



**PREVALENCE OF HUMAN *Taenia solium* CYSTICERCOSIS
IN MAYO-DANAY DIVISION (CAMEROON)**

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Dedication

This work is dedicated to

- my dearest wife Odile for her love and care to the family
- my dear children Borel, Marlène, Laurence and Hilarie for all the love I owe them. May this serve as source of inspiration and motivation

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List of abbreviations and acronyms

Ab-ELISA	Antibody detection ELISA
Ag-ELISA	Antigen detection ELISA
ASF	African Swine Fever
CR	Case report
CSF	Cerebrospinal Fluid
CT scan	Computerized Tomography scan
EITB	Enzyme-Linked Immuno-electrotransfer Blot
ELISA	Enzyme Linked Immunosorbent Assay
H ₂ O ₂	Hydrogen Peroxyde
H ₂ SO ₄	Sulfuric Acid
IMT	Institut de Médecine Tropicale
ITM	Institute of Tropical Medicine
kDa	kilodalton
LL-GP	Lentil-Lectin Glycoprotein
MoAb	Monoclonal Antibody
MRI	Magnetic Resonance Imagery
NCBS	Newborn Calf Serum
NNC	Neurocysticercosis
OD	Optical Density
OPD	Orthophenylene Diamine
PBS	Phosphate Buffered Saline
TCA	Trichloro-acetic Acid
UDs	University of Dschang
WHO	World Health Organisation

Summary

In order to determine the prevalence of human cysticercosis in Mayo-Danay Division (far north province of Cameroon), a study was carried out in 7 villages during the month of October 2002 including 1317 persons among which 766 males and 551 females. These persons were clinically examined, their serum collected and tested with Ag-ELISA. Circulating antigens of *Taenia solium* metacestodes were detected in 2.05% (0.49-4.52%) of the study population. Because this test detects only living cysts, it is an indication of people with active cysticercosis. Random effects logistic regression showed that age had a significant effect on the percentage of those that were positive whereas sex did not have a significant effect. Apart from Vada village, seropositivity did not significantly differ in the villages. During clinical examinations, 179 persons showed one or more symptoms suggestive of cysticercosis (sub-cutaneous nodules, seizures and chronic headaches) among which 3.9% were seropositives against 1.7% of the 1138 asymptomatic individuals.

This study shows that human 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.

Keywords *Taenia solium*, cysticercosis, enzyme linked immunosorbent assay, circulating antigen, seroprevalence, Cameroon

Résumé

Dans le but de déterminer la séroprévalence de la cysticercose humaine dans le département de Mayo-Danay (province de l'extrême nord du Cameroun), une étude a été menée en Octobre 2002 dans 7 villages, incluant 1317 personnes dont 766 hommes et 551 femmes. Ces personnes ont été cliniquement examinées et leur sérum collecté et testé à l'Ag-ELISA. Il en ressort que les antigènes circulants des metacestodes de *Taenia solium* avaient été détectés chez 2.05% (0.49-4.52%) des personnes examinées. La régression logistique à effet aléatoire avait démontré que l'âge avait un effet significatif sur le pourcentage des personnes séropositives alors que le sexe n'avait aucun effet. En dehors du village Vada, la séropositivité n'a pas été significativement différente entre les villages. Pendant les consultations, des symptômes caractéristiques de la cysticercose (nodules sous-cutanés, crises d'épilepsie et maux de tête chroniques) ont été révélés chez 179 personnes parmi lesquelles 3.9% étaient séropositives. En plus, 1.7% des 1138 personnes asymptomatiques étaient aussi séropositives.

Il ressort de cette étude que la cysticercose humaine est un problème de santé publique dans le département de Mayo-Danay et probablement dans toute la région septentrionale du Cameroun puisque les études antérieures y ont révélé une forte prévalence de la cysticercose porcine, et où la plupart des conditions qui favorisent la complétion du cycle de *T. solium* sont présents.

Mots clés *Taenia solium*, cysticercose, enzyme linked immunosorbent assay, antigène circulant, séroprévalence, Cameroun

Chapter I: INTRODUCTION

Cysticercosis is caused by infection with the larval form of the pork tapeworm *Taenia solium*. In the life cycle of this parasite, man is the only final host and pigs the normal intermediate host. Humans can be infected with both the adult worm and larval forms of the cestode *T. solium*, causing taeniasis and cysticercosis, respectively. Neurocysticercosis is the infection of human brain by the larvae of *T. solium*. According to Tsang & Wilson (1995) cysticercosis is largely under-recognised in many developing countries. This statement was confirmed by the review of Geerts *et al.* (2002) that further precise that it is an important zoonosis in many non-muslims regions of Africa where it is endemic or hyperendemic. This situation is due probably to factors that favour its transmission that are present in these countries such as the lack of personal and environmental hygiene, sanitation, proper meat inspection, proper pig husbandry practises and consumption of raw or insufficiently cooked pork.

The disease is of great economic importance as pig production is severely affected resulting in considerable economic losses due to cysticercosis (Tsang & Wilson, 1995; Zoli *et al.*, 2003). These losses are mainly due to condemnation of live pigs and carcasses at meat inspection or reduction of their commercial value. Cysticercosis is also a serious threat to human health and wellbeing as epilepsy, headache, loss of productivity for persons infected, high cost of drugs and utilization of medical resources in case of neurocysticercosis in humans has been reported (Román *et al.*, 2000).

