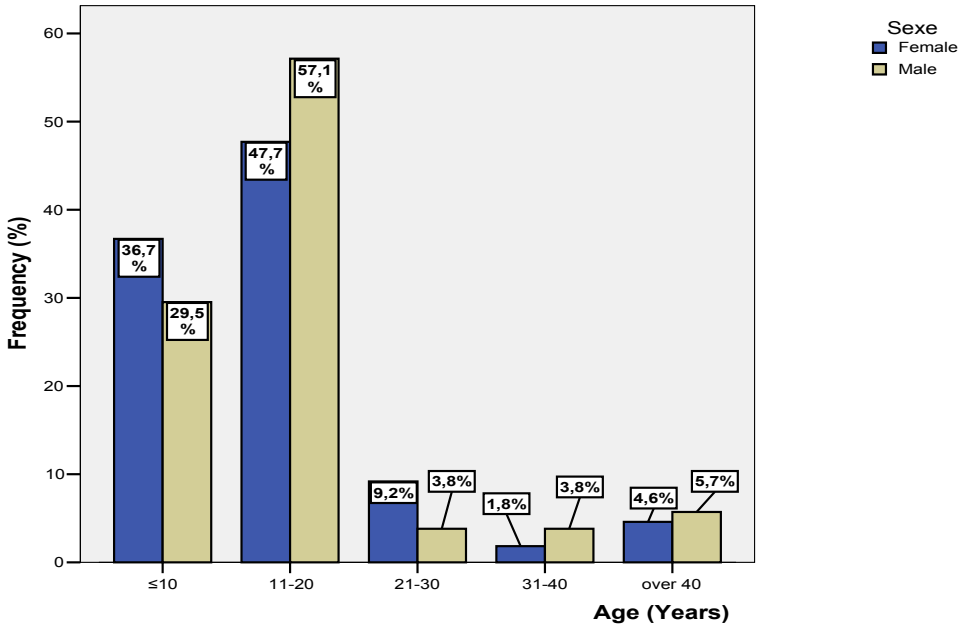

Fig. 2. Distribution of the study population by sex and age

All individuals recruited consumed crabs. Among them, 101(47.2%) consumed crabs roughly on a weekly basis while 64 (29.9%) did so occasionally.

3.2 Clinical Finding

Of the 214 individuals recruited, each had at least one symptom that could suggest paragonimiasis. Cough was present in 213 (99.5%) and was the most frequent symptom. No case of haemoptysis was recorded. Chest pain was the most represented in the 21-30 years age groups with 83.33% of individuals in this group (Fig. 3).

A relationship between the clinical manifestations and the risk factors was studied and no statistical significant difference was noted between clinical manifestations and risk factors.

3.3 Parasitological Findings

Paragonimus eggs were found in four out of the 214 symptomatic individuals recruited, giving a prevalence of 1.9%. Two of the positive cases had paragonimus eggs in their sputa while the remaining two had eggs present in the stools. Three of the positive individuals were aged 10 years and the last one was 9 years old. They all were pupils; they consumed crabs weekly and were from the Manengoteng village.

3.4 Prevalence of Metacercariae in Crabs

Twenty crabs were captured from the Dibombe River and from its tributaries in the surveyed villages. Eight of them were positive for metacercariae giving a prevalence of 40%. However, we noted a very low parasitic load varying between 1-3 metacercaria per infested crab. The metacecariae obtained were all of the species *Paragonimus africanus* (Fig. 4).

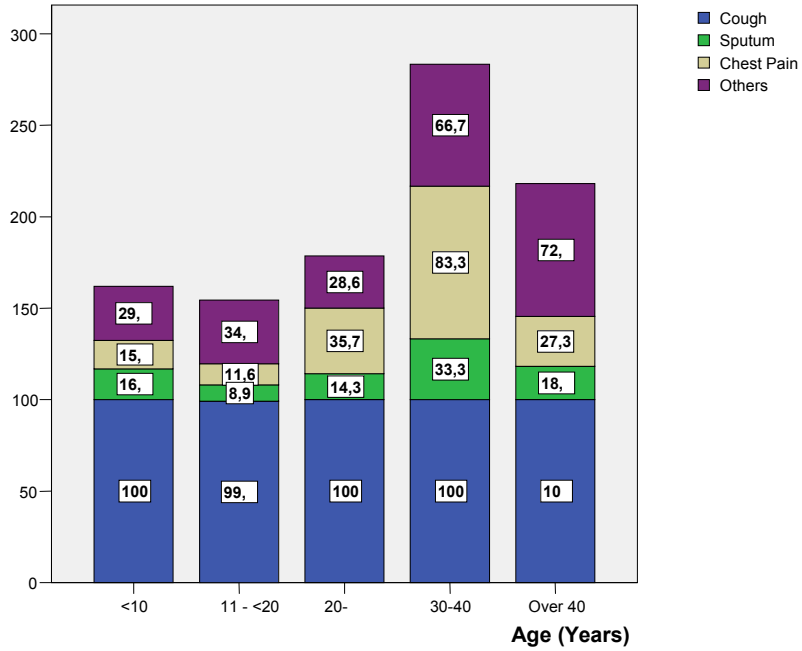

Fig. 3. Distribution of clinical symptoms in different age groups

Eggs of other parasites were identified during stool examination, including *Trichuris trichiura* (23.11%), *Ascaris lumbricoides* (13.68%), *Necator americanus* (6.60%), and *Schistosoma mansoni* (0.94%).

