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TAENIASIS AND CYSTICERCOSIS IN A SELECTED GROUP OF INHABITANTS FROM A MOUNTAINOUS PROVINCE IN NORTH VIETNAM

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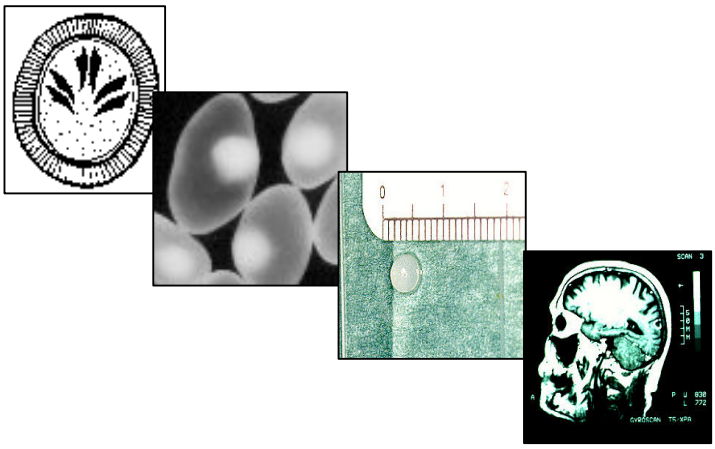

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Dedicated to:

My life-work is today and my daughter is tomorrow

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Acidity	pH
Antibody	Ab
Antigen	Ag
Base pairs	bp
Celsius grade	^{0}C
Centimeter	cm
Central nervous system	CNS
Computer tomography	CT-scan
Deoxyribonucleic acid	DNA
Enzyme-linked immunosorbent assay	ELISA
Enzyme-linked immunotransfer blot	EITB
Institute for Tropical Medicine	ITM
Institutional Review Board	IRB
Kilodalton	kDa
Magnetic resonance imaging	MRI
microliter	µl
Milliliter	ml
Millimeter	mm
Millimol	mM
Mitochondrial genes	mt DNA
National Institute of Malariology, Parasitology and Entomology	NIMPE
Neurocysticercosis	NC
Polymerase Chain Reaction	PCR
Random amplified polymorphic DNA	RAPD
Retriction Fragment Length Polymorphism	RFLP
Reverse Osmotic De-Ionised water	RO-DI water
Ribosomal genes	rDNA
Species (plural)	spp.

Keywords: *Taenia solium, Taenia saginata asiatica, Vietnam,* taeniasis, cysticercosis, antigen detection, ELISA, PCR-RFLP, mitochondrial 12S rDNA gene, gene insertion.

Summary

This work reports on a study on Taeniasis/Cysticercosis in a selected group of inhabitants of a mountainous area in North Vietnam. The participants of the study were not randomly taken from the population but from a group of individuals that was suspected for taeniasis/cysticercosis because they had a reported history of passing proglottids and/or subcutaneous nodules and/or a reported history of seizures. One hundred and three persons participated in the study. They were subjected to a questionnaire, and blood and faeces sampled. A monoclonal antibody-based antigen detecting ELISA was used for serological diagnosis of cysticercosis. The stools were microscopically examined for the presence of helminth eggs and tapeworm carriers were treated and the tapeworms recovered for species identification by PCR-RFLP. The questionnaire survey revealed that a high rate of interviewers had the habit of eating undercooked pork, rare beef and blood pudding (80%, 79% and 78%, respectively). In addition, 13.6% (14/103) of the interviewed persons admitted to have eaten pork containing cysticerci. By Ag-ELISA we detected 13.4% (13/97) seropositive cases. One seropositive case was 14 years old while the 12 other cases were between 22 to 70 years old. There were significantly more sero-positives by Ag-ELISA among the males than in female participants in this study. The results of the examination of gastro-intestinal nematode infection showed high infection rates with *A. lumbricodes* (65.5%), hookworms (39.3%) and *T. trichiura* (23.8%). Taenia eggs were found in the stools of five individuals; among these one person was also positive in the Ag-ELISA, suggesting self- or auto-infection. Treatment of two persons who reported to pass proglottids in theirs stools resulted in the expulsion of tapeworms while the coprological examination was negative. Based on the molecular tests performed on the three Taenia samples that were collected from our survey, two of these could be identified as *T. s. asiatica* by PCR-RFLP; the third one was also tentatively identified as *T. s. asiatica* (percentage homology is 99.5%, 98.2% compared with *T. s. asiatica* and *T. saginata* sequence in the mitochondrial 12S rDNA gene), following repeated PCR tests and RFLP using different primer pairs and restriction enzymes, respectively. This sample appeared to have a gross-alteration in its sequence with the insertion of a 400 bp fragment in the mitochondrial 12S rDNA gene. It was concluded that Taeniasis and cysticercosis are common in the surveyed area and can be associated with clinical symptoms. The impact of *T. solium*, however, remains unknown. Moreover, the epidemiological situation of the different *Taenia* spp. is complex and needs further investigation.

Chapter I Introduction

Taeniasis and cysticercosis caused by *Taenia solium*, the pork tapeworm, is widely prevalent in human and swine hosts in many developing countries of Latin American, Africa, and Asia. Man is not only infected by adult tapeworms but also by the metacestodes of *T. solium*. Neurocysticercosis (NC) is an infection of the central nervous system by the larval stage and a major cause of epilepsy associated with considerable morbidity and mortality in developing countries (Garcia *et al*., 2003a). Cysticerci of *T. saginata*, in contrast, are found exclusively in cattle and do not develop in human.

The disease is often asymptomatic, but can cause headaches, seizures, impairment of higher functions, or hydrocephalus (Medina *et al.*, 1990; Garcia *et al.*, 1993). NC is common in regions where sanitation is poor, particularly in Latin America, Africa and Asia. It is also reported in the United State and Europe, particularly in immigrant populations. The number of cases world-wide appears to be increasing.

There have been reports from Taiwan, Indonesia, Thailand, Korea, China and the Philippines (Bowles & Mc Manus, 1994) on a new form of human taeniid, which has morphological and genetic resemblances with *T. saginata* and is classified as a subspecies of the latter, *Taenia saginata asiatica*. Eom & Rim (1993) described it as *T. s. asiatica* based on the morphology in its adult and larval stage. However, the intermediate host is not cattle but swine (Fan, 1988; Fan *et al*., 1990) and the parasite has a cysticercus more similar to that of *T. solium* (Eom, 2006). In practical terms, the important point is that human constitute the definitive host of three taeniids, *T. saginata*, *T. solium* and *T. s. asiatica* (Galan-Puchades & Fuentes, 2000). For this reason, fast, reliable and specific diagnosis of adult tapeworm infection in human is an important public health priority.

Vietnam has a tropical and sub-tropical climate offering favorable conditions for infection and spreading of parasitic diseases, including taeniasis and cysticercosis. In addition, risk factors for these diseases, such as, open-air defecation, freely roaming of pigs, low sanitation and non-existent slaughterhouse or lack of meat inspection, is still present, mainly in rural areas. Moreover, in some parts of Vietnam, the culinary habit of eating raw or undercooked meat and raw salads and vegetables may favor transmission of the larval stage of tapeworms. In most of the rural areas such as Bac Ninh province consumption of pork from communal markets was 100%, the habit of eating raw pork was 74.3%; the rate of not washing hands after defecating was 53.5%, the rate of feeding pigs free around the house was 6%, the use of fresh faeces for fertilizing crops was 5%. and finally the latrinisation rate was 96% (De *et al.*, 2001) Some local traditions in rural area, such as traditional pig husbandry systems and culinary habits are not easy to change.

The risk factors for taeniasis and cysticercosis have indicated that important factors include consuming undercooked meat (raw pork and not well-done beef), unwashed vegetables, allowing pigs to roam freely and the keeping of pigs under the house that, in some cases, enter homes, and nonexistent or inadequate meat inspection and control. There is poor sanitation due to the absence or lack of latrines; the unfrequent use of the available latrines and defecation next to streams or in the forest. These factors are considerd to be more common in the poor, rural areas of the country, especially in the mountainous region of the North (Willingham *et al*., 2003; De *et al.*, 1999).

Cysticercosis is scattered all over the Vietnamese territory, particularly in the Northern provinces. Figures on porcine cysticercosis are based mostly on passive surveillance, which underestimates the true prevalence. A few community-based studies showed a sero-prevalence of human cysticercosis of around 5% in mountainous rural areas and a much lower prevalence in rural coastal and peri-urban areas (Erhart *et al.*, 2002; Somers *et al.*, 2006a). De *et al.* (2001) reported a seroprevalence of cysticercosis in a community in Bac Ninh province of Vietnam at around 5.7%. Human cysticercosis is well known in northern Vietnam where between 100 and 150 patients are referred to specialized hospitals each year (Dorny, 2004b). At the National Institute of Malariology, Parasitology and Entomology (NIMPE) in Hanoi (a specialized referral hospital for parasitic diseases), between 1996 and 2000, 594 patients with NC were hospitalised (Nguyen, 2001) and in the period 2000 – 2004 the number of inpatients with NC was approximately 500. Surveys on human Taeniasis in Central and Northern provinces indicated a prevalence of 5-7% (De *et al*., 1998; 2001), however, techniques of low sensitivity were used and the results were inconclusive since it was not known with which species of tapeworms these people were infected (Willingham *et al*., 2003). In a study in the northern provinces of Vietnam, De *et al.* (1998) identified most tapeworms infecting humans as *T. saginata* (78-80%), *T. solium* accounting for only 20-22% of the tapeworms recovered. While, in a study by Somers *et al.* (2006b) which collected 65 *Taenia* samples from patients in a referral hospital in Hanoi identified that *T. s. asiatica* was the most common species (55.4%) followed by *T. saginata* (38.5%) and *T. solium* (6.2%)

Diagnosis of taeniasis can be done by recovering eggs or proglottids in the stools of infected hosts or by the copro-antigen test (Allan *et al*., 1990). Three species of *Taenia* infecting humans have been detected in Vietnam, *T. solium*, *T. saginata* and *T. s. asiatica* (Willingham *et al*., 2003; Nguyen, 2004). Infection by the metacestodes can be detected by biopsy of subcutaneous cysts, neuroimaging techniques (computer tomography (CT-scan), or/and magnetic resonance imaging (MRI)) and immunodiagnosis, and can be suspected in endemic regions on basis of neurological symptoms (epilepsy, headache). Accurate diagnosis of NC still requires cranial imaging technology such as CT-scan and MRI, techniques that are expensive and frequently inaccessible to people of endemic areas (Sciutto *et al*., 2000). The diagnosis of NC without imaging studies is difficult because of the highly variable clinical symptoms, ranging from asymptomatic to a very severe neurological syndrome with intracranial hypertension or dementia, with a variety of non-specific mild clinical symptoms in between. For immunological diagnosis, the enzyme-linked immunosorbent assay (ELISA) and the enzyme-linked immunoelectro-transfer blot (EITB) are the most widely used techniques (Rosas *et al.*, 1986; Garcıa *et al.*, 1993). In recent years immunodiagnostic techniques have been improved, including detection methods for specific antibodies and circulating parasite antigen in serum or cerebrospinal fluid and urine. However, until now only few of the current techniques have been standardized and fully validated (Dorny *et al*., 2003). The follow up after treatment of NC cases is usually also done using imaging technology such as CT-scan and MRI, however here also, the costs of treatment and those for the evaluation of the success of treatment are far too high for the people of developing countries that are at risk.

In poor countries, application of cheap techniques for the diagnosis and follow up after treatment of human cysticercosis cases are required, which have high sensitivities and specificities (Erhart *et al.*, 2002). The monoclonal antibody-based Ag-ELISA test is a promising technique to monitor NC patients after treatment; it is much cheaper than CT-scan, which makes it an interesting tool, in particular for patients of developing countries where poverty is an increasing reality (Nguekam *et al.*, 2003). Recently, it was found that antigen

detection may also be performed on urine samples (unpublished results). Since collection of urine is easier than collection of blood samples, this technique offers obvious advantages.

The objectives of this study were:

- To add epidemiological data on Taeniasis and Cysticercosis in a specific region of Northern Vietnam, Hagiang province
- To assess the use of detection of circulating antigens (Ag-ELISA), performed on serum samples
- To determine which species of *Taenia* are present in that area.

The results will provide necessary information for further research in order to prevent and control Taeniasis and Cysticercosis in Vietnam.