Until recently, very few epidemiological data on the disease were available in some parts of Africa (Zoli *et al.*, 1987; Geerts, 1993). During the last decade however, many surveys have been carried out in several countries. The disease is endemic or hyperendemic in many regions of Africa with infection rates close to those of Central and South America, except in Islamic countries where it is almost absent (Preux *et al.*, 1996). Endemic countries include among others Benin (Houinato, 1998; Adjide *et al.*, 1996), Togo (Balogou *et al.*, 2000), Nigeria (Onah & Chiejina, 1995), Mozambique (Vilhena *et al.*, 1999). In Cameroon, many studies have been carried out particularly in the western highlands that used to be the most important pig production area. In 1965, *T. solium* was first signalled by Nelson *et al.* (quoted by Zoli *et al.*, 2003) to be common in the country. In a study carried out in two village communities of Bafou and Bamendou in the Menoua division (West Cameroon), Vondou *et al.* (2002) found 0.13 % of 3109 human fecal samples positive to *Taenia* spp. eggs using flotation technique. Studies have been also carried out in Menoua, Bamboutos, Mifi, Koungkhi divisions (Zoli *et al.*, 1987 and 2004; Vondou *et al.*, 2002; Pouedet *et al.*, 2002; Nguekam, 1998) and West Cameroon has been found to be an endemic region with prevalence figures of up to 38% in pigs. Other studies have been carried out in Batibo in the North-West province and the seroprevalence of cysticercosis found in pigs and epileptic patients was respectively 27.7% (Ag-ELISA) (Shey-Njila *et al.*, 2003) and 44.6% (Ab-ELISA) (Zoli *et al.*, 2004). Up to now, only one large scale survey in man has been carried out by Nguekam *et al.* (2003a) studying the occurrence of human cysticercosis in 4993 individuals from three rural communities of Menoua Division, West Province of Cameroon and circulating antigens of *T. solium* metacestodes were detected in 0.4% (Bafou), 1.0% (Bamendou) and 3.0% (Fonakekeu) of the serum samples.

The Northern part of Cameroon which is currently one of the most important pig producing areas and consequently a potential focus of pig and human cysticercosis, has not yet received such attention as only few studies on the prevalence of the disease in pigs have

been carried out only recently. This situation is due to the fact that despite its actual importance, pig production there is recent. Pig husbandry importance shifted from the Southern and Western parts of the country to the North because of African swine fever (ASF) outbreaks in the former regions. According to Njoya *et al.* (1996) quoted by Assana *et al.* (2001), 60.4% of pig farms in the north and far north provinces were created between 1993 and 1996 and 75.3% of pigs are either on free range or kept in pens during the night and are totally free during the day. These conditions are close to those reported by Zoli *et al.* (1987) in the western province, conditions that can favour cysticercosis.

Awa *et al.* (1999) in an abattoir inspection study in the north of the country (Garoua) reported a cysticercosis prevalence of 12.3%. Furthermore, the survey of Assana *et al.* (2001) in the Mayo-Danay Division (far north of Cameroon) and Mayo-Kebbi district (South west of Chad) has revealed a prevalence in pigs of 20.5% at tongue inspection, 15.7% at carcass inspection and a seroprevalence (Ag-ELISA) of 39.8% coupled to pig rearing techniques and hygienic conditions of the population conducive to heavy infections by *T. solium* cysticerci. These results show that the region is an important focus of porcine cysticercosis.

Since the prevalence of cysticercosis in pigs can be an indicator of the presence of the disease in man, especially when transmission factors are favourable and since the prevalence in humans has not yet been determined in the whole northern area of Cameroon, it will be worthwhile to carry out a survey for human cysticercosis in that region.

The aim of this study was therefore to establish the seroprevalence of active *T. solium* cysticercosis in humans in the Mayo-Danay Division of the Far North Province of Cameroon.

Chapter II: LITERATURE REVIEW

2.1 Taeniasis/cysticercosis complex due to *T. solium*

2.1.1 Biological Cycle

Man, the final host, harbours the adult tapeworm which produces several thousands of eggs daily. The proglottids and eggs are eliminated into the environment through the faeces. The pig, which is the intermediate host, ingests some of these eggs and the latter develop into cysticerci. When man consumes raw or insufficiently cooked pork containing viable metacestodes of *T. solium*, the latter develop into an adult worm inhabiting the human intestine and this completes the life cycle of the parasite. After ingestion of the eggs, the metacestodes can also develop in humans where they constitute a dead-end stage i.e. its life cycle can not progress any further (Pawlowski, 2002). Man can become infected by *T. solium* eggs through external autoinfection (faecal-oral infection with eggs in tapeworm carriers) or when they live in contaminated environment.

2.1.2 Pathology

2.1.2.1 In Pigs

In pigs, the cysticerci of *T. solium* localize most frequently in the skeletal muscles and in the brain (Schantz *et al.*, 1998). Light to moderate infections do not have any clinical effect on pigs. But heavy infections can produce disturbances in appetite and muscular movements, rapid pulse and respiration, vomiting, diarrhoea and increased body temperature up to 41.4 °C (Tyshkevich, 1973). Neurologic symptoms caused by neurocysticercosis are not observed in pigs. This is probably due to the fact that they usually have few cysticerci in the brain and are in general slaughtered when 9-12 months old (Flisser, 1988). It is known that clinical manifestations of neurocysticercosis in man need around 5 years to develop.

2.1.2.2 In humans

In humans, cysticerci occur in skeletal muscles but most clinical manifestations emanate from cysticerci localized in the central nervous system (neurocysticercosis). Less frequently, they may localize in the eyes, subcutaneous tissues and heart (Schantz *et al.*, 1998). Neurocysticercosis is considered to be the most important parasitic infection of the central nervous system (Engels *et al.*, 2003).

Cysticercosis may produce absolutely no clinical signs or symptoms but it may also present with a variety of non-specific clinical manifestations. Therefore defining a typical syndrome of cysticercosis is unrealistic (Tsang & Wilson, 1995). Epilepsy, focal neurological deficit, headache, increased intracranial pressure and intellectual deterioration are the most common clinical manifestations of neurocysticercosis (Del Brutto *et al.*, 1998). Symptoms are more common and often more serious in undernourished, ill, elderly or infant patients (Neafie *et al.*, 2000).

Cysticercosis is one of the leading causes of intracranial hypertension in Mexico along with tuberculosis (Mahajan, 1982) and recent studies suggest that neurocysticercosis may be a risk factor for human cancer (Flisser, 2003). However, there are contradictory opinions about this statement as it has been proven that *T. crassiceps* cysticercosis in mice does not increase the carcinogenic effect of methyl-nitrosourea (Ordoñez *et al.*, 2003) whereas an induction of

DNA instability in human lymphocytes treated with a soluble factor secreted by *T. solium* metacestodes could represent a risk for malignant transformation (Herrera *et al.*, 2003).

