

FIRST FINDINGS ON THE SEROEPIDEMIOLOGY OF HUMAN PARAGONIMOSIS AT THE ANTI-TUBERCULOSIS CENTRE OF DIVO, REPUBLIC OF IVORY COAST (WEST AFRICA)

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Summary:

An epidemiological study was carried out in 2004-2005 at the anti-tuberculosis centre of Divo (Ivory Coast) to collect sera from patients who consulted for tuberculosis suspicion and to estimate the seroprevalence of human paragonimosis in the context of a systematic screening. No *Paragonimus* egg was found in the stools and/or sputa of the 167 persons investigated. In contrast, 41 sera were ascertained with antibodies against *Paragonimus africanus* using ELISA testing. As the optical density (OD) values related to seropositive findings were found under 0.6 (the minimal OD to detect an active paragonimosis), the above antibody titres might originate from patients in chronic or in convalescent stages, or might result of cross reactions with trematodes. Concomitantly, dissection of local crabs (*Callinectes marginatus*) demonstrated the presence of *Paragonimus metacercariae* in six out of 34 examined. The parasite burdens in crabs ranged from two to 35 cysts with a mean diameter of 302 µm. In Ivory Coast, the locality of Divo must be considered an at-risk zone in reason of the presence of anti-*Paragonimus* antibodies in several human sera and the presence of infected crabs at the local market.

KEY WORDS : *Callinectes marginatus*, Africa, Ivory Coast, metacercaria, paragonimosis, seroprevalence.

MOTS CLÉS : *Callinectes marginatus*, Afrique, Côte d'Ivoire, métacercaire, paragonimose, séroprévalence.

Résumé : PREMIERS DOCUMENTS SUR LA SÉROÉPIDÉMIOLOGIE DE LA PARAGONIMOSE HUMAINE AU CENTRE ANTI-TUBERCULEUX DE DIVO, RÉPUBLIQUE DE CÔTE D'IVOIRE (AFRIQUE DE L'OUEST)

Une étude épidémiologique a été réalisée en 2004-2005 au centre anti-tuberculeux de Divo (Côte d'Ivoire) pour recueillir les sérums de patients qui consultaient pour une suspicion de tuberculose et les analyser en utilisant la technique ELISA afin de déterminer la séroprévalence de la paragonimose humaine dans le contexte d'un dépistage systématique. Aucun œuf de *Paragonimus* n'a été trouvé dans les selles ou les expectorations des 167 personnes examinées. Par contre, des anticorps contre *Paragonimus africanus* ont été identifiés dans les sérums de 41 patients. Comme les valeurs notées pour la densité optique des échantillons identifiés positifs étaient au-dessous de 0,6 (la valeur seuil minimale en rapport avec une paragonimose active), ces anticorps pourraient provenir de patients en phase chronique ou en convalescence, ou les résultats pourraient être en rapport avec la présence de trématodes. Par ailleurs, la dissection des crabes locaux (*Callinectes marginatus*) a montré la présence de métacercaires spécifiques de *Paragonimus* chez six crustacés (sur les 34 examinés). Les charges parasitaires se distribuaient entre deux et 35 métacercaires par crabe tandis que le diamètre moyen des kystes était de 302 µm. En Côte d'Ivoire, la localité de Divo doit être considérée comme une zone à risque en raison de la présence d'anticorps anti-P. africanus dans le sérum de certains sujets et surtout la présence de crabes infestés sur le marché local.

INTRODUCTION

Human paragonimosis, also known as endemic haemoptysis, is a food-borne parasitosis, caused by lung flukes of *Paragonimus* genus (World Health Organization, 1995). This disease poses a real public health problem in some parts of the world. In fact, 200 million people are exposed to this

disease (Toscano *et al.*, 1995). It prevails mostly in tropical and subtropical zones, where the environmental and ecological living conditions of the parasite are assured.

In Ivory Coast, the first case was discovered in 1974 at Lakota, in the south-west of the country (Coulibaly *et al.*, 1975). Later, nine cases were also detected in the same town between 1975 and 1983 (Bosse, 1984), while other five human cases were found in other districts of this country: Oumé (one case in 1978), Daloa (one in 1979), Abidjan (three in 1979, 1983 and 1995) (Bosse, 1984; Aka *et al.*, 1996). In 1999, a study of bronchial fluids originating from 142 patients showed the presence of several *Paragonimus* eggs in one sample (Adou-Bryn *et al.*, 1999). 12 cases (out of the 16 listed) came from the south-western region (Fig. 1). In fact, this scattered distribution of human cases supposes that a lot of districts might be affected by this disease. In Ivory Coast as well as in other black African countries, direct examination of stools and/or expectorations