Chapter II Literature review

II.01 Taxonomy

Superreign:	*Eukariota*
Reign:	*Metazoa*
Kingdom:	*Animalia*
Phylum:	*Platyhelminthes*
Class:	*Cestoidea*
Order:	*Cyclophyllidea*
Family:	*Taenidae*
Genus:	*Taenia*
Species:	*solium*
	saginata
Subspecies	*saginata asiatica*

II.02 Geographical distribution

Taenia solium and *Taenia saginata* are distributed worldwide. They can be found more frequently in countries where swine and cattle are raised for human consumption, respectively. Their prevalence has decreased in developed countries owing to stricter meat inspection, better hygiene and sanitary facilities. The distribution of human cysticercosis coincides with the distribution of *T. solium*. In Latin American, the first report of human cysticercosis was published in Mexico in 1901. Recent reports demonstrated the presence of *T. solium* in many Latin American countries. The frequency of clinical cases ranged from less than 1% to 9% (Flisser, 2002). In African countries like Ethiopia, Kenya and the Democratic Republic of Congo, around 10% of the population is infected; in Madagascar even 16% (http://www.who.int/zoonoses/diseases/taeniasis/en/). The infection in pigs and man is more widely distributed in sub-Saharan African than previously assumed. Several rural regions of Africa are now known to be (hyper)-endemic for cysticercosis in pigs and man (Geerts *et al*., 2002). Some countries in Asia, including Vietnam, also appear to be endemic for taeniasis and cysticercosis (Rajshekhar *et al.*, 2003). In 1993 Eom reported a new (sub)-species, *T. saginata asiatica* or Asian *Taenia.* Following this event, there have been several reports on that new form of human taeniid in Taiwan, Indonesia, Thailand, Korea, China, the Philippines, Nepal and Vietnam (Bowles & Mc Manus, 1994; Willingham *et al.*, 2003, Rajshekhar *et al.*, 2003).

II.03 Life cycle

The life cycle of taeniid tapeworms is complex. Swine and bovines are known as the intermediate hosts of human taenidae. Man is the definitive host where the adult tapeworm lives in the intestine. Infected humans shed the proglottids containing the eggs in their faeces. The gravid segments of *T. saginata* are very active and escape through the anus, releasing large numbers of eggs in the peri-anal region or on the ground where they can survive for long periods. The gravid segments of *T. solium* are less active and usually leave the host with the stools; often several attached proglottids may leave at the same time. The gravid proglottids contain approximately 50,000 eggs each. Eggs contain a larva or oncosphere surrounded by several protective layers resistant to adverse conditions. The eggs are ingested by bovines (*T. saginata*) or swine (*T. solium, T.* s. *asiatica*), in which they hatch, each releasing an oncosphere, which migrates through the intestinal wall and blood vessels to reach striated muscle within which it encysts, forming a cysticercus. Humans acquire the infection by ingestion of raw or undercooked beef/pork containing the cysticercus; the oncosphere excysts,

settles in the small intestine and develops there into an adult cestode over the next three months or so. For *T. saginata* humans are the final host. This is also true for *T. solium*, but in *T. solium* humans can also be the intermediate host. This is the stage in which health problems occur. *Cysticerci* appear as translucent cysts with an invaginated scolex, seen in the striated muscles, as well as in the brain, liver and other tissues. Three ways of transmission of this disease have been described. First, self-infection may be a consequence of insufficient personal hygiene and usually occurs through contaminated hands. Second, auto-infection is possible. This means that humans can become infected in the gastrointestinal tract by the presence of a *T. solium* tapeworm. Gravid proglottids are directed to the stomach by antiperistaltic movements of the intestines; oncospheres will be liberated and start their migration. This way of infection has never been confirmed but is suggested by the occurrence of massive infections. Finally, humans can become infected by accidental infection of eggs excreted by tapeworm carriers in the family or by eggs contaminating the environment (on vegetables or in drinking water).
The third form of tapeworm in man, *T. s. asiatica* is as an adult very similar to *T. saginata* (Eom & Rim, 1993). But the life cycle of this cestode is different from classical *T. saginata* in its intermediate host animal as well as in the infected organs (Eom, 2006). The intermediate host is mainly the pig, although cattle can also be infected (but in cattle the parasite does not develop well) (Fan, 1988; Fan *et al*., 1990). *T. s. asiatica* metacestodes are viscerotropic rather than musculotropic in their larval stage; they are found mainly in the liver and visceral organs such as the omentum, serosa, and lungs but not into the muscles of the intermediate host (Eom & Rim, 2001; Eom, 2006). As a consequence, transmission to man is caused by consumption of raw or undercooked liver or viscera.

II.04 Morphology

Taeniids are flat, ribbon shaped and segmented. Adults are hermaphrodite. The body forms three parts:

II.04.01 The scolex or head

The main function of the scolex is attaching the worm to the mucosa of the intestinal wall of its host. It is a very small structure, provided with 4 oval suckers that may or may not possess a muscular rostellum at the apex of the scolex. This rostellum is retractable and may be provided with one or more rings of hooks.

On the basis of morphology and the presence or absence of the hooklets and the rostellum of the scolex, *T. solium* Linnaeus (1758), *T. saginata* Goeze (1782) and *T. s. asiatica* Eom & Rim (1993) are classified as follows (table I).

Table I: Differentiation of *Taenia* spp based on the morphology of the hooklets and rostellum of the scolex (Eom, 2006)

Scolex and hooklets	*Taenia* species	Classified by
Scolex armed	*Taenia solium*	Linnaeus, 1758
Scolex unarmed, rostellum absent	*Taenia saginata*	Goeze, 1782
Scolex unarmed, rostellum present	*Taenia saginata asiatica*	Eom & Rim, 1993

II.04.02 The neck or cervical region

The cervical region is not segmented and has no specific morphological features. It is in this cervical region that strobilization takes place.

The scolex and neck are very important; as long as both structures are present the infection persists, even though the entire rest of the body is eliminated.

II.04.03 The body or strobila

The strobila is composed of a chain of proglottids. These can be classified in 3 groups: immature proglottids in which male organs are first formed; mature proglottids which contain completely developed male and female sexual organs and gravid proglottids in which the primary sexual organs are atrophied and the content consists of the uterus filled with eggs.

T. solium: the adult tapeworm measures 2 to 4 meters and produces 800 to 1000 proglottids. Mature proglottids have 3 ovary lobes and no vaginal sphincter is present. The gravid segment measures about 7 to 20 mm and the lateral uterine branches, visible by the mass of eggs, are less numerous than in *T. saginata*: 7-16 unilaterally. Gravid segments detach from the strobila in groups of 5 to 6, are somewhat motile and are usually expelled with the feces, and contain from 30,000 to 50,000 eggs. Often three or more unseparated proglottids are shed with the feces (Krauss *et al*., 2003).

T. saginata: the adult is 4 to 12 meters long (even up to 25 meters) and composed of 1,000 to 2,000 proglottids. Mature proglottids have 2 ovary lobes and a vaginal sphincter is present. The gravid proglottid is 20-30 mm long, 5-7 mm wide and has in excess of 12 main lateral branches (Bogitsh & Cheng, 1998). Usually, proglottids detach themselves spontaneously from the strobila and are rather motile. Eggs are shed in mature proglottids that contain up to 100,000 eggs with the feces. Four to nine mature proglottids are produced daily (Krauss *et al*., 2003). About 750,000 eggs may be expelled daily (WHO, 1983).

T. s. asiatica: According to Eom & Rim (1993) and Eom (2006) adults are large sized tapeworms (mean 341 cm long and 9.5 mm wide) with on average 712 segments. Proglottids are rectangular. Anterior proglottids are wide and short; the posterior proglottids are long and narrow. Free proglottids bear a posterior protuberance. Mature proglottids have two ovary lobes that are unequal in size. The vaginal sphincter is round to oval. Gravid proglottids are 9.5 – 16.0 mm in length with 4.2 – 5.8 mm in width without pressing. Uterine main branches number between 16 and 2, and uterine twigs are 57-99 on once side. The way of leaving the host is as a single proglottid and spontaneously, independent from defecations as for *T. saginata* (Fan, 1988; Ito *et al*., 2002).

II.04.04 Cysticercus

The cysticercus is a bladder filled with liquid, with a bud on the cyst wall from where a single invaginated scolex will be formed.

The cysticercus of *T. solium* is named *Cysticercus cellulosae*, measures 5 by 8-10mm and has a scolex provided with rostellar hooks (Beaver *et al*., 1984).

The cysticercus of *T. saginata* is called *Cysticercus bovis*. These cysticerci are thin-walled, fluid-containing small bladders and measure 5 by 8 mm. The invaginated single scolex is already the scolex of the future adult stage, thus without hooks (Krauss *et al*., 2003). Human cysticercosis caused by *T. saginata*, either does not occur or is extremely rare (Krauss *et al.*, 2003).

The bladder wall of the *T. s. asiatica* metacestode (named C*ysticercus viscerotropica)* is covered with wart-like formations rather then simple rugae, which are seen in *C. bovis*. These cysts are ovoid; have a milky white bladder, measuring 1.9-3 mm in maximum length with thin and transparent cyst wall when alive. The outer bladder surface is covered with wart-like formations, which are composed of numerous microtriches. Rudimentary hooklets on the rostellum are observed only in a minority (about 17%) with varying numbers between 0 and 37 (Eom & Rim, 1993; Eom, 2006).

II.05 Emergence and burden of disease

Taeniasis is among the zoonoses and zoonotic agents with a current and potentially increasing impact (WHO/FAO/OIE, 2002). Cysticercosis, due to the zoonotic pork tapeworm *T. solium*, has emerged as an important cause of human morbidity and mortality, particularly in parts of Latin America, Africa and Asia. Classified as a List B disease by the OIE, cysticercosis is one of the most important zoonotic diseases in the world. According to Krauss *et al.* (2003) there are 40 millions persons infected with *T. saginata* in the world. The annual loss due to porcine cysticercosis was roughly estimated at US$ 43 million in Mexico and at about 25 million Euro in 10 West and Central African countries (Zoli *et al*., 2003). In Latin America it was estimated to be US$ 164 million (Schantz *et al.*, 1993). Fan (1997) estimated that up to 21% of the inhabitants of rural areas in Taiwan, Korea and Indonesia are infected with *T. s. asiatica*, with the annual economic loss due to taeniasis (including *T. s. asiatica*) in mountainous regions of Taiwan and in Samosir island of Indonesia amounting to US$ 11 million and US$ 2.4 million, respectively. In northern Sumatra, 10% of the population is affected, and 23% of all examined pigs were found to harbour larvae (Murrell *et al.*, 1996)

II.06 Epidemiology

There are 4 main factors in the epidemiology of taeniasis/cysticercosis.

– Environmental contamination with human faeces (poor sanitation)
– Pig management practices (free-range farming)
– Pork consumption habits (undercooked or raw pork)
– Lack or absence of pork inspection and control

According to WHO (1983) *T. saginata* can be classified into 3 groups of endemicity:

i. Countries or regions that are highly endemic, with prevalence rates in the human population that exceed 10%: e.g. Central and East African countries.
ii. Countries and regions with moderate infection rate (0.1-10%): e.g. South East Asia: Thailand, India, Vietnam and the Philippines.
iii. Countries and regions with prevalence below 0.1%: e.g. Western Europe.

The infection rate of *T. saginata* taeniasis in man is closely related to the frequency of eating raw beef. The infection is most common in the 20 - 40 age groups. The risk of being infected

is 5 times greater in members of a carrier's family, 14 times greater in workers that have professional contact with raw meat and 40 times greater in raw beef eaters (WHO, 1983).

Prevalence of *T. solium* infection varies according to the regional levels of sanitation, pig husbandry system and eating habit. It is endemic in most developing countries where pigs are raised and pork is consumed. In Asia, reliable prevalence data on *T. solium* taeniasis and cysticercosis for most countries, except Indonesia, are unavailable (India, Philippines), incomplete or are outdated (Taiwan, Korea, China) (Rajshekhar *et al*., 2003).

Prevalence rates of *T. s. asiatica* in humans have not yet been estimated in the countries where it is present. Factors affecting transmission of *T. s. asiatica* relate to the custom of eating viscera; especially the consumption of the liver and blood of freshly killed animals seems to be contributing to the infection (Fan, 1988; Eom & Rim, 2001).

II.07 Taeniasis

The great majority of *T. saginata* and *T. solium* carriers are unaware of their infection. However carriers of *T. solium* carry a substantial risk of acquiring cysticercosis by self or autoinfection and members of their households are also at increased risk. *T. solium* taeniasis is more rarely clinically apparent than *T. saginata* because its proglottids are less active and therefore, less noticeable to the patient. *T. solium* tapeworms can survive in the human intestine for 25 years (Krauss *et al.*, 2003). *T. saginata* taeniasis is often sub-clinical and is only revealed by faecal examination or when the patient notices the expulsion of proglottids. *T. saginata may* persist for 30 – 40 years (Krauss *et al*., 2003). Symptoms of *T. s. asiatica* are similar to those of *T. saginata*.

II.08 Cysticercosis

Human cysticercosis is caused by the development of *T. solium* cysticerci in tissues of humans. It is not always symptomatic. The most important feature of *T. solium* is the risk of development of neurocysticercosis in case of establishment in the brain. The location that most often prompts a medical consultation is the central nervous system, followed by the eye and its surrounding tissues. (Acha & Szyfres, 2003)

II.09 Clinical signs

Infection with adult tapeworms in human, caused by *T. saginata*, *T. s. asiatica* or *T. solium* rarely produce serious clinical problems: abdominal pain, diarrhoea, nausea, and anal pruritis, except for some cases where the tapeworms may physically block the intestinal tract and cause appendicitis (Acha & Szyfres, 2003). In contrast to taeniasis, caused by infection of the adult tapeworm, human cysticercosis is a serious health problem. The pathology and clinical symptoms associated with *T. solium* cysticercosis depend on which organs are infected and on the number of cysticerci that establish. An infection consisting of a few small cysticerci in the liver or muscles does not cause problem (Garcia *et al*., 2003a). On the other hand, even one or a few cysts, if located in a particularly "sensitive" area of the body, such as the central nervous system or the eye, may result in irreparable damage and affect human health and productivity (Mukaratirwa *et al*., 2003).

