

THE TAENIASIS-CYSTICERCOSIS COMPLEX IN CAMEROON



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1: INTRODUCTION

This book gives a comprehensive overview of the taeniasis-cysticercosis complex due to *Taenia solium* and *T. saginata* in Cameroon. The research results which are presented in this document were obtained within the taeniasis-cysticercosis project in Cameroon (1998-2002), a project which was financed through the framework agreement between the Institute of Tropical Medicine, Antwerp and the Directorate-general for Development Cooperation (DGCD, Brussels). The promoters of this project were Prof. A. Zoli (University of Dschang, Cameroon) and Prof. S. Geerts (ITM, Antwerp). Epidemiological studies were carried out in different regions of Cameroon. Data were collected on the occurrence of cysticercosis in pigs and on the prevalence of taeniasis and cysticercosis in the human population. The role of cysticercosis in the aetiology of epilepsy is also highlighted. *T. solium* cysticercosis remains an under-recognised public health problem, which deserves more attention from the Cameroonian authorities.

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- ASSANA, E., ZOLI, P.A., SADOU, H.A., NGUEKAM, VONDOU, L., POUEDET, M.S.R., DORNY, P., BRANDT, J., GEERTS, S. (2001). Prévalence de la cysticercose porcine dans le Mayo-Danay (Nord-Cameroun) et le Mayo-Kebbi (Sud-Ouest du Tchad). *Rev Elev Méd vét Pays trop* **54**: 123-127.
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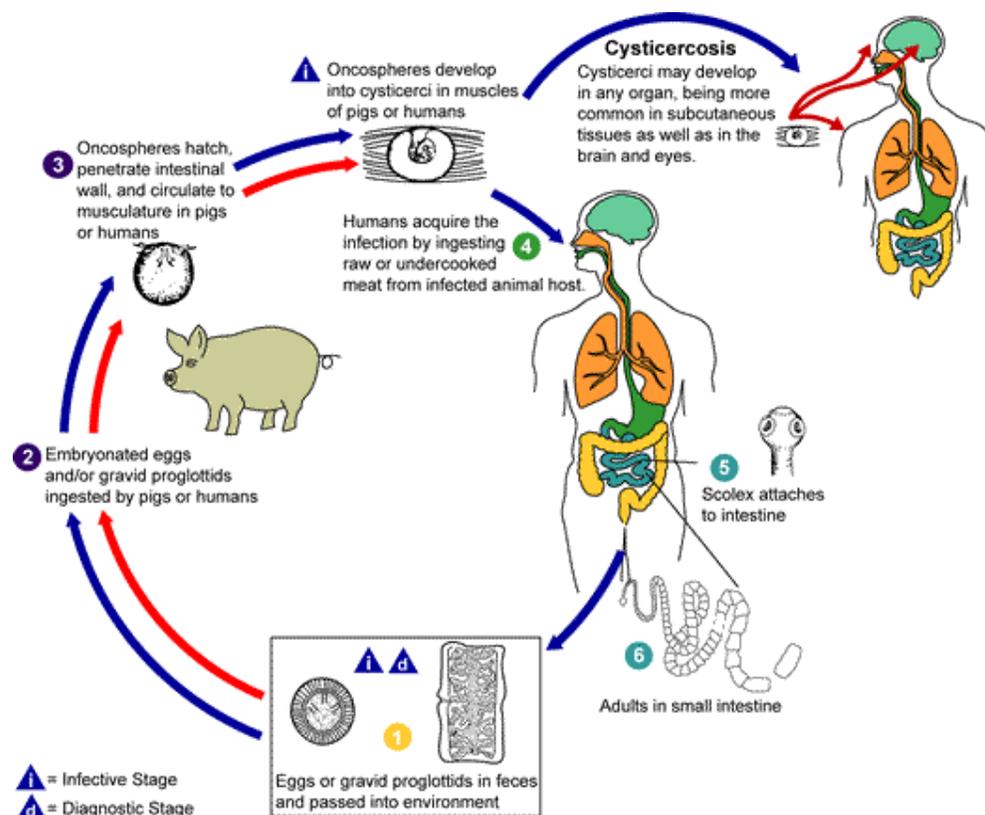
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Life cycle of *Taenia solium* (Source : Centers for Disease Control and Prevention (CDC), www.dpd.cdc.gov/dpdx/HTML/Cysticercosis.htm)

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2: *TAENIA SOLIUM* CYSTICERCOSIS IN AFRICA

2.1. REGIONAL STATUS, EPIDEMIOLOGY AND IMPACT OF *TAENIA SOLIUM* CYSTICERCOSIS IN WESTERN AND CENTRAL AFRICA.

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Abstract

In West Africa, *T. solium* cysticercosis in pigs and man has been reported in Benin, Burkina-Faso, Ghana, Ivory Coast, Senegal and Togo, and although official data are lacking, *T. solium* is anticipated to be present in most of the pig-raising regions of other West African countries as well. In some regions of Nigeria, the prevalence of porcine cysticercosis and human taeniosis is quite high (20.5% and 8.6% respectively). Surprisingly, however, no cases of human cysticercosis have been reported, although epilepsy is very common. Large epidemiological surveys have only been carried out in Togo and Benin, where the prevalence of human cysticercosis was 2.4% and 1.3%, respectively.

In Central Africa, porcine and human cysticercosis are (hyper)-endemic in Rwanda, Burundi, the Democratic Republic of Congo and Cameroon. The parasite also has been reported in pigs in Chad and Angola. Cysticercosis has been shown to be one of the major causes of epilepsy in Cameroon with figures as high as 44.6%. Cameroon is one of the few countries where the taeniosis-cysticercosis complex has been examined more in detail. In the Western province of Cameroon large scale surveys have shown that active cysticercosis is present in 0.4-3 % of the local population and in 11% of the village pigs. However, the prevalence of adult *T. solium* was only 0.1 %, which underscores the frequency of the *T. solium* paradox.

Based on the available information a very conservative economic estimate indicates that the annual losses due to porcine cysticercosis in 10 West and Central African countries amount to about 25 million Euro. The financial losses due to human cysticercosis are very difficult to estimate, but are certainly exceeded by the social impact of the disease, especially because of the particular perception of epilepsy in many African communities.

It is concluded that the true prevalence of *T. solium* cysticercosis in pigs and humans in Central and West Africa remains underestimated because of unreliable slaughterhouse data and the lack of awareness and diagnostic facilities in the public health sector.

Key words: *Taenia solium*; cysticercosis; man; pigs; Africa; Economic burden; review.

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1. Introduction

According to Tsang and Wilson (1995) *Taenia solium* cysticercosis is largely under-recognised in many developing countries. This statement is true for Africa and even more so for Central and Western Africa where –until recently- very few epidemiological data were available (Geerts, 1993,1995). However, during the past decade many surveys have been carried out in several countries. Comprehensive studies on the taeniosis-cysticercosis complex have been done in Benin, Togo and Cameroon.

Except for the Muslim regions, where pigs pork is not eaten for religious reasons, *T. solium* cysticercosis affects virtually all countries in Western and Central Africa. Although official data are often lacking, in most of the countries all necessary conditions are fulfilled for an easy transmission of the parasite from pigs to man and vice versa: open air defecation or deliberate defecation in pig sties, clandestine slaughtering of pigs, lack of trained and qualified meat inspectors, lack of detection and treatment of *T. solium* carriers and consumption of raw or insufficiently cooked pork (Preux et al, 1996; Zoli et al., 1998; Geerts et al., 2002).

Based on the available literature and on personal collection of information, this paper presents an overview of the regional status, the epidemiology and the impact of *T. solium* cysticercosis in Western and Central Africa . It has to be noticed that many data about porcine cysticercosis which were provided by meat inspection services, should be interpreted with caution. It is well known that these figures are not representative for the real situation, since a large proportion of pigs, and certainly the cysticercotic pigs, are slaughtered outside the abattoirs. Furthermore, meat inspection often underestimates the true prevalence of porcine cysticercosis as was clearly demonstrated by Onah & Chiejina (1995), who found 20.5 % of pigs with cysticercosis after detailed inspection of the carcass, whereas the official meat inspection reported only 3%.

2. Regional Status and Epidemiology

2.1. Western Africa

The available data on porcine and human cysticercosis are summarised in table 1. Due to political instability over a long period it is very difficult to collect information in Guinea-Bissau, Liberia, Sierra Leone, but there are strong indications that *T. solium* is equally present in the pig raising regions of these countries. In Nigeria, the prevalence rate of cysticercosis in pigs has been reported to reach up to 20.5% in some regions (Onah and Chiejina, 1995). Taking into account the high numbers of *Taenia* spp. carriers (8.6%) in the same region (Onah and Chiejina, 1995) and the high prevalence of epileptics in the country (37 per 1000), it can be assumed that human cysticercosis is present in Nigeria, although no reports were published up to now.

Table 1. Prevalence of Porcine and Human Cysticercosis in West Africa

Country	Prevalence in pigs ¹		Seroprevalence in man ²	
	%	Reference	%	Reference
Benin	ND		1.3*	Houinato et al., 1998
Burkina Faso	0.6	Coulibaly et al., 2000	CR	Preux et al., 1996
Ghana	11.7	Permin et al., 1999	CR	Odamtten&Laing, 1967
Ivory Coast	2.5	Mishra & N'Depo,1978	CR	Heroin et al., 1972
Nigeria	1.8-18.4	Dada, 1980	ND	Onah & Chiedjina,1995
	20.5	Onah & Chiedjina,1995		
Senegal	1.2	Deme, pers.comm,2000	CR	Collomb et al., 1964
Togo	17	Dumas et al., 1990	2.4*	Dumas et al., 1989

*: % of the general population ; ¹: meat inspection ; ²: antibody detection ELISA; CR: case report; ND, no data available.

In West Africa *T. solium* cysticercosis in man was recorded for the first time in Ivory Coast (Bowesman, 1952). Proctor et al. (1965) identified spinal cysticercosis as a major cause of paraplegia in Ghana. Extensive studies were carried out in Togo and Benin in order to study the prevalence of the disease. In 1989, Dumas et al. reported 2.4% and 29.5% seropositives for cysticercosis in the adult population and in epileptics of northern Togo, respectively. A prevalence of 10.8% was reported in hospitalized epileptic patients in the capital Lome (Grunitzky et al., 1995). In Benin, nation-wide surveys did show that the overall seroprevalence of cysticercosis in the general population was 1.3 % with a significantly higher prevalence in men (1.9 %) than in women (0.8 %) and that the prevalence increased with increasing age (Houinato et al., 1998). Obviously, higher seroprevalence rates were found in non-muslim regions (up to 3.3 %) than in muslim regions (up to 0.8%). The prevalence rate of epilepsy in Benin was 15.2 per thousand which is comparable to the rate in Togo (16.7 per 1000) (Avode et al, 1998; Dumas et al., 1989).

2.2. Central Africa

In 1965 Nelson et al. reported that *T. solium* was common in Cameroon and parts of East Congo (DRC, ex-Zaire). Pandey and Mbemba (1976) confirmed that 0.1-8.1% of pigs in different regions (Shaba, Ituri, Kinshasa and Kivu) of the Democratic Republic of Congo were infested with *C. cellulosae*. In 1990, up to 30% of pigs were infected with *T. solium* cysticercosis in some regions of the DRC (Chartier et al., 1990). Fain (1997) observed a high frequency of epilepsy during colonial times in eastern upper Congo with the presence of cysticerci in about 3 % of the population.

Table 2. Prevalence of Porcine and Human Cysticercosis in Central Africa.

country	Prevalence in pigs		Prevalence in man	
	%	Reference	%	Reference
Angola	0-6.8 ¹	Kama, 1998	?	
Burundi	2-39 ¹	Newell et al., 1997	2.8 ^o	Newell et al., 1997
Chad	6.8 ¹	Graber&Chailloux, 1970	?	
	25.7 ²	Assana et al.,2001		
Congo	0.1-8.1	Pandey & Mbemba, 1976 Chartier et al., 1997	3*	Fain, 1997
Rwanda	10-30 ¹ 20 ¹	Thienpont et al., 1959	7**	Vanderick et al., 1972

¹ Classical meat inspection, ² Tongue examination; *: presence of cysticerci; **:based on autopsies; °:serology

In Chad, Graber and Chailloux (1970) reported a prevalence rate of 6.8% in Fort Lamy's slaughterhouse based on classical meat inspection. Recent studies carried out by Assana et al. (2001) showed that 26.0% and 40.8% of pigs in Mayo Kebbi (South-West of Chad) were positive by tongue examination and an antigen detection ELISA (Ag-ELISA), respectively.

Rwanda has for a long time been considered as being hyperendemic for taeniosis-cysticercosis (Brandt, 1997). The prevalence of *T. solium* cysticercosis in the human population is even higher than in some hyperendemic regions in Latin America. Cysticercosis was present in 7% of 300 autopsies carried out in a region of Butare (Vanderick and Mbonyingabo, 1972) as against 2.4% in Mexico (Sarti et al. 1992). Unfortunately, no recent figures are available for Rwanda, except the high percentage (21 %) of seropositives for cysticercosis among epileptics (Tsang & Wilson, 1995). In Burundi the prevalence of porcine cysticercosis seems to be very different from one region to the other (2-39%) (Newell et al., 1997). The latter authors reported a seroprevalence of 2.8 % in the general population and 11.7 % in epileptics.

Cameroon is the only Central African country in which the taeniosis/cysticercosis complex has been studied in greater detail both in pigs and in man. Since the report of Nelson et al. (1965) that cysticercosis was common in the Cameroons, many surveys were carried out in village pigs, on markets and at slaughterhouses. The results are summarised in table 3 and show that up to 24.6 % of the pigs are positive by tongue inspection. The risk factors for cysticercosis in village pigs in Cameroon were studied by Pouedet et al. (2002) (Figure 1). As expected, animals that were usually confined were significantly less infected than free roaming pigs and infection rates were significantly higher in pigs that had access to human faeces than in those without access. Adult pigs were more frequently infected than young ones. Recent surveys in the western and north-western parts of Cameroon showed that 81.6 % of the village pigs are usually kept in confinement and 18.4 % are free roaming; 53.1 % of these pigs had access to human faeces, i.a. due to the local habit of defecation in the pigsties (Pouedet *et al.*, 2002; Shey-Njila *et al.* 2002, unpublished results).

Table 3. Prevalence of porcine cysticercosis in Cameroon based on different detection methods

Locality	Prevalence (%)				Reference
	Tongue Inspection	Carcass inspection	Serology		
			Ag-ELISA	Ab-ELISA	
Garoua ^a	-	12	-	-	Awa et al., 1999
Menoua ^b	24.6	19.9	-	38	Zoli et al., 1987
	6.3	-	11.0	21.8	Pouedet et al., 2002
Mifi ^b and Bamboutos ^b	-	2.3	11.2	21.8	Nguekam, 1998
Extreme North ^c	20.5	15.7	39.8	-	Assana et al., 2001
North- West ^c	4.4	-	27	-	Unpubl. results, 2002
Ngaoundere ^a	7.8	-	22.4	-	Unpubl. results, 2002

^aTown

^bDepartment

^cProvince

Ag-ELISA

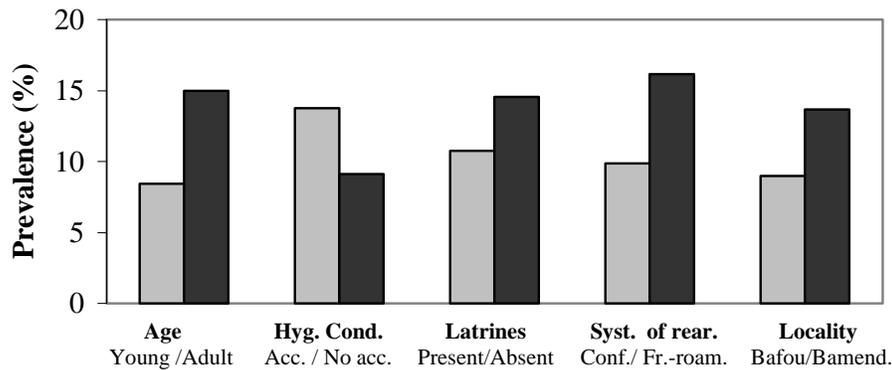


Figure 1: Prevalence of cysticercosis (Ag-ELISA) in function of pig age, rearing system, household hygienic conditions, presence or absence of latrines, and locality in West Cameroon (Pouedet et al., 2002). Hyg. Cond.: Hygienic condition; Acc.: Access to human faeces; No acc.: No access to human faeces; Syst. of rear.: System of rearing; Conf.: Confinement; Fr.-roam.: Free-roaming; Bamend.: Bamendou.

In contrast, in the Far North of Cameroon this habit does not exist. People defecate nearby the farms in open air so that only scavenging pigs have access to the human excrements (Assana *et al.*, 2002). The prevalence of human cysticercosis has been reported to range between 0.7 and 2.4% (Zoli *et al.*, 1987; Nguekam *et al.*, in press) in the West Province of Cameroon. A recent survey amongst Cameroonian epileptic patients revealed a very high prevalence rate (44.6%) using an antibody detection ELISA (Zoli *et al.*, 2002, unpublished results).

Table 4. Prevalence of adult *Taenia* spp. in some Central and West-African countries

Country	Target group	Prevalence (%)	Reference
Burundi	Schoolchildren (n=13,841)	0.22	Newell <i>et al.</i> , 1997
Cameroon	General population (n=3109)	0.13*	Vondou <i>et al.</i> , 2002
Guinée (Conakry)	Schoolchildren (n=800)	3.8	Gyorkos <i>et al.</i> , 1996
Nigeria	Hospital patients (n=1525)	8.7	Onah&Chiejina, 1995
Togo	Adult population (n=1170)	0.09-0.26	Dumas <i>et al.</i> , 1990

*: 75 % were *T. solium*, 25 % *T. saginata*

Furthermore, it was shown that the risk for taeniosis and cysticercosis in butchers and tongue inspectors of the Menoua District was not significantly higher than in people who professionally did not come into contact with pigs or pork (Vondou *et al.*, 2002). Out of 3,109 stool samples examined microscopically, only 4 *Taenia* spp. carriers were found, from which 3 (0.1%) were identified as *T. solium* (Vondou *et al.*, 2002). Similar results have been reported in the few other surveys on *Taenia* spp., which are available (Table 4). However, in Guinée (Conakry) and Nigeria higher prevalence figures have been observed, although no distinction was made between *T. solium* and *T. saginata* in these latter countries.

3. Impact

3.1. Impact on pig production

Porcine cysticercosis is an economically important parasitic disease because it affects a large number of pigs, making their meat unfit for human consumption and thereby incurring sizable economic losses (Acevedo-Hernandez, 1982). According to the legislation in many African countries the meat of infected pigs should be destroyed. However, due to the lack of

well organised meat inspection and to common illegal slaughtering, partial or total seizures due to cysticercosis are rather exceptional and almost all infected carcasses are marketed and/or consumed. Usually a carcass of a pig infected with cysticercosis is sold at a decreased price. This decrease in value is different from country to country. In Cameroon it is on average 30 %, whereas in Benin it is about 25 % or more for carcasses harboring calcified or living cysts, respectively (Codjia, pers. comm.). In Rwanda the losses may reach 50 % of the carcass value (Geysen, pers. comm). Taking into account a pig population of 1,4100,000 (FAO, 1999), an average prevalence of cysticercosis of 9.75 % (based on tongue inspection) and an average value of 50 Euro for an adult pig, the annual economic losses due to cysticercosis in Cameroon can be estimated at minimum 2,062,125 Euro. Table 5 shows that the estimated annual loss due to cysticercosis in 10 West and Central African countries reaches about €25 million based on an average loss of €15 per infected pig (30 % of the value of an adult pig in Cameroon).

Table 5. Estimated economic losses (in Euro) due to pig cysticercosis in 10 West and Central African countries

Country	Porcine Population #	Average prevalence (%) of cysticercosis	Estimated loss* (in Euro)
Angola	800000	3.4	408,000
Burkina Faso	587 000	0.6	52,830
Burundi	71000	20.5	218,325
Cameroon	1410000	9.75	2,062,125
Chad	23000	16.25	56,063
DR Congo	1180000	12.1	2,141,700
Ghana	339000	11.7	594,945
Nigeria	7 600 000	15.3	17,442,000
Senegal	320000	1.2	57,600
Togo	850 000	17	2,167,500
Total	13,180,000	12	25,201,088

FAO, 1999; * 30 % of the value of an adult pig: 15 €

It has to be noticed that in some villages in West Cameroon pork infected with cysticerci is considered to have a better flavour than healthy meat. Therefore, pork harbouring cysticerci is sometimes sold at a higher price than uninfected meat (Zoli and Tchoumboué, 1992).

3.2. Impact on human health

The impact of cysticercosis on human health is difficult to estimate, because of the highly variable clinical picture of the disease, going from asymptomatic to severe headache, epilepsy and even death. Roberts et al. (1994) estimated the average hospitalisation of a patient with cysticercosis at 8 days and the wage loss at 19 days. Furthermore, the costs of several visits to the physician, the costs of serology and/or CT-scan, transport and drugs (anthelmintic and/or symptomatic) have to be taken into account. Although in many African countries patients are not hospitalised during the treatment of neurocysticercosis, the losses due to the disease remain quite important. Djou (2001) estimated the costs (wage losses not included) for treatment of one cysticercosis patient in Cameroon at 170,000 FCFA (260 Euro), which is far beyond the reach of the affected resource-poor population. Based on an average prevalence of active neurocysticercosis in Cameroon of 0.7 % (Nguekam et al, in press) and taking into account a treatment percentage of 50 % of the patients, treatment of about 52,000 people would represent a cost of €13,520,000.