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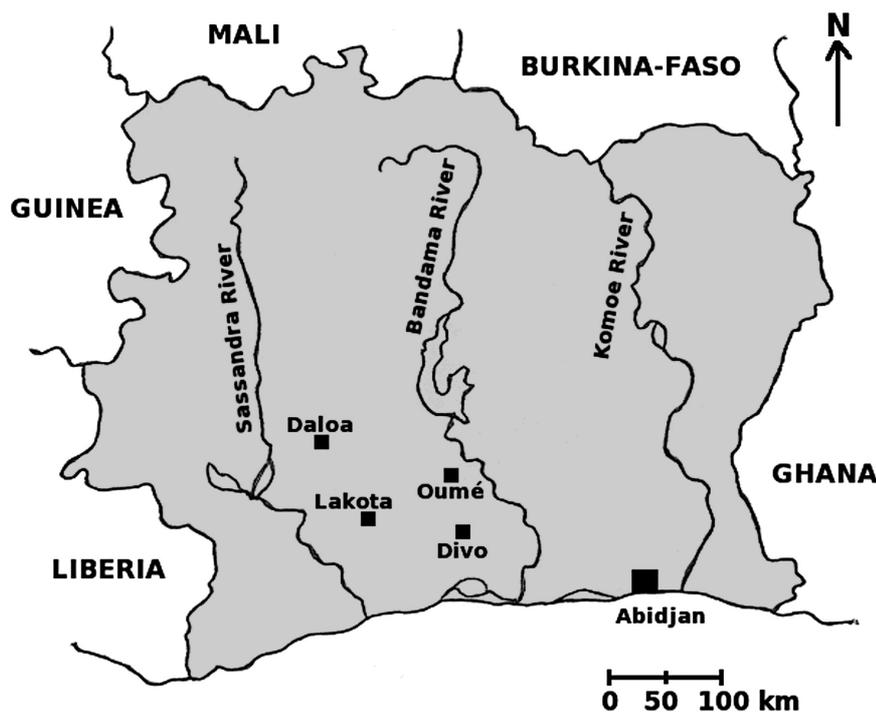


Fig. 1. – Map showing the south of Ivory Coast and the zone of study.

was the most used technical practice to find *Paragonimus* eggs. ELISA testing was still little used, even if this method is essential to detect acute and chronic cases of paragonimosis when eggs are absent in stools and expectorations. As no systematic detection of this parasitosis using serology was still carried out in Ivory Coast, it was interesting to study the conditions under which such a screening might be conducted. To answer this question, a field study was made from July 2004 to January 2005 at the anti-tuberculosis centre of Divo (Ivory Coast) to collect sera from patients consulting for tuberculosis suspicion and to analyze them in a Japanese laboratory specialized in serological detection of paragonimosis. A complementary study was also carried out on local river crabs to search metacercariae of *Paragonimus* spp.

MATERIALS AND METHODS

PATIENTS STUDIED

The centre of Divo (5° 48' N, 5° 15' W) is located at 200 km from Abidjan (Fig. 1). The choice of this centre is based on the fact that every year, a mean of 1,000 persons were examined for tuberculosis. Among them, 500 to 600 patients were *Mycobacterium tuberculosis*-negative in their sputa and several persons have sometimes received an anti-tuberculosis treatment when a chronic pneumonia was noted (unpublished data). The population of the Divo district comprised 534,644 persons (national census of 1998) and

is composed of Dida-, Godie- and Ega-speaking ethnic groups. These medical investigations were made with the agreement of the Ivorian Ministry of Public Health in December 2002.

206 persons between July 2004 and January 2005 were contacted during their medical consultation at the anti-tuberculosis centre of Divo. The purpose and methodology of the survey were explained to them and, finally, 89 men and 78 women had given their informed consent to participate to a paragonimosis survey. Their age was less than 30 years (58 persons), between 30 and 60 years (92), and more than 60 years (17). During examination, each patient was questioned about its past or recent symptoms and its crustacean-eating habits. Five ml of venous blood were collected from each patient and centrifuged during 2 min at 1,500 rpm before the storage of each serum at – 20° C. Stool and sputum samples were also collected to detect the presence of *Paragonimus* eggs under a microscope on direct examination.

SEROLOGICAL TESTS

The sera collected from patients were analyzed at the Laboratory of Parasitology, Faculty of Medicine of Miyazaki Kitoyake (Japan). Microplate ELISA tests using *Paragonimus africanus* antigens were carried out on their sera. Three lyophilized adult worms originating from Cameroon were homogenized in 2 ml PBS before being centrifuged at 15,000 rpm (4° C) for one hour. After collection of the supernatant, the total protein content was determined using a standard curve obtai-

ned by a serial dilution of known concentration of bovine serum albumine. The sensitivity of ELISA tests using *P. africanus* antigens was calculated using three positive sera from Africans patients having *Paragonimus* eggs in their sputa and was 100 %. In the same way, the specificity using negative sera of 30 healthy patients was 97 %.