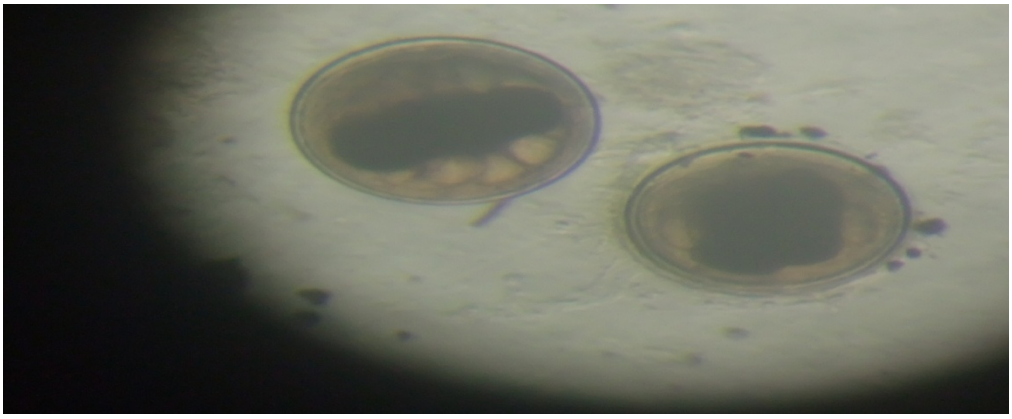

Fig. 4. Metacercariae of *Paragonimus africanus* collected from crabs caught from the Dibombe river at Manengoteng village

4. DISCUSSION

The aim of this study was to determine whether, the Manjo health district is an endemic zone for paragonimiasis. To achieve this, we had to obtain from eligible subjects recruited, many positive cases of paragonimiasis, and also identify metacercariae in fresh water crabs collected from streams and/or from the Dibombe river that cross the health district.

We used parasitological methods for the detection of eggs in stool or in sputum, and to search for metacercariae in freshwater crabs. This procedure is similar to the ones used for previous studies [7,9-12].

All subjects recruited ate crabs. All children examined declared they consumed crabs and that during the cooking, they associated the colour change of the crab shell to its readiness for consumption, the duration of cooking notwithstanding. This appears to support the results of others [7,11-12] and confirms that the major risk factor for pulmonary paragonimiasis is the consumption of uncooked or partially cooked crustaceans.

Every individual in the study population had at least one pulmonary symptom suggestive of paragonimiasis. Cough (99.5%) was the most frequent of all symptoms with an associated sputum production and chest pain accounting for 17.3% of the total population. The symptoms/signs noted in the present study are similar to those reported elsewhere [1,7,12-13]. They mimicked other respiratory diseases like Tuberculosis or lung cancer [14-17].

We noted eggs of paragonimus in stool and/or sputum samples of 1.9% of participants included in the study. This prevalence is lower than the ones obtained from previous studies in Cameroon [3, 6-8,12]. However, it falls within the prevalence range of 0.1-23.75% noted in endemic areas worldwide [1]. The above prevalence was certainly underestimated. This is because the submission of serial samples improves on the sensitivity to as high as 89% with greater than six samples, meanwhile the sensitivity of one sample submitted (as was the

case for this study) is limited, with a range of 37-57% [9]. In addition, a similar study carried out at the Kyusyu Island, Japan [10] detected paragonimus eggs in the sputum and/or stool of only 31% of patients and the remaining 69% were detected either by immunodiagnostic or bronchoscopic examination, which appear to be more sensitive than the standard methods. The molecular techniques are also very sensitive [18-19]. Such sensitive tools were not used in this study due to their high cost.

Fourty percent of crabs dissected were positive for metacercariae. This prevalence is greater than the ones obtained from previous studies in Cameroon [7,12]. However, we had a weak parasitic load (1-3 metacercariae per infested crabs). This weak parasitic load could explain the low prevalence in humans compared to the 40% prevalence in crabs, as infestation needs a long and consistent consumption of crustaceans to show symptoms and signs of paragonimiasis. All metacercariae were of the species *Paragonimus. africanus*. This finding is similar to previous studies in Cameroon [3,6-7].

We detected other intestinal parasites in stools, including eggs of *Trichuris trichiura*, *Necator americanus*, and *Ascaris lumbricoides*. The poor hygienic habits of these inhabitants can explain the presence of these parasites.

5. CONCLUSION

The Manjo Health District appears to be an endemic zone for pulmonary paragonimiasis, and should be considered as the 6th focus of the disease in Cameroon. This is the first report of indigenous cases of pulmonary paragonimiasis in the Littoral Province of Cameroon. With this new focus, Cameroon appears to be the most infected country in Africa. This should be considered during the differential diagnosis of tuberculosis in a country like Cameroon where HIV/AIDS is highly prevalent.

CONSENT

All authors declare that ‘written informed consent was obtained from the patients (or from parents/legal guardians for minors below 15 years) for publication of this paper provided the names of participants do not appear.

ETHICAL APPROVAL

All authors hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. Experiments have been examined and approved by the appropriate ethics committee. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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