Neurocysticercosis (NC) is commonly associated with clinical manifestations such as seizures, headache, and focal neurological deficits, and may lead to long-term neurological sequelae such as epilepsy, hydrocephalus, and dementia. The pleomorphism of NC makes its

diagnosis impossible on clinical grounds alone. An accurate diagnosis is possible only after suspicion on epidemiologic grounds, proper interpretation of clinical data, and synthesis of findings on neuroimaging studies and specific immunologic tests of the cerebrospinal fluid (Carpio *et al.*, 2005)

A widely accepted classification system based on the viability and location of the parasite in the central nervous system (CNS) is presented below:

- Active, when the parasite is alive
- Transitional, if it is in the degenerative phase
- Inactive, if evidence of its death is apparent

Each viability category is subdivided into parenchymal and extraparenchymal forms. On the basis of this classification, relating clinical manifestations to each category of the proposed classification is possible. No definitive data exist regarding the duration of individual stages. Anecdotal evidence indicates that, once the parasite lodges in the brain, it may remain viable from months to years. The transitional phase lasts 4-6 months. Finally, the dead parasite is resorbed or it calcifies (Carpio *et al.*, 2005).

II.10 Diagnosis of taeniasis

Correct identification of *Taenia* spp is important because the consequences of human infection by the different species are very different.

II.10.01 Parasitological diagnosis of taeniasis:

- *Coprological examination:* Microscopic identification of eggs and proglottids in human faeces is the most common diagnostic tool for taeniasis. But this is not possible during the first 3 months following infection, prior to development of the adult tapeworm. Nevertheless, speciation of *Taenia* is impossible if solely based on microscopic examination of eggs, because all *Taenia* species produce eggs that are morphologically identical (Acha & Szyfres, 2003).

- *Morphological examination*: Differential diagnosis between the adult stage of *T. solium* and *T. saginata* is based mainly on morphological criteria on fixed and stained proglottids, mostly based on the number of uterine branches present in well preserved gravid proglottids, or on the presence or absence of hooklets in the scolex of the tapeworms (Mayta *et al*., 2000). However, overlapping between the numbers of uterine branches of these species has been described; it is also relatively uncommon to obtain a whole adult tapeworm with an intact scolex following treatment. Recently, Jeri *et al.* (2004) improved the treatment method to obtain a recognizable tapeworm, making differentiation between *T. solium* and *T. saginata* easier. Proglottids can be stained with the Semichon's acetocarmine stain method to allow morphological differentiation. *T. saginata* and *T. s. asiatica* are difficult to differentiate based on morphological characteristics. However, according to Eom & Rim (1993), the presence of posterior protuberances in gravid proglottids is a useful characteristic for the diagnosis of *T. s. asiatica*.

II.10.02 Immunodiagnosis

II.10.02.01 Coproantigen detection

Immunological methods have been developed for the diagnosis of *Taenia* spp infection. The best available diagnostic assay for taeniasis is the coproantigen detection ELISA. It has a high sensitivity and specificity; however, this test does not provide species-specific diagnosis and there is a potential biohazard of collecting and handling faeces from *Taenia* carriers (Allan *et al.*, 2003).

II.10.02.02 Serological diagnosis of intestinal T. solium infection

The possibility of diagnosing *T. solium* taeniasis by the detection of species-specific circulating antibodies has been demonstrated. This test, in an EITB format, using excretory-secretory derived antigens from non-gravid T. *solium* tapeworms was determined to be 95% sensitive and 100% specific (Wilkins *et al.*, 1999). Its molecular range from 32.7 to 42.1 kDa appeared to be specific for *T. solium* taeniasis infection. This serological test offers the possibility to overcome some of the problem that remain with coproantigen testing. It avoids the potential biohazard of collecting faeces and also offers the possibility of diagnosing both stages of *T. solium* infecting man on a single serum sample, when combined with other immunodiagnostic techniques for cysticercosis, (Allan *et al.,* 2003).

II.11 Diagnosis of cysticercosis

II.11.01 Detection of subcutaneous nodules

By clinical examination, biopsy and radiography subcutaneous nodules can be detected. Ophthalmoscopy contributes to the diagnosis of ocular cysticercosis. However, these tests can only be performed on patients with suspected cysticercosis, while, there is a need to detect the infection as early as possible.

II.11.02 Imaging diagnosis

Infection by the metacestodes can be detected by neuroimaging techniques (CT-scan, or/and MRI) of brain cysts. The diagnosis of NC without imaging studies is difficult because of the highly variable clinical symptoms, ranging from asymptomatic to a very severe neurological syndrome with intracranial hypertension or dementia, with a variety of non-specific mild clinical symptoms in between. CT-scan and MRI have been useful in the study of the evolution of the cysticercus within the brain parenchyma. MRI is more useful than CT-scan in detecting intraventricular and subarachnoidal cysts, as well as the accompanying signs of cyst degeneration and pericystic inflammatory reaction. However, CT-scan is preferred for detection of parenchymal calcifications. These techniques are expensive and frequently inaccessible to people of (hyper)-endemic areas (Sciutto *et al.*, 2000).

II.11.03 Serological tests

II.11.03.01 Enzyme-Linked Immunoelectro Transfer Blot (EITB)

The EITB was described originally by Tsang *et al*. (1989). A mix of glycoprotein antigens, purified by lentil-lectin chromatography, is separated on a gel and transferred to nitrocellulose

paper. The diagnostic bands (GP50, GP42, GP24, GP21, GP18, GP14, GP13) become visible after addition of the species-specific conjugate and the substrate. The EITB was reported to be 100% specific and from 70 to 90% sensitive (Tsang *et al.*, 1989). But according to Wilson *et al*., (1991) a sensitivity of only 28% has been found in cases with single cysts in brain. The EITB assay is more sensitive than the ELISA, especially when serum is being tested (Proaño-Narvaez, 2002).

II.11.03.02 Enzyme-linked immunosorbent assay for antibody detection (Ab-ELISA)

Infection with *T. solium* results in a specific antibody response such as IgG, IgM, IgA and IgE, however, the IgG antibody is more common than other immunoglobulins (Dorny *et al*., 2003). The antigens used in this test are either cyst fluid or crude homogenates of *T. solium* cysticerci. The major components of the *T. solium* metacestode glycoproteins are now well-recognized as hydrophobic ligand-binding proteins. To date, these glycoproteines are the best candidate antigens for detecting specific antibody responses to *T. solium* in infected patients. Antibody assay is suitable for detecting cases of active cysticercosis with and without a history of epilepsy (Ito & Craig, 2004). The limitation of these tests is that antibodies may be detected in a certain proportion of individuals who do not have active disease with calcified lesion. Thus, the presence of antibodies does not constitute direct evidence of a living parasite within the host (Correa *et al*., 2002). The antigen mixture used in the EITB is not applicable for Ab-ELISA because of the presence of non-specific fractions. With potentials as better availability, simplicity, specificity and lower cost compared with EITB, the ELISA test is preferred in developing countries (Rosas *et al.*, 1986; Dorny *et al*., 2003).

II.11.03.03 Enzyme-linked immunosorbent assay for antigen detection (Ag-ELISA)

Specific monoclonal antibodies directed against cysticercus antigen are used in a sandwich ELISA to determine antigen in sera or cerebrospinal fluids. The Ag-ELISA is very specific, and does not cross-react with other parasites (Erhart *et al*., 2002; Dorny *et al.*, 2004).

II.12 Molecular approaches

Nowadays, several Polymerase Chain Reaction (PCR)-based assays for detection and species identification of *Taenia* spp eggs, proglottids and larval material from human and porcine tissues, have been described. Their evaluation has shown these assays to be reliable, sensitive and specific. The PCR can be coupled to restriction fragment length polymorphism (RFLP), which can give more information to distinguish the taeniid species, mutations and help in the identification of "new" species. When infection with *T. solium* is not confirmed by histopathological examination, this molecular diagnosis will be more useful for definitive diagnosis (Yamasaki *et al*., 2005; 2006)

Most studies have focused on the differentiation of *T. solium* from *T. saginata*. Recently, a method based on the thymine-base using the cytochrome c oxidase subunit I (CoxI) and cytochrome b of mitochondrial genes has been developed for comprehensive differential diagnosis of *T. saginata, T. s. asiatica*, and two geographic different genotypes (American and Asian) of *T. solium* in a multiplex PCR (Yamasaki *et al*., 2002)

Flisser *et al.* (1988) used total genomic DNA in a specific DNA hybridization assay based on radioactive and biotinylated DNA for detecting *T. saginata* eggs. In similar experiments, Chapman *et al*. (1995) developed *Taenia* sp. specific probes of *T. solium* and *T. saginata* and used them for *Taenia* eggs with high sensitivity and specificity. The multiplex-PCR and PCR-RFLP protocols were designed by González *et al*. (2000; 2002; 2004) from *T. saginata* HDP2

DNA sequence; these have immediate potential for the specific, sensitive, and rapid identification of *T. s. asiatica.*

Meri *et al.* (1999) designed *T. solium*-specific primers to amplify part of the known 18S ribosomal RNA gene in a PCR assay for cysticercosis. Mayta *et al.* (2000) used PCR-RFLP to differentiate *T. solium* and *T. saginata*. They amplified the 3' region of the 18S and the 5' region of the 28S ribosomal gene (encompassing the 5.8S ribosome gene) and used 3 restriction enzymes (either AluI, DdeI or MboI) for analysis of the PCR amplicons. Each enzyme gave a unique pattern for each species. In this assay, the primers amplified DNA from all cestodes, not only from *Taenia* spp.

In recent years, PCR tests using the cytochrome *c* oxidase subunit I and cytochrome *b* genes as targets for species-specific confirmation of *Taenia* spp have been developed based on the detection of the parasite DNA in faecal samples (copro-DNA), or on cysticerci (Yamasaki *et al*., 2002; 2004) or eggs present in the faeces (Yamasaki *et al*., 2004).

Jardim *et al.* (2006) used primers from the conserved regions of the large subunit ribosomal RNA gene sequence of taeniids. Their study was conducted to evaluate the species-specific identification of *T. saginata* and *T. solium* by PCR and duplex-PCR assays using total DNA extracted from cysticerci and proglottids from both parasites.

Eom *et al*. (2002) amplified the ITS2 region and the complete 5.8S rDNA, using primers from conserved sequences of the 5' end region of 5.8 S and 28S rDNA. The result showed that there is sufficient polymorphism in the ITS2 sequence to distinguish *T. s. asiatica* from *T. saginata*. In this study, random amplified polymorphic DNA (RAPD) assay was used also to amplify the ITS2 region with success to differentiate human *Taenia* species. But this is a cumbersome test that has appeared difficult in getting reproducible results.

Nickisch-Rosenegk *et al.* (1999a) identified the sequence of the mitochondrial 12S rDNA gene, which is frequently used for phylogenetic studies, because it is specific for 21 cestodes species of eight families. A fragment of 311bp of the 12S rDNA, specific for the genus *Taenia*, was amplified by two highly specific primers (Nickisch-Rosenegk *et al.*, 1999b). However, these authors reported 50% sequence homology and 30% sequence conservation among the species. This degree of variability was considered suitable for phylogenetic comparisons in *Taenia*.

Rodriguez-Hidalgo *et al*. (2002) improved the PCR-RFLP assay using the 12S rDNA fragment. The DdeI was used as restriction enzyme to differentiate between *T. solium* and *T. saginata* by developing new primers to reduce non-specific amplification experienced when using field samples.

Nguyen (2004) evaluated the 12S rDNA PCR-RFLP assay for differentiation of *Taenia* spp. in human. The use of Hpy8I and DdeI as restriction enzymes proved to enable to distinguish *T. solium*, *T. saginata*, and *T. s. asiatica*.

II.13 Treatment

II.13.01 Anthelmintic treatment

Human taeniasis may be controlled by detection and treatment of tapeworm carriers or by treatment of the whole population (WHO, 1983). In regions where taeniasis is only due to *T. saginata* and human cysticercosis does not occur, praziquantel can be used. For treatment of human taeniasis in *T. solium* endemic areas bithionol, niclosamide or praziquantel may be used, however, niclosamide is preferred because it is highly effective against the intestinal stage of the parasite and has no effect on the cystic stage (WHO, 1983; Miyazaki, 1991; Allan *et al*., 1997). Mass treatment of the human population may be performed in areas where *T. solium* infection is endemic (Allan *et al*., 1997).

Treatment of cysticercosis is difficult and yields varying success. The drugs used are praziquantel and albendazole; both drugs can be combined. Often antiparasitic treatment is combined with corticosteroids to reduce inflammatory reactions. In some cases surgical removal of the cysts is the only option. In case of epilepsy specific treatment of NC has to be complemented with the use of anti-epileptic drugs (Carpio *et al*., 2005).

