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A seroepidemiological study of human cysticercosis in West Cameroon

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Summary We studied the occurrence of human cysticercosis in 4993 individuals from three rural communities of Menoua Division, West Province of Cameroon. Circulating antigens of *Taenia solium* metacestodes were detected in 0.4%, 1.0% and 3.0% of the serum samples taken in Bafou, Bamendou and Fonakekeu, respectively, and examined using a monoclonal antibody-based enzyme-linked immunosorbent assay. This test detects only carriers of living cysticerci and gives thus a good idea of the presence of active cysticercosis. The percentage of persons infected with cysticercosis increased with age. Twenty-two of the 34 seropositives underwent computed tomography (CT) of the brain. Thirteen of them were CT-scan positive, which shows that neurocysticercosis was present in 59.1% of the tested seropositive persons. No living cysticerci were detected among 20 seronegative people. About 20.6% of the seropositives had a history of or current taeniasis against only 1.9% of the seronegatives. Based on these figures and on the data on porcine cysticercosis (prevalence: 11%) and human taeniasis (prevalence: 0.13%) collected in the same region, we conclude that *T. solium* cysticercosis is an endemic, but overlooked public health problem in West Cameroon.

keywords *Taenia solium*, cysticercosis, antigen-enzyme-linked immunosorbent assay, neurocysticercosis, computed tomography-scan, Cameroon

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Introduction

Taenia solium cysticercosis remains a major public health problem in lower-income and some industrialized countries (Schantz *et al.* 1998). It is reported as one of the major causes of epilepsy in the tropics (Garcia *et al.* 1993; Carpio *et al.* 1998; White 2000). The true impact of this disease, however, has been obscured by the lack of sensitive and specific diagnosis tools for the collection of reliable epidemiological data (Tsang & Wilson 1995). Although computerized tomography (CT scan) and magnetic resonance imaging are useful tools, they are very expensive and generally inaccessible in low-income countries. However, some reliable serological tests for antigen or antibody detection of human and pig cysticercosis [enzyme-linked immunosorbent assay (ELISA), electro-immuno-transfer blot (EITB)] have been developed (Tsang *et al.* 1989; Brandt *et al.* 1992; Ito *et al.* 1998; Garcia *et al.* 2000; Erhart *et al.* 2002).

In Africa, the importance of human cysticercosis is unclear because of the lack of well developed medical infrastructures or diagnostic facilities (Preux *et al.* 1996). But, epidemiological studies of the disease have been conducted in several countries, a. o. Benin, the Island of Reunion, Madagascar, Togo and Burundi (Geerts *et al.* 2002).

In Cameroon, cases of human cysticercosis were first reported in 1985 in the Western Province (Marty *et al.* 1985, 1986). In a sero-epidemiological study carried out in Menoua Division (West Province), Zoli *et al.* (1987) reported a seroprevalence of 2.4%. Today, there are indications that human cysticercosis remains a serious public health problem in this region (Zoli *et al.* 1998). The

purpose of this study was to evaluate the actual importance of the disease in this area using an ELISA for the detection of circulating antigen.

Materials and methods

Study area

The study took place from August 1999 to November 2000 in Menoua Division, one of the important pig breeding regions of Cameroon. African swine fever has decimated pig stock over the last years. Pigs are usually confined during the rainy season and released after harvest or during the dry season. During confinement, pigs are mainly fed farm and kitchen residues, but in some households also human faeces. Free roaming pigs have also access to human faecal material. Many pigs are slaughtered outside the slaughterhouses without any veterinary supervision. Pork is widely consumed in the area especially during funerals and other traditional events.