Besides these purely economic aspects, the social aspects of epilepsy have also to be taken into account. Most communities cast out epileptic patients, because epilepsy is

considered as a contagious and/or a shameful disease (Avode *et al.*, 1996; Preux *et al.*, 2000). Therefore, epileptics are often isolated to prevent the spread of the ailment. According to surveys of Preux *et al.* (2000) in West Cameroon only 27% of epileptics get married and 39% don't get involved in any professional activity. Furthermore, certain practices of traditional healers might be quite harmful to the patients. A majority of healers explained epilepsy as the presence of excess foam in the abdomen. Some patients reported that they were asked to inhale smoke from burnt cola nut leaves and mistletoe as a form of treatment. Others had to restrict some foodstuffs such as meat, eggs, sweet potatoes, sugar cane etc., which paradoxically are highly nutritious foods.

4. Conclusions

The data presented in this review clearly underscore the importance of *T. solium* cysticercosis in man and pigs in West and Central Africa, albeit that the available data are an underestimation of the true prevalence due to the unavailability of diagnostic tools. Therefore, cysticercosis must be considered as one of the most "neglected" parasitoses, rivalling the well-documented significance of this zoonosis in Latin America.

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Heart of a pig infected with cysticerci of *Taenia solium*

3: PORCINE AND BOVINE CYSTICERCOSIS IN CAMEROON

3.1. PREVALENCE OF PORCINE CYSTICERCOSIS IN MAYO-DANAY (NORTH CAMEROUN) AND MAYO-KEBBI (SOUTH WEST TCHAD) (in french)

Prévalence de la cysticerose porcine dans le Mayo-Danay (Nord Cameroun) et le Mayo-Kebbi (Sud Ouest Tchad)

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Abstract: A study was conducted from May to October 1999 in the Far North of Cameroon (Mayo-Danay) and the South West of Tchad (Mayo-Kebbi) in order to determine the prevalence of porcine cysticercosis and to identify the main factors associated with parasite transmission. Hygienic standards and sanitary conditions in pig husbandry appeared to be poor and pigs were highly infected with *Taenia solium* cysticerci. In fact 42 % of 126 households did not have latrines. Free roaming pigs had direct access to human feces. Tongue inspection showed that 20.5% of the 852 pigs examined were infected by the parasite. Meat inspection at local slaughterhouses indicated that 15.7% of 51 slaughtered pigs harboured cysticerci. Analysis of 264 pig sera by an ELISA-test for the detection of circulating antigens showed that 39.8% of animals were seropositive. This study indicates that Mayo-Danay and Mayo-Kebbi are important foci of *Taenia solium* cysticercosis.

Résumé : Une étude conduite de Mai à Octobre 1999 dans le Département du Mayo-Danay (Extrême-Nord du Cameroun) et dans la Préfecture du Mayo-Kebbi (Sud-Ouest du Tchad) avait pour but de déterminer la prévalence de la cysticerose porcine et d'identifier les principaux facteurs qui la favorisent. Elle a montré que les conditions hygiéniques dans lesquelles vit la population ainsi que celles de l'élevage des porcs y sont très médiocres, ce qui a comme conséquences des infestations massives de la population porcine par des cysticerques de *Taenia solium*. En effet 42 % des 126 exploitations

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visitées sont dépourvues de latrines et les porcs, en divagation permanente ou semi-permanente, ont facilement accès aux matières fécales humaines déposées aux alentours des habitations. Le diagnostic clinique effectué par la méthode du “ langage ” a montré que 20,5% des 852 porcs vivants examinés sont porteurs de cysticerques. L'inspection des carcasses réalisée dans les abattoirs locaux a révélé que 15,7% des 51 porcs abattus étaient laders. Parmi les 264 sera de porcs soumis au test ELISA pour la détection des antigènes circulants de cysticerques, 105 soit 39,8% se sont révélés positifs. Ces résultats indiquent que le Mayo-Danay et le Mayo-Kebbi sont d'importants foyers de cysticercose porcine à *Taenia solium*.

Mots-clés: *Taenia solium*, prevalence, Cameroon, Tchad, pig, cysticercosis

Introduction.

La cysticercose, due aux larves du *Taenia solium* ou *Cysticercus cellulosae*, constitue un problème économique et de santé publique important, mais relativement méconnu dans un grand nombre de pays africains (6, 7, 17). Et pourtant, à l'exception des régions où l'élevage et surtout la consommation du porc constituent un tabou religieux, la cysticercose porcine affecte probablement tous les pays au sud du Sahara où les conditions d'apparition et de perpétuation de cette zoonose sont généralement réunies : conditions hygiéniques pauvres, absence quasi généralisée de latrines, surtout dans les zones rurales, divagation permanente ou saisonnière des porcs et absence totale d'inspection de la viande des porcs (16). Mais, contrairement à l'Amérique latine, les données sur la cysticercose porcine sont plutôt rares en Afrique bien que quelques études y aient été menées. En effet, Chambers (4) rapporte qu'au Zimbabwe 67,6 % des carcasses de porcs saisies en un an dans les abattoirs étaient laders. Au Nigeria une prévalence de 20 % a été rapporté par Onah et Chiejina (14) dans l'Etat d'Enugu. En Tanzanie un taux moyen d'infestation de 13,3 % a été déterminé dans trois communes du pays (2). Au Cameroun la fréquence et la distribution de la cysticercose porcine sont également assez mal connues. Les études menées jusqu'ici sur cette maladie ont été focalisées surtout dans la région de l'Ouest (9, 10, 12, 15, 18, 19). Au Nord du pays, Awa et al. (1) ont rapporté que 12,3 % des 750 carcasses inspectées à l'abattoir de Garoua étaient infestées. Au Tchad, Graber et Chailloux (8) ont rapporté des cas de laderie massive chez des porcs abattus à Fort-Lamy. En dehors de ces travaux, il n'existe pas d'autres données sur la cysticercose porcine et humaine dans la partie septentrionale du Cameroun et au Tchad, surtout dans les grandes régions d'élevage porcin de l'Extrême-Nord du Cameroun et du Sud-Ouest du Tchad (13). C'est pourquoi nous avons entrepris une étude dans ces régions afin d'identifier les facteurs susceptibles de favoriser l'infestation des porcs par les œufs de *T. solium*, et d'y déterminer la prévalence de cette zoonose. Les diagnostics ont été réalisés sur les animaux vivants et abattus et également par l'examen sérologique grâce au test ELISA sandwich pour la détection des antigènes circulants des métacestodes du *Taenia spp.*

Materiels et Methodes

L'enquête a eu lieu sur deux marchés du Département du Mayo-Danay (Extrême-Nord du Cameroun) situés à une dizaine de kilomètres chacun de la frontière Tchad-Cameroun d'une part, et dans 126 élevages dont 70 dans le Département du Mayo-Danay et 56 dans la Préfecture du Mayo-Kebbi (Sud-Ouest du Tchad) d'autre part. Ces deux régions d'étude s'étendent entre le 9° et le 11°15' de latitude Nord et le 14° et 16°30' de longitude Est. Les exploitations, choisies au hasard, abritaient un total de 2065 porcs. Un questionnaire a servi pour la collecte des données sur le système d'élevage, les conditions hygiéniques et sanitaires dans les exploitations, le niveau de connaissance des éleveurs par rapport au complexe taeniose-cysticercose. Des observations directes sur l'environnement des exploitations ont permis également de déterminer les habitudes hygiéniques des éleveurs

ainsi que celles de leurs voisins non éleveurs, mais pouvant avoir des contacts, d'une façon ou d'une autre, avec les porcs.

Animaux

Un total de 903 porcs de races locales dont 852 vivants – 566 adultes (> 1 an) et 286 jeunes (< 1 an) et 51 abattus (23 > 1 an et 28 < 1 an) a été examiné par les méthodes de “langueyage” pour les animaux sur pieds, et de l'inspection des carcasses pour ceux qui sont abattus. Le “langueyage” consiste en l'examen et/ou la palpation de la face inférieure de la langue. L'inspection des carcasses a été effectuée sur les marchés mêmes par des infirmiers vétérinaires selon la méthode classique, à savoir des incisions pratiquées au niveau des masséters, du cœur, des muscles fessiers, du diaphragme et de la langue.

Sérums

Des échantillons de sang ont été prélevés sur 255 des 852 porcs vivants qui se sont révélés “indemnes” à l'examen clinique, et seulement sur 9 porcs abattus, également “indemnes” à l'examen ante et post-mortem. Les séra ainsi obtenus ont été congelés à -20°C jusqu'au moment de l'analyse.

ELISA pour la détection d'antigènes circulants de cysticerques de *Taenia solium* (Ag-ELISA)

L'ELISA-sandwich a été fait comme décrit par Dorny et al. (5). Les anticorps monoclonaux (Mab) B158C11A10 et B60H8A4 biotynilé ont été utilisés pour capturer les antigènes circulants. Ces MAb ont été développés contre les produits d'excrétion et de sécrétion de cysticerques de *T. saginata*, mais ils réagissent aussi bien avec ceux de *T. solium* (3). Le complexe streptavidine-peroxydase et l'orthophénylène diamine ont servi respectivement comme conjugué et comme chromogène. La réaction a été lue à l'aide d'un lecteur ELISA (Multiskan RC, Labsystems) à 492 nm. Le seuil de positivité a été déterminé par comparaison de la densité optique (DO) de chaque échantillon avec la moyenne des DO d'une série de 8 échantillons négatifs (provenant de porcs d'un élevage amélioré sans risque d'exposition aux oeufs de *T. solium*), au seuil de probabilité de 0,001 (test de Student modifié).

Resultats et Discussion

Système d'élevage porcin et conditions hygiéniques

Les tableaux 1 à 3 résument les facteurs favorisant l'infestation des porcs par les métacestodes de *T. solium*. Ces facteurs sont : la divagation des animaux, le manque de latrines, les défécations délibérées à l'air libre à des endroits facilement accessibles aux porcs et la méconnaissance par la population du mode d'infestation et des aspects zoonotiques et pathologiques du parasite.

Tableau 1 : Systèmes d'élevage dans les régions de l'enquête

Régions	Elevages enquêtés		Elevages en divagation permanente			Elevages en divagation saisonnière			Elevages en clausuration permanente		
	No.él.	Eff. porcs	No. él.	Eff. porcs	% él.	No. él.	Eff. porcs	% él.	No. El.	Eff. porcs	% él.
Mayo-Danay (Cameroun)	70	1083	26	525	37,1	38	460	54,3	6	98	8,9
Mayo-Kebbi (Tchad)	56	982	31	463	55,4	25	519	44,6	0	0	0

No. : Nombre. El. : élevages. Eff. : Effectifs

Tableau 2 : Niveaux hygiéniques et sanitaires dans les exploitations porcines.

Régions	Total exploitations enquêtées	Exploitations avec latrines		Exploitations où la défécation se fait dans			
		No	%	Porcherie		Autres lieux *	
		No.	%	No.	%	No.	%
Mayo-Danay (Cameroun)	70	57	67,1	0	0	23	32,9
Mayo-Kebbi (Tchad)	56	26	46,4	0	0	30	53,6

No. : Nombre

*Autres lieux : Champs, environs des habitations, touffes d'arbustes qui longent les pistes des bétails ect...

Tableau 3 : Niveau de connaissance des éleveurs du complexe Tæniose-Cysticerose

Régions	Chefs d'exploitations porcines enquêtés	Chefs d'exploitations connaissant la cysticerose porcine		Chefs d'exploitations connaissant la relation entre la Tæniose et la Cysticerose		Chefs d'exploitations connaissant la Cysticerose humaine	
		Nombre	%	Nombre	%	Nombre	%
Mayo-Danay (Cameroun)	70	54	77,1	9	12,9	0	0
Mayo-Kebbi (Tchad)	56	31	55,4	7	12,5	0	0

Bien que la majorité de la population musulmane du Cameroun se trouve dans les 3 provinces septentrionales du pays (Adamaoua, Nord et Extrême-Nord), elle y constitue cependant moins de la moitié de la population locale. Ce fait est encore plus marqué au

Nord et à l'Extrême-Nord. En effet la majorité de la population dans ces deux provinces y est de religion chrétienne ou animiste. C'est la raison pour laquelle l'élevage du porc y est non seulement présent, mais depuis quelques années, il y prend un développement toujours croissant. Les grandes métropoles de Yaoundé et de Douala sont régulièrement approvisionnées en porcs plus à partir de ces deux provinces que de la province de l'Ouest qui a vu son cheptel porcin se réduire drastiquement depuis le début des années 80 suite à l'épizootie de peste porcine africaine (PPA) restée enzootique dans la région (11). Les trois provinces du nord sont pour l'instant indemnes de la PPA (1) et il est à souhaiter que la barrière sanitaire reste suffisamment étanche pour les préserver de cette épizootie. Toutefois d'autres entraves à l'essor de l'élevage porcin existent dans le Nord. Il s'agit notamment des pathologies parasitaires externes (poux, gâles etc...) et internes (helminthoses, etc...) et surtout nutritionnelles qui entraînent des mortalités de 30 à 42% chez les porcelets avant l'âge d'un mois (1).

En plus, comme le montrent les tableaux 1 et 2, les porcs sont élevés dans des conditions hygiéniques très déplorables. En effet 32,9% et 53,6% des exploitations porcines visitées au Cameroun et au Tchad respectivement, sont dépourvues de latrines. Même si ici, contrairement à ce qui se passe dans la province de l'ouest (18), les porcheries ne servent pas de lieux de défécations, celles-ci se font le plus souvent à l'air libre dans des endroits facilement accessibles aux porcs dont à peu près 95% sont en divagation permanente ou saisonnière (Tableau 1). Les centres urbains n'échappent pas non plus à cette règle dans la mesure où il y existe des terrains vagues qui servent de lieux de défécation et où les porcs, en divagation permanente ont l'habitude de se rassembler.

La structure même des porcheries, généralement construites en matériaux locaux peu résistants, ou le fait que les porcs soient maintenus au piquet, permettent à ceux-ci de s'échapper facilement, ce qui favorise leur divagation et les expose ainsi aux infestations diverses dont l'infestation par les œufs de *T. solium* contenus dans les matières fécales humaines déposées à l'air libre.

En ce qui concerne le niveau de connaissance des éleveurs au sujet du complexe taeniose-cysticercose, le tableau 3 montre qu'il y a encore un grand effort à faire dans le domaine de l'éducation sanitaire. Bien qu' environ deux tiers des chefs d'exploitation connaissent la cysticercose porcine, il semble y avoir très peu de gens, qui se rendent compte de la relation avec la taeniose et avec la cysticercose humaine.

Cysticercose porcine

Les résultats du langage et de l'examen post-mortem sont consignés dans les Tableaux 4 et 5 dont il ressort qu'en moyenne 20,5% des porcs vivants sont porteurs de cysticerques et que 15,7 % des porcs abattus sont laders. Le nombre plus faible de porcs parasités observés lors de l'inspection de viande est dû au fait que seuls les porcs, qui sont négatifs au langage, sont abattus. Ce chiffre de 15,7 % est deux fois supérieur à celui observé par Graber et Chailloux (8) dans la même région du Tchad et presque identique à celui d'Awa et al. (1) dans le Nord-Cameroun. En 1984-85 Zoli et al. (18) ont trouvé une prévalence plus élevée (24,6% au langage) dans le département de la Ménoua, même si Nguékam (12) et Pouedet (15), suite à une certaine amélioration du système de l'élevage, ont montré qu'entre 1997 et 2000 la prévalence de la cysticercose porcine a sensiblement diminué dans la province de l'ouest (respectivement 2,3 et 6,1%).

Tableau 4 : Prévalence de la cysticercose porcine dans les zones d'étude (langage).

Régions	Nombre de porcs examinés	Nombre de porcs ladres	Prévalence (%)
Mayo-Danay (Cameroun)	441	68	15,4
Mayo-Kebbi (Tchad)	411	107	26,0

* : 155 et 20 respectivement chez des porcs adultes (n=566) et jeunes (n= 286)

Tableau 5 : Prévalence de la cysticercose porcine chez les porcs abattus (inspection de viande)

Catégories	Nombre total des porcs inspectés	Porcs infestés	
		Nombre	Prévalence (%)
Porcs adultes	23	7	30,4
Porcs jeunes	28	1	3,6

Les résultats de l'Ag-ELISA (Tableau 6) réalisé sur 264 porcs "indemnes" à l'examen clinique montre que 38,9% et 40,8 % étaient positifs respectivement dans le Mayo-Danay et le Mayo-Kebbi. Comme il a été démontré par Brandt et al. (3), cela indique que ces animaux sont des porteurs de cysticerques vivants, parce que l'Ag-ELISA ne détecte pas des cysticerques morts. Ce test a une sensibilité de 84,6 % et une spécificité de 99,1 % (12). Bien que la divagation des porcs et la défécation en dehors des latrines soit nettement plus fréquente au Mayo-Kebbi qu'au Mayo-Danay et par conséquent aussi le taux de positifs au langage soit plus élevé dans la première région (Mayo-Kebbi: 26 % contre 15,4 % au Mayo-Danay), les séroprévalences observées dans les deux régions étaient fort similaires. Ceci pourrait s'expliquer par le fait que l'Ag-ELISA détecte aussi bien les infestations légères que massives, tandis que le langage détecte plus facilement les infestations massives. On peut donc supposer que le nombre de porcs faiblement positifs au Mayo-Danay soit plus important qu'au Mayo-Kebbi à cause du fait que dans la première région la divagation des porcs est moins fréquente et par conséquent aussi l'accès aux proglottis entiers de *T. solium* (qui est à la base d'infestations massives).

Tableau 6: Séroprévalence de la cysticercose porcine (Ag-ELISA).

Régions	Nombre de sérums Examinés	Nombre de sérums positifs	Séroprévalence (%)
Mayo-Danay (Cameroun)	139 (9)	54 (2)	38,9
Mayo-Kebbi (Tchad)	125	51	40,8

(9) et (2) : Neuf (9) sera sur 102 de Mayo-Danay ont été prélevés sur les porcs abattus, mais indemnes à l'inspection ante et post-mortem. Deux (2) sera parmi les neufs ont réagi positivement à l'analyse sérologique.

Conclusion

Cette étude, réalisée sur un nombre relativement restreint de porcs ainsi que celle de Njoya et al. (13) menée dans la région de Garoua (province du Nord) indiquent que le Nord-Cameroun et le Sud-Ouest du Tchad sont des régions hyperendémiques pour la cysticercose porcine. Cela s'explique par le fait que les conditions d'infestation des porcs par les œufs de *T. solium* et des hommes par les métacestodes sont réunies dans ces régions. En effet : 1°) l'inexistence ou la rareté des latrines, même dans les centres urbains, et les défécations à l'air libre, 2) la divagation permanente ou semi-permanente des porcs, 3°) les abattages des porcs qui se font en général à domicile et/ou dans des endroits inaccessibles aux services vétérinaires, 4°) l'inspection de la viande de porcs qui est quasi inexistante et 5°) la méconnaissance des aspects zoonotiques du parasite constituent des conditions idéales pour l'accomplissement du cycle biologique du parasite et la perpétuation de la zoonose dans les deux régions.

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***T. solium* cysticerci in the liver of a pig**

3.2. EPIDEMIOLOGICAL SURVEY OF SWINE CYSTICERCOSIS IN TWO RURAL COMMUNITIES OF WEST-CAMEROON

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Abstract:

To determine the prevalence of porcine cysticercosis, a survey was carried out in 27 villages belonging to two rural communities of West-Cameroon (Bafou and Bamendou). Between January and August 2000, a total of 707 pigs were examined serologically and by tongue inspection. Serum samples were examined for circulating parasite antigen using a monoclonal antibody-based sandwich enzyme-linked-immunosorbent assay (Ag-ELISA) and for antibodies against cysticerci (Ab-ELISA). Seventy-eight samples (11.0%) were found positive in the Ag-ELISA and 154 (21.8%) in the Ab-ELISA, while by tongue inspection on the same animals cysticerci were detected in forty-three pigs (6.1%). Gibbs Sampling using results of these three tests indicated that the estimated prevalence of porcine cysticercosis was 10.9%. Analysis of the Ag-ELISA results demonstrated that adult pigs showed a significantly higher seroprevalence (15%) than young ones (8.4%). There was no statistical difference in cysticercosis prevalence in pigs raised in households with or without a latrine. Animals that were reported to be usually confined were significantly less infected (9.9%) than free roaming pigs (16.2%). Infection rates were significantly higher in pigs that had access to human faeces (13.8%) than those which did not have access (9.1%). This study has identified some community behavioural and environmental practices that should be modified to prevent continuous transmission of porcine cysticercosis.

Key words: Pig; Cestoda; *Taenia solium*; Cysticercosis; ELISA; Epidemiology; Cameroon

1. Introduction

Cysticercosis in man and swine is an infection caused by the larval stage of *T. solium*. In the life cycle of this parasite, humans are the definitive hosts because they harbour the adult tapeworm in the small intestine whereas pigs are the normal intermediate hosts. Humans can also serve as the intermediate host by accidental ingestion of *T. solium* eggs. Infection in pigs is facilitated by their coprophagic habits (Sarti et al., 1992).

In many developing countries, this disease constitutes a serious but sometimes under-recognised public health problem (Tsang & Wilson, 1995) and causes important economic losses because of condemnation of infested pork (Roberts et al., 1994). Poor sanitation and lack of veterinary control provide the conditions to sustain the life cycle of *T. solium* (Garcia et al., 1998).

In Latin America (Flisser, 2002), and some parts of Asia (Ito et al., 2002) and Africa (Geerts et al., 2002), cysticercosis has been reported as endemic. In Cameroon, few reports have been published about cysticercosis in pigs (Zoli et al., 1987; Nguekam, 1998; Awa et al., 1999). Recently, Assana et al. (in press) described a new focus of cysticercosis in the far-

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north province of the country. All these studies in Cameroon have been carried out in local slaughterhouses or in market places. However, little or no data exist on the epidemiology of cysticercosis of village pigs in smallholders' farms. We used three different diagnostic techniques in order to reliably estimate the real prevalence of porcine cysticercosis in two rural communities of West-Cameroon (Bafou and Bamendou). We also analysed the effects of the age of the pigs, presence or absence of latrines in the household, access to human faeces, system of pig rearing, and locality on the seroprevalence of porcine cysticercosis.