II.13.02 Surgical Care

Surgical treatment should be restricted to removal of the parasite located in the subarachnoid (racemose form) or ventricular area, and to ventriculoperitoneal shunting for the treatment of decompensated hydrocephalus. Surgery should not be considered for parenchymal cysts without regard to location, size, or stage of evolution, because this form of NC can be controlled only by symptomatic treatment (or presumably by etiologic treatment). In addition, surgical sequelae could result in more brain damage than the parasite itself. Transitional or degenerative cysts, regardless of their size or location should not be biopsied or removed since the parasite is dead and will disappear or be calcified spontaneously (Carpio *et al.*, 2005).

II.14 Prevention and control of taeniasis/cysticercosis in man

Prevention is based on strict meat inspection, health education, cooking pork and beef well, hygiene, and widespread sanitary installations. Health education may play an important role by helping to change public awareness of food safety (eating habits needed for thorough cooking of pork) and personal hygiene practices (toilet use, hand-washing) and to promote the self-diagnosis of *T. saginata* infection as well as to improve sanitation, animal husbandry system and meat inspection in slaughterhouses (Cao *et al.*, 1997; Acha & Szyfres, 2003). Control measures for cysticercosis consist of interrupting the chain of transmission of the parasite at any of the following intervention points: the production of eggs by an infected person, the dissemination of the eggs to the environment, the ingestion of the eggs by the intermediate host, the development of the cysticercus in the intermediate host, and the dissemination of the cysticerci to the definitive host (Schantz, 1993). Community cooperation is very important for any intervention to achieve sustainable control (WHO/FAO/OIE, 2004). Vaccination of pigs against *T. solium* to control human NC was investigated by Gonzalez *et al.*, 2005. According to these authors with this strategy as an additional tool to control *T. solium* transmission, the goal of disease eradication should be considered achievable. But it still seems a realistic preventive measure to be implemented in the near future (Krauss, 2003).

Chapter III Materials and methods

III.01 Study area

The study was conducted in Hagiang, a highland province in the North-East of Vietnam (318 km from Ha Noi). It is bordered by Cao Bang, Tuyen Quang, Lao Cai, and Yen Bai provinces and shares a border of 274 km with China. The total surface of the province is 7 831.1 km^2, including 130 435 ha (21%) of cultivated land. This province is centered on the town of Hagiang itself and is divided into 10 administrative districts (figure 1 and table I), which are each subdivided in several communes. These are the smallest administrative units. The area has two distinct climatic seasons (rainy and dry seasons) with average temperature between 24 and 28°C; the lowest temperature can reach -5°C on high altitude.

Table II: 10 districts and their population of Hagiang province, Vietnam.

Districts	Population
Dong Van	59 931
Meo Vac	61 542
Yen Minh	69 653
Quan Ba	41 325
Bac Me	42 321
Hoang Su Phi	55 412
Vi Xuyen	88 638
Xin Man	51 791
Bac Quang	104 932
Quang Binh	55 252
Hagiang town	42 634
Total	**673 431**

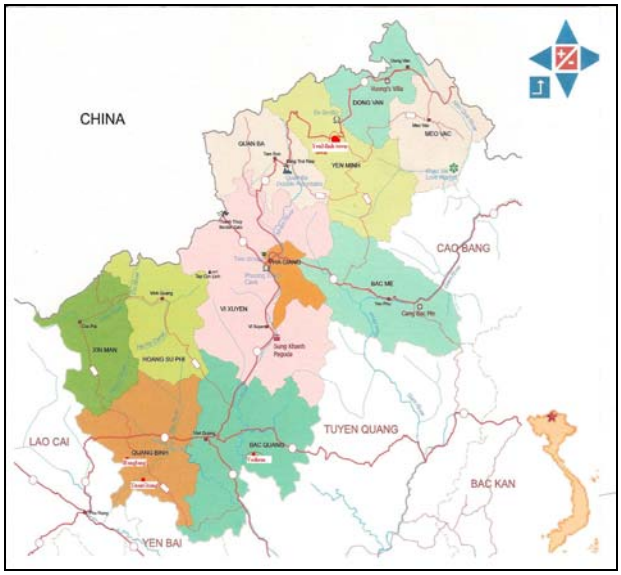


Figure 1: Hagiang province map

Hagiang counts 673 431 inhabitants (table II) including Viet/Kinh, Cao Lan, Dao, H'mong, Chinese, Nung, San Diu, Giay, Tay and some other ethnic groups. It is one of the poorest provinces of Vietnam. Traditionally, the vast majority of its economic activity revolved around agriculture and forestry, but in recent years, there have been attempts to establish a manufacturing industry. Infrastructure in Hagiang has seen improvement, but remains poor. Roads, schools, and health services are less developed than in other parts of Vietnam (http://en.wikipedia.org/wiki/Ha_Giang_province; http://www.gso.gov.vn; Vietnam Administrative Atlas, 2003).

III.02 Experimental design

The study sites were selected based on previous reports of clinical cases of cysticercosis from the Clinical Department of NIMPE.

The selection of participants was based on data of a helminthiasis survey carried out in 4 communities (Yen Minh, Bac Quang, Quang Binh, Hagiang town) of Hagiang province from

10 – 20 January 2005 by cooperation between NIMPE and the Centre of Malaria disease control (CMDC) of Hagiang province. That survey was based on the participation of volunteers, man and women of more than 5 years old, from 4 districts (Bac Quang, Quang Binh, Yen Minh, Hagiang town) of the province.

The inhabitants from that survey that had suspected Taeniasis or cysticercosis (by reporting a history of having seen proglottids in their stools, having eaten meat with cysticerci, having observed subcutaneous nodules and/or having reported to be epileptic) were informed and invited to participate in the study by the communal health services. These volunteers were clinically and coprologically examined, blood sampled, and submitted to a questionnaire in March and May 2005 at the community health centers by NIMPE and Hagiang's CMDC staff.

The protocol of this study was approved by the IRB (Institutional Review Board) of NIMPE, Hanoi. Informed consent was received from all village leaders and People Committees after explaining the study objectives and methodology. Selected individuals were informed about the purpose of the study and invited to be part of the survey some days in advance, but were free to refuse. Other people not selected but showing symptoms of disease at the time of the survey were examined and treated accordingly but were not included in the survey database.

III.03 Sample collection

III.03.01 Stool sampling

Approximately 2 grams of stool samples were collected from consenting individuals in plastic bags in January 2005. When the material for collecting stool samples was distributed, participants were carefully instructed about adequate hygiene to avoid contamination, and were given toilet paper and soap. A microscopic examination for the presence of *Taenia* and nematode eggs was done on the same day of collection of stool samples at the community health centers.

III.03.02 Blood sampling

Approximately 5 ml of venous blood was drawn from the volunteers. The samples were kept in cool boxes and transported to the laboratory of the Parasitological Department in NIMPE, Ha Noi. Blood samples were allowed to clot and then centrifuged to obtain serum which was aliquoted in two sets of 1.5 ml vials and stored at -20°C. They were sent to the Department of Animal Health, the Institute for Tropical Medicine in Antwerp (ITM), Belgium to be assayed by Ag-ELISA in March 2006.

Individuals with a positive result for *Taenia* eggs by coprological examination and/or a positive serological test will be revisited and offered a visit to NIMPE to confirm their disease status by CT-scan or MRI. The clinician will decide on whether these patients should be hospitalized and given anti parasitic treatment.

III.03.03 Proglottids and tapeworms

Following a positive result in the coprological examination, these persons were treated with 15 mg/kg praziquantel (600mg table, Shinpoong-Korea) and a laxative (Magnesium sulphate, 30 gram) and monitored during 3 days at the health unit of their community for recovery of

tapeworm fragments in the stools. The recovered worms and proglottids were fixed in ethanol 70% and sent to the Department of Animal Health of ITM, Belgium in March 2006 for molecular identification.

III.04 Questionnaire survey

The questionnaire form for inhabitants included personal information (code, interview date, full name, age, gender, address, and occupation) and simple, direct questions in local terms about history of passing tapeworm proglottids, headaches, seizures, epilepsy, and about habits related to infectious taeniasis and cysticercosis, such as culinary habits (eating of raw pork, undercooked beef, blood pudding) and history of eating infected pork (Annex 3).

III.05 Diagnostic tools

III.05.01 Coprological examination

In order to determine the presence of eggs of *Taenia* spp. and other helminth eggs faecal materials were examined with the Kato thick smear method following the recommendations of WHO (1991). Approximately 0.5 g faecal material was filtered and put on a slide (75 x 25 mm) and then covered by a cellophane paper slice (25 x 35 µm), which was impregnated into Malachite blue solution (0.3% w/v Malachite blue and glycerin 50% v/v). Next, thick smears were made using a rubber cork with a flat face. These slides were kept at room temperature about 30-60 minutes before being analysed by microscopy.

Morphological identification of *Taenia* and other helminth eggs in faecal material was done according to the guidelines of the WHO (1994).

III.05.02 Enzyme-linked immunosorbent assay for antigen detection (Ag-ELISA) of cysticercosis

The Ag-ELISA test was performed as described by Brandt *et al*. (1992) and improved by Dorny *et al.* (2000). In this test, pre-treatment of serum samples was done using trichloroacetic acid (TCA) to break up immune complexes. The serum was mixed with TCA (1/1) and incubated for 20 min at ambient temperature, after which it was centrifuged for 9 min at 12 000 rpm (Eppendorf 5415D). Following centrifugation, the supernatant was collected and mixed with neutralisation buffer (1/1).

The plate (Maxisorp F96 4394454) wells were coated with 100 µl Mab (monoclonal antibody) B158C11A10 0.0172v/v. After incubation during 30’ at 37°C on a shaker the plate was washed once with PBS-T (0.05% v/v Tween 20 in phosphate buffered saline). All the wells were blocked with 150 µl of blocking buffer (1% v/v new born calf serum in phosphate buffered saline) and incubated for 15’ at 37°C while shaking. Pre-treated serum samples were added to the wells and after shaking for 15’ at 37°C the plate was washed 5 times with PBS-T. The next step consisted of adding 100 µl of diluted Mab B60H8A4 0.0011 v/v-BIOT to each well and incubation for 15’ at 37°C on a shaker. The plate was washed 5 times with PBS-T. Then 100 µl of diluted streptavidin-HRP (Jackson) (1/1000) was added to the well and again incubated for 15’ at 37°C on a shaker, followed by washing 5 times with PBS-T. Colour development was done with OPD substrate solution (100µl/well) at room temperature in the dark. Colour reaction was stopped after 15’ by adding 50 µl of H_2SO_4 (4N) to each

well. Finally, the optical densities were measured at 490 / 655 nm with an ELISA reader (Thermo labsystems multiskan ex model 335)

Each ELISA run included 8 negative reference sera and 2 positive reference sera (Nguekam *et al.*, 2003). The cut-off value was determined by comparing the optical density (OD) of each sample with the mean of the series of 8 negative reference samples using a modified Student's T test (Sokal & Rohlf, 1981) at a probability level of P = 0.001. 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 positive.

III.06 Molecular biology

III.06.01 Extraction of DNA

The DNA extraction protocol was slightly modified from Boom *et al.* (1990). The segments were cut into 1-3 mm^3 pieces and transferred into a 1.5 ml tube. 250 µl of the lysis buffer 2X (consists of 60 mM Tris-HCl, pH 7.4, 60 mM EDTA, 10% Tween, 5 mM MgCl2, 1% Triton X-100, and 1.6 M Gu-HCl), 250 µl of RO-DI water (ITM, Antwerp, Belgium) and 50 µl of Proteinase K (Sigma, P2308) were added into the tube. Samples were incubated in a thermoblock (Eppendorf, Thermomixer Compact) overnight at 56^0C and shaken at 1400 rpm. Then, 40 µl of diatomaceous earth suspension (Sigma, D5384) was added, mixed and incubated at 37^0C for one hour while shaking at 900 rpm. The suspension was centrifuged at 20,800*g* for 20 seconds and the supernatant discarded. The pellet was washed with 900 µl of 70% ethanol (v/v) at 4^0C and centrifuged at a speed of 15,300 rpm for 20 seconds, next the supernatant was discarded. This washing step was done twice. Then, the pellet was washed with acetone, centrifuged and the supernatant was discarded. Subsequently, the pellet was dried in the "thermoblock" at 50^0C for 20 minutes and 90 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8) was added to the pellet and incubated for 20 minutes at 60^0C while shaking at 1000 rpm in the "thermoblock". Finally, 50 µl of the supernatant was transferred to a new PCR tube after centrifuging at 20,800*g* for 40 seconds, and stored at -20^0C. This tube was used in PCR assays.

III.06.02 Polymerase Chain Reaction (PCR)

III.06.02.01 Primers

The primers (table III) were based on comparison of the conserved areas of the 12S rDNA fragments from *T. saginata*, *T. s. asiatica* and *T. solium;* these were obtained from the Genbank. The following primers were chosen because it was found that these would hybridise universally with all *Taenia* species. Specificity of the primers against all DNA sequences in the Genbank is checked by using the nucleotide-nucleotide BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/).

The thoretical length of PCR products, obtained by the different pair primers are shown in table III.