Sampling

The study was conducted in the three rural communities of Bafou, Bamendou and Fonakekeu near the city of Dschang. An information campaign was initially organized in these localities with the support of local traditional authorities to sensitize the populations about the objectives of the study. A total of 4993 people were included in the survey: 2628 of an estimated number of 58 000 inhabitants of Bafou, 2299 of about 32 000 in Bamendou and 66 of approximately 360 inhabitants in Fonakekeu. All individuals who showed up when the sampling team visited the villages were included. Nobody has been refused. During the campaign, informed consent was obtained from every adult volunteer and in case of children, from their parents. Name, age, gender and village were recorded and everybody was questioned about his/her consumption habits (pork, fruits and raw vegetables, origin of drinking water), hygienic behaviour (place of defecation), and neurological history (epilepsy, chronic headache, mental disorder). Epilepsy was considered present if at least one epileptic crisis had occurred during the past year. No difference was made between late onset epilepsy and other types of epilepsy. Mental disorders were defined as the presence of any abnormal behaviour (other than epileptic seizures), whereas chronic headache was defined as recurrent severe headache refractory to treatment with the usual analgesics. All participants were examined by a physician (search of nodules under the tongue, the subcutaneous tissue and in the eyes) and a blood sample was collected for serological examination. A confirmatory biopsy of the nodules was only carried out when the person reacted positive in the antigen (Ag)-ELISA. Cysticercosis was considered to be present when the ELISA value of the person examined was significantly different from the average of a series of reference negative Cameroonian people. Multivariate logistic regression adjusted for clustering on village was used to determine the significance of the different variables (localities, ages and sexes). Analyses were conducted in Stata (StataCorp 2001).

ELISA for detection of circulating antigen of *T. solium* (Ag-ELISA)

A monoclonal antibody (MoAb) based ELISA was used to detect circulating antigens of *T. solium* metacestodes in serum. The Ag-ELISA was performed as described by Dorny *et al.* (2000) with a slight modification. Briefly, the sera were pre-treated using trichloroacetic acid and used in ELISA at a final dilution of 1/4. Two MoAbs were used in a sandwich ELISA. MoAb B158C11A10 was diluted at 5 μg/ml in carbonate buffer (0.06 M/pH 9.6) for coating and a biotinylated MoAb B60H8A4 (1.25 μg/ml in phosphate-buffered saline containing 0.05% Tween 20 and 1% new-born calf serum) was included as detector antibody. The incubation was carried out at 37 °C on a shaker for 30 min for the coating of the first MoAb and for 15 min for all subsequent steps. The chromogen/substrate solution consisting of *o*-phenylene diamine (Dako, Glostrup, Denmark, #S2045) and H_2O_2 was added and incubated without shaking between 30 and 33 °C for 15 min. To stop the reaction, 50 μl of 4 N H_2SO_4 was added to each well. The plates were read using an ELISA reader (Labsystems Multiskan RC. Brussels, Belgium) at 492 nm.

Eight negative reference control sera from local people of the region of Dschang and one reference positive serum from a Cameroonian patient with confirmed cysticercosis (by CT-scan) were included in each ELISA run. The optical density (OD) of each serum sample was compared with the mean of the eight negative reference sera at a probability level of $P = 0.001$ to determine the result using a modified Student's t test (Sokal & Rohlf 1981). The ELISA ratio was calculated by dividing the OD of the sample by the calculated cut-off value of the eight negative controls. An ELISA ratio of >1 was considered as positive.

CT scan

Twenty-two of the 34 seropositive individuals underwent a cerebral CT scan (eight people refused the examination and four individuals, who were already sick at the time of sampling, died before) and a similar number ($n = 20$) of seronegative persons. The scans were done at Yaounde

Central Hospital using a Somatom AR Star, VB41A apparatus (Berlin, Germany). Ioxitalamic acid (Telebrix® 35, Laboratoire Guerbet, Roissy, France) was used as intravascular contrast agent. Each patient was examined before and after contrast injection and two image series on slides were made.

Results

Serological results (Ag-ELISA)

A total of 4993 persons (1792 men and 3201 women), representing about 5.5% of the estimated population of the study area, were examined. Table 1 shows the baseline characteristics of the people. The proportion of females in each village was very similar. The percentage of seropositive persons per locality, age group and sex is presented in Table 2. It varied from 0.4% to 3% in the three communities. However, if only the adult population (>15 years) is taken into account, the figures are 1.4%, 0.8% and 6.7% in Bamendou, Bafou and Fonakekeu, respectively. Multivariate logistic regression (clustering on village) adjusted for age and sex showed that the number of seropositives in Fonakekeu was significantly higher than in Bamendou (odds ratio: 4.7; $P < 0.001$) and Bafou (odds ratio: 9.6; $P < 0.001$). There was no significant difference, however, between the number of seropositive men and women (odds ratio: 0.58; $P = 0.125$). There was a significant effect (odds ratio: 1.05; $P < 0.001$) of age (years).