2. Materials and Methods

2.1. Animals

Two hundred and ninety families, representing about 80% of the total number of pig raising households in two rural communities (Bafou and Bamendou, Menoua Division) in the western highlands of Cameroon were selected on the basis of accessibility. All pigs belonging to these households – except pregnant and nursing sows – i.e. 707 pigs (184 males and 523 females) were included in this study. The survey was performed between January and August 2000 in 27 villages of these two communities (altitude: 1800 m above sea level; mean annual rainfall: 1872 mm; temperature: 16-25°C).

2.2. Tongue inspection

The tongue test consisted of palpation and visual identification of nodules on the tongue. The pig was placed on its side, held by the neck and firmly restrained. A hard wooden rod was used to keep the mouth open. The tongue was pulled out, examined, and palpated throughout its base for the presence of cysticercosis nodules.

2.3. Serological tests

2.3.1 Serum samples

Blood samples were collected from 707 village pigs. The serum was separated and stored at -20°C until tested. Each sample was tested in duplicate, and on each ELISA plate two positive reference serum samples from local naturally infected pigs (*T. solium* cysticercosis confirmed at slaughter) and eight serum samples from *T. solium* cysticercosis-free pigs (negative at tongue palpation and originating from a local farm with good hygienic conditions and without any history of cysticercosis) were included.

2.3.2 Enzyme-Linked Immunosorbent Assay for the detection of circulating antigens (Ag-ELISA)

The Ag-ELISA, which was initially developed for *T. saginata* cysticercosis (Brandt et al., 1992), was performed as described by Dorny et al. (2000) with slight modifications. The sera were pre-treated using trichloroacetic acid (TCA) and used in ELISA at a final dilution of 1/4. Two monoclonal antibodies (MoAb) were used in a sandwich ELISA. MoAb B158C11A10 was diluted at 5µg/ml in carbonate buffer (0.06 M/pH 9.6) for coating and a biotinylated MoAb B60H8A4 (1.25µg/ml in PBS-T20/NBCS) was included as detector antibody. The incubation was carried out at 37 °C on a shaker during 30 min. for the coating of the first MoAb and during 15 min. for all subsequent steps. The chromogen/substrate solution consisting of orthophenylene diamine (DAKO, #S2045) and H₂O₂ was added and incubated without shaking between 30-33°C for 15 min. To arrest the reaction 50µl of 4N H₂SO₄ was added to each well. The plates were read using an ELISA reader (Labsystem Multiskan RC) at 492 nm.

2.3.3 Enzyme-Linked Immunosorbent Assay for the detection of antibodies against *T. solium* cysticerci (Ab-ELISA)

Fresh cysticerci were collected from massively infected local pigs and were carefully dissected from host tissues. Then, they were washed repeatedly and stored at -20°C . After thawing, the material was centrifuged at $3217g$ for 30min at 4°C . The supernatant (cyst fluid) was collected and used as antigen in the ELISA. The optimal dilution of the antigen, serum and conjugate was determined by “checker-board titration”.

The assay involved coating polystyrene ELISA plates (Nunc® Maxisorp) with $100\mu\text{l}$ per well of cyst fluid antigen diluted at $1/2000$ in carbonate buffer ($0.06\text{ M/pH } 9.6$), and incubating on a shaker during 30 min. at 37°C . The plates were washed once with PBS-Tween-20 (Phosphate Buffered Saline + 0.05% Tween-20). Blocking was done by adding $150\mu\text{l}$ per well of PBS-Tween-20 + 1% New Born Calf Serum (PBS-T20/NBCS), and then the plates were incubated on a shaker during 15 min. at 37°C . Plates were emptied and $100\mu\text{l}$ of test sera diluted at $1/300$ in PBS-T20/NBCS was added (without washing) and incubated on a shaker at 37°C for 15 min. After washing (three times), $100\mu\text{l}$ of peroxidase conjugate (Rabbit anti-Pig IgG, SIGMA) diluted $1/30\ 000$ in PBS-T20/NBCS was added and incubated on a shaker for 15 min. at 37°C . The wells were washed (3 times), and the final steps were executed as described for the Ag-ELISA. For both Ag- and Ab-ELISA, the optical density (O.D.) of each serum sample was compared with a series of reference negative serum samples ($n = 8$) at a probability level of $p = 0.001$ to determine the cut-off using a modified Student Test (Sokal & Rohlf, 1981).

2. 4. Data collection and analysis

Information on the system of pig rearing, faecal access, and presence or absence of latrines was provided by the heads of the households and verified by direct observation. Pigs were classified as adult (≥ 1 year) or young (< 1 year).

For the analysis of the effects of pig age, presence or absence of latrines in the household, access to human faecal materials, system of pig rearing, and locality on the seroprevalence (Ag-ELISA) of porcine cysticercosis, the z-test for the equality of two proportions (Kanji, 1999) was used with a probability level of $P = 0.01$.

A Bayesian approach was used to draw inferences about cysticercosis prevalence and test properties (Sensitivity, Specificity) of the three tests (tongue inspection, Ag-ELISA and Ab-ELISA). A Gibbs sampling programme (Gamerman, 1997) was developed in Winbugs (available upon request to D. Berkvens). Gibbs sampling is a Monte Carlo Markov Chain technique, whereby the transition makes use of the full conditional distributions. This technique allows the simultaneous estimation of prevalence and test characteristics, combining the prior knowledge (previous surveys, expert opinion or simply a non-informative distribution) with the present survey results to obtain the posterior distribution for each of the parameters.

3. Results

Of the 707 pigs examined, 577 (81.6%) were usually kept in confinement and 130 (18.4%) were free roaming. Among the 290 pig-owning households visited (174 in Bafou and 116 in Bamendou), 33 (11.4%) did not have latrines, and in 154 (53.1%) of the households pigs had access to human fecal material.

Table 1 summarizes the data on the villages visited, and on the number of pigs found positive for cysticercosis by either tongue inspection, Ab-ELISA or Ag-ELISA. By tongue inspection, there was no statistical difference between the number of animals found positive in Bafou and in Bamendou. The Ag-ELISA and Ab-ELISA, however, detected significantly higher number of positive cases in Bamendou than in Bafou. In four out of 27 villages, infected pigs could not be detected.

Table 1: Prevalence of porcine cysticercosis by tongue inspection, Ag-ELISA and Ab-ELISA in Bafou and Bamendou (West Cameroon)

Localities		Number of samples collected	Positive at tongue inspection	Positive in Ag-ELISA	Positive in Ab-ELISA
Bafou Community	Bawouwoua	27	2	4	1
	Lepia	28	0	2	0
	Doumbouo Centre	7	0	0	0
	Bassessa	8	0	0	0
	Bafou Sp.	13	0	0	0
	Fokamezo	7	0	1	0
	Mezet	32	0	7	0
	Tsingbeu	20	2	2	8
	Tchoutsi	72	9	8	3
	Fombet	29	3	3	14
	Batsingla	27	1	3	11
	Fonakeu	6	0	1	5
	Bafou chefferie	60	0	4	5
	Lepouo	25	0	0	12
Melouong	39	5	1	2	
Sub-total		400	22 (5.5%)	36 (9%)	61(15.3%)
Bamendou community	Bamendou Lumière	4	0	0	3
	Mbouo	37	7	7	1
	Tchueffi	33	4	0	3
	Mentsa	30	2	4	2
	Bamendou Chefferie	21	0	2	13
	Dedeng	28	0	3	2
	Bamendou Sp.	19	0	0	0
	Balefock	25	2	6	9
	Nkotsa	21	1	2	12
	Batoula	41	1	7	24
	Metchou	24	4	10	16
Léo	24	0	1	8	
Sub-total		307	21 (6.8%)	42 (13.7%)	93 (30.7%)
Total		707	43 (6.1%)	78 (11.0%)	154 (21.8%)

Nine animals were positive for cysticercosis by tongue inspection, but were negative in the Ag-ELISA. Twenty-seven animals positive in the Ag-ELISA were negative in Ab-ELISA while antibodies were absent in the serum of seventeen pigs positive by tongue inspection (Table 2).

Table 2: Comparison of three different tests two by two used for the detection of cysticercosis in village pigs (n=707).

		Tongue inspection		Ab-ELISA	
		+	-	+	-
Ag-ELISA	+	34	44	51	27
	-	9	620	103	526
Ab-ELISA	+	26	128		
	-	17	536		

Table 3 shows that twenty-two animals were simultaneously declared positive in the three tests while 521 were negative in all the tests used in this study.

Table 3: Correlation between the results obtained by three different tests for the detection of cysticercosis in village pigs (n=707).

Number of samples	Tests		
	Tongue inspection	Ag-ELISA	Ab-ELISA
521	-	-	-
99	-	-	+
29	-	+	+
15	-	+	-
5	+	-	-
4	+	-	+
12	+	+	-
22	+	+	+

The results of the Gibbs Sampling applied to the data obtained from the three tests used (Ag-ELISA, Ab-ELISA and tongue inspection) are shown in Tables 4a and 4b. The estimated prevalence with or without prior information about the sensitivity and specificity of the 3 tests is 10.9 and 12 %, respectively.

Table 4a : Results of the Gibbs Sampling without any prior information about the sensitivity and the specificity of the three tests (Ag-ELISA, Ab-ELISA and tongue inspection).

Node	Mean	Standard deviation
P	0.120	0.017
Se1	0.465	0.064
Se2	0.858	0.071
Se3	0.683	0.060
Sp1	0.992	0.005
Sp2	0.989	0.008
Sp3	0.844	0.015

Table 4b : Results of the Gibbs Sampling with prior information on the sensitivity and the specificity of the tongue inspection (Se: 70% et Sp: 98%, slightly modified according to Gonzalez et al. (1990)), the Ab-ELISA (Se: 67.6% et Sp: 98.2%, Nunes et al. (2000)) and the Ag-ELISA (Se: 86% et Sp: 99%, slightly modified according to Nguekam (1998)).

Node	Mean	Standard deviation
P	0.109	0.015
Se1	0.618	0.041
Se2	0.872	0.061
Se3	0.716	0.061
Sp1	0.992	0.005
Sp2	0.981	0.009
Sp3	0.860	0.014

Test 1: Tongue inspection; Test 2: Ag-ELISA ; Test 3: Ab-ELISA ; P: Prevalence ; Se: sensitivity and Sp: Specificity

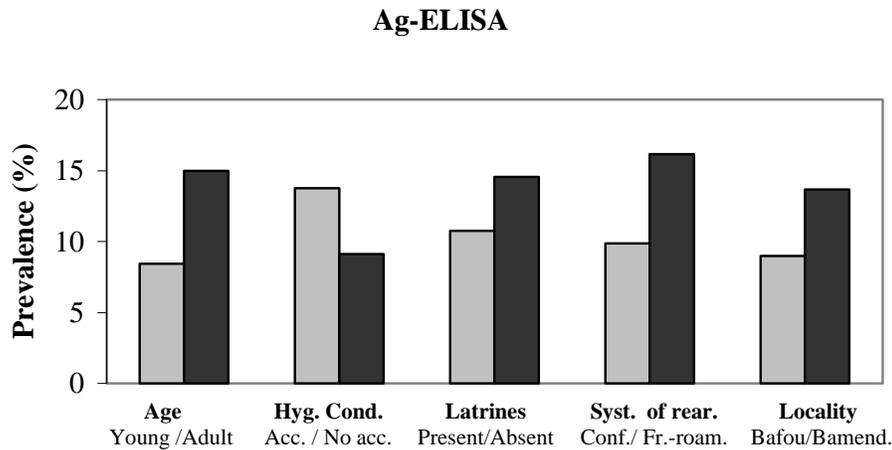


Figure 1: Prevalence of cysticercosis (Ag-ELISA) in function of pig age, rearing system, household hygienic conditions, presence or absence of latrines, and locality (Hyg. Cond.: Hygienic condition; Acc.: Access to human faeces; No acc.: No access to human faeces; Syst. of rear.: System of rearing; Conf.: Confinement; Fr.-roam.: Free-roaming; Bamend.: Bamendou).

Figure 1 presents the percentage of positive samples (Ag-ELISA) in function of pig age, system of rearing, hygienic conditions of the household, presence or absence of latrines, and locality. There was no statistical difference in cysticercosis seroprevalence of pigs raised in households with or without latrines. Adult pigs showed a significantly higher seroprevalence (15%) than young ones (8.4%). Pigs that were reported by their owners to be usually confined were significantly less infected (9.9%) than those that free-roamed (16.2%). Animals which had access to human faeces were significantly more infected (13.8%) than those which did not (9.1%).

4. Discussion

The prevalence of porcine cysticercosis using tongue inspection was 6.1%. This result is distinctly higher than the 2.3% rate reported by Nguekam (1998) in the bordering Divisions of Bamboutos and Mifi (West-Cameroon). In contrast, it is lower than the 24.6% reported by Zoli et al. (1987) in the Menoua Division.

However, the prevalence of porcine cysticercosis estimated by Ab-ELISA (21.8%) and Ag-ELISA (11.0%) was almost trice or double, respectively than by tongue inspection. In this study we used an antigen detection ELISA which has proved to be highly specific (99.1%) and sensitive (84.6%) for pigs infected with cysticercosis (Nguekam, 1998). The cut-off level for the Ag-ELISA was calculated on the basis of the average O.D. of serum samples of 8 pigs which originated from a farm without history of cysticercosis and which were negative at tongue palpation. Although these pigs have not been autopsied, the O.D. of their sera was very low (< 0.02), so that it can be assumed that they were free of cysticercosis. However, the use of a larger number of sera from uninfected control pigs, which are representative for the local pig population, would undoubtedly increase the reliability of the Ag-ELISA. Since the detection limit of this test is unknown, it is probable that the nine animals found positive by tongue inspection but negative by Ag-ELISA harboured a number of cysts below the detection limit of this technique. Another explanation might be that the nodules discovered on the tongue of those animals were not caused by cysticerci but by other lesions (e.g. scars after mechanic injuries). These arguments might also be valid for the seventeen positive cases by tongue inspection but negative in the Ab-ELISA. With the Ab-ELISA, antibodies were detected in 154 (21.8%) sera representing the number of animals in both communities, which had a history of contact with *T. solium* oncospheres. According to Nunes et al. (2000) the Ab-

ELISA using cyst fluid of *T. solium* cysticerci as antigen had a sensitivity of 67.6% and a specificity of 98.2%. The fact that 27 animals, which were positive in the Ag-ELISA and thus probably harbouring living cysts, were not identified in the Ab-ELISA is very surprising. This confirms the low sensitivity of the antibody detection ELISA. However, it might also indicate that some false positive reactions occurred in the Ag-ELISA.

The Gibbs Sampling programme was used assuming that the three tests were statistically independent conditional on the true disease status of the subject. With or without prior information on the sensitivity and specificity of the three tests used the programme gave an estimated prevalence of cysticercosis of 10.9 and 12.0%, respectively. The advantage of this approach is that it allows a much more reliable estimate of the disease prevalence than a survey based on the results of a single test. It is striking that the obtained figures were very close to the prevalence figures recorded in the Ag-ELISA (11.0%). These results indicate the endemicity of the disease in both rural communities (Bafou and Bamendou) and complement previous reports based on data collected at slaughterhouses and market places (Zoli et al., 1987; Awa et al., 1999; Nguekam, 1998). The latter results, however, have to be interpreted with caution due to the high mobility of animals between markets of different localities within the country.

The seroprevalence (Ag-ELISA) of porcine cysticercosis in Bamendou (13.7%) was significantly ($p < 0.01$) higher than in Bafou (9%) and at least 15.3% of pigs examined in Bafou and 30.3% in Bamendou were carrier of antibodies against cysticerci. The higher figures in Bamendou are probably due to the high percentage of people who used the pigpen as a toilet. In this area human defecation along the roads and in crop fields was also common. Total seroprevalence by Ab-ELISA (21.8%) was lower than the result (38%) reported by Zoli et al. (1987) in a survey in the same Menoua Division. This decrease of the seroprevalence (Ab-ELISA) indicates that the general hygienic conditions in this area have been improved by the time although insufficiently.

For the epidemiological analysis, the data of the Ag-ELISA were used because these were very close to the estimated prevalence using Gibbs Sampling. The results of the present study demonstrated that adult pigs showed a significantly ($p < 0.01$) higher seroprevalence (15%) than young ones (8.4%). One obvious reason is that old animals have been more exposed to the infection than young ones. These results are in agreement with those reported by Sarti et al. (1992) in Mexico. In contrast, in some studies no relationship between age and prevalence of infection with cysticerci of *T. solium* was recorded (Sakai et al., 1998; Nguekam, 1998). There was a significant difference ($p < 0.01$) in cysticercosis prevalence between pigs that were usually confined to pens (9.9%) and those which were free roaming (16.2%). These figures are similar to those obtained by inquiries about the hygienic conditions of the households, which showed animals with access to human faeces to be more infected (13.8%) than those without access (9.1%). Although most households had latrines, they were often not constructed in a manner that excluded access of pigs. A similar situation was reported by Schantz et al. (1998) in Mexico, Guatemala and Peru. The high percentage (53.1%) of households visited where pigs had access to human faecal material was remarkable.

5. Conclusion

The results of this study conducted in two rural communities (Bafou and Bamendou) demonstrated that porcine cysticercosis still remains an important problem in West-Cameroon. Bamendou appeared to be the most infected community. Multiple factors, including pig husbandry practices, household sanitation, and hygiene were shown to be associated with parasite transmission. In order to reduce the infection risk it is necessary to intensify meat inspection, improve sanitary infrastructure, and educate the population. Recent surveys showed that many inhabitants of both communities either ignore or are ignorant of the

danger to which they expose themselves by eating meat with cysticerci and by using pigpens as a toilet. Most of them did not understand the association between the presence of cysticerci in the animal and the tapeworm infection in man (Djou, personal communication).

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3.3. PORCINE CYSTICERCOSIS IN VILLAGE PIGS OF NORTH-WEST CAMEROON

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Abstract:

A study was carried out in two villages and one marketplace of the Batibo sub-division in North-West Cameroon to determine the prevalence of porcine cysticercosis. The results showed that 4.44% of 383 pigs were positive at tongue examination whereas ELISA detected circulating antigens in 27.7% of 271 pig sera. A questionnaire survey in 140 pig raising households indicated that 59.3% of them lacked latrines while in 75.7% of the households members defecated directly into pigpens. The seroprevalence of porcine cysticercosis was significantly higher in households without latrines than in those with latrines. Similarly, significantly more seropositive pigs were present in households that defecated in the pig pens (35.5%) than in those that did not (14.4%). Although 91.4% of pig raising households did know of pig cysticercosis, only 28.6% were aware of the link with human taeniasis and only 10.7% were aware of human cysticercosis.

Introduction

Taenia solium cysticercosis is an under-recognized, economic and public health problem in many developing countries, especially in Africa (Tsang & Wilson, 1995; Geerts *et al.*, 2002). According to Preux *et al.* (1996) cysticercosis is almost non-existent in Muslim countries but affects almost all sub-Saharan countries. The *T. solium* taeniasis/cysticercosis complex is associated with poor sanitation and hygiene, poor methods of pig husbandry and poor meat inspection and control. The occurrence of the taeniasis /cysticercosis complex has been reported already in some parts of Cameroon (Marty *et al.*, 1985, 1986; Zoli *et al.*, 1987; Awa *et al.*, 1999; Assana *et al.*, 2001; Pouedet *et al.*, 2002). In order to obtain baseline data on the prevalence of porcine cysticercosis in the North-West province of Cameroon and to determine factors associated with the transmission of this zoonosis, an epidemiological study was undertaken in the Batibo sub-division of this province.

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Materials and Methods

Study site

The study was carried out between April and August 2001 in three localities (Ashong, Central Batibo and Guzang) of Batibo (North-West province of Cameroon). The area is situated between latitude 5° and 6°N and longitude 9° and 10°E. The altitude varies from 1600 m to 2000 m above sea level. There are two seasons: the rainy season (March to November) and the dry season (November to February). An average annual precipitation of 2500 mm is recorded in the area.

According to the Veterinary Services in Batibo, the pig population in the area is estimated at about 10,000. Pigs play a very important social, cultural, religious and economic role. Many families keep a few pigs, which are usually confined to pens and are commonly fed human faeces. During some periods of the year some pigs are allowed to roam freely.

Survey on pig cysticercosis

All piggeries in Ashong (n=125) and 15 (selected on the basis of accessibility) out of 27 piggeries in Batibo were visited. All pigs were sampled except pregnant sows, nursing sows with litters less than 2 month old and piglets younger than two months. Only five farmers refused collaboration. A total of 271 animals were examined in both localities, 195 of which were males and 76 females. The majority of the animals (214 of 271) was younger than 12 months. Blood samples were taken from 219 pigs in Ashong, whereas only 140 were examined by tongue inspection. At Central Batibo 52 pigs were examined by tongue inspection and blood sampled. Blood was collected from the jugular vein using a vacutainer. The serum was conserved at -20°C until laboratory analysis was done.

Besides this village survey, a total of 191 pigs were examined at the Guzang market for cysticercosis by tongue inspection, 120 of which were males and 71 females. The majority of these animals (156 of 191) were younger than one year. Blood samples were not collected from these animals.