Table III: The length of PCR products obtained with primer pairs annealing at specific temperatures

Primers	PCR products	Temperature	Developed by
ITMF/ITMTnR	373 bp	61°C	Rodriguez-Hidalgo *et al*. (2002)
TAEnF/ITMTnR	898 bp	57°C	Rodriguez-Hidalgo *et al*. (2002)
nTAE/ITMTnR	796 bp	57°C	Geysen *et al.* (2006)
TsolFip/ITMTnR	1113 bp	50°C	Geysen, ITM., pers.comm
LampF3/ITMTnR	1124 bp	50°C	Geysen, ITM., pers.comm
LampF3/LampB3	395 bp	50°C	Geysen, ITM., pers.comm
TaenF2/LampB3	215 bp	50°C	Geysen, ITM., pers.comm
TaenF/LampB3	169 bp	50°C	Geysen, ITM., pers.comm
nTAE/LampB3	64 bp	50°C	Geysen, ITM., pers.comm

III.06.02.02 Amplification by nested PCR

A set of primers (table III) was used for the amplification of the PCR products of the conserved areas of the 12S rDNA fragments from *T. saginata*, *T. s. asiatica* and *T. solium.* PCR was performed using 5µl of the extracted DNA as template and 20 µl of PCR solution containing 1 µl YellowSub^{TM} 12.5 µl of PCR-buffer (20 mM Tris-HCl, pH 8.4, 100 mM KCl, 0.2%v/v Triton X-100, 3.3 mM $MgCl_2$), 3.3 µl of RO-DI water, 2 µl of a mix of the 4 deoxynucleotide triphosphates 2.5mM (dNTP, final concentration 0.2 mM), 0.4 µl of each primer and 0.4 µl of *Taq*-polymerase (50 U/µl, Eurogentec, Searing, Belgium). *T. crassiceps* DNA was used as the positive control and RO-DI water was used as the negative control. The PCR conditions for the primary PCR were as follows: the DNA was denatured at 92°C for 4 min then followed by 35 cycles of 92°C for 45 sec, 61, 57, 55 or 50°C for 45 sec and 72°C for 1 min. The final elongation was performed at 72 °C for 8 min. The success of the amplification was confirmed by running the PCR product on a 2% w/v agarose gel in TAE 0.5x for 20 min. The gel was placed in an electrophoresis chamber (Mupid-21 system UL® Cosmo Bio Co) for 20 minutes at 100 V. A DNA size marker was included on each gel (Gene Ruler™, MBI Fermentas #SM0241). The gel was then stained in 0.1% w/v Ethidium bromide in TAE 0.5x solution for 30 min. The result was visualised under ultraviolet light and a picture was taken by digital camera and analyzed by Photostudio software (http://www.arcsoft.com/products/photostudio/).

III.06.03 Restriction Fragment Length Polymorphism (RFLP)

III.06.03.01 Restriction enzymes

DdeI enzyme (10U/µl, Biolabs) were chosen to distinguish between *T. solium* and *T. saginata* and HinfI (10 U/ µl, New England BioLabos® Inc) were chosen to distinguish between *T. saginata* and *T. s. asiatica* according to the protocol described by Rodriguez-Hidalgo *et al*. (2002) and Nguyen (2004).

Theoretically, the target sequences obtained with ITMF/ITMTnR and TAE/ITMTnR primer pairs should be cut in bands of the following length (bp) (table IV and V, respectively).

Table IV: Theoretical band lengths (bp) of the amplicons obtained with ITMF/ITMTnR primers and digested with restriction enzyme DdeI

Taenia species	DdeI
T. solium	325, 46
T. saginata	164, 158, 46, 5
T. s. asiatica	165, 161, 46

Table V: Theoretical band lengths (bp) of the amplicons obtained with nTAE/ITMTnR primers and digested with restriction enzyme HinfI

Taenia species	HinfI
T. solium	613, 290
T. saginata	613, 299
T. s. asiatica	316, 299, 207

III.06.03.02 Restriction

Four µl of the amplified DNA was placed in a tube containing 11 µl of the enzymatic solution (consisting of 9.2 µl of RO-DI water plus 1.5 µl buffer NB3 and 0.3 µl of restriction enzymes (HinfI or DdeI) and put in an incubator at 37^0C overnight for digestion. Samples were loaded onto a 10% polyacrylamide gel. Electrophoresis was performed at 100 V for 2h40. A DNA size marker was included on each gel (Superladder 100bp, Eurogentec, MW-0310-04). The gel was stained for 40 minutes with Sybr Green 0.01% v/v. The picture and further analysis was done as described for agarose gels.

III.06.04 Cloning and sequencing

The 395 bp PCR product was purified using the Invisorb® Spin PCRapid Kit (Invitek GmbH, Berlin). The recovery of the PCR product after purification was determined by running 2 µl of the purified product on a 2% agarose gel. The purified amplicon was cloned into a plasmid vector using the Topo TA Cloning kit (Invitrogen). Cloning was performed as described by the manufacturer. Briefly, 2 µl of the amplicon was used in a ligation reaction at room temperature for 30 min. Two µl of the ligation reaction product was used to transform 50 µl TOP10 F' *E. coli* competent cells. The cells were then put on ice for 20 min and heat-shocked at 42°C for 30 sec, followed by cooling on ice for 2 min. The cells were then incubated at 37 °C for 1 hr before 50 µl was spread on LB agar plates containing 0.33 ml X-gal (40mg/ml) in dimethylformamide, 0.5 ml ampicillin (50mg/ml) and 0.2 ml IPTG (100mM) per 100 ml LB agar. The plates were then incubated at 37 °C overnight. Blue and white screening was used to identify clones containing the insert. Colony PCR was used to screen ten colonies for recombinants with the desired insert. The recombinants of interest were sent to the Genetic Service Facility, Antwerp University for sequencing. The M13 forward (5'-GTAAAACGACGGAGT-3') and M13 Reverse (5'-CACACAGGAAACAGCTATGACCAT-3') primers were used for sequencing.

III.07 Statistical analysis

Databases were analyzed using SPSS 11.5 version. Direct age and sex standardization was used to compare frequency among individuals. Associations among categorical variables are tested by χ^2 and among continuous variables by Student's t test and Correlation. A series of random effects logistic regression models was fitted to evaluate the presence of in-house

clustering of seropositive cases (by estimating the cluster-level variance as a proportion of the total variance) and the contribution of selected covariates. All confidence intervals (CIs) are at the 95% level.

Chapter IV Results

IV.01 Questionnaire survey

IV.01.01 Age and sex distribution of individuals in the study

The questionnaire survey was done on a total of 103 individuals. The mean age of the interviewed persons was 39.6 ± 13.6 years (range 69, minimum 6, and maximum 75). Age distribution in the study is shown in table VI. More than 50% of the interviewed persons were in the 30-44 years age category. There was an uneven distribution in female/male ratio 29(28%)/74(72%).

Table VI: Age distribution in the study

Age groups	Number	Rate (%)
0->14	5	4.85
15->29	14	13.59
30->44	52	50.49
45->59	22	21.36
>=60	10	9.71

IV.01.02 General results from the questionnaire survey

The 103 individuals in this study came from Yenminh town of Yen Minh (32), Vo Diem of Bac Quang (24), Xuan Giang (12), Xuan Dinh (5), and Bang Lang (27) of Quang Binh and Hagiang city (3) (Annex 1).

The participants of this study consisted of farmers (69,9%), health service workers (6.8%), students (5.8%), policemen (5.8%), staff of people committee (4.9%) and other professions (6.8%).

The survey on eating habits indicated that most people had eaten food made from raw/rare pork (77.7%), rare beef (76.7%), and blood pudding (75.7%). In this study area it appeared that people have quite a good knowledge on taeniasis and cysticercosis: the number of persons who declared to know about the disease, to have seen porcine cysticercosis, and to know about the origin of proglottids were 64% (66/103), 64% (66/103), and 48.5% (50/103), respectively. Among the interviewed persons, 89.3% (92/103) people have pigs in their house and 13.6% (14/103) had seen lesions attributed to cysticercosis in their pigs (Annex 1).

Sixteen individuals (15.3%) reported to suffer from late onset epilepsy. Twenty-three persons reported that they had or have subcutaneous nodules. Thirty-nine participants declared to have a history of passing proglottids. Thirty-six individuals did not declare to have any of the three symptoms that are indicative for taeniasis/cysticercosis (epilepsy, subcutaneous nodules, and history of passing proglottids).

IV.02 Stool examination

A total of 84 stool samples could be collected from the 103 individuals. Among these 77 (91.7%) were positive for eggs of at least one parasite species. Eggs of *Taenia* spp. were found in 5 samples (6.0%). The human roundworm *Ascaris lumbricoides* was the most common parasite (65.5%) followed by hookworms (39.3%) and *Trichuris trichuira* (23.8%).

Fasciolopsis buski was found in 1 sample. A total of 34 and 3 samples contained eggs of two or three parasites, respectively. The results of the stool examinations are presented in table VII.

Table VII: Results of the faecal examinations

Parasite	No.	%
at least one parasite	77/84	91.7
at least two parasites	34/84	40.5
at least three parasites	3/84	3.6
Taenia spp.	5/84	6.0
Ascaris lumbricoides	55/84	65.5
Trichuris trichuira	20/84	23.8
Hookworms	33/84	39.3
Fasciolopsis buski	1/84	1.2

IV.03 Results of treatment

To date three persons from this study received a cestodicidal treatment with praziquantel, among these was one person (HG29) that was positive on stool examination. The other two persons (HG19 and HG30) that were treated were negative on stool examination but had reported to pass proglottids in their stools. .Tapeworm fragments could be recovered in the stools collected post treatment of all these persons following the therapeutic expulsion. Only one proglottid was recovered from the stools of HG29.

IV.04 Ag-ELISA results

A total of 103 blood samples were taken but only 97 samples could be processed in the Ag-ELISA. Among these, 13 samples (13.4%) gave a positive result. The mean ratio of the positives was 1.88 ± 21.19 (range 76.4, minimum 1.1, and maximum 77.5).

All of the positive samples in this study were from males, none from females. There was a strong correlation between sex and positivity in the Ag-ELISA ($\chi 2 = 5.8$, $p = 0.016$) (Figure 2). The mean age of the positives was 37.00 ± 16.8 years (range 56, minimum 14 and maximum 70) (Annex 2).

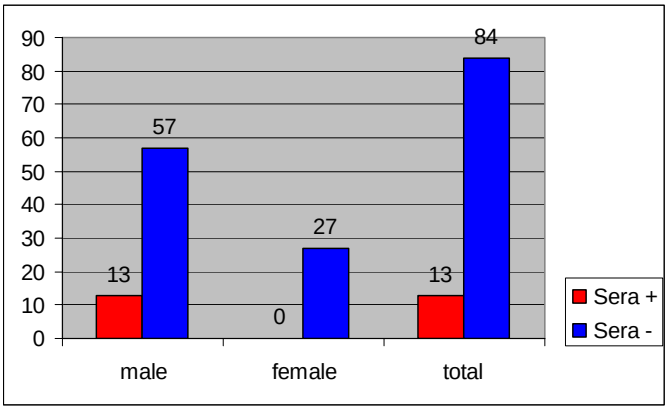


Figure 2: Relationship between sex and serological result by Ag-ELISA

IV.05 Associations between results of stool examination, Ag-ELISA and questionnaire survey

Among the 16 individuals that reported to suffer from late onset epilepsy, two were positive in the Ag-ELISA. In one of these persons taeniasis was identified by stool examination. Among the 23 individuals reporting the presence of subcutaneous nodules, three were positive in the Ag-ELISA while one of these was positive for *Taenia* eggs In the group of 39 that had declared to have a history of passing proglottids, four were positive for *Taenia* eggs on stool examination, while 6 were positive in the Ag-ELISA. Among the 36 participants without symptoms suggesting taeniasis or cysticercosis one was positive for Taeniasis on stool examination and in the Ag-ELISA; four other cases were positive in the Ag-ELISA (Table VIII).

Table VIII: Results of stool examination for taeniasis and Ag-ELISA for cysticercosis according to disease history*.

Symptoms	Stool examination		Ag-ELISA	
	Positive	Negative	Positive	Negative
Epilepsy (16 cases)	1	15	2	14
Subcutaneous nodules (23 cases)	1	22	3	20
History of passing proglottids (39 cases)	4	35	6	33
No any symptom* (36 cases)	1	35	5	31

* The individuals that did not declare to have any of these three symptoms are not included in this table.

Seropositivity in the Ag-ELISA was similar in the persons declaring that they had eaten undercooked meat and those who did not (Figure 3).