Table 1 Characteristics of the examined population in the three rural communities

	Bamendou	Bafou	Fonakekeu
No. of people examined	2299	2628	66
Percentage of males	36	36	35
Percentage of females	64	64	65
Age groups			
≤ 15 years	39	54	55
16–45 years	29	20	21
≤ 46 years	32	26	24

Table 2 Occurrence of cysticercosis (Ag-ELISA) according to age, sex and locality

	No. examined	No. of seropositives (%)	95% (Wilson)* C.I.
Locality			
Bamendou	2299	22 (1.0)	0.63, 1.46
Bafou	2628	10 (0.4)	0.20, 0.71
Fonakekeu	66	2 (3.0)	0.28, 11.15
Sex			
Male	1792	16 (0.9)	0.54, 1.46
Female	3201	18 (0.6)	0.35, 0.90
Age group (years)			
≤ 15	2352	3 (0.1)	0.03, 0.40
16–45	1212	2 (0.2)	0.01, 0.65
≥46	1429	29 (2.0)	1.41, 2.92

* Agresti and Coull (1998).

Clinical examination and cerebral CT scan of seropositive persons

The percentage of seropositives among the individuals showing clinical history or symptoms suggestive of *T. solium* cysticercosis is given in Table 3. Positive results were also obtained in 28 of 4785 (0.6%) asymptomatic individuals. The ELISA ratios of the 34 seropositive persons varied from 1.2 to 69.2 with a mean of 13.5. Their median age was 60 years (range: 5–85). Of the 34 seropositive individuals, six had a history of tapeworm and one was currently carrying a tapeworm (confirmed as *T. solium* on the basis of morphological criteria of the proglottids). Another seropositive person was the neighbour of the tapeworm carrier and a last one lived in a family where one of the members was infected with *T. solium*. The percentage of seropositives with a history of or with current taeniasis was 20.6% (7 of 34) *vs.* 1.9% (95 of 4959) for the seronegative individuals.

Neurocysticercosis was detected by CT in 13 (59.1%) of the 22 seropositive persons who accepted to undergo the examination. Calcifications as well as living cysts were observed in the brain, sometimes in association. Only one of the two seropositive epileptic patients was CT-scan positive, showing more than 25 calcifications in the brain. All three seropositive patients suffering from chronic headache were CT-scan positive. Living cysts were not detected on CT-scan images of the brain of 20 seronegative (ELISA ratio <1) persons examined.

Table 3 Number (%) of seropositives for human cysticercosis in relation with clinical history or symptoms suggestive of cysticercosis

Clinical history or symptoms	No. examined	No. of seropositives* (%)
Mental disorders	33	(0)
Epileptic crisis	79	2 (2.5)
Chronic headache	81	3 (3.7)
Subcutaneous nodules	13	1 (7.7)
Asymptomatic	4785	28 (0.6)
Total	4993	34 (0.7)

* Ag-ELISA.

Questionnaire about dietary and hygienic customs

Table 4 summarizes the data on the hygienic and dietary customs of the study population ($n = 4993$), which were elicited with a questionnaire. Only 4.9% of the people declared not to eat pork, of whom seven were epileptic patients and three had mental disturbances. However, none of them was positive in the Ag-ELISA. All but two of the 34 seropositives were pork consumers. These two were epileptics and stopped eating pork when their epileptic crises started.

Discussion

The antigen detection ELISA revealed the presence of active cysticercosis in 0.4–3% of the people examined in the three rural communities. If we take into account only the adult population (>15 years) the figures double (0.8–6.7%). It is indeed well known that children are usually much less affected by cysticercosis than adults. In this study a total of approximately 5000 people were screened, which represented about 5.5% of the population of three rural communities of the Menoua region. As a result of the fact that the sampling was not carried out at random, but on a voluntary basis at village level, this sample cannot be considered as representative for the general population of these communities. Females were over-represented in this survey, which might also have introduced some bias. However, as the true proportion of females in the population study is not known, no attempt could be made towards using weights to correct for this bias. It is expected that the bias because of a possible oversampling of females, is not too important as a result of the large number of people which has been tested. The oversampling of females is also similar in all three localities used in this study.

The Ag-ELISA used in this study has a sensitivity of 94.4% and a specificity of 100% (Erhart *et al.* 2002), whereas a similar antigen detection ELISA as reported by Garcia *et al.* (2000) showed a sensitivity of only 85% and a specificity of 92%. The former test is known to detect only living cysticerci as has been clearly shown in cattle (Brandt *et al.* 1992), in pigs (J. P. Nguekam, unpublished results) and in men (Erhart *et al.* 2002). Therefore, it gives a better idea of the prevalence of active cysticercosis than antibody detection tests, which often detect persons with transient infections, i.e. persons who have been exposed to eggs of *T. solium*, but did not develop a viable infection of the parasite and remain seropositive for a short period (Garcia *et al.* 2001). On the other hand, the test certainly underestimates the prevalence of cysticercosis in a community because all patients harbouring only calcified cysticerci are not detected.