Household questionnaire

A questionnaire survey on the socio-economic and technical characteristics of pig production and occurrence and transmission of taeniasis/cysticercosis due to *T. solium* was carried out in 140 pig raising households in Batibo and Ashong, in which pigs were sampled as described above. Information on the awareness of cysticercosis in pigs and the relationship between human taeniasis and pig and human cysticercosis was also collected. Hygienic and sanitary conditions were inquired and confirmed by direct observation. The respondent in each household was the person taking care of the pigs or the head of the household, although sometimes intermediaries were required.

Enzyme-linked immunosorbent assay for detection of circulating antigens (Ag-ELISA)

The Ag-ELISA was performed as described by Dorny *et al.* (2000) with slight modifications. The serum samples were pre-treated using trichloroacetic acid (TCA) and used in ELISA at a final dilution of 1/4. Two monoclonal antibodies (MoAb) were used in a sandwich ELISA. MoAb B158C11A10 was diluted at 5µg/ml in carbonate buffer (0.06 M/pH 9.6) for coating and a biotinylated MoAb B60H8A4 (1.25µg/ml in PBS-T20/NBCS) 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 orthophenylene diamine (DAKO, #S2045) and H₂O₂ was added and incubated without shaking between 30-33°C for 15 min. To stop the reaction, 50µl of 4N H₂SO₄ was added to each well. The plates were read using an ELISA reader (Labsystem Multiskan RC) at 492 nm. Each sample was tested in duplicate, and on each ELISA plate two positive reference serum samples from local naturally infected pigs (*T.*

solium cysticercosis confirmed at slaughter) and eight serum samples from *T. solium* cysticercosis-free pigs were included. These reference negative samples were taken from pigs, which did not show nodules at tongue palpation and did originate from a local farm with good hygienic conditions and without any history of cysticercosis.

The mean optical density (O.D.) of each serum sample was compared with a series of reference negative serum samples (n = 8) at a probability level of p = 0.001 to determine the cut-off using a modified Student Test (Sokal & Rohlf, 1981).

Statistical analysis

The Chi Square test (X^2) as described by Steel & Torrie (1980) was used to compare differences of significance between proportions at a probability level of 5%.

Results

Table 1 summarizes the results of the tongue inspection and the Ag-ELISA. Of the 383 pigs subjected to tongue examination 17 (4.4%) were found infested with cystic lesions, whereas 75 of 271 (27.7%) were seropositive.

Table 1. Prevalence of porcine cysticercosis in Batibo sub-division, Cameroon by tongue inspection (T) and Ag-ELISA (E).

Locality		Pigs examined	Infected pigs	
			Number	Prevalence (%)
Ashong	E	219	72	32.9
	T	140	1	0.7
Central Batibo	E	52	3	5.8
	T	52	0	0.0
Guzang Market	E	ND	ND	ND
	T	191	16	8.4
Total	E	271	75	27.7
	T	383	17	4.4

ND =Not Done

Table 2 shows the comparison between the results of the Ag-ELISA and the tongue inspection. Whereas only one of 192 animals was found positive by tongue inspection, the Ag-ELISA allowed the detection of another 74 infected animals.

Table 2. Comparison of the results of tongue inspection and Ag-ELISA in village pigs in Cameroon

	T+/E+	T+/E-	T-/E+	T-/E-	Total
Ashong	1	0	71	68	140
Central Batibo	0	0	3	49	52
Total	1	0	74	117	192

T: tongue inspection; E: Ag-ELISA

One hundred and twenty eight (91.4%) of the 140 households were aware of the existence of porcine cysticercosis against only 10.7% for human cysticercosis. Also, 28.6% of the households knew that a direct relationship exists between human taeniasis and pig cysticercosis. The percentage of households which did not possess a latrine was 59.3%, while

75.7% declared that members of the households deliberately defecated in piggens. The absence of latrines and deliberate defecation in piggens was significantly higher ($p < 0.05$) in Ashong than in Central Batibo.

The seroprevalence of porcine cysticercosis was higher ($p < 0.05$) in households without latrines (34.1%) than in households with latrines (21.6%) (table 3). The prevalence rate was equally higher ($p < 0.05$) in households that defecated in their piggens (35.5%) than in those that did not (14.1%). There were two predominant pig husbandry systems practised in Batibo sub-division: permanent confinement (60.7%) and partial confinement (39.3%). The proportion of pigs infected with cysticercosis was slightly higher in permanently confined (29.0%) than in partially confined pigs (25.3%). This difference, however, was not statistically significant ($p > 0.05$).

Table 3. Factors associated with porcine cysticercosis (Ag-ELISA) in Ashong and Central Batibo, Cameroon.

	No. of pigs examined	No. (%) of infected pigs
Type of management		
Partial confinement	95	24 (25.3)
Permanent confinement	176	51 (29.0)
Household hygiene		
No latrine	132	45 (34.1)
Presence of latrine	139	30 (21.6)
Defecation in pig pen	172	61 (35.5)
Effective use of latrine	99	14 (14.1)

Discussion

Although tongue nodules were detected in only 4.4% of the examined animals, the seroprevalence of 27.7% obtained in this study suggests that porcine cysticercosis is widespread in the Batibo subdivision of North-West Cameroon. These results confirm the observations of Nguekam *et al.* (2003) and Pouedet *et al.* (2002) that the monoclonal antibody based Ag-ELISA is much more sensitive than tongue inspection. Since this antigen detection ELISA is known to detect only living cysticerci both in cattle (Brandt *et al.*, 1992) and in pigs (Nguékam *et al.*, 2003), the number of seropositive pigs is a good indication of animals which present a risk to the consumer. According to Pouedet *et al.* (2002) the sensitivity and specificity of the Ag-ELISA for the detection of porcine cysticercosis as derived from Gibbs sampling analysis is 85.8-87.2 and 98.1-98.9%, respectively. Although data on the occurrence of *Cysticercus tenuicollis* in Cameroonian pigs are not available, it cannot be excluded that some cross-reactions might occur with the metacestodes of *T. hydatigena*.

Even though 28.6% of the households were aware of the direct relationship between human taeniasis and porcine cysticercosis, clandestine trade in infected pigs is still common in the study area. Infected live pigs and carcasses are cheaper on the clandestine market. It is common practice by consumers of measily pork to salt, dry or cook the meat at high temperatures for a long time. Although the latter measure is undoubtedly very effective in destroying the cysts, pork consumed during most traditional ceremonies is usually not adequately cooked because of large amounts of meat that have to be prepared during a short period of time. One of 10 people interviewed was aware of the existence of cysticercosis in humans from observation of the cysts during traditional autopsies. It is customary in these

villages that autopsies of persons who die suddenly are carried out by the villagers themselves.

The absence of latrines in 59.3% of the households and the direct defecation of 75.7% of the interviewed people in pigpens were important factors associated with the transmission of cysticercosis in the area. The latter practice has also been reported to be common in the West province of Cameroon (Marty *et al.*, 1986; Zoli *et al.*, 1987; Pouedet *et al.*, 2002). However, this is not the case in the Far North of Cameroon, where inhabitants are in the habit of defecating near the farms in the open air and where pigs scavenge the human excrement (Assana *et al.*, 2001). In Batibo sub-division, the local authorities obliged the inhabitants to construct latrines. However, this does not mean that these latrines are also effectively used, because there is a strong belief that pigs fed human excrement produce better quality pork than those fed otherwise. Defecation in the pigpen is therefore not only a means of faecal disposal in the area but also a cheaper way of feeding pigs.

Conclusion

This study clearly shows that in low-input pig farming - as is the case in the Batibo subdivision of the North-West province of Cameroon - all the conditions are present for an effective transmission of *T. solium* from man to pigs and vice versa. The region can be considered as endemic for *T. solium* cysticercosis since prevalence figures were similar to those obtained in other known endemic regions in Mexico or Peru (Sarti *et al.*, 1992, 1994; Garcia *et al.*, 1999). Very often good correlations have been observed between the presence of porcine and human cysticercosis in endemic regions (Flisser *et al.*, 2002). Studies are currently ongoing in order to examine the prevalence of human cysticercosis in this area.

The results of the present study have also identified certain community behavioural and environmental practices that must be modified in order to prevent the continued spread of this zoonosis. Given the fact that the majority of the people interviewed were very much aware of cysticercosis in pigs but that only about one in four knew of the association of the latter with taeniasis in man, efforts need to be made in the field of health education in order to clarify these issues and to bring about the necessary changes in knowledge and behaviour of the human population

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3.4. KINETICS OF CIRCULATING ANTIGENS IN PIGS EXPERIMENTALLY INFECTED WITH *TAENIA SOLIUM* EGGS

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Abstract : Three groups of 4 piglets were experimentally infected with different doses (10^3 , 10^4 and 10^5) of *Taenia solium* eggs whereas a fourth group of 2 pigs received gravid proglottids. At autopsy 6 months post infection, the two latter pigs were heavily infected with more than 3000 living cysts per kg of muscle. Ten of the 12 other pigs harboured light infections, i.e. between 2 and 107 cysticerci, 42.4 % of which were degenerated. The two remaining pigs had no detectable cysts at post mortem examination. Circulating antigens (CA) were detected in the sera of all pigs harbouring living cysticerci using a monoclonal antibody based ELISA. CA were first detected between 2 and 6 weeks post infection and remained present generally throughout the entire observation period even in pigs carrying only 5 to 8 living cysts, although strong fluctuations of the level of CA were observed in some pigs. In animals without living cysts at post mortem CA were only detected for a short period and disappeared presumably when the cysticerci became degenerated. The minimum number of living cysts, which could be detected using this ELISA, was 1.

Key-words: Pig-cestoda; *Taenia solium*; cysticercosis; ELISA; experimental infection; circulating antigen

1. Introduction

Taenia solium cysticercosis is a parasitic infection which affects mainly pigs and human beings following the ingestion of eggs of this cestode. Skeletal muscles and brain are the main predilection sites of the cysticerci in pigs, although the cysts can also develop in the heart, lungs, kidney, liver and subcutis of the host (Hall, 1977). Up to now, only few reports have been published about experimental infection of pigs with *T. solium* among others because of, as yet unexplained, low infection rates (Verastegui & al., 2000). Mainly, the parasitic burden and the antibody response have been studied in experimentally infected pigs (Tsang & al., 1991; Aluja & al., 1996; Aluja & al., 1999). Only few attempts have been reported to examine the presence of circulating antigens in serum of experimentally-infected animals (Sciutto et al., 1998a & b). The purpose of this study was to investigate the kinetics of circulating antigens in pigs experimentally infected with different doses of *T. solium* eggs, using a monoclonal antibody-based antigen detection ELISA (Brandt et al., 1992; Dorny et al., 2000). Since circulating antigens have been demonstrated using this

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technique in patients infected with (neuro)-cysticercosis (Erhart et al., 2002), the pig might prove an interesting model to study the evolution of circulating antigens in human cysticercosis.

2. Materials and Methods

2. 1. *T. solium* eggs

T. solium eggs were collected from a 7 year old girl. After treatment with 2 g Niclosamide (Yomesan[®], Bayer) and administration of a laxative (Skilax, PRODES, S.A. Laboratory) the gravid proglottids were collected and thoroughly washed. Eggs were removed from the proglottids in a watch glass, washed and stored in physiological saline at 4°C for 24 hours. No assessment of egg viability was made.

Ten proglottids were stained with India ink. *T. solium* was identified by the average number of 8 unilateral uterine branches and by the scolex bearing 2 rows of hooks.

Just before the infection, the egg suspension was homogenized and eggs were counted in a series of 10 replicates of 10 µl in order to calculate the average number of eggs per ml and to prepare the infection doses.

2.2. *Experimental infection of pigs*

Fourteen crossbred pigs originating from 2 semi-intensive pig farms (without history of cysticercosis) in the villages of Bamendou and Bafou were used in the experiment. Four groups of pigs were infected with different doses of *T. solium* eggs. Group I, II and III, which consisted of 4 piglets each (about 2 months old), were orally infected using a stomach tube with 10³, 10⁴ and 10⁵ of *T. solium* eggs, respectively. The eggs were suspended in a standard volume of 10 ml of water, which was the same for all pigs. Afterwards the stomach tube was flushed with additional water. The 2 pigs of group IV (about 6 months old) were fed with faecal material containing gravid proglottids of *T. solium* which originated from the same girl. A total of 13 proglottids were administered during 3 consecutive days to both pigs, which were housed together. Assuming an average number of 60,000 eggs per proglottid, these two pigs received together a total of 780,000 eggs.

Blood samples were collected from each pig before infection and starting from the day of infection (d₀), every 2 weeks during a period of 6 months. Blood was kept at 4°C overnight after which the serum was separated and stored at -20°C.

2. 3. *Detection of circulating antigen by sandwich-ELISA (Ag-ELISA)*

The serum samples were tested in duplicate for the detection of circulating antigens of *T. solium* using a sandwich-ELISA as described by Dorny et al. (2000) and slightly modified by Pouedet et al. (2002). The serum samples were pre-treated with trichloro-acetic acid (TCA) to dissociate immune-complexes according to Dorny et al. (2000). Briefly, sera were treated with 5 % TCA and incubated during 20 min at room temperature. After centrifugation the supernatant was neutralised using a 0.6 M sodium carbonate/bicarbonate buffer (pH: 10). The circulating antigens present in the serum were captured in a sandwich ELISA by two monoclonal antibodies (MoAb): 158C11A10 and a biotinylated MoAb 60H8A4. These MoAbs were developed against the excretory-secretory products of *T. saginata* cysticerci, but were shown to cross-react with *T. solium* (Brandt et al., 1992; Van Kerckhoven et al., 1998). The epitopes recognised by the MoAbs are repetitive and have a carbohydrate or partly

carbohydrate/partly protein nature. The molecular weight of the antigenic components with which the MoAbs ranges from 40,000 to 200,000 (Draelants et al., 1995). The conjugate (Streptavidine Horseradish Peroxidase 'Jackson', Lucron) was diluted 1:10000, and orthophenylene diamine (OPD)/H₂O₂ was used as chromogen/substrate. The plates were read at 492 nm with a Labsystems Multiskan RC reader.

2.4. Detection of antibody by ELISA (Ab-ELISA)

Antibodies against *T. solium* were only examined in a pre-infection serum sample of the pigs in order to verify whether antibodies, maternal or otherwise, were present. The ELISA was carried out as described by Pouedet et al. (2002). Cyst fluid of *T. solium* cysticerci was used as antigen. The sera and conjugate (rabbit anti-pig IgG/peroxidase, Sigma) were diluted at 1/300 and 1/30,000, respectively. OPD/ H₂O₂ was used as chromogen/substrate.

2.5. Determination of cut-off values

The cut-off values for both Ag-ELISA and Ab-ELISA were calculated as the average optical density (OD) of 8 negative controls using a modified Student test at a probability level of P=0.001 (Sokal & Rohlf, 1981). These negative control sera came from pigs from a local farm with good hygienic conditions and without any history of cysticercosis. The pigs were negative on tongue inspection. The ELISA ratio was calculated by dividing the OD of the sample by the calculated cut-off value. ELISA ratios equal to or higher than 1 were considered positive.

2.6. Necropsy

At the end of the experiment (182 days post infection), all pigs were humanely killed and the entire musculature, the brain and visceral organs (spleen, liver, kidneys and lungs) were cut into thin slices (about 0.5 cm) and examined for cysticerci. Since the 2 pigs of group IV were heavily infected, only the brain, tongue, masseter, heart, diaphragm, the above mentioned visceral organs and 1 kg of the muscles of the thigh were sliced and examined. The number of cysticerci was counted and they were classified as viable (clear cyst fluid present) or dead (caseous or calcified) based on their macroscopic appearance.

3. Results

3.1. Necropsy of the experimentally-infected pigs

The results of post-mortem examination are summarized in Table 1. Cysticerci were found in 10 of the 12 piglets of groups I, II and III infected with *T. solium* eggs. The recovery rate of cysticerci decreased with increasing doses of eggs (Table 1). Average recovery rates of 3.2, 0.12 and 0.03 % were found after infection with 10³, 10⁴ and 10⁵ eggs, respectively. A total of 255 metacestodes were counted in the pigs of these 3 groups, 57.6% of which were viable and 42.4% degenerated. No cysticerci were recovered from one animal in each of groups I and II. The number of cysts in the remaining infected animals varied from 2 until 107.

The 2 pigs of group IV, which received whole gravid proglottids, were heavily infected. They harboured more than 3000 viable metacestodes per kg of thigh muscles. Nodules were also observed at ante-mortem tongue inspection from 10 weeks post infection onwards, which was not the case in any of the other infected animals.

Table 1: Number and status of cysticerci in 4 groups of pigs experimentally-infected with *T. solium* eggs or proglottids

Groups	Pig No.	Total no. of larvae	Status of cysts		Presence of CA*
			V	Cs/Ca	
Group I (1,000 eggs)	1	27	18	0/9	+
	2	66	8	20/38	+
	4	3	0	0/3	-
	mean no. \pm SD	32 \pm 31.8	8.7 \pm 9	23.3 \pm 30.2	
mean RR (%)		3.2			
Group II (10,000 eggs)	2	19	0	12/7	+
	3	3	0	0/3	-
	4	14	0	0/14	-
	mean no. \pm SD	12 \pm 8.2		12 \pm 8.2	
mean RR (%)		0.12			
Group III (100,000 eggs)	1	6	5	0/1	+
	2	107	107	0/0	+
	3	8	8	0/0	+
	4	2	1	0/1	+
mean no. \pm SD	30.8 \pm 50.9	30.3 \pm 51.3	0.5 \pm 0.6		
mean RR (%)		0.03			
Group IV (intact proglottids)	1	>3000/kg			+
	2	>3000/kg			+

V: viable; Cs: caseous; Ca: calcified; RR: recovery rate; *: at necropsy

The distribution of the cysticerci in select sites of the pigs is presented in Table 2. In all pigs cysts were predominantly found in skeletal muscles. The number of cysts in the brain was very small, even in the 2 massively infected pigs of group IV. All but one of the 16 brain cysticerci (93.8 %) were viable. Cysticerci were not detected in any visceral organs of the infected pigs except in one of the pigs of group IV. This animal harboured 6 living cysts in the stomach wall and 19 cysts in the liver, four of which were degenerate (caseous).

Table 2 : Distribution of *T. solium* cysticerci in select sites of experimentally-infected pigs

Pig no.	Group I			Group II			Group III				Group IV	
	1	2	4	2	3	4	1	2	3	4	1	2
Muscles	15	60	3	17	2	13	5	88	8	0	>3000	>3000
Brain	4	1	0	0	0	0	1	0	0	1	8	1
Heart	1	0	0	1	0	0	0	7	0	1	200	71
Tongue	4	3	0	1	0	0	0	9	0	0	612	50
Masseter	3	2	0	0	0	0	0	1	0	0	448	27
Diaphragm	0	0	0	0	1	1	0	2	0	0	350	97

3. 2. ELISA

At the start of the experiment all the pigs were negative for both circulating Taenia antigens and antibodies against *T. solium*. The Ag-ELISA results during the course of the infection are presented in Figures 1 and 2. Circulating antigens were detected in all pigs harbouring viable cysts. In the heavily-infected animals (group IV), they were first detected at week 2, and in the lightly infected animals (groups I to III), at week 4 to 6 post infection and

remaining present generally throughout the entire period of observation. However, in some pigs a strong fluctuation in circulating antigen levels was observed. As well, the presence of circulating antigens was only detected during limited periods in 2 animals with only dead cysts (II-2 and II-3) and in one pig, in which no cysts were identified at necropsy (II-1). The other pig in which no cysts were recovered at necropsy (I-3) as well as two pigs (I-4 and II-4) with 3 and 14 calcified cysts, respectively, remained sero-negative during the whole experiment.

4. Discussion

Although several authors (Verastegui et al., 2000; Aluja et al., 1996) reported difficulties in infecting pigs experimentally, we succeeded in infecting 12 out of 14 pigs with *T. solium* in this experiment. The number of cysticerci recovered after experimental infection with a suspension of *T. solium* eggs, however, was rather low (0.03 to 3.2 %), whereas after infection with fresh, whole proglottids (in faecal material) the number of cysts recovered was very high. This suggests that there might be a factor in faecal material and/or proglottids, which enhances the infectivity of the eggs, although it should be noted that Sciutto et al. (1995) obtained a rather low number of cysts even after infection with 3 gravid proglottids. The cyst recovery rates in this study were very similar to the ones reported by Santamaria et al. (2002). Using *T. solium* eggs with a viability of 80 % (based on trypan blue staining) the latter authors obtained recovery rates varying between 0.45 and 2.5 % after experimental infection with 1,000 to 100,000 eggs. Difficulties in infecting pigs with the eggs of *T. solium* might be caused by the presence of maternal antibodies, but this possibility was excluded in this trial, because all the piglets were negative for antibodies against *T. solium* metacestodes prior to infection. The absence of cysticerci in two piglets (I-3 and II-1) is therefore difficult to explain. In one of them (II-1) the presence of circulating antigen during a short period suggests that some initial development of oncospheres into cysticerci had taken place, but had not progressed.

Previously, it has been shown in cattle infected with *T. saginata* cysticerci that circulating antigens can be detected in the serum of animals carrying living cysticerci (Brandt et al., 1992), which is not surprising since the Moabs used were directed against metabolic products of the metacestodes. Consequently the Ag-ELISA was able to distinguish animals which harbour living cysts from those harbouring only dead cysts. This was confirmed in this study by the overall association between the presence of circulating antigen and the presence of living cysts. A general trend exists which suggests that circulating antigens disappeared when cysticerci started to degenerate. Pig II-3 (3 calcified cysts) showed a few positive ELISA values corresponding probably with a certain development of the cysticerci, which died afterwards. In pig II-2 (19 cysticerci, from which 12 caseous and 7 calcified), the positive values remained present over a longer period presumably because viable cysticerci persisted longer. The fact that the cysticerci were still caseous and not yet calcified at the time of the autopsy supports this.