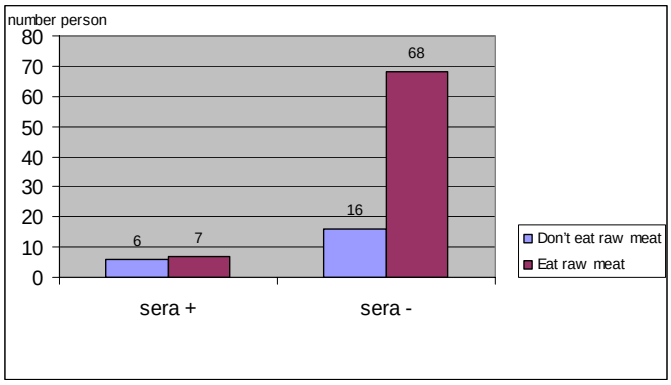


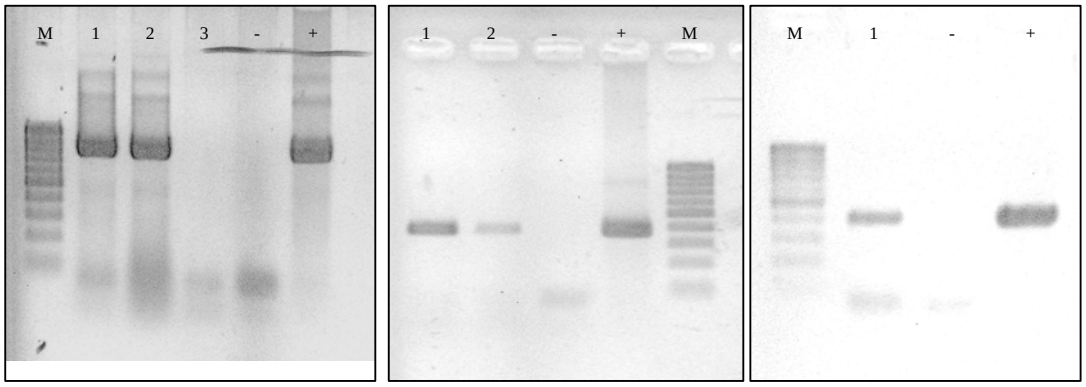
Figure 3: Relationship between habit of eating undercooked meat and serological result by Ag-ELISA

IV.06 Molecular results

IV.06.01 PCR results

While nTAE/ITMTnR primers detected *Taenia* DNA in HG19 and HG30 samples, giving a fragment of approximately 800bp (Figure 4.a), no signal was obtained with the HG29 using the same primers (Figure 4.a). Because of this negative result PCR using other primer pairs were done. No signal was obtained using primer pairs primer pairs TAEnF/ITMTnR,

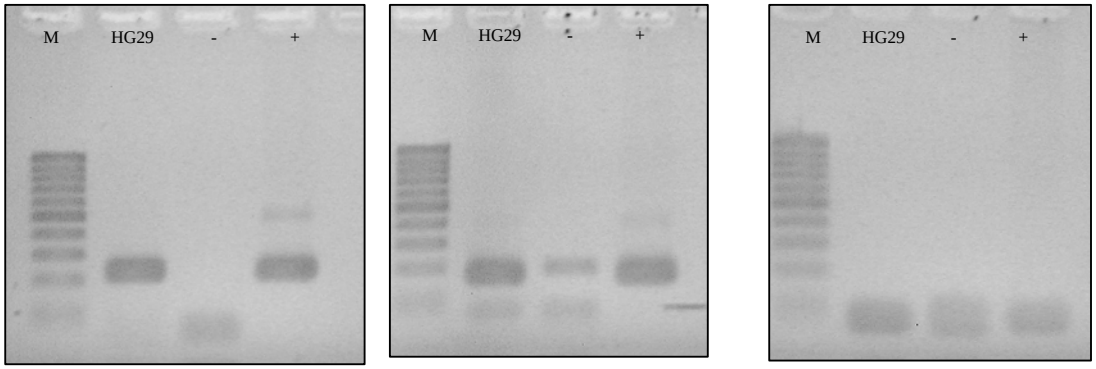
TAEnF2/ITMTnR, TsolFip/ITMTnR and LampF3/ITMTnR. However, using the ITMF/ITMTnR (Figure 4.b) and LampF3/LampB3 (Figure 4.c) primers *Taenia* DNA could be detected in the HG29 sample; the molecular size of the amplified product was approximately 380bp and 400bp, respectively.



(4.a) (4.b) (4.c)

Figure 4: PCR products from the proglottid samples by amplification of nTAE/ITMTnR primers (4.a), ITMF/ITMTnR primers (4.b), and LampF3/LampB3 primers (4.c). Analysis is performed on a 2% agarose gel and stained with ethidium bromide. lanes M: DNA sizer 1000bp marker ; lanes +: *T. crassiceps* profile as positive control ; lanes -: negative control ; lanes 1, 2 in (4.a): profile of *Taenia* spp. approximately 800bp (HG19, HG30); lane 3 in (4.a): negative for *Taenia* spp. (HG29); lanes 1 (HG29 ratio 1/1) and 2 (HG29 ratio 1/10) in (4.b) and lane 1 in (4.c): profile of *Taenia* spp. with molecular size of approximately 380bp and 400bp, respectively.

Also, with the TaenF2/LampB3, TaenF/LampB3, nTAE/LampB3 primers (Figure 5.a, 5.b, and 5.c) DNA from *Taenia* could be detected in the HG29 sample; the molecular size of the amplified product was approximately 169 bp, 216 bp, and 69 bp, respectively.



(5.a) (5.b) (5.c)

Figure 5: PCR products from the proglottid of HG29 sample by amplification of TaenF2/LampB3 (5.a), TaenF/LampB3 (5.b), nTAE/LampB3 (5.c) primers. Analysis is performed on a 2% agarose gel and stained with ethidium bromide. Lanes M: DNA sizer 1000bp marker; lanes +: *T. crassiceps* profile as positive control; lanes HG29 in (5.a, 5.b, and 5.c): profile of *Taenia* spp. with molecular size approximately 169 bp, 216 bp, and 69 bp, respectively.

Finally, with the TaenF/ITM nTR primer pair (Figure 6) DNA from *Taenia* could be amplified in the HG29 sample. However, the molecular size of the amplified product was approximately 1300 bp, which is longer than the 900bp to be expected on samples from *T. solium, T. saginata* and *T. s. asiatica.*

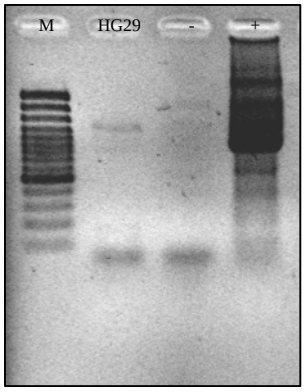


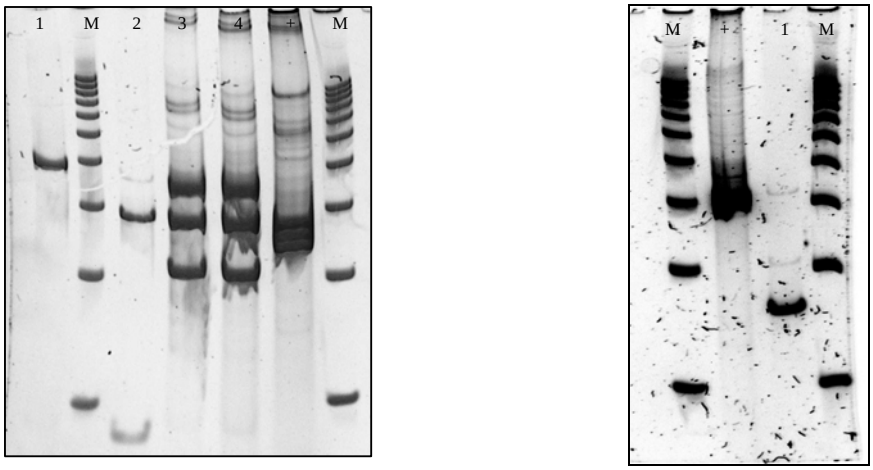
Figure 6: PCR products from the proglottid of HG29 sample by amplification of TaenF/ITMTnR primers by long Taq polymerase. Analysis is performed on a 2% agarose gel and stained with ethidium bromide. Lane M: DNA sizer 1500bp marker; lane +: *T. crassiceps* profile as positive control; lane -: negative control

IV.06.02 RFLP results

Enzymatic restriction with HinfI of the amplicons from the nTAE/ITMTnR PCR on the HG19 and HG 30 samples gave bands of 316, 299 and 207 bp (Figure 7.a) corresponding with *T. s. asiatica* (Somers *et al*., 2006b).

Enzymatic restriction with HinfI of the amplicons from the ITMF/ITMTnR PCR on the HG29 sample gave bands of 299, 81 bp (Figure 7.a). It was not possible to make a distinction of this sample between *T. solium, T. saginata* and *T. s. asiatica*.

Figure 7.b shows the Ddel enzymatic digestion of the HG29 sample obtained with the ITMF/ITMTnR PCR giving a band approximately 160 bp. No distinction can be made between *T. saginata* and *T. s. asiatica*.



(7.a) (7.b)

Figure 7: RFLP profiles following digestion of 12S rDNA amplicons with HinfI (7.a) and Ddel (7.b) on a polyacrylamide gel. Lanes M: DNA size marker; lanes +: T. crassiceps profile as positive control; In 7.a: lanes 1: HG29 sample isolate amplicon by LampB3/LampF3 primers, band approximately of 400bp; lane 2: HG29 sample isolate amplificon by ITMF/ITM TnR primers bands of 299, 81bp; lanes 3, 4: profile of T. s. *asiatica* (HG19, HG 30), amplicon by nTAE/ITM TnR primers at bands of 316, 299, 207 bp. And in 7.b, lane 1: HG29 sample isolate amplicon by ITMF/ITM TnR primers at band approximately of 160bp

IV.06.03 Result of HG29 sample's sequencing

The sequencing result of the 392 bp PCR product from the proglottid of the HG29 sample by amplification with LampF3/LampB3 primers showed 2 nucleotides difference compared with the sequence of *T. s. asiatica* (at 9194 (C-T) and 9244 (G-T)) in the mitochondrial 12S rDNA gene. This gives a homology ratio of 99.5%. There was a 7 nucleotides difference compared with the mitochondrial 12S rDNA gene of *T. saginata* (Genbank at 65 (C-T), 94 (A-T), 105 (G-T), 199 (A-G), 203 (G-A), 213 (G-A) and 344 (A-G)) giving a homology ratio of 98.2%.

Chapter V Discussion

This study reports on a survey on taeniasis and cysticercosis in a selected group of inhabitants from a mountainous province in North Vietnam. The present study sites were selected because of low living standards of the population and because this province was reported to be one of the areas in the country with high endemicity of *T. solium* (Willingham *et al.*, 2003). The socio-economic situation in this province is among the poorest in the country and this may be conductive for the transmission of taeniasis and cysticercosis. Indeed, at these study areas the use of latrines was found to be very limited and the habit of feeding pigs free around the house very common. Moreover, pork is mostly sold uninspected on the communal market. The consumption of traditional dishes containing raw pork and blood pudding made from fresh blood of pigs, is considered to be another major risk factor for acquiring taeniasis in Vietnam (De *et al*., 1999 & 2001; Dorny *et al*., 2004a). Our study revealed that a high rate of interviewers had the habit of eating undercooked pork, rare beef and blood pudding (80%, 79% and 78%, respectively). In addition, 13,6% (14/103) of the interviewed persons admitted to have eaten pork containing cysticerci.

We used a monoclonal antibody-based sandwich ELISA for serological diagnosis of cysticercosis (Brandt *et al.*, 1992; Dorny *et al.*, 2002 & 2004a; Erhart *et al.*, 2002). The Ag-ELISA was originally designed to detect the excretory-secretory products of viable cysticerci of *T. saginata* in cattle (Brandt *et al.*, 1992), but appeared to also detect circulating antigen of *T. solium* in pigs (Dorny *et al.*, 2002) and human (Erhart *et al.*, 2002). The specificity of this assay was shown to be very high (Erhart *et al.*, 2002) and the Ag-ELISA was able to identify patients with only two viable cysticerci and four cysts with ring enhancement in the brain (Nguekam *et al.*, 2003), demonstrating a high sensitivity too. In this study we detected 13.4% (13/97) seropositive cases by Ag-ELISA. We repeat that in this survey the samples were not randomly taken from the population but from a group of individuals that was suspected for taeniasis/cysticercosis because they had a reported history of passing proglottids and/or subcutaneous nodules and/or a reported history of seizures. This may explain why the percentage of positives in our survey is higher than that of other studies in some mountainous provinces in the northern Vietnam (5.3% in Backan province (Somers *et al.*, 2006a), 7% in Yen Bai province (De *et al.*, 2003) and 5.7% in BacNinh province (Erhart *et al.*, 2002)).

There were more sero-positives by Ag-ELISA among the males than in female participants in this study ($\chi^2 = 5.8$, $p = 0.016$), suggesting that males were more at risk for cysticercosis in this area than females. This result agrees with other studies in Vietnam (Willingham *et al*., 2003; De *et al.*, 2001; Somers *et al.*, 2006a) but not with the study of Erhart *et al.*, 2002. The reason for this higher percentage of positives in males is unknown but might be related to higher exposure risks. There were no significant differences in infection rates between individuals who declared not to eat undercooked meat (raw pork, rare pork, rare beef and blood pudding) and individuals who consumed these dishes. According to Nguyen, 1996 more than 57% of cysticercosis patients in Vietnam are in the age class between 26 and 45 years and no cases were recorded in age group less than 15 years. However, in our study there was one seropositive case in a 14 years old (male) while the 12 other cases were between 22 to 70 years old.