Table 4 Results of a questionnaire about hygienic and dietary customs of the study population

	Yes (%)	No (%)
Pork consumption	95.1	4.9
Latrine in house	93.1	6.9
Defecation in open air	93.6	6.4
Defecation in pigsty	20.1	79.9
Consumption of untreated water*	81.7	18.3

* Water from rivers or non-controlled water sources.

As such, data related to immune responses of the host can hardly be compared with those directly related to living parasites. Nonetheless, in the mid 1980s, antibody detection ELISA showed the presence of cysticercosis in the same region of Cameroon with 2.4% seropositivity, in a survey of 764 people with 95% adults (Zoli *et al.* 1978). This corresponded well with observations in large surveys held in other regions of West and Central Africa using antibody (Ab)-ELISA and/or EITB, i.e. 1.3% seropositives in Benin (Houinato *et al.* 1998) and 2.4% in Togo (Dumas *et al.* 1989).

Contrary to the observations of Erhart *et al.* (2002) in Vietnam, which showed that eight of nine positives in Ag-ELISA were confirmed using a cerebral CT scan, brain lesions suggestive of cysticercosis were found in only 13 (59.1%) of 22 seropositives in this study. This indicates that cysticerci might be present elsewhere (e.g. muscles). However, subcutaneous nodules (confirmed as *T. solium* cysticerci by biopsy) were observed in only one of 34 positives in Ag-ELISA (2.9%), which is much lower than the figures from other endemic regions of Africa and Asia (10–30%, Dumas *et al.* 1990; Schantz *et al.* 1998) and more similar to what is reported in South America (2.9–6%) (Cruz *et al.* 1994). This might be partially because of the fact that the region is endemic for onchocercosis and subcutaneous nodules might be caused as well by *Onchocerca volvulus* as by *T. solium* cysticerci.

It was very striking that 20.6% (7 of 34) of the seropositive people were harbouring a tapeworm (one) or had harboured one in the past (six) whereas only 1.9% of seronegative individuals had a history of or were carrying a tapeworm. Two other seropositive persons were living together either with a family member or with a neighbour, who did carry a tapeworm. As these tapeworms were identified as *T. solium* (based on the number of uterine branches of a gravid segment) and because three of four tapeworms collected in another study in the same region were shown to be *T. solium* (Vondou *et al.* 2002), it is obvious that these people lived in a highly contaminated environment. This confirms previous observations that

living in a household of persons with a history of or current taeniasis is one of the most important risk factors for cysticercosis (Sarti *et al*. 1992; Schantz *et al*. 1998).

The number of epileptics who were seropositive for cysticercosis was rather lower (2.5%) in this study than in other reports (Preux *et al*. 1996; Schantz *et al*. 1998). However, it has to be noticed that epilepsy is usually caused by degenerating or calcified cysticerci in the brain, which are not detected by the Ag-ELISA. The observation that the occurrence of cysticercosis increases with age is in agreement with many other studies in Africa and elsewhere (Sarti *et al*. 1992; Houinato *et al*. 1998). It is not surprising that the longer the people are exposed to *T. solium* eggs in an endemic environment, the greater are the chances of them being infected, although more information is needed on the survival of metacestodes and on the possibility of re-infection.

We conclude that taeniasis–cysticercosis complex caused by *T. solium* remains an overlooked public health problem in the Menoua division although human cysticercosis, locally called *fo'o-kassan*, seems to be well known by the local population. It is regularly reported during traditional autopsies performed on human corpses in the villages. The data collected in this study complement the data about porcine cysticercosis (prevalence of 11%, Pouedet *et al*. 2002) and human taeniasis (prevalence of 0.13%, Vondou *et al*. 2002) in the same localities. They clearly prove that *T. solium* cysticercosis is endemic in the region. As long as defecation in open air or even in the pigsties remains a common practice (even if latrines are available as is shown by the results of the questionnaire, see Table 4) and as long as many pigs roam freely – temporarily or continuously (Pouedet *et al*. 2002), all the conditions are present for a very efficient parasite transmission.

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