Taeniasis is usually asymptomatic but can be responsible of abdominal discomfort, diarrhoea, anorexia, malaise, weight loss, indigestion and even constipation (Palmer & Reeder, 2001).

2.2 Epidemiology of *Taenia solium* Taeniasis/Cysticercosis

2.2.1 Transmission

Pigs become infested after ingestion of viable proglottids or eggs of *T. solium* released in faeces of the human tapeworm carrier. Humans are infected when they eat raw or undercooked mealy pork (Tsang & Wilson, 1995). Humans are the only natural definitive host of *T. solium*, implying that they are the most important multiplier, reservoir and disseminator of the infection to himself and to pigs (Pawlowski, 2002).

Man can also become infected with the metacestodes by drinking contaminated water, eating uncooked vegetables contaminated with eggs or by contact with the faeces containing eggs (Mahajan, 1982), implying that one does not have to be in contact with pigs or consume pork to become infected by cysticercosis (Schantz *et al.*, 1992).

Transmission from human to human occurs when human faeces which contain eggs contaminate the environment and food under poor hygienic conditions (Krauss *et al.*, 2003).

2.2.2 Factors affecting transmission

The coprophagous nature of pigs is an important factor in the transmission (Sarti *et al.*, 1992). The greatest risk for pigs of acquiring cysticercosis comes from livestock raising practises that allow the animals to roam freely, absence of latrines in most households of rural areas of developing countries, use of pigsties as a toilet and indiscriminate defecation along the roads and in crop fields, situations that give them access to human faeces (Pouedet *et al.*, 2002). Human migration and increased consumption of pork increases the spread of the disease from the endemic rural areas into urban areas (Pawlowski, 1994).

Other factors such as the lack of personal and environmental hygiene, proper meat inspection, clandestine slaughtering of pigs also favours the transmission.

The global malnutrition and deficiencies in proteins, minerals and vitamins increase the receptivity (Euzeby, 1998). In addition, swine raised on small family farms where they have a greater opportunity to ingest human faeces are generally slaughtered by their owners without veterinary inspection or are often sold without restriction in local markets (Acha & Szyfres, 2003).

2.2.3 Distribution of *Taenia solium* cysticercosis

Table I summarises some data on *T. solium* cysticercosis prevalence in pigs and man in Africa.

Taenia solium is endemic in many less developed countries, both in highlands and tropical areas of Central and South America and non-Muslims populations of Asia and Africa (Garcia & Del Brutto, 2000). It is a disease related to underdevelopment because it is mostly found in countries that lack adequate sanitary infrastructure and health education (Flisser & Lightowlers, 2001).

In Japan and Singapore, increasing economic prosperity and accompanying infrastructure, have made the disease almost non-existent, while in other countries, such as the Islamic countries of the Middle East, West Asia and North Africa, religious proscription of the consumption of pork has had a similar result (Rajshekhar *et al.*, 2003). In other regions of Asia, however, the disease is known to occur with varying prevalence (Rajshekhar *et al.*, 2003).

Table 1: Prevalence of porcine and human cysticercosis in Africa

Country	Prevalence in pigs		Prevalence in man	
	%	References	%	References
Benin			1.3	Houinato <i>et al.</i> (1998)
Burkina Faso	0.57	Coulibaly & Yameogo (2000)	CR	Preux <i>et al.</i> (1996)
Burundi	2-39	Newell <i>et al.</i> (1997)	2.8	Newell <i>et al.</i> (1987)
Chad	6.8	Graber & Chailloux (1970)		
	25.7	Assana <i>et al.</i> (2001)		
Congo	0.1-8.1	Pandey & Mbemba (1976)	3	Fain (1997)
	10-30	Chartier <i>et al.</i> (1990)		
Ivory Cost	2.5	Mishra & N'depo (1978)	CR	Heroin <i>et al.</i> (1972)
Madagascar			18	Michel <i>et al.</i> (1993)
Mozambique	6.5-33.3	Afonso (2001)		
Nigeria	1.8-18.4	Dada (1980)		
	20.5	Onah & Chiejina (1995)		
Togo	17	Dumas <i>et al.</i> (1990)	2.4	Dumas <i>et al.</i> (1989)
			3.8	Balagou <i>et al.</i> (2000)
Zambia	20.6	Phiri <i>et al.</i> (2003)		

CR= Case Report

Source: Extracted from Zoli *et al.* (2003) and Phiri *et al.* (2003)

2.3 Diagnosis

2.3.1 In humans

2.3.1.1 Diagnosis of taeniasis

a) Coprological method

The diagnosis of adult tapeworm is based on the demonstration of proglottids or ova in the patient's faeces. On the basis of the eggs, coprological techniques did not allow differentiation of *T. solium* and *T. saginata* infections (Grove, 1990) because their eggs are morphologically identical. Due to the low sensitivity of these techniques (Flisser, 2003), many infected persons are not detected.

b) Serology

Coproantigens can also be detected. The microtiter plate coproantigen assay that has been recently simplified to a dipstick-based assay can be used, the former being more sensitive. The disadvantage is that it cannot distinguish between taeniasis caused by *T. solium* or *T. saginata* (Schantz *et al.*, 1998). Wilkins *et al.* (1999) developed a serologic assay to identify adult *T. solium* tapeworm carriers using excretory/secretory antigen collected from in vitro cultured *T. solium* tapeworms and the test was identified to be 95% sensitive and 100% specific.

2.3.1.2 Diagnosis of cysticercosis

2.3.1.2.1 Clinical signs

Diagnosis of neurocysticercosis on clinical grounds is difficult because signs and symptoms are non-specific (Carpio, 2002). Cysticerci under the skin form visible subcutaneous nodules which are largely asymptomatic (Smyth, 1994), that can be diagnosed by biopsy of these nodules.