Circulating antigen remained present throughout almost the whole observation period of 6 months even in pigs infected with only 5 to 8 living cysts. Similar observations were reported by Sciutto et al. (1998a). The minimum number of viable cysts detected by the ELISA in this experiment was 1, assuming all cysts in the carcass were detected, which indicates the high sensitivity of the test. It remains to be proven, however, whether the test will be able to detect such low parasite burdens in naturally-infected animals.

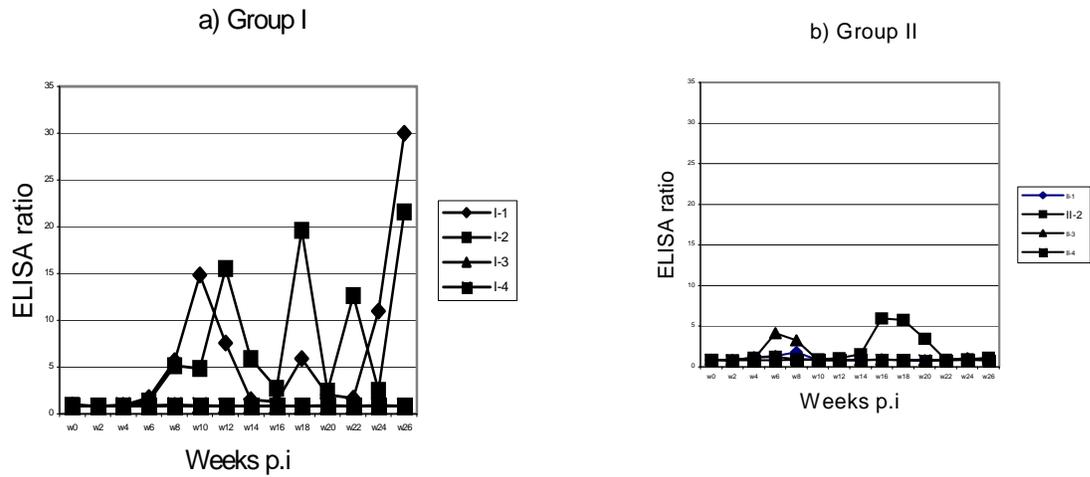


Fig. 1. Kinetics of circulating antigen in pigs infected with 1,000 (group I) or 10,000 eggs (group II) of *T. solium*

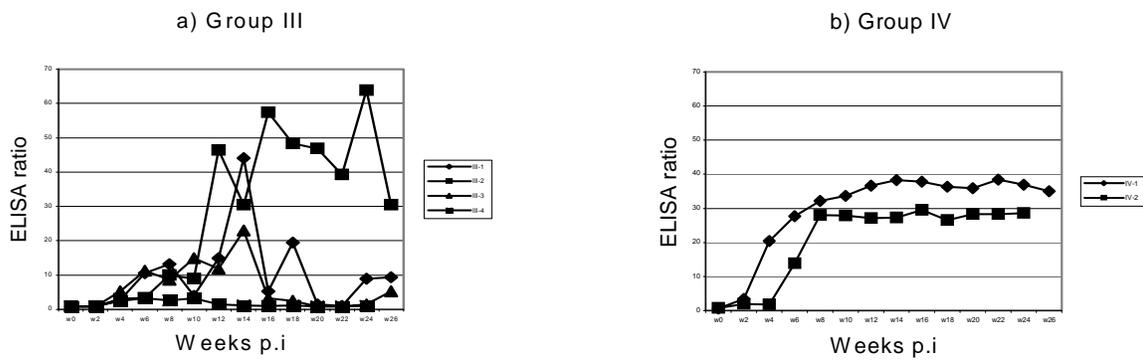


Fig. 2. Kinetics of circulating antigen in pigs infected with 100,000 eggs (group III) or whole proglottids (group IV) of *T. solium*

The strong variation in the level of circulating antigen in some of the pigs, e.g. I-1, I-2 and III-2, are somewhat surprising. This might be due to varying metabolic activity of the cysticerci and/or to differences in the permeability of the connective tissue capsule around the cysts. This phenomenon was only observed in pigs with a small number of living cysts (<100). In pigs III-2 (107 living cysts) or IV-1 and IV-2 (both heavily infected), the level of circulating antigen remained relatively constant at a high level. This suggests that numbers of viable cysts exceeding this threshold value are required to consistently produce ES antigen at detectable levels. Contrary to the report of Sciutto et al. (1998a) we did not observe a systematic drop in circulating antigen levels in the later phase of infection. Sciutto et al. (1998a) ascribed this phenomenon to antigen-antibody complex formation. In our study dissociation of these complexes was accomplished by the trichloroacetic acid treatment of the sera prior to the ELISA.

In conclusion, the results of this study show that there is a clear association between the presence of living cysts and the detection of circulating antigen in pigs experimentally infected with *T. solium* cysticerci. On the basis of these results and the results obtained by Erhart et al. (2002), who demonstrated circulating antigen in 8 out of 9 patients with confirmed cysticercosis, it is anticipated that the monoclonal antibody-based Ag-ELISA used in this study might be a valuable tool to detect active human cysticercosis as well

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3.5. BOVINE CYSTICERCOSIS IN SLAUGHTER CATTLE OF CAMEROON

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(unpublished results)

Abstract

Surveys were carried out at the abattoirs of Maroua and Dschang in Cameroon in order to assess the prevalence of *Taenia saginata* cysticercosis by conventional meat inspection methods and by an antigen detection ELISA (Ag-ELISA). A total of 1241 slaughter cattle between 1 and 9 years old were examined. The meat inspectors did not detect any single infected animal whereas the Ag-ELISA identified 13.0 and 16.9% seropositive cattle at the abattoirs of Dschang and Maroua, respectively. Since it is known that the Ag-ELISA is more sensitive than the 'knife and eye' method and since *T. saginata* carriers have been reported in both regions, this study confirms that the results of the conventional meat inspection do not reflect the real prevalence of bovine cysticercosis.

Keywords: Cattle-cestoda; *Taenia saginata*; ELISA; Meat inspection; Cameroon

Introduction

Bovine cysticercosis is a cosmopolitan parasitic zoonosis, which is particularly common in some East and Southern African countries (Murrell et al., 2005). It is caused by the metacestode stage of the human intestinal cestode, *Taenia saginata*. Very few data, however, are available for West and Central Africa. The last published reports on bovine cysticercosis in Cameroon concern meat inspection figures from 1979-80 (Nfi & Alonge, 1987) and 1981 (Thys et al., 1983). Thys et al. (1983) reported a prevalence of 4.78% in the abattoir of Maroua whereas Nfi & Alonge (1987) observed prevalences of 0.44 (1979) and 3% (1980) in 4 slaughterhouses of the Fako division of the South-West province of Cameroon. The purpose of this study was to assess the prevalence of cysticercosis in slaughter cattle at two abattoirs in Cameroon, one in the western part of the country (Dschang) and one in the far north (Maroua). Given the lack of sensitivity of the conventional meat inspection technique, the antigen detection ELISA (Dorny et al., 2000) was also used in order to obtain more reliable results.

Materials and methods

Abattoir surveys

The first survey was carried out at the abattoir of Dschang (West Province of Cameroon; Fig. 1) between August 2002 and July 2003. A total of 785 animals between 1 and 9 years old were examined for the presence of cysticerci of *Taenia saginata* by an overall inspection of the carcass and by making an incision in the tongue, the masseter and the myocard. Incisions in the thigh and shoulder muscles were not made systematically. Blood samples were taken every day animals were slaughtered (i.e. 5 days per week). Since only a small number of cattle were slaughtered on a daily basis, these 785 animals represent about two thirds of the cattle slaughtered during the study period. All cattle belonged to the Red

(Djafoun; n=689) and White Fulani (Akou; n=96), Bororo crossbreds. The majority came from the North West Province (region of Bamenda) and the Adamaoua whereas only a small number came from the region of Dschang.

The second survey took place between 15 February and 15 March 2005 at the abattoir of Maroua (Far North Province of Cameroon; Fig. 1). All cattle slaughtered during this period (n=456) were included in the study. They were zebu cattle (2-9 years old) belonging to the Goudali and Mbororo breeds and originated from the Far North and North provinces. The meat inspection consisted of an incision in the tongue, masseters and myocard and a visual inspection of the oesophagus, the liver and the psoas.

In both abattoirs the inspection of the carcasses was carried out by the local team of meat inspectors (in the presence of some of the authors). Age and sex of all animals included in both surveys were recorded.

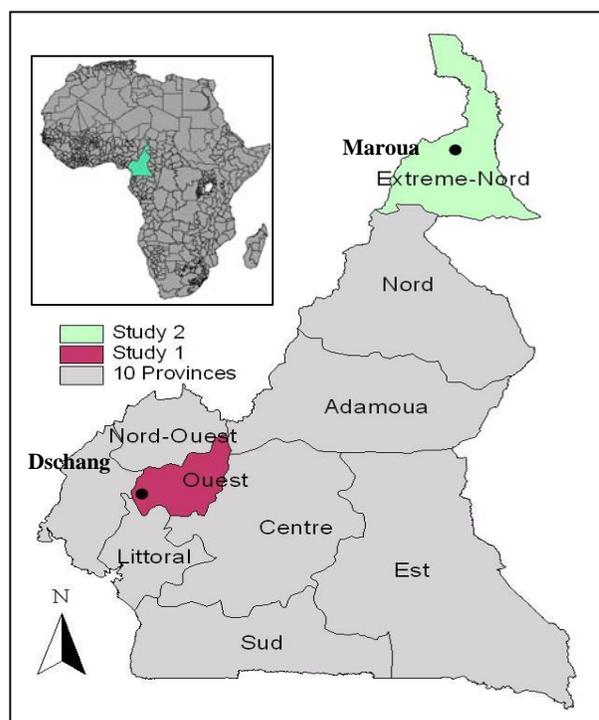


Figure 1. The cities of Dschang and Maroua in Cameroon, where the abattoir surveys on bovine cysticercosis took place

ELISA for the detection of circulating antigen of Taenia saginata (Ag-ELISA)

Blood was taken from all animals involved in both surveys at the abattoirs of Dschang and Maroua. The serum was examined using a monoclonal antibody based antigen detection ELISA as described by Brandt et al. (1992) and modified later on by Dorny et al. (2000). A positive reference serum (experimentally infected animal) and 8 negative reference sera (originating from a Cameroonian farm without a history of bovine cysticercosis) were included on each ELISA plate.

Data analysis

The optical density (OD) of each serum sample was compared with a set of 8 negative reference samples at a probability level of $P = 0.001$ to determine the result in the test (Sokal & Rohlf, 1981). In order to facilitate the comparison between the different plates, all results

have been expressed as a ratio calculated by dividing the OD of each sample by the OD of the cut-off value. All results superior to one were considered as positive.

Logistic regression was used to analyse the effect of age and sex on the seroprevalence of cysticercosis (Stata, 6.0, StataCorp., 2001)

Results

Neither at the slaughterhouse of Dschang, nor at the abattoir of Maroua cysticercosis was detected in any of the cattle by the meat inspectors. However, the Ag-ELISA detected 13.0 % (IC95: 10.64-15.35) and 16.9 % (IC95: 13.43-20.33) seropositive cattle at the abattoir of Dschang and Maroua, respectively. The seroprevalence of bovine cysticercosis according to the sex of the animals in the two studies is presented in table 1.

Table 1. Seroprevalence (ELISA) of bovine cysticercosis in the abattoirs of Dschang and Maroua according to the sex of the animals

Abattoir	Total No. of animals examined	No. of females examined	No. of males examined	No. (%) of seropositives	
				Females	Males
Dschang	785	509	276	57 (11.2)	45 (16.3)
Maroua	456	376	80	60 (15.9)	17 (21.3)
Total	1241	885	356	117 (13.2)	62 (17.4)

Seventy one percent (71 %) of the investigated animals were female and only 29% were males. At Dschang the males were significantly more infected than the females ($P < 0.043$). In the Maroua dataset, however, there was no significant difference between the seroprevalence in both sexes ($P > 0.05$).

The mean age of the slaughter cattle in Dschang was 5.16 year, while it was 6.1 in Maroua. In both studies the seroprevalence of cysticercosis decreased with increasing age of the animals (table 2), but the effect of age was not significant ($P > 0.05$).

Table 2. Seroprevalence of bovine cysticercosis in the abattoirs of Dschang and Maroua according to the age of the animals

Abattoir	No. of cattle examined			% seropositives		
	< 4 y	4 – 6 y	> 6 y	< 4 y	4 – 6 y	> 6 y
Dschang	54	612	119	14.8	13.7	8.4
Maroua	37	106	313	24.3	17.9	15.7
Total	91	718	432	18.7	14.3	13.7

Discussion

This study showed a very striking discrepancy between the results of the conventional meat inspection and the Ag-ELISA, which detected 0 and 13.0-16.9% infected cattle, respectively. According to the local inspection services, cases of bovine cysticercosis have not been detected in Maroua since more than 10 years and in Dschang cysticercosis was never reported. Although the sensitivity of the Ag-ELISA is quite high (92.3%) in animals carrying

more than 50 cysticerci, the sensitivity is very low (12.8%) in animals with less than 50 cysts (Van Kerckhoven et al., 1998). Furthermore, the test detects only living cysts and not the degenerated cysts (Brandt et al., 1992), which implies that the seroprevalence figures obtained in this study underestimate the real prevalence. On the other hand, the specificity of the Ag-ELISA is 98.7%, but some cross-reactions with cysticerci of *Taenia hydatigena* might occur (Dorny et al, 2005). Data on the prevalence of the latter parasite in cattle, however, are not available in Cameroon.

Similar studies in which the classical meat inspection results were compared to the same Ag-ELISA as used in this study were carried out in Belgium (Dorny et al., 2000) and in Nigeria (Faleke et al., 2004). In Belgium the Ag-ELISA and meat inspection detected 3.09 and 0.26% positive animals, respectively which means a factor of difference of 11.9. In Nigeria the Ag-ELISA (12.8%) did find 3.8 times more animals with cysticercosis than the meat inspectors (3.4%).

The fact that no single animal was found positive by the classical 'knife and eye' method is very surprising given the fact that *Taenia saginata* is known to exist in both regions of Dschang and Maroua. Vondou et al. (2002) carried out a large survey in two village communities Bafou and Bamendou, near to Dschang, and found 0.13 % tapeworm carriers. Both *T. solium* and *T. saginata* were reported. During a visit of one of the coauthors (AZ) to three localities in the far north, Yagoua, Tokombere and Maroua itself, persons with worm infections were treated with niclosamid (Yomesan[®]) and 20 tapeworms were collected, which were all confirmed as *T. saginata* using PCR as described by Rodriguez et al.(2002.) (unpubl. results). A possible explanation of the poor performance of the meat inspection might be sought in the economic crisis and the subsequent devaluation the local currency (FCFA) in 1994. Indeed, significant decreases of salaries occurred during this period which resulted in a demotivation of many civil servants, including meat inspectors. It is well known, however, that the operator's physical and psychological condition influences the rate of detection of cysticercosis (Walter & Koske, 1980).

In this study there was a borderline significant effect of sex on seroprevalence of cysticercosis in Dschang, but the effect was not significant in Maroua. Contradictory results have been reported about the impact of sex on the occurrence of cattle cysticercosis, but large scale epidemiological surveys usually confirm that there is no effect of sex (Dorny et al., 2000). There was no significant effect of age on seroprevalence of cysticercosis, which is in contradiction to the results of Dorny et al. (2000) who found an increasing number of seropositive cattle with increasing age in Belgium. On the other hand, in some African studies an inverse relationship between age and prevalence was reported (Onah and Chiejina, 1986; Okafor, 1988).

Conclusion

For the abattoir of Maroua meat inspection figures are available over a period of about 40 years. In 1963 Graber & Thome (1964) reported a prevalence of approximately 30 %. This figure dropped to 4.78 in 1981 (Thys, 1983) and to 0 in 2005 (this study). In the slaughterhouse of Dschang no single case of bovine cysticercosis has ever been reported. These abattoir data have to be interpreted with caution given the results of the Ag-ELISA and the presence of *Taenia saginata* carriers in the region. This study confirms that the conventional meat inspection is insensitive and does not reflect the real prevalence of bovine cysticercosis.

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4: HUMAN TAENIASIS AND CYSTICERCOSIS IN CAMEROON.

4.1. AN IMPORTANT FOCUS OF PORCINE AND HUMAN CYSTICERCOSIS IN WEST CAMEROON

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*'Helminth zoonoses' (Eds Geerts, S., Kumar, V. & Brandt, J.),
Martinus Nyhoff, Dordrecht, p. 85-91, 1987**

Abstract:

Since there were several indications that *T. solium* cysticercosis is an important zoonosis in the Menoua region (West Cameroon), a more detailed study was carried out to get a better idea of the prevalence of the parasite in pigs and men from this area. Ante mortem inspection of about 600 pigs on several local markets showed that 24.6% harboured cysticerci. By classical meat inspection of 151 pigs, taken at random at 5 different slaughterhouses in the region, 19.9% of the pigs were found positive. Using Elisa, however, antibodies against *T. solium* metacestodes were shown at least in 38% of these pigs.

A sero-epidemiological survey of 764 human serum samples, taken from apparently healthy people at different villages in the region, showed that 15.1% reacted positively in Elisa with a *T. crassiceps* metacestode antigen. After absorption of these sera to eliminate cross-reactions with filariasis and echinococcosis and comparative evaluation of the reactions with different other parasite antigens, at least 2.4% of the samples still remained positive for cysticercosis. These high levels of infection as well in men as in pigs prove that the Menoua region is an important focus of *T. solium* cysticercosis.

Introduction

Cysticercosis caused by the metacestodes of *Taenia solium* is a serious health and economic problem in some regions of Africa. Up to now, however, very few data are available about the distribution of *T. solium* cysticercosis in the latter continent. Nelson et al. (10) reported in 1965 that *T. solium* was common in Madagascar, the Cameroons, parts of East-Congo (Zaire) and South Africa. Few research has been done since then. Pandey and Mbemba (11) confirmed in 1976 that 0.1-8.1% of the pigs in different regions of Zaire were infected with *Cysticercus cellulosae* and there are records of human cysticercosis as well (12) The disease in man was recorded for the first time in West Africa in Ivory Coast (1). Proctor et al.(13) identified spinal cysticercosis as a major cause of paraplegia in Ghana. In Tchad (7) and Nigeria (3, 4) high prevalence figures in pigs were mentioned in some regions, although

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no information is available on human cysticercosis. In Cameroon 2 short reports (8, 9) have been published on taeniasis-cysticercosis due to *T. solium*. Since there were several indications that *T. solium* cysticercosis is an important zoonosis in the Menoua division (West Cameroon) this study was undertaken to collect more detailed information on the prevalence of porcine and human cysticercosis in this area.

Material and Methods

Detection of T. solium cysticercosis in pigs

Ante mortem inspection: was carried out from September 1984 until May 1985 on 607 pigs, selected at random on 6 livestock markets (Dschang city, Bafou, Baleveng, Penka-Michel, Fongo-Tongo and Fongo-Ndeng) and in 5 slaughterhouses (the same localities, except Penka-Michel) of the Menoua division. The animals were examined for the presence of cysticerci by inspection of the inferior side of the tongue and of the conjunctiva of the eyes (18).

Post-mortem inspection of 151 out of the 607 above mentioned pigs was carried out by making incisions in the internal and external masseters, the heart, the tongue, the diaphragm, the muscles of the forelegs and hind legs and also the psoas (18).

Serology: Two hundred serum samples were taken from pigs, kept by smallholders according to local customs (traditional husbandry). The blood was taken in the same abattoirs, which were mentioned above.

The sera were examined by Elisa and compared with the sera of 32 pigs, originating from the farm of the University Centre of Dschang, where the pigs were kept inside throughout the year (according to modern husbandry practices), so that infection with cysticercosis was unlikely. A serum sample was considered as positive when the optical density (O.D.) exceeded the threshold value, which was calculated on the basis of the mean O.D. + 3 x the standard deviation (S.D.) of the pig sera from the university farm. The optimal dilutions and incubation times of antigen, serum and conjugate were determined by checkerboard titration. Delipidised hydrosoluble extracts of *T. crassiceps* and *T. solium* metacestodes were used as antigens. The latter were recovered from local naturally infected pigs. The antigens were prepared according to the method used by Geerts et al. (6), except that the cyst fluid of the *T. solium* metacestodes was not incorporated in the antigen, as suggested by Flisser & Larralde (5).

The Elisa-test was performed essentially as described by Geerts et al. (6). Briefly the antigens (5 μ protein/ml) were incubated overnight at 4°C. Serum was diluted 1/200 and conjugate (R a SW IgG (H + L)/PO, Nordic) 1/500 in PBS-Tween 20 with addition of respectively 2 and 4% normal horse serum. Orthophenylene diamine was used as substrate.

Detection of T. solium cysticercosis in men

A total of 764 serum samples were taken

- a) in the following villages of the Menoua-region : Baleveng; Bafou-road, -Chefferie, -Souschefferie, -Pastorale and -Cooperative; Bamendou II (n=675);
- b) from the workers and students on the university farm (n=63) and
- c) from patients (n=26) at the hospital of Dschang, who were not suspected for cysticercosis.

The majority of the examined people were adults (725 or 94.9%). Only 39 children were included. The number of samples taken from males and females was respectively 324 (42.4%) and 440 (57.6%).

Twenty serum samples from black Africans without parasitic infections were used as negative controls. These were used to calculate the threshold value (mean O.D. + 3 x S.D.). The Elisa was carried out using serum diluted 1/200 and conjugate (GaH IgG (H + L)/PO, Miles), diluted 1/5000 in PBS-Tween 20. A fraction (F₁) of *T. crassiceps* metacestodes, prepared by gel filtration on Ultragel (AcA 34; LKB) was used as antigen (at a concentration of 5µg protein per ml) in order to improve the specificity of the test.