Due to the possibility of cross reactions with other trematodes which were usually found in Ivory Coast, antibody titres against *Fasciola hepatica* (in the case of fasciolosis) and *Schistosoma japonicum* (in the case of schistosomosis) antigens were also investigated. These antigens were kindly provided by the Laboratory of Parasitology, Faculty of Medicine of Miyazaki Kitoyake. All antigens were kept at -20°C until their use.

Three ELISA tests (one for each antigen) were realized for each patient. First, the antigen concentration of each parasite was adjusted at $10\ \mu\text{g/ml}$ by diluting it with a carbonate-bicarbonate buffer, pH 9.6. $50\ \mu\text{l}$ of this antigen solution were placed in each well and the plate was incubated overnight at 4°C before being washed three times with $200\ \mu\text{l}$ of PBS containing 0.05 % Tween-20. Secondly, $200\ \mu\text{l}$ of 0.1 % casein in PBS were added to each well and the plate was again kept two hours at room temperature to block non-specific binding sites. Thirdly, the serum of each patient was diluted to 1:500 with 0.1 % casein/PBS and $50\ \mu\text{l}$ of this dilution were deposited in each well. The plate was then incubated one hour at 37°C before being washed as above. Fourthly, $50\ \mu\text{l}$ of horseradish peroxidase-labelled rabbit anti-human IgG (μ -chain specific) (Dako Group, Troy, Michigan, USA) diluted to 1:2000 in PBS, pH 7.4 were added to each well and the microplate was again incubated 1 hour at 37°C before being washed as above. Lastly, $50\ \mu\text{l}$ of ABTS-substrate solution (Kirkgaard and Perry Laboratories, Gaithersburg, Maryland, USA) were added to each well. The plate was incubated 10 min at 37°C and the reaction was stopped by adding $50\ \mu\text{l}$ of 1 % SDS to each well. The optical density (OD) of each well was read using an EL \times 800 Universal Microplate Reader (Bunkyo-Ku, Tokyo, Japan) at 405 nm.

The negative cut-off values were calculated using sera originating from 29 healthy persons without trematode infection. Based on the following formula: mean OD + 3 standard deviations, the values were 0.088, 0.20 and 0.41, respectively, for *P. africanus*, *S. japonicum* and *F. hepatica* antigens.

INVESTIGATIONS IN CRABS

A total of 34 crabs (*Callinectes marginatus*) were bought at the local market of Divo. Soft masses of every crustacean were then crushed in a meat mill before being placed in one litre of tap water. After agitation for 3 min,

the mixture was placed for 10 min to deposit sediment. This process was repeated several times until the supernatant became transparent and the sediment was subsequently collected for examination under a microscope.

RESULTS

PATIENT EXAMINATION

Out of the 167 persons, cough was noted in 159 patients (95 %) and chest pain in 133 (79 %). One hundred and five persons (62 %) complained of weight loss and 91 (54 %) of fever. A part from 11 persons (6 %), the general health status was good. 44 patients (26.3 %) had a pulmonary condensation syndrome and eight (4.7 %) showed a fluid collection syndrome. The history of 79 patients (46 men, 33 women) included crab/crayfish-eating habits. No *Paragonimus* egg was found in their stool and sputum samples.

Out of the 167 sera tested against *Paragonimus* antigens, 60 had their ODs above 0.09 (data not shown). These 60 sera were also analyzed to detect antibodies against *F. hepatica* and *S. japonicum* antigens. Among them, 19 had positive cut-off values for either of both above parasites so that they were excluded. In the other 41 sera, cut-off values for *F. hepatica* and *S. japonicum* were negative. Figure 2 shows that cut-off values of *Paragonimus* ranged between 0.09 and 0.20 for 38 patients and over 0.20 for the three others so that patients with greater than 0.09 ODs were suspected to be infected with *Paragonimus* spp. Clinically, 12 were also affected by tuberculosis and 31 were known to regularly eat freshwater crabs and/or help for crab cooking.

INVESTIGATIONS IN CRABS

Out of the 34 crustaceans studied, six *C. marginatus* (17.6 %) harboured *Paragonimus*-specific metacercariae, as described by Blair *et al.* (1999). The parasite burden ranged from two to 35 cysts per crab and their mean diameter was $302\ \mu\text{m}$ (SD: $11.5\ \mu\text{m}$). The mean thickness of metacercaria wall was $17\ \mu\text{m}$.