2.3.1.2.2 Imaging techniques

Although to date there are no trustworthy data on the specificity and sensitivity of neuroimaging diagnostic studies, computed tomography (CT scan) and magnetic resonance imagery (MRI) are the main tools in neurocysticercosis diagnosis (Carpio, 2002) but the interpretation of the result may be difficult. These techniques permit the diagnosis of cysticerci in the brain. They are useful diagnostic tools but a major problem is that in many developing countries, neuro-imaging methods are inaccessible and/or too expensive for the rural population at risk (Dorny *et al.*, 2003). Studies comparing the two imaging techniques have concluded that MRI is more sensitive and specific for identifying most forms of neurocysticercosis with the exception of microcalcifications (Schantz *et al.*, 1998).

2.3.1.2.3 Serology

Complement fixation, indirect haemagglutination, ELISA and EITB are among the types of immunodiagnostic assays that have been developed to detect human cysticercosis (Carpio, 2002). Western blot and enzyme linked immunosorbent assay have good sensitivity and specificity, the former being more reliable (Flisser, 2003).

a) Antibody detection

Antibody detection tests (ELISA and EITB) are the most appropriate tools for measuring exposure to *T. solium* in sero-epidemiological surveys and confirmation of *T. solium* as aetiological agent of epilepsy (Dorny *et al.*, 2004b). Some studies have found that serum EITB is highly sensitive (more than 90%) and specific for the diagnosis of human cysticercosis, but in patients with a single brain cyst, the sensitivity was only 25 % (Carpio, 2002). In cases where two or more cysts are present, EITB is very sensitive, 100 % and 95% using serum or cerebrospinal fluid (CSF) respectively and 99% specific for either sample (Wilkins *et al.*, 2002). In addition, an EITB is more likely to show a positive result in patients with calcified lesions than in those with active or transitional cysts and EITB may become negative after the cysticercus dies.

Because all three taeniid (*T. solium*, *T. saginata* and *T. asiatica*) infections are related, there is considerable similarity in the antigenic profile of the different metacestodes, implying cross reactions in areas where more than one species is present (Rogan & Craig, 2002).

Although EITB is accepted as the best immunological test available today, ELISA continues to be used extensively for epidemiological surveys mainly because it is technically simpler than the EITB (Carpio, 2002). In developing countries, ELISA is preferred because of its better availability, simplicity and lower cost compared with immunoblot (Rosas *et al.*, 1986).

b) Antigen detection

To overcome the fact that the presence of antibodies does not constitute direct evidence of a living parasite, several attempts have been made to develop antigen-based assays based on polyclonal and monoclonal antibodies (Carpio, 2002). The application of sandwich ELISA for detection of circulating parasite antigens may present some diagnostic advantages since it does not demonstrate exposure but identifies active infections (Dorny *et al.*, 2003) although Ito *et al.* (2004) believe that there is no evidence to suggest that the Ag-ELISA is better than Ab-ELISA for the detection of active cysticercosis. Some ELISA tests that detect *T. solium* in humans are based on monoclonal antibodies that were developed for the detection of circulating antigens of *T. saginata* in cattle (Brandt *et al.*, 1992). This shows that these antigen detection assays are only genus specific and their use is limited in areas where hydatidosis and cysticercosis are co-endemic (Correa *et al.*, 2002). Since *T. hydatigena* does not occur in humans, the monoclonal antibody-based ELISA can be used for the diagnosis of human cysticercosis (Dorny *et al.*, 2004b).

Pardini *et al.* (2001) in an evaluation of enzyme-linked immunosorbent assay (ELISA) using polyclonal sera of rabbit anti-*T. solium* cysticerci (anti-Tso) and anti- *T. crassiceps* cysticerci vesicular fluid (anti-Tcra or anti-Tcra <30 kDa) in NCC (neurocysticercosis) patients discovered that the test carried out with the anti-Tso, anti-Tcra, and anti-Tcra <30 kDa showed sensitivities of 81.2, 90 and 95.8% and specificities of 82, 98 and 100% respectively.

Because the detection of antigens correlates with the presence of live cysticerci, it has been shown that Ag-ELISA is useful in monitoring disease progression and anticysticercal therapy (Garcia *et al.*, 2000; Nguekam *et al.*, 2003c).

2.3.2 In pigs

2.3.2.1 Tongue palpation

Diagnosis of swine cysticercosis can be made ante mortem by palpation of the tongue. The mouth is opened and gagged by a piece of wood and the tongue pulled out and examined visually and by palpation of the ventral surface for evidence of cysticerci (Boa *et al.*, 2002). It is the most commonly used method as it is simple and can be used by everybody with some experience. Tongue examination for nodules is widely used by farmers in South America to detect *T. solium* cysticerci in pigs (Gonzalez *et al.*, 1990). These authors also found in an evaluation study conducted in Peru that the tongue examination test had a sensitivity of 70% and a specificity of 100% in a study when compared to ELISA and to enzyme-linked immunoelectrotransfer blot assay (EITB). Dorny *et al.* (2004a) found a much lower sensitivity (21%) and equal specificity in a more reliable study in Zambia using a Bayesian approach. Tongue palpation is also widely used in Africa by farmers and pig traders.

2.3.2.2 Meat inspection

This method consists of incision of the predilection sites in slaughter houses in order to look for cysticerci. Boa *et al.* (2002) studied the predilection sites of cysticerci in pigs and found in order of importance the following sites: psoas muscles, internal masseter, external masseter, triceps brachii, forelimb, head muscles, tongue, hind limb, diaphragm, heart, abdominal muscles, trunk muscles, brain and oesophagus. D'souza & Hafeez (1999) compared ELISA with meat inspection and found that 33.33% of pigs, in which infection could not be detected at meat inspection, were positive by ELISA. Dorny *et al.* (2004a) reported a sensitivity of 22.1% and a specificity of 100% in the abovementioned Zambian study.