All positive sera were further tested against a battery of 7 different antigens: total extracts of *Ascaris suum*, *Fasciola hepatica*, *Litomosoides carinii*, *Cysticercus cellulosae* and *Schistosoma mansoni*; hydatid fluid of *Echinococcus granulosus* (horse) and excretion-secretion products of *Toxocara canis*. In order to differentiate between filariasis and echinococcosis absorption tests were performed essentially following the technique described by Speiser (14). For the confirmation of true cysticercosis of echinococcosis cases absorption of the sera was done with *T. crassiceps* metacestode or *E. granulosus* hydatid fluid antigens. Indirect haemagglutination was carried out with this latter antigen using a commercial kit (Fumouze, France). A titre of 1/512 was considered as positive.

Results and Discussion

Porcine cysticercosis

Tables 1 and 2 show the result of the ante-mortem and post-mortem inspection of the local pigs.

Table 1. Prevalence of *T. solium* cysticercosis in pigs of the Menoua division as detected by ante-mortem inspection.

	Total no. of pigs examined	Pigs infected with <i>T. solium</i>	
		number	%
Adult pigs (> 1 year)	409	102	24.9
Young pigs (< 1 year)	198	47	23.7
Total no. of pigs	607	149	24.6

Table 2. Prevalence of *T. solium* cysticercosis in pigs of the Menoua division as detected by post-mortem inspection.

	Total no. of pigs examined	Pigs infected with <i>T. solium</i>	
		number	%
Adult pigs (> 1 year)	142	27*	19
Young pigs (< 1 year)	9	3*	33.3
Total no. of pigs	151	30	19.9

*: 24 out of 27 adult pigs and 3 young pigs were negative at ante-mortem inspection.

From the tables 1 and 2 it is clear that there is a high prevalence of pig cysticercosis in the

Menoua region. The results of the meat inspection are comparable to the highest figures (18.4%) found in Nigeria (3, 4) and are even higher than those found in hyperendemic areas in Mexico (15). The fact that the figures from ante-mortem inspection are higher than those of post-mortem inspection is a common feature in many countries since the owners bring their pigs to the official abattoir only when no cysts are present in the mouth.

Table 3. Presence of antibodies in pig sera against homologous and heterologous taeniid antigens (Elisa).

Metacestode antigen	Total no. of sera examined	Pigs infected with <i>T. solium</i>	
		number	%
<i>T. crassiceps</i>	200	106*	53
<i>T. solium</i>	200	89*	44.5

*: 76 (38 %) sera were positive for both antigens simultaneously.

Table 3 summarizes the serological results of 200 pig sera in Elisa. As expected these figures are still much higher than those from ante- or post-mortem inspection. Three reasons can be advanced to explain this:

1. there are surely a number of cross-reactions with other parasites,
2. some pig sera may contain antibodies, although no cysticerci are present (any more) and
3. a number of infected pigs are not detected by meat inspection (WHO, 1983). It can be concluded that the figure of 24.6% of infected pigs, as evidenced by ante-mortem inspection is certainly a conservative figure and the real infection rate lies probably between 24.6 and 38%, the lowest figure of the serological results.

Human cysticercosis

Out of 764 serum samples 115 (15.1%) were shown to be positive in Elisa using fraction F₁ of a *T. crassiceps* metacestode antigen and a threshold level with a 99.7% confidence limit (mean O.D. + 3 S.D. = 0.286 + 3 x 0.127 = 0.667). The majority of these positive sera, however, were identified as cross-reactions after comparative evaluation against different parasite antigens and absorption tests with heterologous antigenic material (Table 4).

Table 4. Serodiagnosis of 115 human sera, positive* for cysticercosis, after comparative evaluation against different parasite antigens in Elisa.

	<i>T. solium</i> cysticercosis	Filariasis	Hydatidosis	Ascariasis and/or visceral larva migrans	Dubious	Negative
antigens	<i>C. cellulosa</i>	<i>Litomosoides</i> <i>carinii</i>	<i>E.</i> <i>granulosus</i> hydatid fluid	<i>Ascaris suum</i> <i>Toxocara canis</i> (E.S.)		
No. of sera	17°	76°	1**	3	10	8°

*: positive in ELISA with *T. crassiceps* metacestode antigen

** : confirmed by indirect haemagglutination test and absorption tests

° : confirmed by absorption tests

Table 4 shows that at least 17 sera, i.e. 2.4% of the total number of tested sera can be considered as true positives for cysticercosis. Since it is known that at least 10 to 20% of known positive cases are not detected by a sensitive technique like Elisa (5), the real frequency of human cysticercosis in the region is certainly higher than 2.4%. Another reason

why the figure of 2.4% is probably an underestimation of the reality is that only apparently healthy people have been sampled and not those showing characteristic symptoms of neurocysticercosis. When this figure is compared with the results from other sero-epidemiological studies it is very similar to the figures from hyperendemic areas (Central America, some regions in the Far East). In a large survey in Mexico only 0.45% of about 20,000 examined serum samples were found positive with immunoelectrophoresis, but it is known that this technique is not very sensitive and detects only half of the cases (16). In another serological survey in several areas of South East Asia the percentages of positive reactions in Elisa varied between 3 and 21% according to the region (2). These latter figures, however, were not corrected for cross-reactions.

The results of this study confirm the observations, made by Marty et al. (9) on 95 people living in the same region of Cameroon. These authors also detected antibodies against *T. solium* metacestodes in 2% of the examined population. In order to obtain a more complete picture of the situation concerning human cysticercosis in the Menoua division, it is necessary, however, to collect more data about the number of *T. solium* carriers among the local population and also the number of people showing the typical symptoms of cysticercosis (epilepsy, psychiatric symptoms etc.). Data about the presence of cysticerci at routine autopsy would also be very interesting. Based on the currently available figures, however, it can be concluded that the Menoua region is a very important focus of *T. solium* cysticercosis. This can easily be explained since all the conditions are present for a direct human-to-pig transmission of the parasite:

1. Open air fecalism or deliberate defecation in the pig sties are common practices in the region.
2. Well trained and qualified meat inspectors are lacking and clandestine slaughtering of pigs is common.
3. Consumption of raw or insufficiently cooked pork is common in the region.
4. Detection and treatment of human *T. solium* carriers is often lacking.

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4.2. A SEROEPIDEMIOLOGICAL STUDY OF HUMAN CYSTICERCOSIS IN WEST CAMEROON

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*Trop Med Int Health 8: 144-149 (2003) **

Abstract:

The occurrence of human cysticercosis was studied in 4993 persons belonging to 3 rural communities of the 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 ELISA. 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 was shown to increase with age. Twenty two of the 34 seropositives underwent computed tomography of the brain. Thirteen of them were CT-scan positive, which shows that neurocysticercosis was present in 59.1 % of the tested seropositive persons. Living cysticerci were not detected among 20 seronegative people. About one fifth (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 it is concluded that *T. solium* cysticercosis is an endemic, but overlooked public health problem in West Cameroon.

1. Introduction

Taenia solium cysticercosis remains a major public health problem in developing and some developed countries (Schantz et al., 1998). It is reported as one of the major causes of epilepsy in the tropics (Carpio et al., 1998; Garcia et al., 1993; 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 developing countries. However, some reliable serological tests for antigen or antibody detection of human and pig cysticercosis (ELISA, EITB) have been developed (Brandt et al., 1992; Tsang et al., 1989; Ito et al., 1998; Erhart et al., 2002; Garcia et al., 2000).

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In Africa, the importance of human cysticercosis is not well known because of the lack of well developed medical infrastructures as well as diagnostic facilities (Preux & al., 1996). However, epidemiological studies of the disease have been done in several countries, i.a. 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 the 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 & 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.

2. Material and Methods

2.1. Study area

The study was carried out from August 1999 to November 2000 in the Menoua Division (Western Province, Cameroon). This region is one of the important pig breeding regions of Cameroon. Due to African swine fever, however, the number of pigs has been decimated over the last years. Although pigs are usually confined during the rainy season, they are released after harvest or during the dry season. During confinement, pigs are mainly fed with farm and kitchen residues, but in some households also with 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.

2.2. Sampling

The study was carried out in 3 rural communities (Bafou, Bamendou and Fonakekeu) near to the city of Dschang (Menoua Division, Cameroon). An information campaign was initially organised in these localities with the support of local traditional authorities to sensitise the populations about the objectives of the study. A total of 4993 people were included in the survey: 2628 out of an estimated number of 58,000 inhabitants of Bafou, 2299 out of about 32,000 in Bamendou and 66 out of approximately 360 inhabitants in Fonakekeu. All persons, 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, sexe 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 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 (see 2.3). 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).

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

A monoclonal antibody 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) slightly modified. In short, the sera were pre-treated using trichloroacetic acid and used in ELISA at a final dilution of 1/4. Two monoclonal antibodies (MoAb) were used in a sandwich ELISA. MoAb B158C11A10 was diluted at 5µg/ml in carbonate buffer (0.06 M/pH 9.6) for coating and a biotinylated MoAb B60H8A4 (1.25µg/ml in PBS 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 during 30 min for the coating of the first MoAb and during 15 min. for all subsequent steps. The chromogen/substrate solution consisting of orthophenylene diamine (DAKO, #S2045) and H₂O₂ was added and incubated without shaking between 30-33°C for 15 min. To stop the reaction 50µl of 4N H₂SO₄ was added to each well. The plates were read using an ELISA reader (Labsystem Multiskan RC) 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 8 negative reference sera at a probability level of p=0.001 to determine the result using a modified Student test (Sokal & Rohlf, 1981). The ELISA ratio was calculated by dividing the OD of the sample by the calculated cut-off value of the 8 negative controls. An ELISA ratio of > 1 was considered as positive.

2.4. CT-scan

A cerebral CT-scan was done for 22 of the 34 seropositive (8 persons refused the examination and 4 individuals, who were already sick at the time of sampling, died before) and a similar number (n=20) of seronegative persons. The scannings were carried out at the Yaounde Central Hospital using a SOMATOM AR. STAR, VB41A apparatus. Ioxitalamic acid (Telebrix^R 35) was used as intravascular contrast agent. Each patient was examined before and after contrast injection and 2 image series on slides were made.

3. Results

3.1. 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 examined in each of the 3 localities. The proportion of females in each of the three localities was very similar.

Table 1. Characteristics of the examined population in the 3 rural communities

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

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

	No. examined	No.	% seropositive	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				
- ≤ 15 years	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 & Coull (1998)

3.2. Clinical examination and cerebral CT-scan of seropositive persons

The percentage 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 out of 4785 (0.6 %) asymptomatic individuals. The ELISA ratio's 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). Out of the 34 seropositive individuals 6 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 % (7/34) versus 1.9 % (95/4959) for the seronegative individuals.

Neurocysticercosis was detected by computed tomography 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 2 seropositive epileptic patients was CT-scan positive, showing more than 25 calcifications in the brain. All 3 seropositive patients suffering of 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	N° Examined	Number	(%) 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

3.3. 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 collected using a questionnaire. Only 4.9% of the people declared not to eat pork. Among the latter 7 were epileptic patients and 3 had mental disturbances. However, none of them was positive in the Ag-ELISA. All but 2 of the 34 seropositives were pork consumers. These two were epileptics, who did cease pork consumption when their epileptic crises started.

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

Discussion

The antigen detection ELISA revealed the presence of active cysticercosis in 0.4 to 3% of the people examined in the 3 rural communities. If we take into account only the adult population (> 15 years) the figures double (0.8 to 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 3 rural communities of the Menoua region. Due to the fact that the sampling was not done 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 due to a possible oversampling of females, is not too important due to 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 and specificity of only 85 and 92 %, respectively. The former test is known to detect only living cysticerci as has been clearly shown in cattle (Brandt et al, 1992), in pigs (Nguekam, unpubl. 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.

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 eighties, 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., 1987). This corresponded well with observations in large surveys held in other regions of West and Central Africa using Ab-Elisa and/or EITB i.e. 1.3 % seropositives in Benin (Houinato et al., 1.3%) and 2.4% in Togo (Dumas et al., 1989).

Contrary to the observations of Erhart et al. (2002) in Vietnam, which showed that eight out of 9 positives in Ag-ELISA were confirmed using a cerebral CT-scan, brain lesions suggestive of cysticercosis were found in only 13 (59.1 %) from 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 1 out 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 due partially to 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 % (7/34) of the seropositive people were harbouring a tapeworm (1) or had harboured one in the past (6) 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. Since these tapeworms were identified as *T. solium* (based on the number of uterine branches of a gravid segment) and since three out of 4 tapeworms collected in another study in the same region were shown to be *T. solium* (Vondou et al., 2002), it is obvious that these persons 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 which were seropositive for cysticercosis was rather low (2.5 %) in this study as compared to 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 (Houinato et al, 1998, Sarti et al, 1992). It is not surprising that the longer people are exposed to *T. solium* eggs in an endemic environment the more chances they have to become infected, albeit that more information is needed on the survival of metacestodes and on the possibility of re-infection.

It can be concluded that taeniasis-cysticercosis complex due to *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 1. 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 2. many pigs are temporarily or continuously free roaming (Pouedet et al., 2002), all the conditions are present for a very efficient parasite transmission.

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Subcutaneous cysticercus (arrow) on the arm of a Cameroonian woman



4.3. PREVALENCE OF ACTIVE CYSTICERCOSIS IN THE FAR NORTH PROVINCE OF CAMEROON

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(unpublished results)

Summary

In order to determine the prevalence of active cysticercosis in the Mayo-Danay Division (far north province of Cameroon), a study was carried out in 7 villages during the month of October 2002. Thousand three hundred and seventeen persons were clinically examined and serologically tested using an antigen-detection ELISA. Circulating antigens of *Taenia solium* metacestodes were detected in 2.05% of the study population. The percentage of seropositives varied from 0.49 to 4.52% according to the village. Because this test has been shown to detect only persons carrying living cysts, it gives a good indication of the occurrence of active cysticercosis. However, the true prevalence of cysticercosis is probably much higher since people who have been exposed to the parasite and/or who carry only dead cysts are not detected by the test. Random effects logistic regression showed that significantly more seropositive people were present in the age group of above 45 years, whereas sex did not have a significant effect. This study shows that cysticercosis is a public health problem in Mayo-Danay Division and probably the whole northern region of Cameroon as previous studies have revealed that the region has a high porcine cysticercosis prevalence and many favouring factors to the completion of *T. solium* cycle are present.

1. INTRODUCTION

Taenia solium cysticercosis is endemic to hyperendemic in several African countries (Geerts, 1993; Zoli *et al.*, 2003). Case reports on human cysticercosis are available for many countries, but there is a lack of large-scale surveys in order to demonstrate the real impact of the disease. In the western part of Cameroon a high prevalence of porcine and human cysticercosis has been reported (Zoli *et al.*, 1987; Pouedet *et al.*, 2002; Nguekam *et al.*, 2003a; Shey-Njila *et al.*, 2003). It has also been shown that the Mayo-Danay district in the far north province of Cameroon is hyperendemic for *T. solium* cysticercosis in pigs (Assana *et al.*, 2001). Therefore, the purpose of this study was to examine the prevalence of cysticercosis in man in the same district where the survey of Assana *et al.* (2001) was carried out.

2. PATIENTS AND METHODS

2.1 Study area

The study was carried out during the month of October 2002 in 7 villages (Zouaye, Bangana, Hougno, Vada, Vounaloum, Vélé and Datcheka) of the Mayo-Danay division in the far north province of Cameroon (Fig. 1). The selection of these villages was based on the survey of porcine cysticercosis carried out by Assana *et al.* (2001). These authors found a prevalence of 15.4 and 38.9% using tongue inspection and antigen detection ELISA, respectively. The villages are located in the southern part of the division where pig production is more important. The Mayo-Danay division covers an area of 6730 km² with a population of approximately 600.000 inhabitants.

Pigs are reared either according to the traditional system, in which they roam freely without any particular care, or according to the semi-traditional system, in which they are kept in pens part of the time. Very few people keep their pigs permanently in pigsties with adequate animal health care. Most of pigs are fed with kitchen remains. Free roaming pigs have access to human faeces.

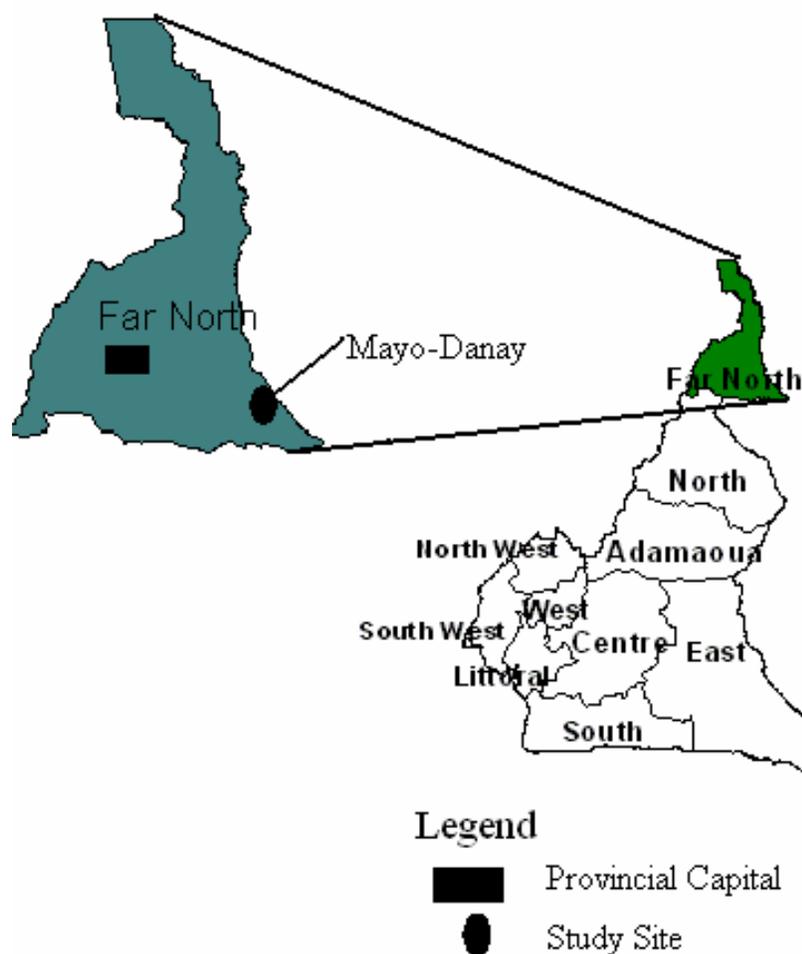


Figure 1: Map of Cameroon and Far North province showing Mayo-Danay Division.

2.2 Data collection

In each locality, the whole population was informed about the objectives of the study and invited to collaborate by the chief of the village. A total of 1526 volunteers showed up for

the examinations and 1317 individuals, for whom complete data were available, were included in the study. The total population of the 7 villages is estimated at about 65000 inhabitants.

The volunteers in the 7 villages were clinically examined by some of the co-authors (F.P.R or W.A.). The following parameters were recorded: sex, age, village, presence/absence and localisation of sub-cutaneous nodules, presence/absence and frequency of seizures, headache and any other symptom that could be linked to cysticercosis.

After informed consent was obtained from the adult volunteers or from their parents in case of children, blood was collected. The sera were stored at -20°C prior to serological examinations.

2.3 Detection of circulating antigens by the Enzyme-Linked Immunosorbent Assay (Ag-ELISA)

Circulating antigens of *T. solium* metacestodes were detected using a monoclonal antibody based ELISA (Brandt *et al.*, 1992). The Ag-ELISA was carried out as described by Nguekam *et al.* (2003b). One positive reference serum sample from a Cameroonian with confirmed cysticercosis (by CT scan) and eight negative reference serum samples from healthy Cameroonian people were used as controls.

The plate was read with the help of an automated spectrophotometer (Multiskan RC version 6.0) at the wavelength of 492 nm. A serum was considered as positive if the value of its optical density (OD) was significantly different from the average OD of eight negative reference sera (cut-off) at a probability level of 0.001 (modified Student *t* test; Sokal and Rohlf, 1981).

2.4 Statistical analysis

The random-effects logistic regression with locality as random effect was used to detect any significant difference between the localities, gender and age at the probability level of 5%. These analyses were conducted using the Stata Statistical Software.

3. RESULTS

3.1 Characteristics of the study population

The characteristics of the study population are presented in table I. Of the 1317 persons who participated in the survey (representing about 2 % of the total population of the study area), 766 (58%) were male and 551 (42%) female. The age distribution ranged from 1 to 100 years with an average age of 26.7 years.

3.2 Cysticercosis prevalence

Of 1317 serum samples tested, 27 (2.05%) were positive in the Ag-ELISA (Table II). The percentage of seropositive individuals varied from 0.49% to 4.52% in the 7 villages. The highest rate was recorded in Vada and the lowest in Zouaye. Random effects logistic regression with locality as random effect showed that apart from Vada village, seropositivity did not significantly differ in the villages. A significantly higher number of seropositive persons was found in Vada than in Zouaye ($p=0.032$) and Datcheka ($p=0.025$).