In this study, clinical signs, such as nervous symptoms, subcutaneous nodules, and seizures were found in 8.7%, 22.3%, and 11.7% of the samples, respectively. Here again we repeat that these rates are higher than in other studies (Willingham *et al*., 2003; De *et al.*, 2001;

Somers *et al.*, 2006a; and Erhart *et al.*, 2002) because this was not a random sample but a sample from a selected group for suspected taeniasis or cysticercosis.

The results of the examination of gastro-intestinal nematode infection showed high infection rates with *Ascaris lumbricodes* (65.5%), hookworms (39.3%) and *Trichuris trichiura* (23.8%). These figures are in agreement with the studies of Van der Hoek *et al.* (2003) and Verle *et al*. (2003) and suggest a very high environmental contamination with soil-transmitted nematodes in Vietnam.

Taenia eggs were found in the stools of five individuals; among these one person was also positive in the Ag-ELISA, suggesting self- or auto-infection. Microscopic examination was reported to underestimate the prevalence. In our study this hypothesis was confirmed as the treatment of two persons who reported to pass proglottids in their stools resulted in the expulsion of tapeworms while the coprological examination was negative.

Based on the molecular tests performed on the three *Taenia* samples that were collected from our survey in Hagiang province, two of these could be identified as *T. s. asiatica* by PCR-RFLP. The identification of the third sample (HG29) was more cumbersome. After having performed several PCR and RFLP assays with several primer pairs and restriction enzymes, respectively, we were not able to identify the species of this sample. By DdeI enzymatic restriction of the amplicons from the ITMF/ITMTnR PCR a band of 160 bp suggested that HG29 was not a *T. solium* parasite. The primers, nTAE/ITMTnR were designed to amplify a conserved gene area of *Taenia* spp, with a 800 bp fragment. However this test could not detect the fragment in the HG29 sample. Therefore, other primer pairs, TaenF/ITMTnR, were used in several PCR on the HG29 sample. It appeared that the molecular size of the amplified product was approximately 1300 bp, while a product of approximately 900bp would be theoretically expected for *Taenia* spp. This DNA product was cloned and sent for sequencing, the result showed that it had a sequence resembling a *T. s. asiatica* sequence (percentage homology 99.5%) and not *T. saginata* (homology ratio 98.2%). It was concluded that the sample HG29 belongs to *T. s. asiatica* and appears to have a gross alteration in its sequence with the insertion of a 400 bp fragment.

The results of species identification of the recovered *Taenia* samples are rather unexpected. According to Willingham *et al.* (2003) northern Vietnam is considered to be endemic for *T. solium*, but in our study *T. solium* was not found. Our result suggests that in the study areas both *T. solium* and *T .s. asiatica* are endemic. In Vietnam, the first *T. s. asiatica* case was reported and confirmed by molecular analysis by De *et al.* (2001) and Le *et al.* (2002). In a recent study on 65 *Taenia* samples Somers *et al*. (2006b) detected *T. s. asiatica* in 55.4% of the samples. However, according to Willingham *et al.* (2003), *C. viscerotropica* has until now never been detected at slaughter, neither in pigs nor in cattle. Therefore, the finding of a high proportion of Asian *Taenia* in studies in Vietnam is puzzling. *T. s. asiatica* is thought to be transmitted only through the consumption of liver and visceral organs, and not through the muscle of pigs (Eom & Rim, 2001; Eom, 2006). According to Ito & Craig (2004) *T. s. asiatica* is endemic in remote areas where local people eat undercooked minced pork with fresh blood and viscera. While the habit of eating raw meat, either pork or beef is very common in northern Vietnam (Willingham *et al.*, 2003; Somers *et al.*, 2006a), liver and visceral organs are usually well cooked before consuming.

Whether or not *T. s. asiatica* can cause human cysticercosis has been a matter of debate among scientists. In some countries that are considered endemic for *T. s. asiatica* (i.e. Taiwan

and Indonesia) the number of patients with human cysticercosis or NC is very low suggesting that cysticercosis in human is not attributable to *T. s. asiatica* (Galan-Puchades & Fuentes, 2000; Ito *et al.*, 2003). In northern Vietnam, human cysticercosis is relatively common, with prevalence of more than 5% of the population (Erhart *et al.* (2002) in Bacninh; Willingham *et al.* (2003) in Yenbai; Somers *et al.* (2006a) in BacKan). A hospital-based study indicated that the most common clinical signs of human cysticercosis in North Vietnam are the presence of subcutaneous nodules and neurological symptoms (Nguyen *et al.*, 2003). In addition, taeniasis is also common among cysticercosis patients (Willingham *et al.*, 2003). It is remarkable that cysticercosis in Asia manifests as both NC and subcutaneous cysticercosis (Ito *et al.*, 2003), while subcutaneous cysticercosis is rarely reported in Africa and South America. Molecular analysis of subcutaneous cysts should be done to confirm that these are caused by *T. solium* in all cases and to rule out that *T. s. asiatica* might be involved. In our study the three tapeworm carriers were identified to be infected with *T. s. asiatica* but none of these individuals showed clinical signs (neurological or subcutaneous cyst) apart from a history of passing proglottids. That result agreed by Galan-Puchades & Fuentes (2000) that there is no clear scientific data to support that human cysticercosis can be caused by *T. s. asiatica.*

Chapter VI Conclusions and recommendations

The results of this study conducted on a group of persons that showed symptoms suggestive for taeniasis and cysticercosis confirmed that these infections are common in the surveyed area. Risk factors for acquiring taeniasis/cysticercosis are presented, however, in our small survey these factors were not found to be significant. The relatively low sero-prevalence of circulating cysticercus antigens in the individuals who declared to have subcutaneous nodules or epilepsy, suggest that other causes for these symptoms are present. A case-control study to measure the proportion of NC patients within the group of epileptics should be conducted. It must also be mentioned that symptomatic NC usually occurs when the cysts start to degenerate and that this coincides with a decrease in circulating antigens. Therefore, it is possible that the proportion of NC cases among the epileptics is higher than found by antigen detection. Neuroimaging and/or antibody detection would be more appropriate to confirm this. Finally, the finding of only *T. s. asiatica* in the *Taenia* samples is intriguing. However, it is impossible to draw any conclusions on prevalence and epidemiology of *Taenia* species based on 3 samples only. More samples should be examined. The failure of the routinely practiced PCR on one *Taenia* sample was unexpected. It shows the high genetic variation within the *Taenia* species.

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Annex

Annex 1: Results of the questionnaire surveys

	Communities	**Districts**	**No. of participants**
Study sites	Vo Diem	Bac Quang	24
	Xuan Giang	Quang Binh	12
	Bang Lang	Quang Binh	27
	Xuan Dinh	Quang Binh	5
	Yen Minh sub-town	Yen Minh	32
	Hagiang city		3
	Total		**103**
colume name	**available**	**No.**	**%**
Job	farmer	72	69.90
	butcher	1	0.97
	health services worker	7	6.80
	student	6	5.83
	trader	2	1.94
	teacher	2	1.94
	policeman	6	5.83
	Staff of people committee	5	4.85
	driver	1	0.97
	retired	1	0.97
Do you have habit of eating raw pork?	yes	80	77.67
rare pork?	yes	80	77.67
rare beef?	yes	79	76.70
blood pudding?	yes	78	75.73
Do you know about Cysticercosis/Taeniasis			
	Yes	66	64.08
	heard about it	8	7.77
Have you ever seen swine cysticercosis?			
	Yes	66	64.08
	no answer	1	0.97
Do you have any pigs in your house?			
	Yes	92	89.32
	no answer	1	0.97
Have your pigs ever had cysticercosis?			
	Yes	14	13.59
	don’t answer	2	1.94
When did your pigs have cysticercosis?			
	now	1	0.97
	5 year ago	3	2.91
	10 year ago	5	4.85
	no answer	5	4.85
Have you ever eaten cysticercus infected pork?			
	Yes	14	13.59
	no answer	4	3.88
Do you know if you eat cysticercus pork you will be infected taeniasis/cysticercosis?			

	Yes	6	5.83
	heard about it	3	2.91
	no answer	7	6.80
Do you know about the proglottids?			
	Yes	50	48.54
	heard about it	0	0.00
	no answer	6	5.83
Have you ever found proglottids in your faeces?			
	No	50	48.54
	Yes	39	37.86
	I don't remember	10	9.71
	no answer	4	3.88
When did you find proglottids in your faeces?			
	1-2 year ago	10	25.64
	3-5 year ago	13	33.33
	6-10 year ago	2	5.13
	over 10 year ago	9	23.08
	don't remember	3	7.69
	no answer	2	5.13
Do you have late onset epilepsy?(in adult age)			
	No	87	84.47
	Yes	16	15.53
	no answer	0	0.00
How is your epilepsy?	It is a seizure	12	11.65
	or convulsion	4	3.88
Clinical signs		9	8.74
	headache	4	
	lisp	2	
	headache, less reflex, lisp	1	
	no information	2	
Subcutaneaous nodules?	positive	23	22.33
	negative	80	77.67

Annex 2: Results of the questionnaire survey in relation to results of the Ag-ELISA for cysticercosis and stool examination including molecular diagnotis of Taeniasis

Code	Age	Sex	Occupation	Community	Ag-ELISA Ratio	HE	HP	ES	SCN	Pigs*	*Taenia* eggs	PCR
HG7	37	male	farmer	Xuan Giang	1.54	a, b, c	+	-	0	+	Neg	ni
HG13	55	male	farmer	Xuan Giang	1.88	a, b, c	+	+	0	+	Neg	ni
HG14	14	male	student	Xuan Giang	25.41	-	**	-	+	+	Neg	ni
HG15	53	male	Staff of people committee	Xuan Giang	13.31	a, b, c	+	+	+	+	Neg	ni
HG28	36	male	farmer	Vo Diem	77.49	a, b, c	-	+	+	+	Neg	ni
HG37	41	male	farmer	Vo Diem	8.13	a, b	+	+	0	+	Neg	ni
HG40	27	male	farmer	Vo Diem	1.64	a, b, c	-	-	0	+	Neg	ni
HG43	61	male	farmer	Yenminhtown	1.45	-	-	-	0	+	Neg	ni
HG46	22	male	policeman	Yenminhtown	2.99	a, b, c	-	-	0	-	Pos	ni
HG47	24	male	policeman	Yenminhtown	1.08	a, b, c	-	-	0	+	Neg	ni
HG48	51	male	farmer	Yenminhtown	3.54	-	+	-	0	+	Neg	ni
HG61	31	male	farmer	Yenminhtown	1.63	-	+	-	0	+	Neg	ni
HG60	70	male	healthy service	Yenminhtown	1.50	-	-	-	0	+	Neg	ni
HG19	38	male	authourity worker	Xuan Giang	0.34	a, b, c	+	-	-	+	Neg	*T s. asiatica*
HG29	36	female	farmer	Xuan Giang	0.62	a, b, c	+	-	-	+	Pos	*T.s. asiatica &* gross-alteration
HG30	42	male	farmer	Xuan Giang	0.26	a, b, c	+	-	-	+	Neg	*T. s. asiatica*

HE: Habit of eating undercooked meat
RP: Reported history of passing proglottids
ES: Epilepsy symptoms
SCN: Subcutaneous nodes
Pigs*: Have pigs in house
Neg: Negative
Pos: Positive

+: yes
-: no
**: don't concentrate
*** : for this sample to clone and sequence of DNA
a: raw pork
b: undercook beef
c: blood pudding

Annex 3: Questionnaire of Taeniasis/Cysticercosis survey

For interview:

1. Personal information:

Code (list number and code of the field): [/]
Date(yy/mm/dd): _____ /____./_____
Full name:……
Sex: 1 = male; 2 = female [ ]
Age: (birth year): [ ]
Address: (province, district, community, village) [ ], [ ], [ ], [ ]
Job: [ ]
No = 0; Farmer = 1; Butcher = 2; health service = 3; other = 4
if your job is 4, please answer:……

2. Information related to transmission Taeniasis/Cysticercosis:

2.1 Do you have habit of eating raw pork?
0 = No; 1 = Yes; 2 = Don't remember; 9= Don't answer [ ]
If your answer is Yes, please go to 2.2 question
2.2 Which have you eaten undercook meat?
Rare pork: 0 = No; 1 = Yes; 2 = Don't remember; 9= Don't answer [ ]
Middle beef: 0 = No; 1 = Yes; 2 = Don't remember; 9= Don't answer [ ]
Blood pudding: 0 = No; 1 = Yes; 2 = Don't remember; 9= Don't answer [ ]
2.3 Do you have any knowledge (understanding) on Cysticercosis? [ ]
0 = No; 1 = Yes; 2 = heard about it; 9= Don't answer
2.4 Have you ever seen any swine cysticercosis?
0 = No; 1 = Yes; 2 = Don't remember; 9= Don't answer [ ]
2.5 Do you have any pigs in your house?
0 = No; 1 = Yes: 2= Don't answer [ ]
2.6 Have your pigs ever had any cysticercosis?
0 = No; 1 = Yes; 2 = Don't remember; 9= Don't answer [ ]
If your answer is Yes, please go to let me When?.....
2.7 Have you ever eaten cysticercus infected pork ?
0 = No; 1 = Yes; 2 = Don't remember; 9= Don't answer [ ]
If your answer is Yes, please go to let me When?.....
2.8 Do you know if you eat cysticercus pork you will be infected cysticercosis?
0 = No; 1 = Yes; 2= heard about it; 9= Don't answer [ ]
2.9 Have you known the proglottids?
0 = No; 1 = Yes; 2= heard about it; 9= Don't answer [ ]
2.10 Have you ever seen the proglottids in your faeces?
0 = No; 1 = Yes; 2 = I did not pay attention; 9= Don't answer [ ]

3. Information related to Taeniasis/Cysticercosis:

3.1 Do you have the lateral epilepsy (in adult age)?
0 = No; 1 = Yes; 9 = Don't answer [ ]
If your 3.1 answer is yes, please go to 3.2, 3.3 and 3.4 questions
3.2 When did you know about your epilepsy? [ ]
1 = for 1-2 year ago; 4 = over 10 year before;
2 = for 3-5 year ago; 5 = I don't remember;
3 = for 6-10 year ago; 6 = don't answer
3.3 How often do you have epilepsy?