2.3.2.3 Serology

a) Antibody detection

Many tests using homologous (*T. solium*) or heterologous antigen (*T. crassiceps*) have been developed to detect antibodies against *C. cellulosae* in pigs. The mostly used are ELISA and EITB. Antigens used in these tests are either cyst fluid or crude homogenates of *T. solium* cysticerci (homologous) or crude preparations of the related parasite *T. crassiceps* (heterologous) (Pardini *et al.*, 2001). There is no reason to believe that components from heterologous species are more reliable than those from homologous species (Ito *et al.*, 2004).

Biondi *et al.* (1996) evaluated the detection of *T. solium* in an indirect ELISA test using heterologous metacestode antigen from a laboratory-adapted murine *T. crassiceps* strain and reported a specificity of 97-100% and sensitivity of 100%. The limitation of the use of heterologous antigens of *T. crassiceps* are cross-reactions with hydatidosis and ascariasis (Nguekam, 2003) but its advantage is that it can be maintain in laboratory rodents (Rogan & Craig, 2002). However a sensitivity of 35.8% and a specificity of 91.7% were obtained by Dorny *et al.*(2004a) when evaluating a Ab-ELISA performed with crude metacestode antigen of *T. crassiceps* in the estimation of the prevalence of porcine cysticercosis in comparison with 3 other tests (tongue palpation, meat inspection and Ag-ELISA).

Kumar *et al.* (1987) evaluated the ELISA for the diagnosis of *T. solium* cysticercosis using *T. solium* larval scoleces. A sensitivity and specificity of 91.6% and 92.3% respectively were obtained, but crossreactions occurred with *T. hydatigena*.

In order to increase the performance of tests, purified antigens have been developed. Purification of glycoproteins from cyst fluid by single-step preparative isoelectric focusing was shown to produce very specific antigens which are applicable both in immunoblot and ELISA (Ito *et al.*, 1998). In the same study where Kumar *et al.* (1987) evaluated the ELISA for the diagnosis of *T. solium* cysticercosis using *T. solium* larval scoleces, its purified fractionated first and second peaks were also used as antigens and much better results were obtained with the first peak. Sensitivity and specificity of 95.8 and 96.2% respectively were obtained for first peak and 70.8 and 92.3% respectively for the second peak. In addition, no cross-reaction was observed using first peak antigen.

An immunoblot assay designed to diagnose human cysticercosis based on purified antigenic glycoproteins by Lentil-Lectin (LL-GP) was evaluated for efficacy in pigs by Tsang *et al.* (1991) and they found that the test performance was 100% sensitive and 100% specific.

Since the preparation of purified antigens relies on the availability of parasitic material and may be subject to the quality of this material, attempts were made to produce recombinant

antigens but while their specificity is reported to be high, the sensitivity is generally lower than with the native antigens (Dorny *et al.*, 2003).

b) Antigen detection

The test developed by Brandt *et al.* (1992) using monoclonal antibodies for the detection of *T. saginata* in cattle is also effective for the detection of swine cysticercosis. According to Pouedet *et al.* (2002) the sensitivity and specificity of Ag-ELISA for the detection of porcine cysticercosis in Cameroon as derived from Gibbs sampling analysis using three tests is 85.8-87.2 % and 98.1-98.9 % respectively. Very similar results were obtained by Dorny *et al.* (2004a) when evaluating the Ag-ELISA in the estimation of the prevalence of porcine cysticercosis in comparison with 3 other tests (tongue palpation, meat inspection and Ab-ELISA) using Gibbs sampling analysis.

. The sensitivity of antigen detecting ELISA is reported to be very high as it can detect a single-cyst infection in a pig model (Nguekam *et al.*, 2003b). The genus specificity of the tests does not allow differentiation between metacestodes infections of *T. solium* and *T. hydatigena* in pigs (Dorny *et al.*, 2003).

2.4 Control

Cysticercosis is a potentially eradicable disease as it was demonstrated in most European countries that were endemic for cysticercosis at the turn of the previous century and are now almost free from the disease (Del Brutto *et al.* 1998) through implementation of meat inspection and confinement of pigs, effective use of toilets and strict personal hygiene (Pawlowski, 1990; Plancarte *et al.*, 1999).

2.4.1 Prevention

Health education for at-risk population is the foundation of cysticercosis prevention (Acha & Szyfres, 2003). However, health education must be provided by well trained personnel and will be more effective if it is associated with identification and treatment of tapeworm carriers (Flisser & Lightowers, 2001).

2.4.2 Vaccination

An alternative approach for the control of taeniasis and cysticercosis due to *T. solium* is the use of vaccines in pigs. Some progress has already been made in this domain.

The final objective is to develop inexpensive and highly effective vaccine which can prevent *T. solium* in neonates as well as in old pigs, and that can be administered orally without the need for equipment or trained personnel (Lightowers, 2003). Up to now, such a vaccine is not yet available, but repeated intramuscular injection of recombinant *T. solium* antigens achieved excellent results. A recombinant protein (TSOL18) used in a vaccination trial against challenge infection with *T. solium* in pigs resulted in 100% protection (Pers. com., Geerts S., 2004), whereas Nascimento *et al.* (1995) got a much lower protection level (71.43%) when vaccinating pigs using *T. solium* scolex extracts.

2.4.3 Treatment

2.4.3.1 In pigs

Oxfendazole has been shown to be highly effective against porcine cysticercosis in muscles, when given as a single dose at 30 mg/kg bodyweight (Gonzalez *et al.*, 1997).