Table I: Characteristics of the study population of 7 villages of the Mayo-Danay division in the far north province of Cameroon

Village	No. examined	% male	% female	age group (%)		
				<16	16-45	>45
Zouaye	205	65	35	25	61	14
Bangana	173	46	54	25	55	20
Hougno	140	58	42	28	48	24
Vada	199	48	52	49	39	12
Vounaloum	211	72	28	31	49	20
Vele	101	74	26	27	51	22
Datcheka	288	52	48	44	44	12
Total	1317	58	42	34	49	17

Table II: Seroprevalence of cysticercosis per locality, age group and sex in the Mayo-Danay division, Cameroon

Parameter		No. examined	No. positive*	% positive
Locality	Zouaye	205	1	0.49
	Bangana	173	5	2.89
	Hougno	140	2	1.43
	Vada	199	9	4.52
	Vounaloum	211	4	1.90
	Vele	101	3	2.97
	Datcheka	288	3	1.04
Age	<16	452	8	1.77
	16-45	645	10	1.55
	>45	220	9	4.09
Sex	Male	766	16	2.09
	Female	551	11	2.00
TOTAL		1317	27	2.05

*: Ag-ELISA

3.3 Influence of age and sex on the prevalence of cysticercosis

The highest percentage of seropositive persons (4.09%) was found in the older age group (above 45 years) whereas in the adults (16-45 year) and the young people (<16 years) 1.55 and 1.77 % were seropositive, respectively (Table II).

The seroprevalence in males and females was very similar with 2.09 and 2.00 %, respectively. Random effects logistic regression showed that age had a significant effect on the percentage of those that were positive ($p=0.009$) whereas sex did not have a significant effect ($p=0.996$).

3.4 Correlation between symptoms recorded and seropositivity

The percentages of seropositive individuals showing symptoms suggestive of *T. solium* cysticercosis are presented in table III.

A total of 179 individuals presented at least one symptom that could be linked to cysticercosis among whom 7 (3.9%) were seropositive. Of the two persons, who were affected simultaneously by headache, subcutaneous nodules and seizures, one was seropositive.

Among the 1138 individuals that were asymptomatic, 20 (1.76%) were seropositive whereas 7 (29.53%) of the 27 persons with positive serology, had some sign suggestive of cysticercosis (Table III).

Table III: Correlation between symptoms recorded and seropositivity for cysticercosis (Ag-ELISA)

Symptoms or clinical history	No. examined*	No. positive**	% positive
Headache	34	1	2.94
Subcutaneous Nodule	143	7	4.90
Seizures	19	1	5.26
Asymptomatic	1138	20	1.76

*: some persons showed more than one symptom

** : One individual showed all 3 symptoms

4. DISCUSSION

In this study, the Ag-ELISA was used in order to estimate the prevalence of active cysticercosis in the Mayo-Danay division of the Far North province of Cameroon. Of the 1317 persons that were included in the study, 2.05 % were seropositive. It can be assumed that this figure underestimates the true prevalence of cysticercosis because the persons carrying only dead cysts were not detected. Indeed, it has been shown that the Ag-ELISA detects only living cysts as well in man (Erhart *et al.*, 2002) as in pigs and cattle (Brandt *et al.*, 1992; Nguekam *et al.*, 2003b). On the other hand, the inconvenient of an antibody detection ELISA as compared to the Ag-ELISA is that the former overestimates the true prevalence of cysticercosis, because antibodies seem to disappear from the serum within 1 to 3 years in 30 to 40 % of patients seropositive for *T. solium* in endemic countries (Garcia *et al.*, 2001; Dorny *et al.*, 2004).

The presence of human cysticercosis in the far north of Cameroon is not surprising as Assana *et al.* (2001) discovered a high seroprevalence in pig (39.8%) in the same study area. Since the sampling was not done at random, but based on voluntary presentation of the village people to the medical team, the results of this study have to be interpreted with some caution.

Males were over-represented in some of the villages in this survey. However, as the true proportion of males in the study population is not known, no attempt could be made towards using weights to correct for this bias. It is expected that the bias due to oversampling of males is not too important as a result of the large number of people, which have been tested (2% of the total study population).

A lower seroprevalence of 0.7% was obtained by Nguekam *et al.* (2003a) in a similar study using Ag-ELISA in 3 communities of the Menoua Division in the Western province of Cameroon which is known to be endemic for human and porcine cysticercosis. The two areas have similar conditions that favour the transmission of cysticercosis such as free roaming pig rearing system combined to open air defecation. The prevalence of 2.05% (0.49-4.52) found in this study was lower, however, than the seroprevalence of 5.7% obtained by Erhart *et al.* (2002) in Northern Vietnam or 5.7-9.1% in Central-West Venezuela (Ferrer *et al.*, 2003) using a similar test for circulating antigen detection.

The diagnostic sensitivity and specificity of the Ag-ELISA used in this study are 94.4 and 100%, respectively (Erhart *et al.*, 2002), which implies that the predictive value of a positive test result is 100%. Although no further confirmation test (imaging techniques or biopsy of subcutaneous nodules) was performed, it can be assumed that more than half of the 27 individuals, who were seropositive, were affected by brain cysticercosis. Indeed, in similar studies Nguekam *et al.* (2003a) found a confirmation rate of Ag-ELISA positive cases by cerebral CT scan of 59.1% whereas Erhart *et al.* (2002) reported 89 %.

The occurrence of cysticercosis in the 7 villages varied from 0.49% to 4.52% with the highest prevalence in Vada and the lowest in Zouaye. Since these 2 localities are very close to each other and their populations have very similar living conditions, this difference is probably due to the presence of one or more tapeworm carriers in Vada as it has been demonstrated by Sarti *et al.* (1992) that seropositive persons are clustered within households in which a member has been reported of having passed tapeworm proglottids.

No significant difference was found between the prevalence in males and females. This finding is in accordance with Nguekam *et al.* (2003a) and Ferrer *et al.* (2003) in studies carried out in West Cameroon and a rural area of Venezuela, respectively. A different prevalence according to gender, however, was found by Garcia *et al.* (1998).

The fact that only a small percentage of seropositives was found among the people with headache or seizures is not surprising, since the former symptom is not very characteristic for cysticercosis and the latter is mainly due to dead or degenerating cysticerci, which are not detected by the Ag-ELISA.

Of the 143 persons with subcutaneous nodules only 7 (4.90%) were seropositive. Since the facilities were not available to carry out biopsies, the nodules might also be due to *Onchocerca volvulus* which is endemic in the region (Wahl *et al.*, 1998; Seiderfaden *et al.*, 2001).

The highest prevalence of cysticercosis was found in the oldest age group (> 45 years), which is in agreement with the results of many other authors (Sarti *et al.*, 1992; Houinato *et al.*, 1998; Nguekam *et al.*, 2003). This can be explained by the fact that the longer people are exposed to *T. solium* eggs in an endemic environment, the more chances they have to become infected. In a hyperendemic area of Venezuela, however, Ferrer *et al.* (2003) did find more cases of active cysticercosis in individuals aged ≤ 30 years.

It can be concluded that cysticercosis is a public health problem in the Mayo-Danay Division and probably in the whole northern region of Cameroon, since Awa *et al.* (1999) showed a high occurrence of porcine cysticercosis (12.3%) in an abattoir study in Garoua. This study shows again that human cysticercosis is almost unavoidable when a high prevalence of porcine cysticercosis is present in a region characterised by low-input pig husbandry and poor sanitary conditions.

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4.4. TAENIASIS/CYSTICERCOSIS DUE TO *TAENIA SOLIUM* IN THE MÉNOUA (WEST CAMEROUN) (in French)

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Résumé

La présente étude a été conduite de août 1999 à avril 2000 et avait pour objectif de déterminer la prévalence de la taeniose due à *Taenia solium* dans Bafou et Bamendou, deux groupements villageois de la Menoua (Ouest-Cameroun). Quatre (0.13 %) sur 3109 échantillons de matières fécales humaines ont été trouvés positifs pour des œufs de *Taenia* spp. par la technique de la flottaison. Trois des 4 vers expulsés étaient des *T. solium* et un était *T. saginata*. Deux cas de cysticercose étaient présents dans une des familles où vivait un porteur de *T. solium*.

En plus, des enquêtes coprologique et sérologique pour la taeniose et la cysticercose à *T. solium* ont été faites chez un groupe à risque constitué de bouchers et/ou langueyeurs (n=137). Les résultats ont été comparés à ceux d'un groupe témoin (n =198). Aucun porteur de *Taenia* n'a été détecté par l'examen microscopique. Les prévalences de la cysticercose dans les deux groupes ont été respectivement 3.6 and 4.5 %.

Mots Clés : Prévalence, Taeniose, *Taenia solium*, Groupe à risque, Cysticercose, Cameroun.

Abstract

The present study was carried out between august 1999 and april 2000 with the objective of determining the prevalence of *Taenia solium* taeniasis in two village communities of Bafou and Bamendou in the Menoua division (West Cameroon).

Four (0.13 %) out of 3109 faecal samples were positive for *Taenia* spp. eggs using the flotation technique. Three of the 4 worms expelled were *T. solium* whereas the other one was *T. saginata*. Two cases of cysticercosis were diagnosed in one of the families with a *T. solium* carrier.

Furthermore, coprological and serological investigations for *T. solium* taeniasis and cysticercosis were carried out among butchers and/or tongue inspectors (n=137) of the city of Dschang. The results were compared with those of a control group (n=198). *Taenia* spp. eggs were not detected by microscopic examination. The prevalence of cysticercosis in the two groups was relatively similar (3.6 and 4.5 % respectively).

Key Words : Prevalence, taeniasis, cysticercosis, *Taenia solium*, risk group, Cameroon.

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Introduction

Dans plusieurs pays Africains *T. solium* est endémique (Geerts *et al.*, 2002). Les mauvaises conditions hygiéniques et d'élevage (divagation et accès facile des porcs aux matières fécales humaines) et le manque d'inspection vétérinaire pérennisent le cycle biologique de ce parasite.

Au Cameroun, plusieurs zones d'endémicité de la cysticerose ont été rapportées (Zoli *et al.*, 1987 ; Assana *et al.*, 2001 ; Pouedet *et al.*, 2002). Cependant il y a très peu de données sur la prévalence de la taeniose dans ce pays. La taeniose a été mise en évidence dans une petite enquête chez une centaine de personnes à Fombap (Ouest-Cameroun) (Marty *et al.*, 1986) et *T. saginata* a été rapporté à Koza dans les Monts Mandara (Extrême-Nord-Cameroun) (Ripert *et al.*, 1983).

L'objectif de ce travail était de déterminer la prévalence de la taeniose dans Bafou et Bamendou, deux groupements villageois de la Menoua, et d'étudier la prévalence de la taeniose-cysticerose chez un groupe à risque constitué de bouchers et/ou langueyeurs de la Menoua en comparaison avec un groupe témoin.

Matériels et Methodes

Endroit de l'enquête

L'enquête sur la prévalence de la taeniose a été menée dans 13 villages du groupement Bamendou, 14 villages du groupement Bafou et dans la ville de Dschang. L'élevage des porcs y est une activité économique importante. Le nombre de porcs par porcherie varie de 1 à quelques dizaines et souvent ces animaux sont en divagation permanente. Pouedet *et al.* (2002) ont démontré que 10,9 % des porcs étaient infestés de cysticerose dans les villages cités ci-dessus. Dans la plupart des porcheries il n'y a pas de mangeoires et les animaux sont nourris au sol. Très souvent il n'y a pas de latrines dans les habitations, ou bien elles sont situées près des porcheries. Les porcheries servent parfois de lieux d'aisance. L'étude de Pouedet *et al.* (2002) a montré que dans 53,1 % des porcheries les porcs avaient accès aux déjections humaines.

Collecte des échantillons

a. Population de Bafou et de Bamendou

Après une campagne de sensibilisation préalable une équipe médicale a organisé des journées de consultation dans 27 villages des groupements Bafou et Bamendou ainsi que dans la ville de Dschang (août 1999- avril 2000). Chaque personne intéressée était prié de venir avec ± 3 g de matières fécales enveloppées dans une feuille de plante verte. Ainsi des échantillons ont pu être collectés chez 3109 personnes, dont 1677 à Bamendou, 1366 à Bafou et 66 à Dschang. 933 personnes étaient de sexe masculin (dont 518 jeunes et 415 adultes) et 2176 personnes étaient de sexe féminin (dont 2060 adultes et 116 jeunes). Un échantillonnage aléatoire n'a pas été possible à cause de raisons organisationnelles et logistiques.

b. Familles des porteurs de Taenia spp.

Des échantillons de selles et de sang ont été collectés chez 49 membres de famille des porteurs de *Taenia* spp. Les personnes confirmées séropositives ont subi un CT-scan du cerveau. Toutes les personnes séropositives ont été traitées à l'albendazole avec une dose de 15mg/kg/jour pendant une semaine et sous surveillance médicale.

c. Groupe à risque (bouchers et langueyeurs)

Des échantillons de matières fécales et de sang ont été collectés chez 139 bouchers ou langueyeurs de la ville de Dschang (groupe à risque) et chez 198 personnes provenant de 12 familles différentes vivant dans le voisinage mais n'exerçant pas les mêmes métiers (groupe témoin).

Les examens coprologiques

Les matières fécales ont d'abord été examinées de façon macroscopique pour la recherche d'éventuels proglottis de *Taenia* spp. ; ensuite de façon microscopique pour la recherche des œufs de *Taenia* (flottaison sur solution saturée de NaCl) telle que décrite par Thienpont *et al.* (1979).

Les personnes positives à l'examen microscopique ont été traitées au niclosamide (Yomesan, Bayer). Les vers ont été récupérés après expulsion afin d'identifier l'espèce.

Les analyses sérologiques

Le test ELISA pour la détection d'antigènes circulants de cysticerques de *Taenia* (Ag-ELISA) a été exécuté comme décrit par Brandt *et al.* (1992) et modifié par Dorny *et al.* (2000). L'anticorps monoclonal B158C₁₁A₁₀ (IgG1) a servi pour la sensibilisation des plaques et le monoclonal B60H₈A₄ biotinylé de conjugué. Cette technique a été utilisée avec succès pour la détection de la cysticerose humaine (Erhart *et al.*, 2002). La sensibilité et la spécificité de ce test ont été estimés à 94.4 (34/36) et 100 (0/57) % (résultats préliminaires).

Résultats et Discussion

Prévalence de la taeniose dans les groupements de Bafou et Bamendou (Menoua)

Des œufs de *Taenia* spp. ont été observés dans 4 des 3109 échantillons de matières fécales examinées au microscope, soit une prévalence de 0.13%. Comme les échantillons n'ont pas pu être pris de façon aléatoire, il y a eu une surreprésentation des femmes, ce qui pourrait causer un certain biais. D'autre part on peut espérer que le biais ne soit pas trop important à cause de la taille assez importante de l'échantillon (4 % de la population de la zone d'étude). Etant donné que la sensibilité d'un seul examen coprologique est nettement plus faible que celle de 2 ou 3 examens consécutifs (Schantz & Sart-Gutierrez, 1989) ce chiffre de 0.13 % est certainement une sous-estimation de la prévalence réelle. Cependant cette prévalence est comparable à celle de 0.2-0.3% à base d'un examen coprologique rapportée dans plusieurs communautés de l'Amérique Latine (Schantz *et al.*, 1998). Parmi les 4 *Taenia* expulsés il y avait un *T. saginata* (24 branches utérines et un scolex inerme). A base de la présence d'un scolex armé (mis en évidence chez un ver) et/ou le nombre de branches utérines (≤ 10) les trois autres ont été identifiés comme *T. solium*.

Taeniose/cysticerose chez les membres de famille des 4 porteurs de Taenia

Les résultats de l'enquête chez les membres de famille des porteurs de *Taenia* spp. sont résumés dans le tableau I. Aucun œuf de *Taenia* n'a été observé à l'examen coprologique des 49 personnes examinées. Seulement dans la famille F2 deux cas de cysticerose ont été détectés. La porteuse de *T. solium* était une fillette de 7 ans. Elle était séropositive au test ELISA et le CT-scan a confirmé la présence de cysticerques dans le cerveau. L'une de ses sœurs âgée de 5 ans était aussi positive au test ELISA et souffrait d'épilepsie mais le CT-scan était négatif. Un garçon de 8 ans de la même famille était épileptique, mais il était négatif au test ELISA et au CT-scan. Trois des 5 porcs de cette familles étaient ladres (langueyage). Des margelles avaient été aménagées pour permettre à tous les membres de la famille de déféquer dans la porcherie. Les enfants ont affirmé avoir souvent mangé de la viande ladre. Ces observations confirment celles de Cao *et al.* (1997) en Chine et celles de Sarti *et al.* (1992) en

Amérique Latine qui ont trouvé qu'une infection actuelle ou précédente avec le ver adulte (*T. solium*) est un des facteurs de risque les plus importants pour la cysticerose humaine.

Tableau I. Etude de la cysticerose chez les familles des porteurs de *Taenia* spp.

	Nombre de personnes	Espèce de <i>Taenia</i> *	No. d'épileptiques	Nombre de séropositifs (Ag-ELISA)
F1	9(7)	<i>T. solium</i>	0	0
F2	25(2)	<i>T. solium</i>	2	2
F3	3(0)	<i>T. solium</i>	0	0
F4	12(7)	<i>T. saginata</i>	0	0

() : nombre de scolarisés ; * : présente chez le porteur de ver solitaire

Taeniose/cysticerose chez les bouchers et les langueyeurs

Comme les bouchers et les langueyeurs viennent plus souvent en contact avec des porcs ou la viande porcine, la fréquence de la taeniose et de la cysticerose chez ce groupe à risque a été examinée et comparée avec celle d'un groupe témoin. Aucun échantillon de selles n'a été trouvé positif pour les œufs de *Taenia*. Les prévalences de la cysticerose dans les deux groupes étaient relativement comparables : 3.6 et 4.5 % respectivement dans le groupe à risque et dans le groupe témoin.

Dans une étude comparable chez les vendeurs professionnels de viande porcine au Pérou, une prévalence de la taeniose de 8.6% a été trouvée contre 3% chez le groupe témoin (Garcia *et al.*, 1998). Ces auteurs admettaient que ces chiffres sont 4 à 5 fois plus élevés que les valeurs habituellement trouvées. Ces prévalences extraordinaires sont partiellement explicables par le fait que plusieurs tests ont été combinés pour identifier les porteurs de vers solitaires, tandis que dans notre travail seulement un test a été utilisé.

Dans notre étude le pourcentage de séropositifs pour la cysticerose n'était pas significativement différent ($p > 0.05$) chez le groupe supposé à risque (3.6%) et chez le groupe témoin (4.5%). Ceci confirme le fait que la cysticerose n'est pas seulement un problème des personnes opérant dans la filière porcine mais aussi un problème des personnes vivant dans de mauvaises conditions d'hygiène (Flisser 1988). Des observations comparables ont été faites dans l'étude de Garcia *et al.* (1998) citée plus haut où la séroprévalence de la cysticerose dans le groupe à risque et le groupe témoin étaient aussi similaires, respectivement 23.3 et 23.8% (à base de l'immunoblot).

En conclusion, cette étude a démontré une prévalence de *T. solium* de seulement 0.10 % à Bafou et Bamendou. Cette faible prévalence par rapport à une prévalence élevée de la cysticerose porcine (11% à base de l'ELISA pour la détection d'antigènes, Pouedet *et al.*, 2002) et de la cysticerose humaine (3.6 à 4.5 %) a été appelée le "*T. solium* paradoxe" en Afrique du Sud (Joubert et Evans, 1997). Ce même paradoxe semble donc être applicable aussi au Cameroun.

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4.5. FOLLOW-UP OF NEUROCYSTICERCOSIS PATIENTS AFTER TREATMENT USING AN ANTIGEN DETECTION ELISA

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*Parasite 10: 65-68 (2003) **

Summary

Seven patients with active neurocysticercosis (NCC) received an 8 days treatment with albendazole and were followed up using computed tomography (CT-scan) and a monoclonal antibody based ELISA for the detection of circulating antigen (Ag-ELISA). Only three patients were cured as was shown by CT-scan and by the disappearance of circulating antigens one month after treatment. After a second course of albendazole therapy 2 other patients became seronegative. CT-scan showed the disappearance of viable cysts in all persons who became seronegative whereas patients who were not cured remained seropositive. These preliminary results show that this Ag-ELISA is a promising technique for monitoring the success of treatment of NCC patients because of the excellent correlation between the presence of circulating antigens and of viable brain cysts.

Résumé : Suivi de patients atteints de neurocysticercose après traitement en utilisant un test ELISA pour la détection d'antigènes

Sept patients atteints de neurocysticercose active (NCC) ont été traités à l'albendazole pendant 8 jours et ont été suivis en utilisant la tomodensitométrie et un ELISA à base d'anticorps monoclonaux pour la détection d'antigènes circulants (Ag-ELISA). Seulement trois patients ont guéris comme indiqué par le CT-scan et la disparition des antigènes circulants un mois après traitement. Après un deuxième traitement à l'albendazole, deux autres patients sont devenus séronégatifs. Le CT-scan a montré la disparition de tous les cysticerques vivants chez les personnes qui sont devenues séronégatives, tandis que les patients, qui n'étaient pas guéris, sont restés séropositifs. Ces résultats préliminaires montrent que l'Ag-ELISA est une technique prometteuse pour évaluer le succès d'un traitement de la neurocysticercose au vu de la corrélation excellente entre l'Ag-ELISA et la tomodensitométrie.

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Introduction

Neurocysticercosis (NCC) is an infection of the central nervous system by the larval stage of *Taenia solium*. It is recognized as a common cause of neurological disease in developing countries (Schantz *et al.*, 1998). The presence of *T. solium* metacestodes in the nervous system leads to a variety of clinical manifestations depending upon the number, size, viability and location within the brain and the host inflammatory reaction (White, 2000). Albendazole and praziquantel are the drugs of choice for the treatment of NCC. Follow-up after treatment is usually done using computed tomography (CT-scan), which is an expensive technique for the people of developing countries (Tsang & Wilson, 1995). Considering the importance of NCC and the increasing poverty in these countries, it is necessary to investigate alternative techniques, which could reduce at least the cost of evaluation of the efficacy of the treatment. Since an antigen detection ELISA (Ag-ELISA) has been developed, which has a high sensitivity for the detection of human cysticercosis (Erhart *et al.*, 2002) and which allows to distinguish cattle and pigs carrying living cysts from those harbouring only dead cysts (Brandt *et al.*, 1992; Nguekam *et al.*, in press), it was decided to evaluate its use in NCC patients. The purpose of this study was to compare the Ag-ELISA test with CT-scan to evaluate the efficacy of an albendazole treatment of NCC.