Times per year:.....
Times per month, ...
Times per week,.....
Times per day.....

3.4 How is your epilepsy? *(click your answer)*

a. It is a seizure [ ]

b. Your convulsions are only the part of body [ ]

If it is the (b) answer, what part of the body is it?.....

From now for doctor doing:

3.5 nervous system examination: Normal = 1; Abnormal = 2 [ ]

3.6 If Abnormal, writing is very clear the clinical signs.....
.....

3.7 Subcutaneous nodes, positive or negative?

0 = negative; 1 = positive [ ]

3.8 If 3.7 is positive, how many are there? [ ]

4. Parasitological test:

4.1 Stool Examination before treatment: 0 = No; 1 = Yes [ ]

The selected stool date: (yy/mm/dd).....

Tapeworm eggs: 0 = negative; 1 = positive [ ]

Other parasite eggs: [ ]

0 = negative; 1 = *Ascaris lumbricoides*; 2 = *Trichuris trichiura*; 3 = Hook worm; 4= *Clonorsis*; 5= *Giardia*; 6= Fasciola sp; 7=other;

4.4 Treatment (Praziquantel 15mg/kg) 0 = No; 1 = Yes [ ]

The date of treatment :(yy/mm/dd).....

Defecated proglottids: 0 = No; 1 = Yes [ ]

Collected proglottids: 0 = No; 1 = Yes [ ]

Stored samples [ ]

(0= undeveloped proglottids; 1= developed proglottids; 2= both)

4.5 Collected blood sample (3ml): 0 = No; 1 = Yes [ ]

The date doing (yy/mm/dd):

4.6 Ag-ELISA result:

(- = negative; + = possitive T-test [ ]) [ ]

Name and signature:

Interviewer:

Stool tester:

Serum tester:

CT-scan reader:

5. Final result: Is this person Taeniasis or /and Cysticercosis?

1 = Taeniasis; 2 = Cysticercosiss; 3 = 1+2 [ ]

Thank for your attention!

Annex 4: List of the individuals in the study and results of Ag-ELISA and stool examination.

Code	Village	Commune	District	Age	Sex	Worm	Serol_result	*Taenia* egg
HG1	Trung	Xuan Giang	Quang Binh	63	1	ni	negative	0
HG2	Trung	Xuan Giang	Quang Binh	35	2	ni	negative	0
HG3	Trung	Xuan Giang	Quang Binh	27	1	ni	negative	0
HG4	Tinh	Xuan Giang	Quang Binh	56	1	ni	negative	+
HG5	Trung	Xuan Giang	Quang Binh	40	1	ni	negative	0
HG6	Quyen	Xuan Giang	Quang Binh	39	2	ni	negative	+
HG7	Trung	Xuan Giang	Quang Binh	37	1	ni	positive	0
HG8	Kieu	Xuan Giang	Quang Binh	48	1	ni	ni	0
HG9	Trung	Bang Lang	Quang Binh	42	1	ni	negative	0
HG10	Trung	Xuan Giang	Quang Binh	12	1	ni	negative	0
HG11	Trung	Xuan Giang	Quang Binh	66	1	ni	negative	0
HG12	Tinh	Xuan Giang	Quang Binh	60	1	ni	negative	0
HG13	Trung	Xuan Giang	Quang Binh	55	1	ni	positive	0
HG14	XuanMoi	Xuan Giang	Quang Binh	14	1	ni	positive	0
HG15	Trung	Xuan Giang	Quang Binh	53	1	ni	positive	ni
HG16	Trung	Xuan Giang	Quang Binh	59	1	ni	negative	+
HG17	Then	Xuan Giang	Quang Binh	58	2	ni	negative	0
HG18	Dung	VoDiem	Bac Quang	44	1	ni	negative	0
HG19	Dung	Vo Diem	Bac Quang	38	1	*T. s. asiatica*	negative	0
HG20	Thia	Vo Diem	Bac Quang	43	1	ni	negative	0
HG21	Thia	Vo Diem	Bac Quang	46	1	ni	negative	0
HG22	Thia	Vo Diem	Bac Quang	40	1	ni	negative	0
HG23	Dung	Vo Diem	Bac Quang	17	1	ni	negative	ni
HG24	Ka	Vo Diem	Bac Quang	32	1	ni	negative	0
HG25	Dung	Vo Diem	Bac Quang	40	2	ni	negative	0
HG26	Dung	Vo Diem	Bac Quang	53	1	ni	negative	0
HG27	Dung	Vo Diem	Bac Quang	41	1	ni	negative	0
HG28	thia	Vo Diem	Bac Quang	36	1	ni	positive	0
HG29	Dung	Vo Diem	Bac Quang	36	2	*T.s.asiatica?*	negative	+
HG30	Thia	Vo Diem	Bac Quang	42	1	*T. s. asiatica*	negative	0
HG31	Thia	Vo Diem	Bac Quang	10	1	ni	negative	0
HG32	MeHa	Vo Diem	Bac Quang	31	1	ni	negative	ni
HG33	Dung	Vo Diem	Bac Quang	23	2	ni	negative	ni
HG34	Ka	Vo Diem	Bac Quang	39	1	ni	negative	ni
HG35	Thia	Vo Diem	Bac Quang	45	1	ni	negative	0
HG36	Thia	Vo Diem	Bac Quang	45	1	ni	negative	0
HG37	Dung	Vo Diem	Bac Quang	41	1	ni	positive	0
HG38	Dung	Vo Diem	Bac Quang	32	1	ni	negative	0
HG39	Ka	Vo Diem	Bac Quang	43	1	ni	negative	0
HG40	Dung	Vo Diem	Bac Quang	27	1	ni	positive	0
HG42	4 group	HaGiangtown	HaGiangtown	75	1	ni	negative	ni
HG43	NaMa	Yenminhtown	Yen Minh	61	1	ni	positive	0
HG44	3 group	Yenminhtown	Yen Minh	48	1	ni	negative	0
HG45	3 group	Yenminhtown	Yen Minh	39	1	ni	negative	0
HG46	policeman	Yenminhtown	YenMinh	22	1	ni	positive	+
HG47	policeman	Yenminhtown	Yen Minh	24	1	ni	positive	0

HG48	NaQuang	Yenminhtown	Yen Minh	51	1	ni	positive	0
HG49	health center	Yenminhtown	Yen Minh	31	1	ni	negative	ni
HG50	3 group	Yenminhtown	Yen Minh	32	2	ni	negative	0
HG51	3 group	Yenminhtown	Yen Minh	43	2	ni	negative	0
HG52	3 group	Yenminhtown	Yen Minh	41	2	ni	negative	0
HG53	5 group	Yenminhtown	Yen Minh	26	1	ni	negative	0
HG54	2 group	Yenminhtown	Yen Minh	48	1	ni	negative	0
HG55	NaRuou	Yenminhtown	Yen Minh	43	2	ni	negative	0
HG56	NaRuou	Yenminhtown	Yen Minh	43	2	ni	negative	0
HG57	NaCang	Yenminhtown	Yen Minh	42	1	ni	negative	0
HG58	2 group	Yenminhtown	Yen Minh	40	1	ni	ni	0
HG59	4 group	Yenminhtown	Yen Minh	28	1	ni	negative	0
HG60	KhuonAng	Yenminhtown	Yen Minh	70	1	ni	negative	0
HG61	PhacNghe	Yenminhtown	Yen Minh	31	1	ni	positive	0
HG62	KhuonAng	Yenminhtown	Yen Minh	50	1	ni	positive	0
HG63	4 group	Yenminhtown	Yen Minh	50	1	ni	negative	0
HG64	NaQuang	Yenminhtown	Yen Minh	30	2	ni	ni	0
HG65	NaQuang	Yenminhtown	Yen Minh	31	2	ni	negative	0
HG66	NaQuang	Yenminhtown	Yen Minh	37	2	ni	negative	0
HG67	NaQuang	Yenminhtown	Yen Minh	11	1	ni	negative	0
HG68	health center	Yenminhtown	Yen Minh	29	1	ni	negative	0
HG69	NaTen	Yenminhtown	Yen Minh	36	2	ni	negative	0
HG70	NaRuou	Yenminhtown	Yen Minh	63	1	ni	negative	0
HG71	3 group	Yenminhtown	Yen Minh	24	1	ni	negative	ni
HG72	2 group	Yenminhtown	Yen Minh	38	1	ni	negative	ni
HG73	health center	Yenminhtown	Yen Minh	41	2	ni	negative	0
HG74	group 22	HaGiangtown	HaGiangtown	6	1	ni	negative	ni
HG80	BacNgan	HaGiangtown	HaGiangtown	23	1	ni	negative	ni
HG81	KhuayThe	Bang Lang	Quang Binh	37	1	ni	negative	0
HG82	PhuThe	Bang Lang	Quang Binh	33	1	ni	negative	0
HG83	PhuThe	Bang Lang	Quang Binh	41	1	ni	negative	0
HG84	KhuThun	Bang Lang	Quang Binh	54	2	ni	negative	0
HG85	KhuThun	Bang Lang	Quang Binh	54	2	ni	negative	ni
HG86	KhuThun	Bang Lang	Quang Binh	52	1	ni	negative	0
HG87	KhuThun	Bang Lang	Quang Binh	35	1	ni	negative	0
HG88	KhuayThe	Bang Lang	Quang Binh	32	1	ni	negative	0
HG89	Trung	Bang Lang	Quang Binh	40	1	ni	negative	ni
HG90	Trung	Bang Lang	Quang Binh	36	2	ni	ni	ni
HG91	Trung	Bang Lang	Quang Binh	38	2	ni	negative	0
HG92	Trung	Bang Lang	Quang Binh	36	1	ni	negative	0
HG93	TienYen	Bang Lang	Quang Binh	29	2	ni	negative	ni
HG94	Na	Bang Lang	Quang Binh	17	2	ni	negative	0
HG95	Trung	Bang Lang	Quang Binh	46	2	ni	negative	0
HG96	Trung	Bang Lang	Quang Binh	47	1	ni	negative	0
HG97	Trung	Bang Lang	Quang Binh	51	2	ni	negative	0
HG98	Trung	Bang Lang	Quang Binh	51	2	ni	negative	0
HG99	Trung	Bang Lang	Quang Binh	36	1	ni	negative	0
HG100	Khun	Bang Lang	Quang Binh	35	2	ni	negative	0

HG101	Trung	Bang Lang	Quang Binh	28	2	ni	negative	0
HG102	Trung	Bang Lang	Quang Binh	64	1	ni	negative	ni
HG103	Khun	Bang Lang	Quang Binh	60	2	ni	negative	0
HG104	Khun	Bang Lang	Quang Binh	30	1	ni	negative	ni
HG105	Trung	Bang Lang	Quang Binh	32	1	ni	negative	ni
HG106	Na	Bang Lang	Quang Binh	31	2	ni	negative	0
HG107	Trung	Bang Lang	Quang Binh	32	1	ni	negative	ni
HG108	Dung	Vo Diem	Quang Binh	36	1	ni	ni	0
HG109	NaQuang	Yenminhtown	Yen Minh	73	1	ni	ni	0

ni: no infromation

PERSONAL INFORMATION (C.V)

FULL NAME: NGUYEN THU HUONG Female

NATIONALITY: Vietnamese

DATE OF BIRTH: October 14th, 1976 in Hanoi, Vietnam

MAIN DIPLOMAS: Diploma General Medical Doctor
From 1994 to 2000
Hanoi Medical University, Hanoi, Vietnam

PROFESSIONAL EXPERIENCE:

Teaching:	20 %
Management:	20 %
Research:	30 %
Disease control program:	30 %

MAIN FIELD OF EXPERTISE:

I participate in helminthic researches (epidemiology, diagnosis, treatment) and the parasitic diseases control program in Vietnam. Beside, I am involved in implementation management and monitoring of the National Parasitic Diseases Control Program

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