2.4.3.2 In humans

a) Taeniasis

Two anthelmintics, Praziquantel and Niclosamide are currently both indicated and widely available for treatment of human taeniasis (Allan *et al.*, 2002). Albendazole can also be used. Although De Kaminsky (1991) demonstrated it to be very effective against taeniasis, Chung *et al.* (1991) proved the contrary. In general, it has lower efficacy against taeniasis than either Praziquantel or Niclosamide. Praziquantel may be cheaper and more available than Niclosamide (Allan *et al.*, 2002).

b) Cysticercosis

The treatment of human (neuro)cysticercosis is not always indicated because there are immediate risks of neurologic symptoms due to the acute inflammation that results from the death of cysts, and because cysts often die naturally within a short period (Garcia *et al.*, 2004). Therefore, some consensus guidelines were produced which indicate whether or not patients have to be treated (Garcia *et al.*, 2002). These authors found that Albendazole and Praziquantel are larvicidal drugs used in the the treatment of cysticercosis, with Albendazole yielding slightly higher cure rates. The initial focus of therapy for patients with symptomatic neurocysticercosis is suppression of seizures or inflammation with anticonvulsants or corticosteroids respectively (Schantz *et al.*, 1998).

2.5 Impact of the disease

According to the World Health Organisation (WHO), more than 2 million people harbour the adult tapeworm and many more are infected with cysticerci (Garcia & Del Brutto, 2000). These authors also indicated that neurocysticercosis is an important public health problem as it affects people of productive ages and causes an estimated 50.000 deaths every year and many times that number of patients are left with irreversible brain damage.

The disease also causes important economic losses in countries where it is endemic: more than 60 millions dollars per year in Mexico only for condemnation of parasitized carcasses (Euzéby, 1998). According to Zoli *et al.* (2003), economic estimates indicate that the annual losses due to porcine cysticercosis in 10 West and Central African countries amount to about 25 million Euros, among which 2 million for Cameroon. Infected pigs and carcasses are sold cheaper in unofficial meat distribution channels in order to avoid losses from the condemnation of infected carcasses (Tsang & Wilson, 1995; Pawlowski, 1990).

The cost of this parasitosis for humans is very high (treatment, hospitalisation, loss of work days). In 1992, it was estimated at 195 millions dollars in USA and 3700 dollars per case in Mexico (Euzéby, 1998). In addition, it also reduces the availability of proteins to humans as a result of carcass condemnation. The human population that is most exposed to the disease are those living in rural areas where sanitary conditions are not the best. Djou (2001) quoted by Shey-Njila *et al.* (2003) reported that the cost of diagnosis, hospitalisation, and treatment of a human cysticercosis case in Cameroon is 170.000 F CFA (259 €), which is beyond the reach of most rural population.

Chapter III: MATERIALS AND METHODS

3.1 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 Mayo-Danay division in the far north province of Cameroon. The selection of these villages was based on the survey that Assana *et al.* (2001) carried out in 2000 for the detection of porcine cysticercosis. These 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 600.000 inhabitants.

Livestock activities include large ruminants (cattle), small ruminants (sheep and goats) and monogatrics (poultry, pigs, rabbits). Pig production includes the traditional system in which pigs roam freely with no particular care and the semi-traditional system in which pigs are kept in pens part of the time. Very few people keep their pigs permanently in pigsties and with adequate care (health and feeding). Most of pigs are fed with kitchen remains. Free roaming pigs have access to human faeces.

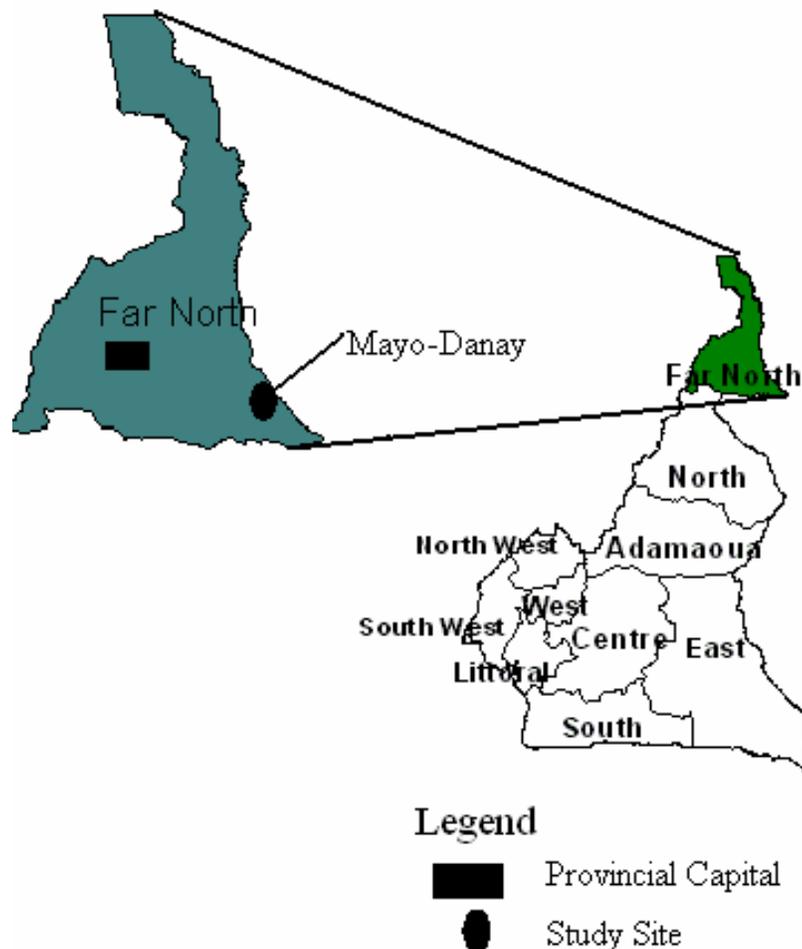


Figure 1: Map of Cameroon and Far North province showing Mayo-Danay Division.

3.2 Data collection

3.2.1 Sensitisation campaign

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 from whom complete data were available were included in the study among which 766 males and 551 females out of a total population of about 65000 inhabitants.

3.2.2 Clinical examinations

The volunteers in the 7 villages were clinically examined by a medical doctor and the following aspects were recorded: sex, age, village, presence/absence and localisation of subcutaneous nodules, presence/absence and frequency of seizures, headache and any other symptom that could be linked to cysticercosis.

3.2.3 Serum collection

After informed consent was obtained from adult volunteer or from their parents in case of children, blood was collected and the samples were kept at ambient temperature to allow coagulation. The sera were then collected and frozen at -20°C prior to serological examinations.