Materials and Methods

Patients

Seven patients with active lesions of NCC from the West province of Cameroon identified by serology (Ag-ELISA) and confirmed by brain CT-scan (in Yaounde Central Hospital) were included in this study. NCC was asymptomatic in all but two of them, who were epileptics (Batoula 94 and KE). Five of them were women and two men. Their age ranged between seven and 73 years (with a mean of 39.9 ± 24.9 years). The patients harboured an average of five viable cysts (range: 1 to 12) and of 7.6 calcified cysts (range: 0 to 25) in the brain. The localisation of the cysticerci was parenchymal and/or subarachnoidal. Informed consent was obtained from each adult and from the parents of the two young girls (seven and 15 years old) included in the study.

Treatment protocol

The patients were treated with albendazole (Alben 400 mg, Smithkline Beecham) at a dosage of 15 mg/kg body weight/day for 8 days as described by Del Brutto *et al.* (1999). To prevent adverse reactions, prednisolone (Solupred 5 mg, Laboratoire Houdé) at 10 mg per person thrice a day was given from one day prior to the albendazole treatment until at least 4 days after the end of it (Groll, 1982). The two epileptic patients continued to receive anti-epileptic treatment (Gardenal[®]).

Six to nine months after the end of the first treatment, the patients who remained seropositive were treated again with the same dose of albendazole for a period of 1 month. In one patient (KE) the first treatment was not a course of eight days, but of one month.

Antigen detection ELISA for cysticercosis (Ag-ELISA)

The patients were sampled before the beginning of the treatment, one and three months after the first treatment and in those patients who received a second treatment, one month later. The serum samples were tested using a monoclonal antibody based antigen detection ELISA as described by Brandt *et al.* (1992) but slightly modified according to Pouedet *et al.* (2002). The sera were pre-treated using trichloroacetic acid and used in ELISA at a final dilution of $\frac{1}{4}$. Two monoclonal antibodies (MoAb) were used in a sandwich ELISA. MoAb B158C11A10 was used for coating and a biotinylated MoAb B60H8A4 was included as detector antibody. Orthophenylene diamine and H_2O_2 were used as chromogen/substrate

solution. After arresting the reaction with 4 N H₂SO₄ the plates were read using an ELISA reader (Labsystem Multiskan RC) at 492 nm. Eight negative reference control sera from local people of the region of Dschang (without any history of taeniasis or cysticercosis in the family) 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 8 negative reference sera at a probability level of $P=0.001$ to determine the result using a modified Student test (Sokal & Rohlf, 1981). The ELISA values were expressed as a ratio by dividing the OD of the test sample by the OD of the cut-off value. An ELISA ratio > 1 was considered as positive.

CT-scan of the brain

The CT-scans were performed in Yaounde Central Hospital using a Somatom AR STAR scanner (Siemens Medical Systems, Erlangen, Germany) before and after contrast fluid injection (Telebrix³⁵, Guerbet, France). Brain image slices of five mm thickness were transferred on negatives and scan reports were produced, indicating the number and types of lesions compatible to NCC. Two CT-scans were made for each patient: one before and one after treatment. For the group of patients who became seronegative after the first treatment, CT-scan was performed six to ten months later, whereas the patients, who received a second treatment, were scanned three to five months later. The radiologist was not aware of the serological results of the patients.

Results

The serological results and brain CT-scan status of each patient before and after albendazole therapy are presented in Table I and Figures 1 and 2.

Ag-ELISA results

One month after the end of the first course of albendazole three patients became negative in the Ag-ELISA (Table I). Patient (KE) received only one treatment course during one month and remained seropositive one month after therapy. A second Ag-ELISA performed on serum samples taken three months post treatment did not show any additional seronegative patients (Figs1 & 2). One month after a second treatment of the three patients, who were not cured, two of them (BL 21 and BMKP 194) became seronegative.

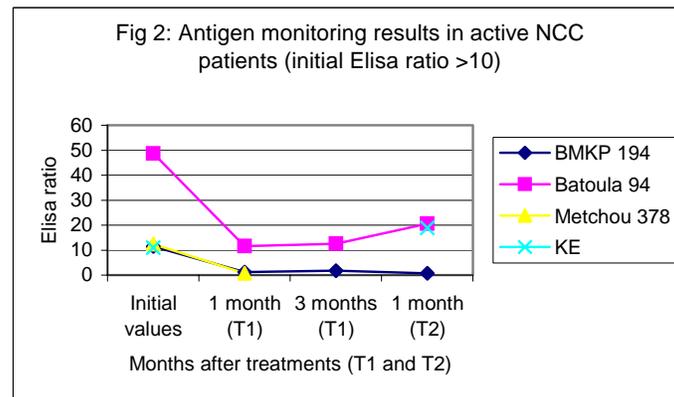
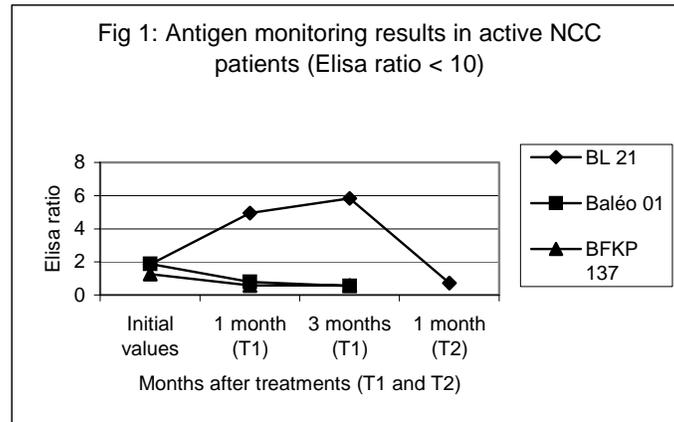
Table I. Ag-Elisa and CT-scan results before and after treatment with albendazole of patients with neurocysticercosis

Patients	Sex/age/epileptic status	Before treatment		After treatment	
		CT-scan no. liv/no. calc	ELISA Ratio	CT-scan no. liv/no. calc	ELISA Ratio
- BL 21	M/73/N	1/1	1.8	0/1	0.7*
- Baleo 01	F/07/N	2/0	1.9	0/0	0.8
- BFKP 137	M/60/N	3/0	1.3	0/0	0.6
- BMKP 194	F/60/N	1/6	11.5	0/3	0.8*
- Batoula 94	F/32/E	6°/25	48.7	2/25	20.6*
- Metchou 378	F/15/N	12°/6	12.5	0/12	0.7
- KE	F/32/E	10/15	11.0	4°/15	18.9

*: after a second treatment; °: including ring enhanced cysts; M: male; F: female; E: epilepsy; N: no epilepsy

CT-scan results

CT-scan of the brain after one or two courses of albendazole therapy showed that viable cysts had completely disappeared in five out of seven (71.4%) patients. In the two others, either a reduction of the number of viable cysts (Batoula 94) or the presence of ring enhanced cysts indicating a process of degeneration (KE) was observed after treatment (Table I). In all patients where the viable cysts disappeared after treatment a negative ELISA result (ratio < 1) was obtained whereas a persistence of even few living or degenerating cysts resulted in positive ELISA values.



Comparative costs of Elisa and CT-scan techniques in the follow-up of NCC patients after treatment

Up to now, CT-scan in Cameroon can only be carried out in Yaoundé or Douala. Besides the costs of scanning and the contrast agent, the total cost of a scan includes therefore also the travel and food expenses (for two days). This cost was estimated for each patient involved in the present study to approximately 152.2 € (100,000 CFA) whereas the cost of a test of an Ag-ELISA (for 40 samples or one plate) was about 17 €, i.e. 0.425 € per patient. The salaries of the medical doctor or the laboratory technicians are not included in this calculation.

Discussion

Computed tomography is an useful imaging technique for the diagnosis of human neurocysticercosis and the assessment of the efficacy of anthelmintic drugs in the treatment of this disease (Padma et al., 1994; Garcia et al., 1997; White, 2000). Its high cost, however, and the fact that it is often unavailable in rural regions of developing countries, where the prevalence of NCC is high (Tsang & Wilson, 1995), constitute a limitation for its wide use. In this study, we compared this technique with an Ag-ELISA as an alternative method for the

follow-up of neurocysticercosis patients after cysticidal treatment. The Ag-ELISA has been shown to detect the excretory-secretory products of viable cysticerci in cattle (Brandt *et al.*, 1992), pigs (Nguekam *et al.*, in press) and man (Erhart *et al.*, 2002). These latter authors reported a sensitivity of 94.4 % and the absence of cross-reactions with sera from human patients infected with *Schistosoma*, hydatid cysts, *Ascaris*, *Trichuris*, filaria, *Entamoeba*, *Plasmodium* and *Trypanosoma*.

Although the follow-up period was not the same for the two techniques, the results obtained in this study were very promising. There was 100 % agreement between the CT-scan and the Ag-ELISA results. The five patients, who became seronegative one month after one or two albendazole courses, showed a complete disappearance of the viable cysts in the brain whereas the two remaining seropositive patients (Batoula 94 and KE) showed respectively two living cysts and four cysts with ring enhancement.

The sensitivity of our Ag-ELISA was better than that of another monoclonal antibody-based Elisa used by Garcia *et al.* (2000) in monitoring neurocysticercosis patients after treatment. This latter Ag-ELISA could not detect patients with only one viable cyst and/or enhancing lesion whereas in this study two patients with one single living cysticercus in the brain could be identified.

These preliminary results clearly show that this monoclonal antibody-based Ag-ELISA is a promising technique to monitor neurocysticercosis patients after treatment. In addition, it is much cheaper than CT-scan in particular for patients of developing countries where poverty is an increasing reality. Currently, studies on a larger number of NCC patients are going on in order to validate the Ag-ELISA as an alternative for CT-scan

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4.6. NEUROCYSTICERCOSIS AND EPILEPSY IN CAMEROON

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Abstract:

The frequency of *Taenia solium* cysticercosis was studied in a series of 504 epileptic patients from 3 rural localities in the West and North-west provinces of Cameroon using ELISA both for circulating antigen (Ag-Elisa) and antibody detection (Ab-Elisa). *T. solium* antigens were detected in the sera of 1.2% of the epileptics whereas specific antibodies against the parasite were present in 44.6% of patients. Significantly more seropositives in Ab-Elisa were recorded in Batibo than in Bandjoun and Batibo whereas a borderline significant difference was recorded with increasing age. Furthermore, 50% of patients with late-onset epilepsy showed antibodies against cysticercosis. *T. solium* cysticercosis appears to be an important cause of epilepsy in Cameroon.

Key-words: *Taenia solium*, cysticercosis, epilepsy, Cameroon, ELISA, circulating antigen, antibody

Introduction

Epilepsy is a major problem in tropical developing countries (de Bittencourt et al., 1996). The incidence and prevalence of the disorder in these countries are high because of poor standards of neonatal care and high rates of infectious and parasitic diseases (Senanayake & Roman, 1993). Among parasitic infections, neurocysticercosis (NCC) which is an infection of the central nervous system by *Taenia solium* larvae, has been reported as a major cause of epilepsy in many Latin American and African countries (Dumas et al., 1990; Van As et Joubert, 1991; Del Brutto et al., 1992; Garcia et al., 1993; Garcia-Noval et al., 2001).

The prevalence of active epilepsy in tropical countries as a whole is between 10 and 15 per 1000 inhabitants (International League Against Epilepsy, 1994). According to the African Declaration on Epilepsy adopted during the Dakar conference (5 and 6 May 2000), epilepsy is the most common serious chronic brain disorder, estimated to affect at least 50 million people in the world of which 10 million live in Africa alone (WHO, 2000). Studies in Africa have shown that the prevalence of epilepsy on the continent ranges between 15 and 25 per thousand and may be as high as 40 per thousand in some regions (Preux et al., 2000).

Although epilepsy is known as a frequent condition in Cameroon, data on its prevalence and on the etiologic factors are unavailable. Given the fact that *T. solium* is endemic in the western highland region of the country (Nguekam et al., 2003b), we studied

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the frequency of cysticercosis in epileptic patients attending health centres of the region using two serological tests (Elisa for antigen and for antibody detection).

Materials and Methods

Study Area and Patients

In the rural localities of Batibo (North-West Province), Bamendjou, and Bandjoun (West Province), epilepsy is known as a serious health problem. Epileptic patients attending the health centres in the region were sensitised about the study by the medical authorities. After this information campaign, the study was carried out in the first half of January 2002 in the health centres of these localities.

During consultation, the patients were clinically examined. A history of epilepsy was obtained from each patient or from the person who accompanied him (in case of young or mentally handicapped patients) and a form was completed (Name, sex, age, village, year of first seizure, relative frequency of seizures). A patient was considered as epileptic when he fulfilled the epilepsy case definition of the International League against Epilepsy (1993), i.e. two or more epileptic seizures occurring more than 24 h apart and not post-partum or caused by fever, cranial trauma or metabolic disorder. Burns and injuries related to seizures were also inclusion criteria. After having obtained informed consent, blood samples were collected and the serum was frozen for further analysis.

Antibody detection Elisa (Ab-Elisa)

An antibody detection ELISA using a recombinant antigen was carried out according to Sako et al. (2000). Recombinant *T. solium* antigens (1.0 µg/ml) were loaded onto 96 well microplates (Maxisorp, Nunc, Copenhagen). Peroxidase labeled goat anti-human IgG (H+L) (10967133, Zymed Laboratories, Inc. California, USA) was used as the secondary antibody in a dilution of 1:5000. ABTS (2,2'-azino-di(3-ethyl-benzthiazoline-6-sulfonate)) (KPL, USA) was used as peroxidase substrate (Sako et al., 2000). Negative control sera from uninfected humans were obtained from 40 healthy people from a non-endemic area. Serum samples used as positive controls were from confirmed cysticercosis cases that were positive by immunoblot (Ito et al., 1999). The cut off point was established as the mean + 4 SD of the values from the 40 individuals. This corresponded to about three times the optical density of the pooled negative control sera.

Antigen detection Elisa (Ag-Elisa)

Serum samples were examined in duplicate using a monoclonal antibody based Elisa for the detection of circulating *T. solium* antigen (Brandt et al., 1992) slightly modified according to Nguekam et al. (2003b). Each Elisa run included 8 negative reference sera and one reference positive serum from the region of Dschang in Cameroon (Nguekam et al., 2003b). The cut-off value was determined by comparing the optical density (OD) of each sample with the mean of a series of 8 negative reference samples using a modified Student test (Sokal & Rohlf, 1981) at a probability level of P=0.001.

Statistical analysis

Random Effect Logistic Regression with as random effect the locality was used to determine the significance of localities, sex and age. In this way possible clustering effects of the results within locality were allowed for. Analyses were conducted in Stata using the binary Ab-Elisa results as a response (Statacorp, 2001).

Results

Based on the clinical history of each patient, 504 epileptics from the 3 localities were included in the study. Their age ranged from 3 to 73 years with a mean of 20.0 ± 10.5 years. Data on the initial episode of epilepsy were available only for 460 patients, of which only 4.3% had late-onset epilepsy (i.e. epilepsy after the age of 30 years as defined by Palacio et al., 1998). Burns and injuries due to epileptic crises were recorded in 11.3% of patients. Reliable information was not available in order to allow a classification into generalised or partial seizure types.

Table 1: Frequency of seropositives for cysticercosis (Ag and Ab-ELISA) according to locality

Localities	No. examined	Ag-ELISA		Ab-ELISA	
		Positives	%	Positives	%
Batibo	345	4	1,2	121	34,8
Banendjou	98	2	2	65	66,3
Bandjoun	61	0	0	39	63,9
Total	504	6	1,2	225	44,6

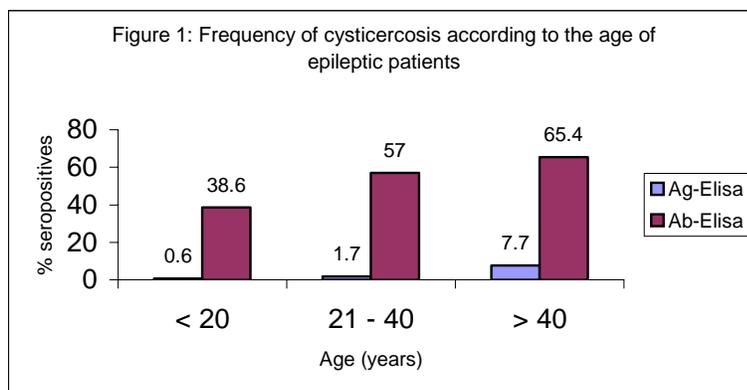
Antigens of *T. solium* metacestodes were detected in only 6 (1.2%) epileptic patients whereas antibodies against this parasite were present in 225 (44.6%) patients. Results of both antigen and antibody detection Elisa according to age, locality and sex are presented in figure 1 and tables 1 and 2. Ab-Elisa results were significantly different between Batibo and localities of Bamendjou and Bandjoun ($P < 0.001$) (Table 1). No significant differences in Ab-Elisa were present between sexes (table 2) and with increasing age of epilepsy onset (Table 3). However, a borderline significant difference ($P: 0.072$) was observed with increasing patient ages (Figure 1)

Table 2: Frequency of seropositives for cysticercosis (Ag and Ab-Elisa) according to the sex of the patients

Sex	No. examined.	Ag-ELISA		Ab-ELISA	
		Positives	%	Positives	%
Male	281	4	1,4	123	43,8
Female	223	2	0,9	102	45,7

Table 3: Frequency of seropositives for cysticercosis (Ag and Ab-ELISA) according to the age of onset of epilepsy

Age of onset	no.examined	Ag- ELISA		Ab-ELISA	
		positives	%	positives	%
≥ 30	20	2	10	10	50
20 - 29	35	1	2,9	21	60
< 20	405	2	0,50	164	40,5
Unknown	44	1	2,30	30	68,20



Comparison of the results of Ag-ELISA and Ab-ELISA showed that out of 6 positive sera in the Ag-ELISA three tested positive in the Ab-ELISA, whereas the 3 others were negative in the Ab-ELISA.

Discussion

In this study, 504 epileptic patients were examined using an Ag- and an Ab-Elisa for the detection of *T. solium* cysticercosis. The results showed that 1.2% of the epileptic patients harboured circulating antigens, which strongly indicates that viable cysts were present in only a very small number of people. Using the Ag-ELISA a strong correlation has been shown to be present between living cysts and circulating antigen as well in cattle (Brandt et al., 1992), in pigs (Nguekam et al., 2003a) as in humans (Erhart et al., 2002). In this group of epileptics, cysticercosis was obviously more linked to the presence of dying or dead cysticerci, since the Ab-ELISA detected 44.6% seropositives among the examined people. This confirms the observations of many different authors that dying and/or degenerated cysticerci are very common in patients with cysticercosis (Sotelo et al., 1985; Garcia-Noval et al., 1996; Nash et al., 2001).

The Ab-ELISA using recombinant antigens, which was used in this study, has been shown to be highly sensitive (89.7 %) and 100 % specific (Sako et al., 2000). However, the figure of 44.6 % should be interpreted with caution, since it has been shown that transient antibodies against *T. solium* occur quite frequently (Garcia et al., 2001). The latter authors did show that about 40 % of seropositive people became seronegative when resampled after one to 3 years. This phenomenon, which was ascribed to the exposure to eggs of the parasite, which did not develop into a viable infection, might also be present in the study area, which is hyperendemic for *T. solium* (Pouedet et al., 2002; Vondou et al., 2002; Nguekam et al., 2003b).

Taking into account this nuance, it can nevertheless be assumed that cysticercosis is clearly an important cause of epilepsy in this area of Cameroon. Although unfortunately no CT-scan could be performed to confirm the parasites in the brain, this study confirms previous observations by other authors, that NCC is one of the most important causes of epilepsy in developing countries (de Bittencourt et al., 1996; Carpio et al., 1998). The frequency of epileptics with antibodies against *T. solium* cysticercosis is twofold the 22.3% reported using immunoblot (EITB) in Madagascar (Andriantsimahavandy et al., 1997). It also exceeds the figures reported in northern Togo (29.5%) using an Elisa test (Dumas et al., 1990) and those in Peru (12%; Garcia et al., 1993) and in Colombia (9.82%; Palacio et al., 1998) using EITB .

Only 4.3% of patients involved in the study had late-onset epilepsy. Of them, 10% and 50% were positive in Ag and Ab-Elisa, respectively. Seropositivity increased - although only

borderline significantly - with increasing age in both Ag- and Ab-Elisa (Figure 1). This is in agreement with the observations of Sarti et al. (1992; 1994) in community-based studies on taeniasis and cysticercosis in Mexico.

No significant difference was found between sexes in this study. This finding is in contrast with the observation by Cruz et al. (1999) who found a higher proportion of female epileptics with positive Ab-Elisa results and who attributed it to food handling activities and their relationship with infection with cysticerci.

It was surprising that 3 out of 6 positive serum samples in Ag-Elisa tested negative in Ab-Elisa. This might be due to the sensitivity of this Ab-ELISA (89.7 %) and more probably to the presence of single cysts (Sako et al., 2000). The latter authors reported that serum samples originating from NCC patients harbouring solitary cyst might escape detection. Negative results in ELISA or EITB in cases of single cyst infections have been reported by several authors (Lara-Aguilera et al., 1992; Wilson et al., 1991).

On the basis of these results it can be concluded that *T. solium* cysticercosis is an important cause of epilepsy in the localities where the study was conducted. A particularly high frequency of antibodies against the parasite was detected in epileptic patients. Additional studies using both serological and imaging techniques are necessary to investigate the association between this neurological disorder and cysticercosis.

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