DISCUSSION

The results reported in the present study confirm the low sensitivity of examinations performed on stools and/or sputa to detect *Paragonimus* eggs, as already documented by other authors (Mukae *et al.*, 2001; Moyou-Somo *et al.*, 2003). This fact might be explained by the irregularity of parasite egg-laying

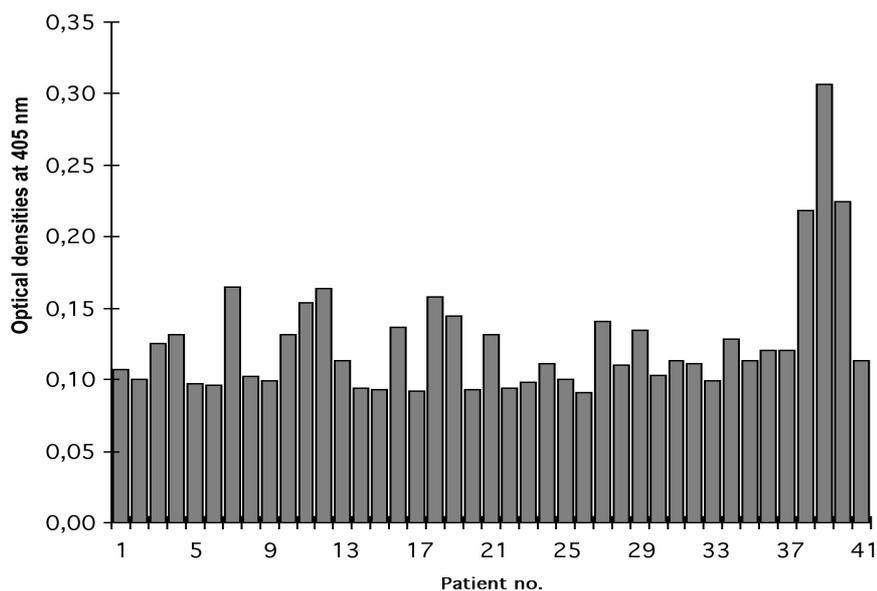


Fig. 2. – Optical densities of antibodies against *P. africanus* antigens in the sera of 41 patients.

over time. In spite of the absence of *Paragonimus* egg, the use of serology against *P. africanus* antigens demonstrated the presence of higher than 0.09 ODs in the sera of 41 patients (frequency, 24.5 %). This percentage is slightly lower than that (31.2 %) reported by Ripert *et al.* (1981) in Cameroon but it is difficult to compare these results, as these last authors had considered greater than 0.12 ODs as positive for paragonimosis. As the minimal OD for considering a human serum as positive for *Paragonimus* antigens, whatever species, is currently estimated at 0.6, particularly when the disease is in active stage, two likely complementary hypotheses to interpret results found in the 41 patients might be proposed. The first was to consider that several of these patients would be in chronic or in convalescent stages and this assumption is supported by the past history of paragonimosis-evocating symptoms found for these persons. In addition, ectopic infection cases cannot be excluded, as hypothesized by Cho *et al.* (1983) for serological results they have obtained in Korea. The second assumption would be the possibility of cross reactions between *P. africanus* and trematodes other than *F. hepatica* and/or *S. japonicum*. As these parasites did not exist in Ivory Coast, these cross reactions might be due to *Fasciola gigantica* and/or local schistosomes (*S. japonicum* imperfectly crossed with *S. haematobium* and *S. mansoni*). Another African paragonimid species: *P. uterobilateralis*, might also be the cause of these cross reactions, although Slemenda *et al.* (1988) had reported that antibody detection for *Paragonimus westermani* did not allow that of other *Paragonimus* species. Further clinical and/or serological investigations in these 41 patients would be necessary to verify either of these both hypotheses.

Two arguments supporting the abovementioned first

hypothesis were: *i*) the finding of *Paragonimus* spp. metacercariae in crabs bought at the local market of Divo, and *ii*) the crab-eating habits for 31 persons (out of the 41 patients with greater than 0.09 ODs). A serious risk of paragonimosis was thus currently present in the district of Divo and this interpretation is sustained by the fact that sold crustaceans came from Grand-Lahou, a town situated in the littoral of Ivory Coast, according to crab sellers. The same fact was already seen in Benin (West Africa) where crabs sold in local markets have been collected from Nigeria (Aka *et al.*, 1999). To tackle this disease in Ivory Coast, the best solution would be the control of crab business in markets within the country.

Because of political and military instability prevailing in Ivory Coast since 2002, some patients have changed their residence and their eating habits, so that it is difficult to currently identify the zones of *Paragonimus* infection. Also, an outbreak of paragonimosis might occur, as that reported by Nwokolo (1972) in eastern Nigeria following the civil war. A surveillance of local anti-tuberculosis centres to detect *Paragonimus*-infected patients and a simultaneous control of local markets to limit selling of infected crustaceans would be effective to ascertain and control the extension of this anthroponozoonosis in Ivory Coast.

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