3.3 Detection of circulating antigens by the Enzyme Linked Immunosorbent Assay (ELISA)

Circulating antigens of *T. solium* metacestodes were detected using a sandwich ELISA as described by Dorny *et al.* (2000) with some slight modifications. 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 control. The complete procedure of the sandwich ELISA was as follows:

Coating

Polystyrene plates (Nunc-Immuno modules F₈ Maxisorb #469949) were coated with 100 µl per well of monoclonal antibody (MoAb) B₁₅₈C₁₁A₁₀ at 5 µg/ml in carbonate 0.06M buffer pH 9.6. The plate was incubated for 30 minutes at 37°C under agitation and then washed once with freshly prepared phosphate buffered saline (PBS)-Tween 20.

Blocking

The wells were blocked with 150 µl per well of freshly prepared PBS-Tween 20 and 1% new-born calve serum (NBCS). The plate was incubated for 15 minutes at 37°C under agitation. No washing was done at the end of the incubation.

Pre-treatment of sera

Sera (150µl) were diluted using an equal volume of Trichloro acetic acid (TCA) 5%. The diluted sera were then incubated at room temperature for 20 minutes. Incubation was followed by centrifugation at 12000 rpm for 9 minutes. The pH of the supernatant was then raised by addition of an equal volume of carbonate buffer 0.6M at pH10.

Incubation of pre-treated sera

Without washing the plate but emptying thoroughly after blocking, 100 µl per well of pre-treated sera were added to the wells in single or in duplicate. The plate was incubated for 15 minutes at 37 °C under agitation and then washed five times with freshly prepared Phosphate Buffered Saline (PBS) Tween20.

Second monoclonal antibody

Afterwards, 100 µl per well of biotinylated MoAb B₆₀H₈A₄ (1.25 µg/ml in PBS Tween 20 and 1% NBCS) were added to each well. The plate was covered and incubated for 15 minutes at 37 °C under agitation and then washed five times with freshly prepared PBS-Tween20.

Conjugate

To each well was added 100 µl of Streptavidine Horseradish Peroxydase (diluted at 1/10 000 in PBS-Tween20 + 1% NBCS). The plate was incubated for 15 minutes at 37 °C under agitation and then washed five times with freshly prepared PBS-Tween20.

Substrate/Chromogen

To each well was added 100 µl of substrate/chromogen (2 tablets of Orthophenylene Diaminine (OPD) in 12 ml of distilled water and 5 µl H₂O₂ at 30%). The plate was incubated for 15 minutes in darkness without agitation. The reaction was stopped with 50 µl of H₂SO₄.4N per well.

Reading of the microplate

The plate was read with the help of an automated spectrometer (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 test of Sokal and Rohlf,1981).

3.4 Statistical analysis

The random-effects logistic regression with locality as group variable was used to detect any significant difference between the localities, gender and age at the probability level of 5% (Pers. com., Speybroeck N., 2004). These analyses were conducted using the Stata Statistical Software.

Chapter IV: RESULTS

4.1 Characteristics of the study population

The characteristics of the study population are presented in table II. Of the 1317 persons that participated in the survey (representing about 2 % of the total population of the study area), 766 (58%) were male and 551 (42%) female. There were 452 children (34.32%) of less than 16 years, 645 (48.97%) adults between 16 and 45 years and 220 (16.7%) old of more than 45 years. The age distribution ranged from 1 to 100 years with an average age of 26.7 years. In all the villages, the proportion of males in the sample population was higher than that of women, except for Bangana and Vada where the proportion of males and females were similar.

Table II: Characteristics of the study population

Locality	No. examined	Male		Female		Age group		
		Number	%	Number	%	<16	16-45	>45
Zouaye	205	134	65.37	71	34.63	52	124	29
Bangana	173	80	46.24	93	53.76	43	96	34
Hougno	140	81	57.86	59	42.14	39	67	34
Vada	199	95	47.74	104	52.26	98	77	24
Vounaloum	211	151	71.90	60	28.10	65	104	42
Vele	101	75	74.26	26	25.74	27	52	22
Datcheka	288	150	52.08	138	47.92	128	125	35
TOTAL	1317	766	100	551	100	452	645	220

4.2 Cysticercosis prevalence per locality and in the division

Of 1317 serum samples tested, 27 (2.05%) were positive in the Ag-ELISA (Table III). 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 group variable showed that apart from Vada village, seropositivity did not significantly differ in the villages. Vada village had significantly higher seropositives persons than Zouaye ($p=0.032$) and Datcheka ($p=0.025$).

4.3 Influence of age and sex on the prevalence of cysticercosis

The most affected age group was that of more than 45 years. The percentage of seropositive persons varied from 1.55% (16-45 years group) to 4.09% (>45 years group) (Table III). 1.77% of the youngs (<16 years) were seropositives.

2.09% of the males were seropositive against 2% of the females. 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 ($p=0.996$).

Table III: Seroprevalence of cysticercosis per locality, age group and sex.

Parameter		No. Examined	No. Positives	%
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

4.4 Correlation between symptoms recorded and seropositivity

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

A total of 179 individuals presented at least one symptom that could be linked to cysticercosis among which 7 (3.9%) were seropositives. Two persons had at the same time headache, subcutaneous nodules and seizures and one of them was seropositive.

Among the 34 individuals that had at least headache, only one (2.94%) was positive. Seven (4.9%) of the 143 individuals who had at least subcutaneous nodules were positive whereas one (5.26%) of the 19 who had at least seizures was positive.

All the individuals that presented 2 of the 3 symptoms were all seronegatives. They were 6 persons with seizures and headache, 6 with nodules and headache and one with seizures and nodules. Among the 134 individuals that presented only nodules, 6 were seropositives whereas all the individuals that presented only seizures (10) or only headache (20) were all seronegatives.

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

Table IV: Correlation between symptoms recorded and seropositivity

Symptoms	No. Examined	No. Positives*	%
Headache	34	1	2.94
Subcutaneous Nodule	143	7	4.90
Seizures	19	1	5.26
Asymptomatic	1138	20	1.76

* Some patients had more than one symptom

4.5 ELISA ratio of seropositive persons

The ELISA ratio (quotient between the OD of the sample and the OD of the cut-off value) varied from 1.02 to 48.18 with a mean of 5.17 (Table V). 67% of them had a ratio of less than 2.

Table V: ELISA ratio of the seropositive persons

ELISA Ratio	No. of persons
1 - 1.99	18
2 - 4	3
> 4	6

Chapter V: DISCUSSION AND CONCLUSION

In this study, the Ag-ELISA was used in order to estimate the prevalence of active human 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 % (0.49-4.52%) were seropositive. These figures are probably underestimated because the persons that had only dead cysts were not detected as Ag-ELISA detects only living cysts (Ehrt *et al.*, 2002). Consequently, the true prevalence of cysticercosis could be much higher. No similar study has yet been carried out in the region but the presence of human cysticercosis there is not surprising as Assana *et al.* (2001) discovered a high seroprevalence in pig (39.8%) in the same study area.

In this study, the sampling was not done at random, but based on voluntary presentation of the village people to the medical team, and as such should be interpreted with caution.

A lower seroprevalence of 0.7% (0.4-3%) was obtained by Nguetkam *et al.* (2003a) in a similar study but with 4993 individuals in 3 communities (Bamendou, Bafou and Fonakekeu) 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% found in this study was lower than the seroprevalence of 5.7% obtained by Ehrt *et al.* (2002) in Northern Vietnam using the same test.

Although no further confirmation test (imaging techniques or biopsy of subcutaneous nodules) was performed, it can be assumed that at least 60% of the 27 individuals that were seropositives are true cases of cysticercosis. In similar studies Nguetkam *et al.* (2003a) found a confirmation rate by CT scan of 59.1% whereas Ehrt *et al.* (2002) obtained 89 % by CT scan and/or biopsy.

The occurrence of cysticercosis in 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 can probably be due to the presence of one or many 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. This could also be due to the bias that was introduced by the sampling method.

No significant difference was found between the prevalence in males and females. This finding is in accordance with Nguetkam *et al.* (2003a) and Ehrt *et al.* (2002) in studies carried out in West Cameroon and a rural area of northern Vietnam respectively using the same test.

Of the 143 who had subcutaneous nodules, only 7 (4.90%) were seropositive. Since biopsy was not done, the nodules might also be due to *Onchocerca volvulus* that has been reported to be endemic in the region (Pers. com., Zoli A.P., 2004). The presence of a lake and river Logone and flooding of the area during the rainy season are favouring factors.

Out of 19 people that had seizures, only one (5.26%) was seropositive which is higher than the 2.5% observed by Nguetkam *et al.* (2003a) in Menoua. This result confirms that viable cysts are present in only a very small number of people having seizures (Zoli *et al.*, 2004). These authors when comparing seropositivity in epileptic patients in West and North West provinces of Cameroon found 1.2 % with Ag-ELISA against 44.6 % with Ab-ELISA in

the same population sample. Such difference is because the Ag-ELISA detects only living cysts whereas Ab-ELISA detects dead cysts which are more often responsible for epileptic seizures (Zoli *et al.*, 2004).

Only one person (2.94%) out of the 34 persons who reported to have headache was seropositive. Although chronic headache is a symptom suggestive of cysticercosis, it is also the symptom of many other diseases.

The prevalence was much influenced by age and the highest prevalence was obtained in the group of more than 45 years. These results are in accordance with the study of Nguekam *et al.* (2003a). 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. Erhart *et al.* (2002) however found no influence of age.

Twenty (1.76%) of the 1138 asymptomatic individuals tested positive to Ag-ELISA which is higher than the 0.6% obtained by Nguekam *et al.* (2003a). Most of these persons were probably at the early stage of the disease or harbouring few cysts as 16 of them had a very low optical density (OD). Their ELISA ratio was less than 1.5. The ELISA ratio of the 4 remaining seropositives varied between 2.36 and 17. It should also be noted that neurocysticercosis manifestations vary according to location, viability and number of cysts and the presence, type and degree of host response (Nash *et al.*, 2003). The person that had the highest ELISA ratio (48.18) had subcutaneous nodules.

It can be concluded that human cysticercosis is a public health problem in Mayo-Danay Division and probably the whole northern region as previous studies have revealed that Mayo-Danay, Mayo-Kebbi in Chad (Assana *et al.*, 2001) and Garoua region in North Cameroon (Awa *et al.*, 1999) have a high prevalence of porcine cysticercosis.

Many favouring factors to the completion of *T. solium* cycle are present in the region, for instance absence or scarcity of adequate faeces disposal systems as latrines, free roaming of pigs, open air defecation, clandestine slaughtering of pigs, absence of adequate meat inspection and lack of adequate knowledge on the transmission mechanisms of the parasite.

As long as nothing is done to break the cycle of the parasite by finding appropriate solutions to most of the above mentioned factors, the region will remain endemic for porcine and human cysticercosis.

***Chapter VI:* RECOMMENDATIONS**

The following recommendations can be given:

- Further studies should be carried out in other parts of the region to confirm the findings of this study. These studies have to be well designed using a randomly selected sample that is representative of the study area population in order to obtain more accurate and reliable data.
- The population of the area should be sensitised on the transmission factors that maintain cysticercosis in the population and even increase its spreading. For example, mass communication and education campaign towards appropriate personal hygiene, proper confinement of pigs, safe disposal of human faeces, rapid treatment of tapeworm carriers and proper environmental hygiene are to be well known by the population if the reduction of the prevalence and why not eradication is to be achieved.
- Medical doctors should be made aware of the problem so that they can consider cysticercosis in the differential diagnosis of sub-cutaneous nodules and neurological disturbances, particularly epilepsy.

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Annexe 1

Principle of sandwich ELISA for the detection of circulating antigen

